Multivariate analysis to discriminate species of phlebotomine sandflies. (Diptera: Psychodidae) *Lutzomyia cayennensis* and *Lutzomyia yencanensis*

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Abstract

Following multivariate analysis, ihe morphometric differentiation among males ofLutzomyia yencanensis and L. cayennensis, is described. By means of principal component analysis, canonical discriminant analysis and cluster analysis, it was shown that L. yencanensis and L. cayennensis are two well-defined species. In addition, by calculating canonical discriminant functions it is possible to identify precisely unknown specimens. The usefulness of the data obtained, arid its application to epidemiologial studies, is discussed. The general use of numerical taxonomy to homogenize the methodology br sandfly identification, is recommended.

Key words: Sandfly, Numerical Taxonomy

119

Med-ULA, Revista de la Facultad de Medicina, Universidad de los Andes. Vol 1 Nº 3. Mérida, Venezuela

Resumen
Análisis multivariante para discriminar especies de flebotominos (Diptera:Psychodidae). *Lutzomyia cayennensis y Lutzomyia yencanensis*

Siguiendo análisis multivariantes se describe la diferenciación morfométrica entre especimenes machos de las especies *Lutzomyia yencanensis* y *L. cayennensis*. Mediante la utilización de análisis de agrupamiento, de componentes principales y de discriminantes canónicos, se demuestra que *L. yencanensis* y *L. cayennensis* son dos especies flebotominas muy bien definidas. Se muestra que calculando la función discriminante canónica, es posible identificar de manera precisa especimenes desconocidos de estas dos especies. Se discute la utilidad de la información obtenida y su aplicación en estudios epidemiológicos, recomendándose, a su vez, el uso general de la taxonomía numérica para homogeneizar la metodología en la identificación de flebotominos vectores de leishmaniasis.

Palabras claves: Flebotominos, Taxomonía numérica.

INTRODUCTION

Lane & Ready (1985) first used the multivariate discrimination analysis in sandfly taxonomy. These authors have stated that "simultaneous manipulations of several quantitative characters by statistical techniques permit the assignment of individuals to *a priori* groups with a known probability". These analysis would increase the use of the numerical taxonomy in the identification of phlebotomine sanflies due to its greater scientific objectivity and taxonomic precision.

In leishmaniasis the correct identification of sandfly species incriminated in the transmission, is an essential factor for epidemiological studies, since the vectorial capacity of member of a species complex can vary significantly from one region to another.

Ortiz (1965) described *Lutzomyia yencanensis* as a new species after misidentifying it as *L. cayennensis* (Scorza & Ortiz, 1960; Ortiz and Scorza, 1963; Scorza et al., 1963). However, Forattini (1973) did not recognize its specific status, making it synonymous with *L. cayennensis*.

In the present paper the morphometric differentiation among males of both species is described, using multivariate analyses, in order to elucidate their taxonomic status.

MATERIALS AND METHODS

A total of 27 operative taxonomic unities (OTU) were analyzed in the present study. Nineteen males of *L. cayennensis* were captured in the locality of Cases (1,000 m.a.s. 1.), Mérida State, Venezuela. In addition 8 males of *L. yencanensis* were collected in their type locality at "Rancho Grande Station", Aragua State, Venezuela. Ah specimens were collected from tree holes. Detajis of specimen preparations arid mounting were previously described in Añez et al., (1988).

Prior to this study, 25 morphological characters were examined by principal components analysis (PCA) in order to choose those with the greatest differences between the two species. From these, 13 characters gave significant differences, including: Labrum length (1); length of wing vein sections: alpha (2), delta (3), gamma (4); genital pump length (5); genital filament length (6); style (7); coxite length (8); paramere length (9); head height (10); eye height (11); eye width (12) and interocular space (13). Measurements of these characters with means and range, are given in Table 1.

Differences between mean vectors of both groups of OTU's, were tested by multivariate analysis of variance (T² Hotteling). Based on a covariance matrix, chosen so that all charac-

120

Med-ULA, Revista de la Facultad de Medicina, Universidad de los Andes. Vol 1 Nº 3. Mérida, Venezuela

ters were measured using the same unit similar variances, the PCA was used to establish the difference among the 27 OTU's.

The discrimination among specimens of the two species was made by a canonical discriminate analysis (CDA). In addition, the similarity between each pair of OTU was determined by the Manhattan Distance Coefficient, resulting in a distance matrix of 27x27 OTU's. From these analysis a phenogram was built using a simple linkage cluster analysis, which permitted the observation of the phenetic relationships among the 27 OTU's. In order to increase the resolution of the phenetic affinities among the 27 OTU's, a shadow diagrammatic representation was made, in which the distance matrix values were transformed into percentages of similarity.

Details on the used methods have been given by several authors (Cooley & Lohnes, 1971; Sneath & Sokal, 1973; Morrison, 1967; Chatfield & Collins, 1980 and Crisci & López, 1983). Computational work for PCA, Manova and CDA, was made using the SAMULA program System developed by Márquez (1986).

Table 1.- Morphometric variation in 13 morphologic characters in males of *Lutzomya cayennensis Lutzomyia cayennensis*

Characters (*)	*Lutzomya cayennensis*			*Lutzomyia cayennensis*		
		RANGE			RANGE	
	x	min-máx	S.D.	x	min-máx	S.D.
1	0.162	0.151-0173	0.006	0.134	0.121-0.143	0.007
2	0.337	0.284-0.378	0.029	0.279	0.245-0.305	0.023
3	0.175	0.133-0.237	0.031	0.057	0.034-0.079	0.022
4	0.184	0.161-0.208	0.016	0.259	0.226-0.275	0.015
5	0.114	0.091-0.124	0.008	0.138	0.132-0.143	0.004
6	0.268	0.230-0.296	0.015	0.415	0.399-0.426	0.014
7	0.103	0.093-0.117	0.007	0.077	0.075-0.079	0.002
8	0.196	0.181-0.217	0.009	0.135	0.124-0.143	0.006
9	0.161	0.142-0.177	0.009	0.134	0.128-0.139	0.005
10	0.332	0.313-0.360	0.014	0.299	0.283-0.305	0.007
11	0.180	0.098-0.155	0.010	0.151	0.143-0.158	0.006
12	0.217	0.166-0.200	0.014	0.080	0.072-0.090	0.007
13	0.091	0.075-0.106	0.010	0.239	0.117-0.136	0.009

X= Arithmetic mean.
S.D,= Standard Deviation.
*= For carácter identification see text.
**= All measurement given in mm.

RESULTS
Principal component analysis (PCA)

PCA revealed a marked separation between individuals of L.yencanensis and L. cayennensis. Fig. 1, shows tile location of specimens in the first 3 principal components. In Table 2 the correlation indices obtaines for the 13 characters with the first 4 principal components are presented, along with autovalues and trace percentages. The highest percentage of variance was detected in

121

Med-ULA, Revista de la Facultad de Medicina, Universidad de los Andes. Vol 1 Nº 3. Mérida, Venezuela

the first component (85,8%), in which al1 the chosen characters showed highly significant correlation indices (P< 0.001).
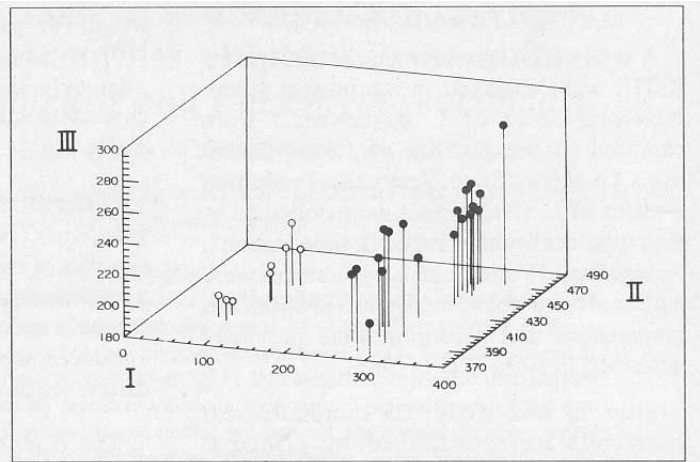

Fig. 1.— Location of individuals of *Lutzomyia cayennensis* (•) and *Lutzomyia yencanensis* (o) in the three principal components.

Table 2.- Correlation among 13 morphometric characters and the first four principal components in males of Lutzomya cayennensis and Lutzomya yencanensis.

PRINCIPAL COMPONENTS *

Character**	I	II	III	IV
1	0.91	0.02	0.11	0.28
2	0.82	0.54	-0.07	0.04
3	0.96	0.26	0.02	-0.08
4	-0.89	0.36	-0.17	0.14
5	-0.82	0.34	0.08	-0.11
6	-0.97	0.20	0.16	0.00
7	0.90	-0.04	0.21	0.27
8	0.95	-0.08	0.13	0.20
9	0.86	0.01	0.11	0.32
10	0.84	0.05	0.37	-0.19
11	0.86	-0.13	0.35	0.02
12	0.86	-0.03	0.25	0.21
13	0.89	-0.04	-0.05	-0.32
Autovalue	12695.34	1108.48	346.80	200.48
% trace	85.80	7.49	2.34	1.36

* Correlation among raw character and principal components
** For character identification see text

Canonical discriminat analysis (CDA)
Prior to CDA, a multivariate analysis of variance (T² Hotteling) revealed highly significant differences among mean   vector

(f=142,7; P 0.001), indicating that the 13 chosen characters are good for discriminating between L. yencanensis arid L. cayennensis
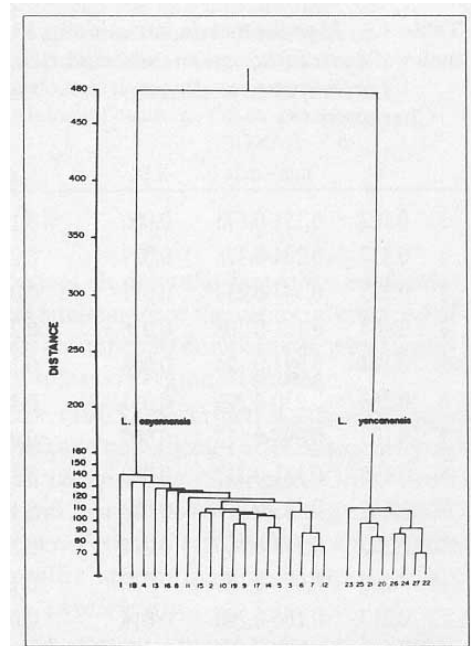

Fig. 2.— Phenogram of individuals of *Lutzomyia cayennensis* (OTU's 1-19) and *Lutzomyia yencanensis* (OTU's 20-27) resulting from the simple linkage cluster analysis.

Clusters analysis (CA)

Fig. 2 shows the results of CA, in which two well defined phenetic clusters are detected: L. cayennensis (OTLJ's 1-19) and L. yencanensis (OTU's 20 - 27). In adittion a diagramatic representation of the distance matrix, is shown in Fig. 3, which demonstrates that in temis of overalí similarity, the individuals examined in this study feil into two well separated phenetic groups.

DISCUSSION

Davis (1973) states that taxonomy is highly subjetives arid dependent upon the skills of individual taxonomist, developed through years of experience. In relation to phlebotomine sandflies vector of leishmaniasis, Ready et al., (1980) recognized that numerical taxonomy based on original data is needed to supplement classical methods of identification. However, apart from

122

Med-ULA, Revista de la Facultad de Medicina, Universidad de los Andes. Vol 1 Nº 3. Mérida, Venezuela

the results reported by Lane & Ready (1985) using multivariate discrimination, few publications have been reported.

In the present work, it was possible to perform a total and correct identification among males of *L. cayennensis* and *L. yencanensis* using only 13 morphometric characters, by following PCA, CDA and CA, respectively.
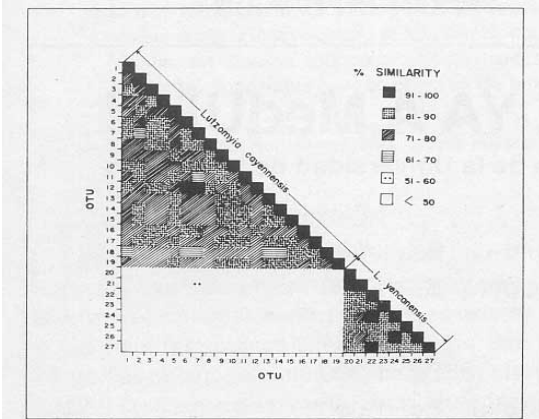

Fig. 3.— Diagramatic representation of the phenetic relationschips among males of *Lutzomyia cayennesis* (OTU's 1-19) and *Lutzomyia yencanensis* (OTU's 20-27). Based on the distance matrix.

The high significance obtained with PCA and CDA (P <0.001 and P<0.001), as well as the clear separation given by the CA and the shadowing diagrammatic representation, demonstrated that *L. yencanensis* and *L. cayennensis* are two well defined species and not synonymous as reported by Forattini (1973). In fact, the estimated discriminant functions gave a high level of accuracy in specimen identification (Table 3), showing a very high canonical correlation (0.996) and a significant discriminative power (P<0.00001).

Table 3.- Discriminates analysis

Discriminat Function	1
Eingenvalue	143.2
Relative Percentage	100
Canonical Correlation	0.99653
Functions	0

Derived	
Chi-square (X²)	91.97
Degree of Fredom	13
Significance Level	0.00001

The resultant discriminant rule is as follows: classify an individual as *L. cayennensis* if Y<5.137 and as *L. yencanensis* if YS±5.137; where y= -9.30458+0.00704 labrum length - 0.01332 alpha length + 0.01585 delta length + 0.01936 gamma length - 0.02141 genital pump length + 0.10190 genital filament length - 0.05480 style length -0.07444 coxite length - 0.05188 paramere length - 0.01266 head height - 0.08539 eye heigth + 0.07988 eye width + 0.03461 interocular space.

This estimate is particularly useful to compare and identify unknown specimens of these two sandfly species in those endemic areas where they cohabitate.

Finally, the general use of numerical taxonomy methods is recommended in order to homogenize a methodology for sandfly identification and also to solve in an objective way the taxonomic problems presented in closely related species.

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123

Med-ULA, Revista de la Facultad de Medicina, Universidad de los Andes. Vol 1 Nº 3. Mérida, Venezuela

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