A MULTIVARIATE ANALYSIS OF *Anocentor nitens* (IXODOIDEA: IXODIDAE): NON-PARASITIC PHASE

R. de la Vega* and G. Díaz**

**LABIOFAM, Apartado 34, General Peraza, CP-19210, Ciudad Habana, Cuba, FAX: (537)334857,
E-mail: delavega@infomed.sld.cu; **Dpto. Biología Animal y Humana, Facultad de Biología,
Universidad de La Habana, E-mail: gdacosta@fbio.uh.cu*

ABSTRACT: **Multivariate statistical methods are useful tools complementary to classic univariate methods. In biology, they are used in several scenarios where data structure, classification and grouping are necessary. Nevertheless, it seems they have not been employed in the study of non-parasitic phase of ticks or any other arthropod. 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, 26, 28, 30, 32, and 34ºC and four relative humidities 100, 80, 75.5 and 70% over the cycle variables of *Anocentor nitens* looking for establishing the most suitable environmental conditions to increase or decrease ticks performance and to apply these knowledges to zoogeography. According to the results, it seems that the best conditions to raise these ticks in laboratory are between 26-30ºC and 100% relative humidity.**

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

ANÁLISIS MULTIVARIADO DE *Anocentor nitens* (IXODOIDEA: IXODIDAE)*:* FASE NO PARASITARIA

RESUMEN: **La estadística multivariada es una poderosa herramienta complementaria de la clásica estadística univariada. En biología ha sido empleada en diferentes situaciones cuando se hace necesario conocer la estructura de los datos, agruparlos y clasificarlos. Sin embargo, al parecer no se ha empleado con anterioridad en el estudio de la fase no parasitaria de alguna garrapata o de algún otro artrópodo. 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, 26, 28, 30, 32, and 34ºC y cuatro valores de humedad relativa 100, 80, 75.5 y 70%, sobre las variables del ciclo de *Anocentor nitens*, con el fin de establecer cuáles son las condiciones más o menos adecuadas para el desarrollo de esta garrapata y aplicar estos conocimientos a la zoogeografía. De acuerdo con los resultados experimentales, las condiciones más favorables para criar estos ácaros en el laboratorio están entre 26-30ºC y 100% de humedad relativa.**

(Palabras clave: garrapatas; Ixodidae; Análisis Multivariado; fase no parasitaria)

INTRODUCTION

Multivariate statistical methods are useful tools complementary to classic univariate methods. In biology, they are used in several scenarios where data structure, classification and grouping are necessary (1, 2, 3, 4, 5, 6,7). Nevertheless, it seems they have not been employed in the study of non-parasitic phase of ticks or any other arthropod. In spite of some articles concerning the influence of environmental conditions over the non-parasitic phase of *A. nitens* (8,9,10,11,12), objective conclusions about the general effects of these factors were not been obtained yet employing univariate statistical methods alone. In this paper, the influence of different incubation conditions over the cycle variables of *Anocentor nitens* were studied looking for establishing the most suitable environmental conditions for increasing or decreasing

ticks performance and to apply these knowledges to zoogeography.

MATERIALS AND METHODS

Procedure

Engorged *A. nitens* female ticks raised on bovines were incubated in groups of 12-15 individuals in each of the 24 combinations of six temperatures (TEMP) 24, 26, 28, 30, 32, and 34ºC and four relative humidities (RH) 100, 80, 75.5 and 70%. The variables recorded were: Female Weight (FW), Laying Weight (LW), the onset of oviposition or Preoviposition (PREOV) and the onset of eclosion or Minimun Time of Eclosion (MTE), the Conversion Efficiency Index or CEI (13) and the Laying Fertility (LF), that is, number of larvae/ number of eggs. Only 5 layings by group were employed to estimate LF because this procedure is cumbersome.

Statistical Analysis

Principal Component Analysis (PCA) was employed descriptively for data analysis and dimensional reduction. The arcsin transformation is 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 well-known technique mainly used to represent group means as points in a two dimensional space. It is traditional to draw confidence circles around these points. These circles are only approximations. Some authors advocated those ellipses, usually having higher inclusion rates (14, 15, 16).

RESULTS

1. Principal Component Analysis.

- All Variables

Table 1 shows the correlation matrix of variables. It is evident that FW and LW are closely related, indeed there is a linear regression between them. Both variables have some degree of correlation with ARSLF. These correlations exist because the bigger the egg mass is, the higher protection from desiccation is accomplished and thus a higher fertility is obtained. Also, there is not significant correlation between these variables and CEI. Actually, the variable CEI is made from both, FW and LW, so the covariance between them does not have to be corrected (13). They are uncorrelated with the rest of the variables. For these reasons, authors decided to draw out the variables FW and LW for further analysis.

- Analysis of Remaining Variables

The eigenvalues of the first three axes are shown in Table 2. 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 (17) to determine the number of valid axes in PCA. So authors decided to include the third axis in the following procedures.

The correlation circle of first-second axis (Fig. 1) shows a high correlation between temperature and the variables related with phase duration (PREOV and MTE). The influence of temperature over the variable CEI is small; this last variable is better represented in the second axis and related with relative humidity. The variable ARSLF is shared between the two axes (for more details on factor-axes correlations, see Figure 1 and Table 3).

TABLE 1. Correlation matrix of all studied variables. See text for abbreviation meanings. *P<0.05./ *Matriz de correlación de todas las variables estudiadas. Ver el texto para el significado de las abreviaturas. *P<0.05*

	FW	PREOV	MTE	LW	CEI	ARSLF	TEMP	RH
FW	1.00	0.03	0.05	0.81*	0.12	0.20*	-0.05	0.14
PREOV	0.03	1.00	0.82*	0.09	0.05	0.54*	-0.83*	0.11
MTE	0.05	0.82*	1.00	0.10	0.06	0.36*	-0.89*	-0.06
LW	0.81*	0.09	0.10	1.00	0.65	0.28*	-0.10	0.23*
CEI	0.12	0.05	0.06	0.65	1.00	0.18*	-0.06	0.21*
ARSLF	0.20*	0.54*	0.36*	0.28*	0.18*	1.00	-0.58*	0.23*
TEMP	-0.05	-0.83*	-0.89*	-0.10	-0.06	-0.58*	1.00	0.01
RH	0.14	0.11	-0.06	0.23*	0.21*	0.23*	0.01	1.00

TABLE 2. Eigenvalues of first three axes./ *Valores propios de los primeros tres ejes*

	Eigenvalue variance	Per axis in %	Cumulative	Cumulative %
1	3.117933	51.96555	3.117933	51.9655
2	1.348116	22.46861	4.466049	74.4342
3	0.750419	12.50698	5.216468	86.9411

Projection of the variables on the factor-plane (1 x 2)
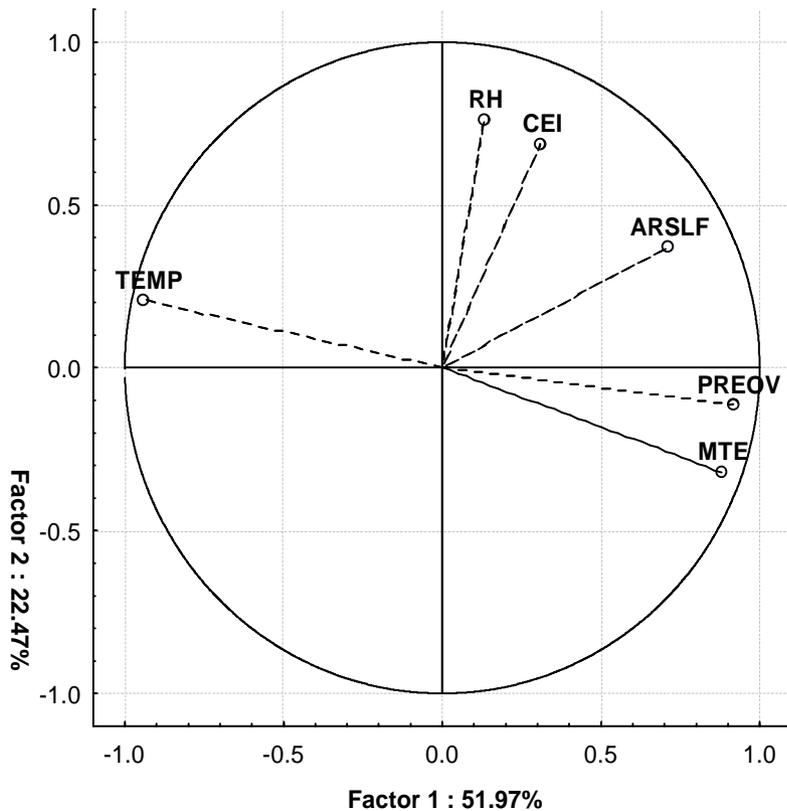
FIGURE 1. Graphic representation of variables over the first-second axis plane. See text for abbreviation meanings./ *Representación gráfica de las variables sobre el plano formado por el primero y segundo eje. Ver el texto para el significado de las abreviaturas.*

TABLE 3. Factor-variable correlations./ *Correlaciones de cada variable por factor*

	Factor 1	Factor 2	Factor 3
PREOV	0.916709	-0.112731	-0.129558
MTE	0.880573	-0.317759	-0.013322
CEI	0.309079	0.688132	0.598728
ARSLF	0.708596	0.370965	0.072006
TEMP	-0.941593	0.206718	0.025583
RH	0.134170	0.762045	-0.607368

TABLE 4. General results of MANOVA./ *Resultados generales del MANOVA*

Effect	Wilks Lambda	Ho Df	Error Df	F	Probability
TEMP	0.003	20	299	73.268	0.000
HR	0.378	12	238	8.827	0.000
TEMP*HR	0.112	60	354	4.423	0.000

Canonical Variate Analysis

- By temperature:

Figure 2 shows the relations between the six temperature groups and the variables studied. Temperatures of 28 and 30°C have an overlap of the confidence circles. These two variates are the best if it is desired to obtain a high laying efficiency and fertility.
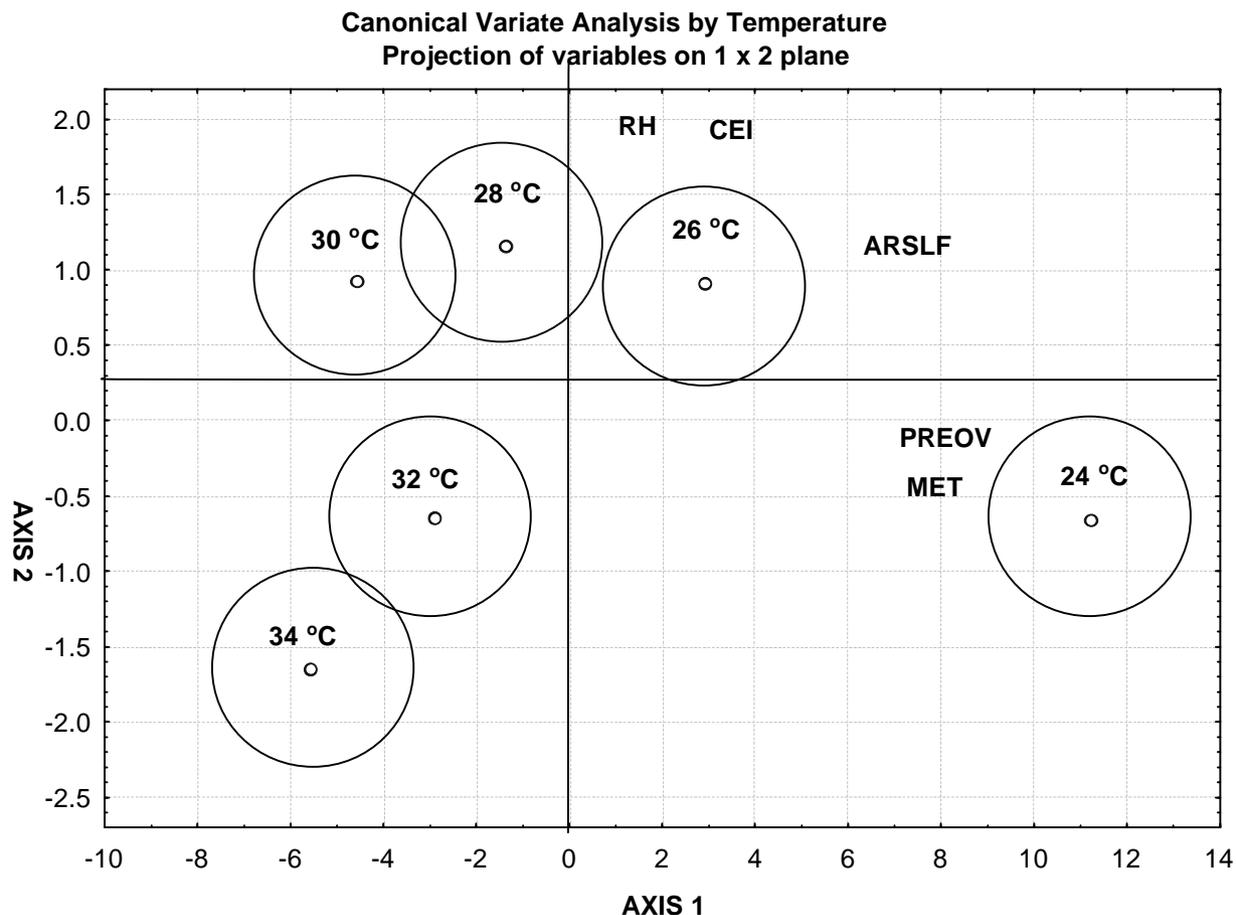
2. Multivariate Analysis of Variance

The general results of MANOVA (Table 4) show that the two factors and their interaction are highly significant.

Canonical Variate Analysis by Temperature
Projection of variables on 1 x 2 plane

FIGURE 2. Canonical Variate Analysis by temperatures. 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.*

The duration of cycle periods is not too long, as it is seen at 24°C. Contrarily, temperatures of 32 and 34°C are harmful for ticks performance.

- By Relative Humidity:

Figure 3 expresses the relationship between groups of ticks incubated at four different relative humidities and the variables studied. Here it is clear the fact that the best perfomance is obtained when ticks are incubated at 100% RH and the worst at 70% RH.

- By Interaction Temperature X Relative Humidity

As each of the 24 groups has a small number of observations (4 or 5) it was decided not to employ data regarding ARSLF to make the procedure, and left for the future the addition of further observations in order to add more data allowing to increase accuracy and precision of results.

DISCUSSION

Temperature was the most important factor acting over the *Anocentor nitens* cycle variables. On the contrary, relative humidity between 70 and 100 % has little importance over the same variables. The new canonical variables show in a more objective way which are the best and the worst incubation conditions for the species.

These results give the suggestion that the best incubation conditions for raising *A. nitens* are: temperatures between 26-30°C and relative humidity of 100%. Higher temperatures and lower relative humidities are harmful for it (Fig. 2 and 3). Results of Despins (11) working on non-parasitic phase of *A. nitens* raised on calves, show congruency when results shown in this paper are compared. Best results for CEI and LF are obtained at temperatures near 30°C
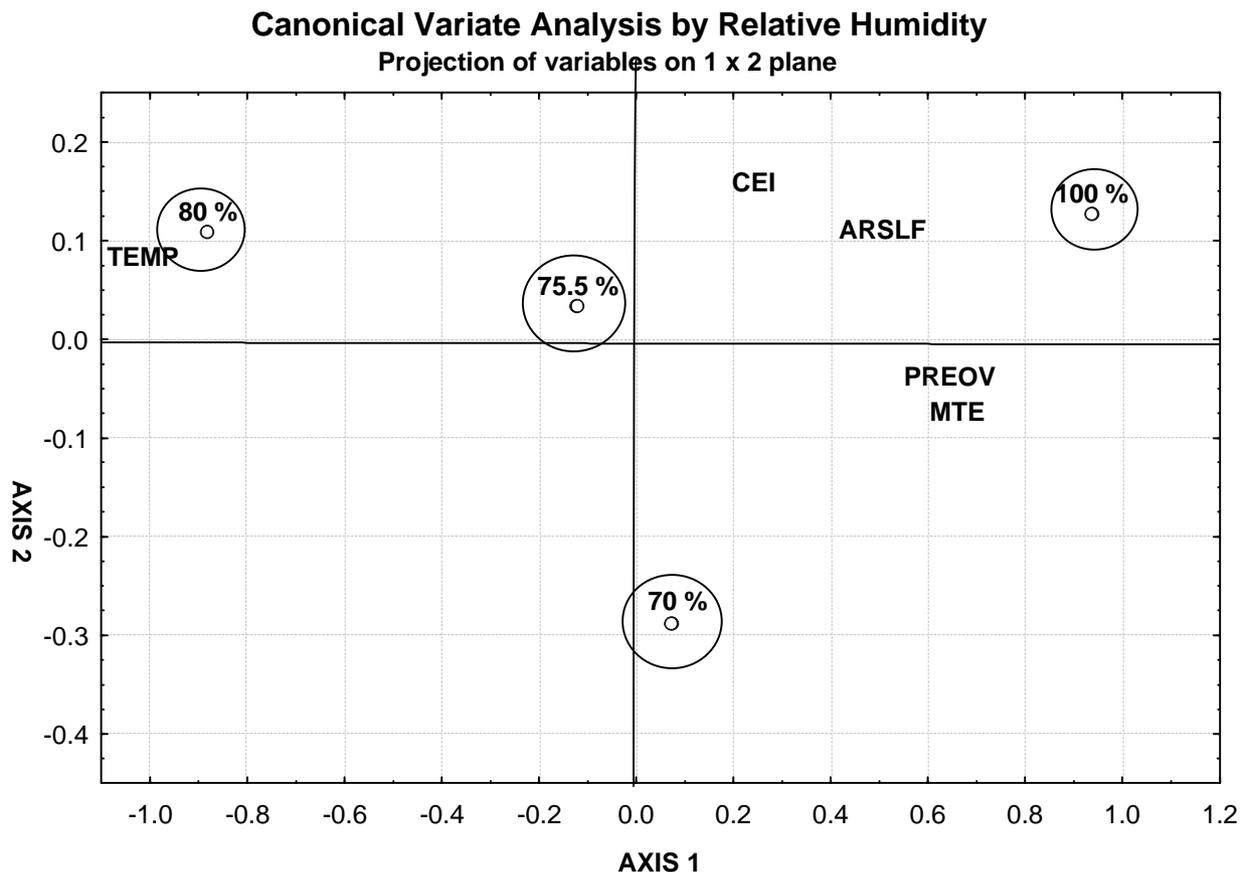
Canonical Variate Analysis by Relative Humidity
Projection of variables on 1 x 2 plane

FIGURE 3. Canonical Variate Analysis by relative humidities. 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.*

and RH of 91%, the highest RH employed in this paper; at 30°C and 61% relative humidity, the mean percentage of egg hatch was only 43.4 % and in the case of 35°C and 40% relative humidity there was no hatch. In the present paper using 34°C and 70% relative humidity, there was the minimum value of fertility (60.3%). Despins (11) considers that data obtained at 35°C indicates that this constant temperature is near the upper lethal temperature for *A. nitens.* The same approximation to the results of the present article is shown in Bastos *et al.* (8) in relation to incubation temperature. The fact that relatively high temperatures and relative humidity as used in the present paper, are suitable to *A. nitens* performance suggests that this tick is well adapted to tropical conditions. In a previous work applying univariate methods (12), authors of this paper have suspected these same conclusions, according to the obtained data about laying fertility (Table 5) and CEI (Table 6). Multivariate statistic methods give this

information in an explicit way even for those with little or no statistical knowledge.

Furthermore, in *A. nitens,* the minimum thermal threshold and the thermal constant for eclosion (10) were 15.25°C and 354°C-day respectively, data that are alike with values of *Boophilus microplus*, 14°C and 289.9°C-day, in the laboratory (18) under natural simulated conditions (19) and validated on conditions near to production (20). This tick is well adapted to tropical conditions. Finally, Borges *et al.* (9) showed that *A. nitens* is not present in the Brazilian states of Santa Catarina and Río Grande do Sul, whose winters are colder than in its northern states.

ACKNOWLEDGEMENTS

TABLE 5. CEI by temperature and relative humidity./ *Indice de eficiencia de la puesta por temperatura y humedad relativa*

TEMP (°C)	Relative Humidity (%)				
	100	80	75.5	70	Mean $_T$
24	59.4^c	43.4^{abc}	49.6^{abc}	54.3^{abc}	51.7
26	58.2^{abc}	42.0^a	49.5^{abc}	49.8^{abc}	49.9
28	51.6^{abc}	53.4^{abc}	54.9^{abc}	51.9^{abc}	53.0
30	53.3^{abc}	46.7^{abc}	52.8^{abc}	46.7^{abc}	49.9
32	59.3^{bc}	47.1^{abc}	43.8^{abc}	51.6^{abc}	50.4
34	51.9^{abc}	49.5^{abc}	47.4^{abc}	46.7^{abc}	48.9
Mean $_{HR}$	55.6	47.0	49.7	50.2	

Means with different superscripts differ with P<0.05

TABLE 6. Laying fertility by temperature and relative humidity./ *Fertilidad de la puesta por temperatura y humedad relativa*

TEMP (°C)	Relative Humidity (%)				
	100	80	75.5	70	Mean $_T$
24	93.4^{ab}	88.7^{ab}	93.2^{ab}	97.0^a	93.1
26	96.7^a	88.6^{ab}	97.1^a	93.4^{ab}	94.0
28	92.8^{ab}	92.9^{ab}	90.4^{ab}	94.3^{ab}	92.6
30	97.3^a	76.2^{ab}	84.0^{ab}	70.9^b	82.1
32	95.3^{ab}	88.6^{ab}	87.0^{ab}	85.6^{ab}	89.1
34	71.4^b	66.9^b	68.5^b	60.3^b	66.8
Mean $_{HR}$	91.2	83.7	86.7	83.6	

Means with different superscripts differ with P<0.05

REFERENCES

1. Hutcheson, H.J.; Oliver, J.H.Jr. (1996): Multivariate morphometrics of larvae of the blacklegged tick (*Ixodes scapularis*) with notes on nomenclature. *J. Parasitol.* 82(6): 916-925

2. Houck, M.A.; OConnor, B.M. (1998): Morphological variation in *Hemisarcoptes* (Acari: Hemisarcoptidae): Application of multivariate morphometric analyses. *Ann. Entomol. Soc. Am.* 91(3): 335-349.

3. Gettinger, D.; Owen, R.D. (2000): *Androlaelaps rotundus* Fonseca (Acari: Laelapidae) associated with akodontine rodents in Paraguay: a morphometric examination of a pleioxenous ectoparasite. *Rev. Brasil. Biol.* 60(3): 425-434.

4. Fernández-Ruvalcaba, M.; Preciado-De la Torre, J. F.; García-Vázquez, Z.; Cruz Vázquez, C.; Saltijeral-Oaxaca, J. (2004): Seasonal assessment of *Boophilus microplus* larvae recovery in four forage legumes in experimental infested plots. *Téc. Pecu.Méx.*42(1): 97-104.

5. Klimov, P.B.; Lekveishvili, M.; Dowling, A.P.G.; OConnor, B.M. (2004): Multivariate Analysis of Morphological Variation in Two Cryptic Species of *Sancassania* (Acari: Acaridae) from Costa Rica. *Ann. Entomol. Soc. Am*. 97(2):322-345.

6. Tolleson, D.R.; Teel, P.D.; Stuth, J.W.; Strey, O.F.; Welsh, T.H.Jr.; Carstens, G.E. (2007): Fecal NIRS: Detection of tick infestations in cattle and horses. *Vet. Parasitol.* 144: 46-152.

7. Zacharda, M.; Kuèera, T. (2006): Diversity of Predatory Rhagidiid Mites (Acari: Rhagidiidae) Inhabiting Montane Stony Debris in the Ötztal

Alps, North Tyrol, Austria. *Arctic, Antarctic, and Alpine Research* 38(2): 292-300.

8. Bastos, K.M.S.; Daemon, E.; Faccini, J.L.H.; Da Cunha, D.W. (1996): Efeitos de diferentes temperaturas sobre a fase n?o parasitária de *Dermacentor (Anocentor) nitens* (Neumann, 1897) (Acari: Ixodidae) em condiòões de laboratório. *Rev. Bras. Parasitol. Vet.* 5(1): 29-32.

9. Borges, L.M.F.; Oliveira, P.R.; Ribeiro, M.F.B. (1999): Seasonal dynamics of the free-living phase of *Anocentor nitens* at Pedro Leopoldo, Minas Gerais, Brazil. *Vet. Parasitol.* 87: 73-81.

10. de la Vega, R.; Díaz, G. (2000): Thermal Constant Estimation in Tropical Horse Tick, *Anocentor nitens* (Acari:Ixodidae). *Ann. N.Y. Acad. Sci.* 916: 298-302.

11. Despins, J.L. (1992): Effects of Temperature and Humidity on Ovipositional Biology and Egg Development of the Tropical Horse Tick, *Dermacentor (Anocentor) nitens*. *J. Med. Entomol.* 29(2): 332-337.

12. Díaz, G.; de la Vega, R.; Chávez, G. (1991): Influencia de la temperatura y de la humedad relativa en la fase no parasitaria de *Anocentor nitens* (Ixodoidea: Ixodidae). *Rev. Salud Anim.* 13: 124-132.

13. Drummond, R.O.; Whetstone, T.M. (1970): Oviposition of the Gulf Coast Tick. *J. Econ. Entomol.* 63(5): 1547-1551.

14. Krzanowski, W.J. (1989): On confidence regions in canonical variate analysis. *Biometrika.* 76(1): 107-116.

15. Krzanowski, W.J.; Radley, D. (1989): Nonparametric Confidence and Tolerance Regions in Canonical Variate Analysis. *Biometrics.* 45: 1163-1173.

16. Ringrose, T.J.; Krzanowski, W.J. (1991): Simulation study of confidence regions for canonical variate analysis. *Statistics and Computing.* 1: 41-46.

17. Cuadras, C.M. (1991): Métodos de Análisis Multivariante. PPU, Barcelona. Estadística y Análisis de Datos.

18. de la Vega, R.; Díaz, G. (1985): Aplicación de las constantes térmicas en el control de la garrapata del ganado vacuno (*Boophilus microplus*). I. Cálculo de las constantes térmicas. *Rev. Salud Anim.* 7: 141-148.

19. de la Vega, R.; Farradá, F.; Díaz, G. (1988): Aplicación de las constantes térmicas en el control de la garrapata del ganado vacuno (*Boophilus microplus*). VI. Cuarentena. *Rev. Salud Anim.* 10: 71-75.

20. de la Vega, R.; Díaz, G. (1992): Aplicación de las constantes térmicas en el control de la garrapata del ganado vacuno (*Boophilus microplus*). VIII. Validación del programa de cuarentena. *Rev. Salud Anim.* 14: 133-136.