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Artículo original

A MULTIVARIATE ANALYSIS OF *Boophilus microplus* (ACARI: IXODIDAE): NON-PARASITIC PHASE

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ABSTRACT: **Multivariate statistical methods are relatively new tools for data analysis. They have a lot of applications in biological researches: nevertheless, for several reasons, they have not been widely employed in this subject yet. Recently, present authors published a paper applying multivariate statistics on non-parasitic phase of *Anocentor nitens*. In the present article *Boophilus microplus* has received a very similar statistic treatment. In this paper, the influence of different incubation conditions was studied in groups of 12-15 individuals in each of the 24 combinations of six temperatures 24, 27, 30, 32, 34 and 36°C and four relative humidity values, 100, 80, 75.5 and 70% over the cycle variables of *B. microplus*. According to the results, the best conditions are 30ºC and 100% relative humidity and the worse ones were higher temperatures together with lower relative humidity. It was remarked that this ixodid is better adapted to warm humid conditions in tropic than *A. nitens* is. Some other issues have been discussed in this new approach, like the possibility of its applications in prognosing geographical distribution of ixodids.**

(Key words: ticks; Ixodidae; ***Boophilus microplus****; Multivariate Analysis; non-parasitic phase)*

ANALISIS MULTIVARIADO DE *Boophilus microplus* (ACARI: IXODIDAE):FASE NO PARASÍTICA

RESUMEN: **Los métodos de estadística multivariada constituyen herramientas relativamente nuevas para el análisis de datos. Ellos tienen una gran aplicación en las investigaciones biológicas, sin embargo, por diversas razones, aún no han sido ampliamente empleados. Recientemente los autores publicaron un artículo aplicando estadística multivariada a la fase no parasitaria de *Anocentor nitens*. En el presente trabajo la fase no parasitaria de *Boophilus microplus* recibió un tratamiento estadístico similar. En este artículo se estudia la influencia de las condiciones de incubación en grupos de 12-15 individuos en cada una de las 24 combinaciones de seis temperaturas 24, 27, 30, 32, 34 y 36ºC y cuatro valores de humedad relativa 100, 80, 75.5 y 70%, sobre las variables del ciclo de B. microplus. De acuerdo con los resultados obtenidos, las condiciones más favorables fueron 30ºC y 100% de humedad relativa y las condiciones más desfavorables resultaron las temperaturas más elevadas unidas a las humedades relativas bajas. Esto indica que este ixódido está mejor adaptado que *A. nitens* a las condiciones cálido-húmedas del trópico. Se discuten otros aspectos como la posible aplicación de estos métodos al pronóstico de la distribución geográfica de los ixódidos.**

(Palabras clave: garrapatas, Ixodidae; ***Boophilus microplus****; Análisis Multivariado; fase no parasitaria)*

INTRODUCTION

Statistical multivariate methods are relatively new tools for data analysis. The authors consider their generalization as a current statistical procedures depending upon multiple reasons: few appropriate programs on the issue that join a solid scientific support with an easy manipulation, and on the writing, by statistical mathematicians, of books at the level of comprehension for current users. It means an easy understanding for scientific researches, who, otherwise, need to expend some of their time on the study of general ideas about the subject that makes possible a common language to allow a mutual comprehension between them and statisticians, and could profited the advantages that offer these quite powerful, exact and precise procedures in conjunction with current univariate statistics. Besides, time should show the prospective advantages they have, and researchers would be more interested in applying these techniques.

Recently (1), authors employed multivariate methods to study non-parasitic phase of *Anocentor nitens*, and took out conclusions about the best and worst incubation conditions to raise this ixodid in laboratory and about the adaptation of this tick to prevailing tropical conditions. *Boophilus microplus* is a tick species extended in tropical and subtropical areas in Africa, Latin American and northern and eastern Australia (2, 3, 4, 5, 6). It seams interesting to know the adaptation degree of *B. microplus* to this climatic environment. For this reason, an experiment was carried out, under almost the same conditions as the previous one (1) for *B. microplus* in order to know if results of multivariate statistical analysis and other scientific methods, like thermal constant, agree with results obtained under natural and controlled conditions for this tick. In the future, the same procedures could be applied to other species being studied, maybe, as a useful forecast guide in the study of geographical distribution of ticks.

Some authors have proposed taxonomic changes in the genus *Boophilus*, considering it as a subgenus of genus Rhipicephalus (7,8,9,10). As this is still under analysis for international acceptance, it was decided to use the ancient denomination.

MATERIALS AND METHODS

Procedure

Engorged *B. microplus* female ticks raised on bovines were incubated in groups of 12-15 individuals in each of the 24 combinations of six temperatures (TEMP) 24, 27, 30, 32, 34 and 36 ^{o}C and four relative humidity values (RH) 100, 80, 75.5 and 70 %. The variables recorded were: Female Weight (FW), Laying Weight (LW), the onset of oviposition or Preoviposition (PREOV), the onset of eclosion or Minimum Time of Eclosion (MTE), and the Conversion Efficiency Index or CEI (11). It is evident that FW and LW are strong correlated in ticks; indeed there is a linear regression between them (12,13,14,15,16); otherwise, the relation LW/FW is expressed in the CEI, and in this way it is better to use CEI in further data analysis because this eliminate the covariance between FW and LW. Also the Laying Fertility (LF) and number of larvae/number of eggs was recorded. Only five layings by group were employed to estimate LF because this procedure is cumbersome.

Statistical Analysis

Principal Component Analysis (PCA) was descriptively employed for data analysis and dimensional reduction. The arcsin transformation was applied to the variable LF in order to obtain ARSLF and fit the normality hypothesis. After that, a MANOVA was performed: PREOV, MTE, CEI and ARSLF as dependent variables and TEMP and RH as factors. Afterwards, a Canonical Variate Analysis (CVA) was done. This is a technique mainly used to represent multivariate means projected as points in a two dimensional space (centroids). It is traditional to draw confidences circles around these points (17, 18, 19). A more complete description about the issue can be found in the article of Arenas and Cuadras (20).

RESULTS

The total absence of fertility (LF) at 70% RH and 34 and 36°C and at 75.5% RH and 36°C (Table 1) was the first notable result. For this reason all data at these incubation conditions were not considered in further analysis.

TABLE 1. Laying fertility by temperature and relative humidity./ *Fertilidad de la puesta por temperatura y por humedad relativa*

	Relative Humidity (RH) %				
TEMP (°C)	**100**	**80**	**75.5**	**70**	**Mean $_{Temp}$**
24	91.29	75.47	72.94	52.11	75.95
27	95.12	74.31	79.35	70.37	79.78
30	95.86	88.32	74.60	89.43	87.05
32	95.98	85.31	66.54	74.71	80.65
34	88.24	73.85	73.68	0.00	58.94
36	54.10	48.63	0.00	0.00	25.68
Mean $_{RH}$	86.76	74.31	61.19	47.77	

Principal Component Analysis

Table 2 shows the correlation matrix of variables. Temperature is negatively correlated to cycle variables (PREOV and MTE) and also to the ARSLF. The relative humidity only is directly correlated to ARSLF. Another interesting result is the direct correlation between CEI and ARSLF.

TABLE 2. Correlation matrix of all studied variables./ *See text for abbreviation meaning.* *P<0.05./ *Matriz de correlación de todas las variables estudiadas. Ver el texto para el significado de las abreviaturas. *P<0.05*

	PREOV	**CEI**	**MTE**	**ARSLF**	**TEMP**	**RH**
PREOV	1.00	-0.23*	0.59*	0.18	-0.57*	-0.03
CEI	-0.23*	1.00	-0.10	0.34*	-0.08	0.01
MTE	0.59*	-0.10	1.00	0.10	-0.88*	-0.16
ARSLF	0.18	0.34*	0.10	1.00	-0.47*	0.44*
TEMP	-0.57*	-0.08	-0.88*	-0.47*	1.00	-0.01
RH	-0.03	0.01	-0.16	0.44*	-0.01	1.00

The eigenvalues of the first three axes are shown in Table 3. The total variance explained by the first two axes is over 70% but does not reach the 79.16% required by the broken-stick test Frontier (1976), cited by Cuadras (18) to determine the number of valid axes in PCA. Due to this, it was decided to include the third axis in the following procedures.

TABLE 3. Eigenvalues of first three axes./ *Valores propios de los primeros tres ejes*

	Eigenvalues Variance	**Per axis In %**	**Cumulative**	**Cumulative %**
1	2.676567	38.23667	2.676567	38.2367
2	2.349165	33.55950	5.025732	71.7962
3	1.054169	15.05956	6.079901	86.8557

The correlation circle of first-second axis (Fig.1) shows a high inverse correlation between temperature and the variables related to phase duration (PREOV and MTE). The variable ARSLF is shared between the two axes and very influenced by RH (for more details on factor-axes correlations see Table 4).

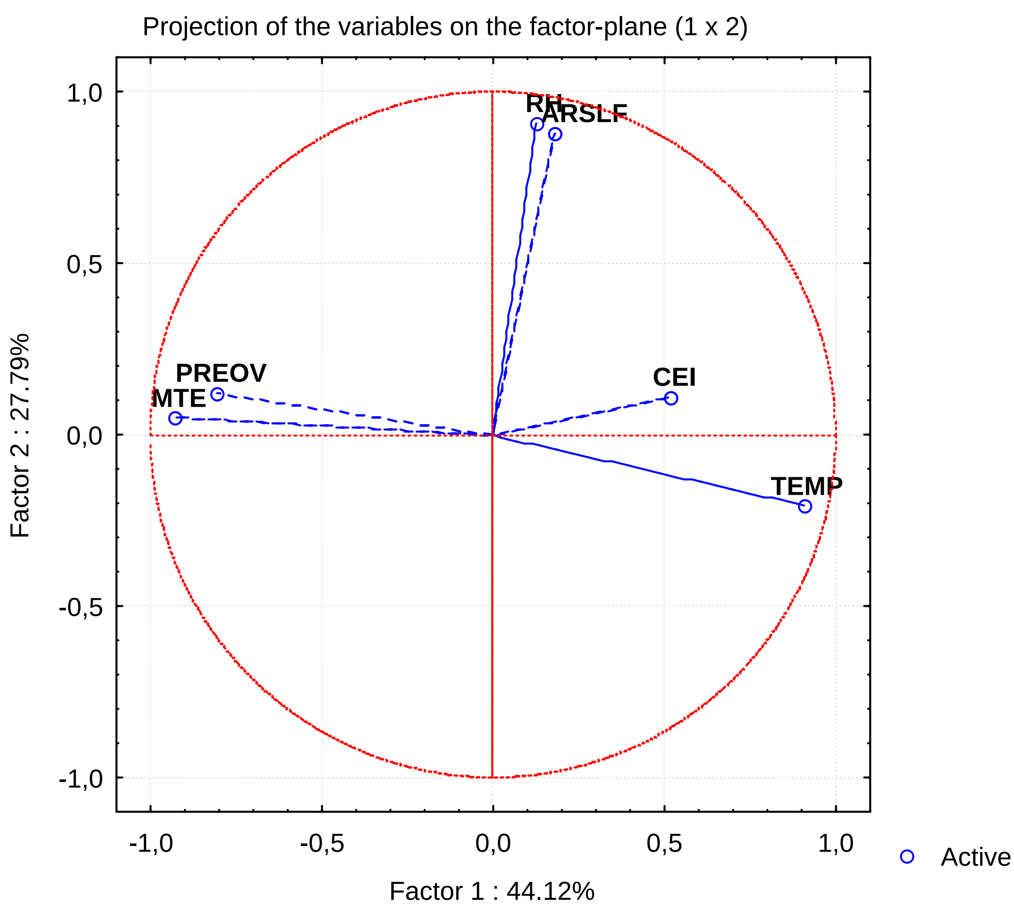

FIGURE 1. Graphic representation of variables over the first-second axis plane. See text for abbreviations meanings./ *Representación gráfica de las variables sobre el plano formado por el primero y segundo eje. Ver el texto para el significado de las abreviaturas.*

TABLE 4. Factor-variable correlations./ *Correlaciones de cada variable por factor*

	Factor 1	**Factor 2**	**Factor 3**
PREOV	-0.805598	0.120673	0.130454
CEI	0.517604	0.109121	-0.822284
MTE	-0.927908	0.047591	-0.271864
ARSLF	0.127540	0.907403	-0.014338
TEMP	0.905652	-0.208941	0.273852
RH	0.180317	0.878399	0.178909

Multivariate Analysis of Variance Canonical Variate Analysis

The general results of MANOVA (Table 5) show that the two factors are highly significant, but their interaction is not.

TABLE 5. General results of MANOVA./ *Resultados generales del MANOVA*

Effect	**Wilks Lambda**	**Ho Df**	**Error Df**	**F**	**Probability**
TEMP	0.070	16	166	14.353	0.000 **
RH	0.325	8	108	10.181	0.000 **
TEMP*RH	0.543	32	201	1.130	0.300

**$P < 0.05$

- By temperature:

Figure 2 shows the relations between the five temperature groups and the studied variables. Temperature of 30°C has the best performance among the temperatures employed in the experiment, the efficiency and the fertility of laying are the best and cycle duration is not too long. Contrarily, temperatures of 32 and 34°C seem to be less favorable for ticks' performance. Temperature of 27°C has less productivity of larvae than 30°C, and 24°C is somehow worse in performance than 27°C. Variables of the cycle, that is, PREOV, and MTE are clearly negatively correlated to temperature, the higher the temperature, the shorter the period and vice versa, see also Fig 1.

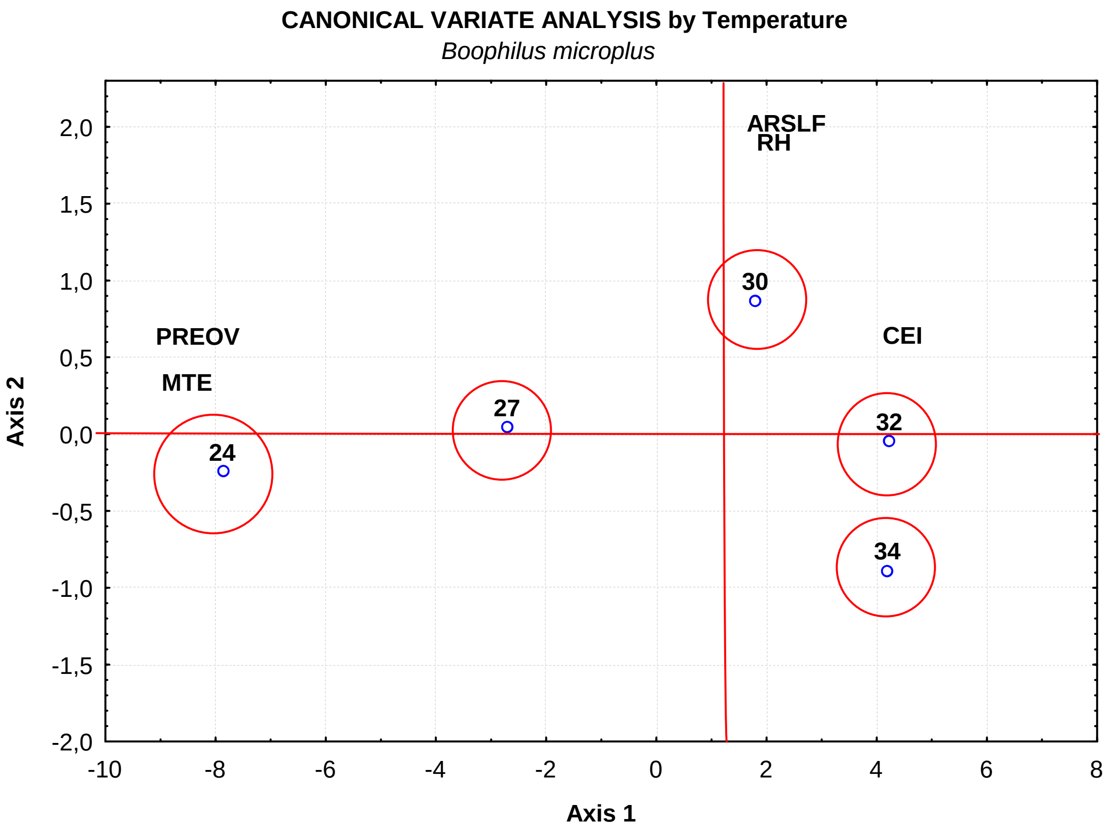

FIGURE 2. Canonical Variate Analysis by temperature. Central points represent the centroids and surrounding circles the 90% confidence intervals. See text for abbreviation meanings./ *Análisis Canónico por temperatura. Los puntos centrales representan los centroides y los círculos el intervalo de confianza para el 90%. Ver el texto para el significado de las abreviaturas.*

-By Relative Humidity:

Figure 3 shows the relationship between groups of ticks incubated at three different relative humidity values and the studied variables. Here it is clear the fact that the best performance (higher CEI and ARSLF) is obtained when ticks are incubated at 100% RH and the worse at 75%.

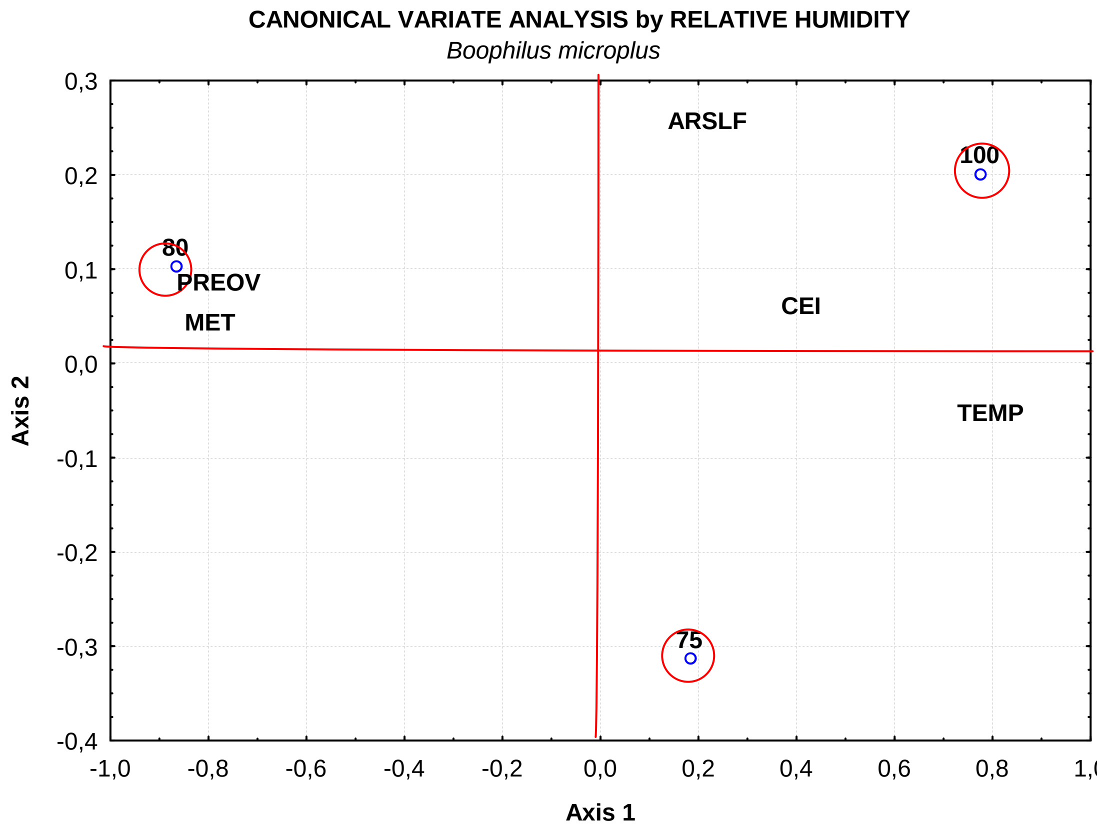

FIGURE 3. Canonical Variate Analysis by relative humidity. Central points represent the centroids and surrounding circles the 90% confidence intervals. See text for abbreviation meanings./*Análisis Canónico por humedad relativa. Los puntos centrales representan los centroides y los círculos el intervalo de confianza para el 90%. Ver el texto para el significado de las abreviaturas.*

DISCUSSION

Temperature is the most important factor acting over the *B. microplus* cycle variables (Figures 1 and 2, Table 4), this fact is already described by other authors (21, 22, 23, 24, 25). The new canonical variables show, in a more objective and easy way, which are the best and the worst incubation conditions for the species, as it was happened in the case of A. nitens (1). These results give the suggestions that the best incubation conditions for *B. microplus* are 30°C and 100% RH; higher temperatures and lower relative humidity are harmful for this ixodid (Figures 2 and 3; Table 1). It looks that *B. microplus* is better adapted for tropical conditions (warm temperatures and high relative humidity) than *A. nitens* (1). In MANOVA the interaction between TEMPxRH is not significant possibly because at lower RHs (70 and 75%) and higher temperatures combinations (34°C and 36°C) data were withdrawn from analysis and number of cases for interactions was too small.

Estrada-Peña *et al.* (26) studied how the climate factors influence geographical distribution of *B. microplus* in Mexico. They concluded that this tick is most commonly found under warm and humid conditions, and it is absent in the central mountainous regions and Mexican plateau, where low temperatures are prevalent. These authors found that in municipalities where yearly mean temperature is 19.97°C and SD is 4.22°C, the species was absent. In this case the confidence interval in their inferior limit is $\mu-2s=19.97-8.44=11.53$°C, in 47.5% of years. This temperature (11.53°C) is below 14°C corresponding to the Minimum Thermal Threshold (MTT) in B. microplus (27,28,29). MTT is the lowest temperature at which development of determined stage could be completed in poikilothermic organisms, first authors working on this subject (30) name this temperature "critical point". Otherwise, this tick is present in municipalities in Mexico (26) where yearly mean temperature is 23.82ºC, and SD is 2.4ºC. The same calculation shows $\mu-2s=23.82-4.8=19.02$°C, this temperature value is over 14°C (MTT of *B. microplus*), and development of the species is always possible. In this direction Alvarez *et al.* (31) in Costa Rica affirm that B. microplus is present in zones were temperatures values are greater than 13°C. All these mentioned papers are congruent with present data and with a series of articles published by authors about thermals constant applied in *B. microplus* (27,28,29,32,33,34,35,36).

The data obtained by those authors (26) could be forecast and/or explained applying thermal constant method (37,38).

Estrada Peña *et al*. (4) said that the collections of Latin American *B. microplus* are very homogeneous according to climate preferences and well separated from the African counterpart. Their graphics show that mean minimum monthly temperature recorded for collection points of Latin American B. microplus are approximately between 12.5°C and 18°C, and the mean maximum temperatures are between 24°C and 30°C. These data are in good correspondence with present results. Mentioned authors (4) proposed the existence of populations (demes) with ecologically requirements within each tick species. It seems interesting to investigate in laboratory about thermal constant parameters in African *B. microplus* because it could be possible that thermal constant would be a conspicuous data in species determination and/or speciation in ticks.

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