Morphometric variations of laelapine mite (Acari: Mesostigmata) populations infesting small mammals (Mammalia) in Brazil

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(With 4 figures)

Abstract

The goal of this study was to evaluate the morphometric variation of laelapine populations (Acari, Mesostigmata) associated with neotropical oryzomyine rodents at different geographic localities in Brazil. Three nominal mite species were selected for study, all infesting the pelage of small mammals at different localities in Rio de Janeiro, Espírito Santo, Bahia, and the Federal District, Brazil. To analyse morphometric characteristics, thirty-seven morphological characters distributed across the whole body of each specimen were measured. We use the Analysis of Principal Components, extracting the three first axes and projecting each mite in these axes. Major species level changes in the taxonomy of the host mammals allows an independent examination of morphometric variation of mites infesting a set of distinctly different host species at different geographic localities. 

Gigantolaelaps vitzthumi and Laelaps differens are associated with oryzomyine rodents of the genus Cerradomys, and consistently showed a tendency to cluster by host phylogeny. 

Laelaps manguinhosi associated with Nectomys rattus in central Brazil is morphometrically distinct from mites infesting N. squamipes in the coastal restingas of Rio de Janeiro and Espírito Santo. The results obtained here indicate that laelapine mite populations can vary among geographic areas and among phylogenetically related host species. Clearly, the study of these mites at the population level can be an important tool for clarifying the taxonomy of both mites and hosts.

Keywords: Brazil, ectoparasites, Gigantolaelaps, Laelaps, rodents.

Variações morfométricas de populações de ácaros lelapíneos (Acari: Mesostigmata) que infestam pequenos mamíferos

Resumo

O objetivo deste estudo foi avaliar a variação morfométrica de populações de lelapíneos (Acari, Mesostigmata) associados com pequenos roedores oryzomíneos neotropicais, em diferentes localidades geográficas do Brasil. Três espécies nominais de ácaros foram selecionadas para este estudo, sendo aqueles que infestam a pelagem de pequenos mamíferos em diferentes localidades nos Estados de Rio de Janeiro, Espírito Santo e Bahia, e no Distrito Federal. Para analisar as características morfométricas, 37 caracteres morfológicos, distribuídos por todo o corpo de cada espécime, foram medidos. Nós usamos a Análise de Componentes Principais, extraíndo os três primeiros eixos e projetando as medidas de cada ácaro nesses eixos. Mudanças importantes na taxonomia dos mamíferos hospedeiros permitem um exame independente da variação morfométrica de ácaros que infestam um grupo de espécies de hospedeiros distintos, em diferentes localidades geográficas. Gigantolaelaps vitzthumi e Laelaps differens estão associados com roedores oryzomíneos de gênero Cerradomys e, consistentemente, mostraram uma tendência a se agrupar pela filogenia do hospedeiro. 

Laelaps manguinhosi associado com Nectomys rattus no Brasil Central é morfometricamente distinto das populações de ácaros que infestam N. squamipes nas restingas costeiras de Rio de Janeiro e Espírito Santo. Os resultados obtidos neste estudo indicam que populações de ácaros lelapíneos podem variar entre áreas geográficas e, especialmente, entre espécies hospedeiras relacionadas filogeneticamente. Claramente, o estudo desses ácaros em nível populacional pode ser uma importante ferramenta para esclarecer a taxonomia tanto dos ácaros quanto de seus hospedeiros.

Palavras-chave: Brasil, ectoparasitos, Gigantolaelaps, Laelaps, roedores.
1. Introduction

The evolutionary expression of individual attributes is variable through time in biological systems. Species change continually, modifying the morphology and physiology of the individuals of each population, in response to characteristics of both the physical environment and the interactions with other organisms (Ricklefs, 1993). Among ectoparasitic arthropods, both short life cycles and reduced time between generations allow strong alterations in the populations and fast differentiation among them, especially when subjected to different selective pressures (Price, 1976). One of the consequences of the isolation of subpopulations and their independent evolutionary change is speciation. The increasing specialisation of diverging host species may increase the level of specialisation of parasites infesting these host groups (Price, 1976; Ricklefs, 1993; Begon et al., 1996).

Mathematical analyses have been widely used in entomology and acarology to detect morphometric variations among populations and consequently, have clarified the dynamics of their speciation (Albrecht, 1979; Footit and Mackauer 1990; Querino and Zucchi 2002). Multivariate statistical techniques are frequently used to order large morphometric datasets. In this way, the biological parameters producing morphological variation among individuals or groups can be resolved (Albrecht, 1979).

In the neotropics, mites of the subfamily Laelapinae (Acari, Laelapidae) are the most abundant arthropod group found in the fur of small terrestrial mammals. Previous studies focusing on ectoparasites of small mammals or in particular on laelapine mites, have described community composition and host associations in different areas and hosts of Brazil (Botelho and Williams, 1980; Linardi et al., 1984, 1985, 1991; Gettinger, 1987; Barros et al., 1993; Martins-Hatano et al., 2000, 2011; Bittencourt and Rocha, 2002; Bossi et al., 2002), or have described new species (Gettinger, 1992b, 1997; Gettinger and Bergallo, 2003). Morphologic variations among mite populations of different areas have rarely been studied (Gettinger, 1992a; Gettinger and Owen, 2000; Botelho et al., 2002).

Species of the genus Gigantolaelaps Fonseca, 1939 are nidicolous mites of wild rodents from Central and South America, characterised by their great size (dorsal shield length more than 1400 µm) (Furman, 1972). This genus shows a predilection for oryzomyine rodents (Cricetidae, Sigmodontinae), and tends to be specific to these hosts (Guitton et al., 1986; Gettinger, 1987; Linardi et al., 1991; Barros et al., 1993; Martins-Hatano et al., 2002; Bittencourt and Rocha 2003).

Gettinger (1992a); Gettinger and Owen (2000) have demonstrated that morphometric variations can occur among laelapine mite populations associated with different host species. This research implies that the morphologic characteristics of these mites can be influenced by the species identity of the host and that morphometric data can provide an important tool to clarify the taxonomy of both mites and hosts.

When this study was carried out, we had strong collections of Gigantolaelaps vitzthumi Fonseca, 1939, Laelaps differens Fonseca, 1936, and L. manguinhosi Fonseca, 1936, from coastal localities of restinga in Rio de Janeiro, Espírito Santo, and Bahia states, from the senior author’s (FMH) doctoral project, and then also, from the cerrados and gallery forests of central Brazil, from another doctoral project (DG), near Brasília. Two host “species” that we had in common were “Oryzomys” subflavus and Nectomys squamipes. The goal of the original study was to evaluate the morphometric variation of laelapine populations associated with a single host species in different geographic areas. At that time, our G. vitzthumi and L. differens collections from all these localities were considered host-specific associates of a single widely distributed host species, Oryzomys subflavus; and Laelaps manguinhosi, a specific associate of Nectomys squamipes, another wide-ranging host. We were interested more in geographic variation in mite populations than genetic variation of the hosts. However, following major revisionary changes in the taxonomy of the rodent tribe Oryzomyini (Wekslar et al., 2006), and the genera Cerradomys (Percequillo et al., 2008; Tavares et al., 2011) and Nectomys (Andrades-Miranda et al., 2001), we can apply this analysis to an equally interesting set of questions:

1) Are there recognisable morphometric differences among populations of Gigantolaelaps vitzthumi, or of Laelaps differens collected from different three distinct host species of the genus Cerradomys, at three different geographic localities?

2) Are there quantitative morphometric differences among populations of Laelaps manguinhosi collected from Nectomys squamipes (Brants, 1827) at two localities in the state of Rio de Janeiro, and those collected from N. rattus (Pelzeln, 1883) in the Federal District of central Brazil?

2. Material and Methods

The mites and hosts were collected in four different geographic areas and at different time periods. The areas were: 1) the Restinga de Jurubatiba National Park, in Rio de Janeiro State (RJ), 2) Restinga of Praia de Neves, in Espírito Santo State (ES), 3) Distrito Federal (DF) and 4) the Chapada Diamantina, in the state of Bahia (BA).

“Restingas” sensu lato are sand dune habitats characteristic of the Atlantic forest (Oliveira-Filho and Fontes, 2000), that occur in the lowlands along the Brazilian coast. Restingas are formed “by sandy substrates resulting from the marine deposits of the Quaternary period and frequently include freshwater or brackish lagoons (Suguio and Tessler, 1984; Turcq et al., 1986). These habitats are relatively open and include herbaceous formations in the beach border, shrub formations, forest formations in depressions and on sand dunes, and vegetation on lagoon margins (Araújo, 1992). The Restinga de Jurubatiba National Park is located in north coast of Rio de Janeiro.
Morphometric variations among laelapine mite populations

State (22° and 22° 23’ S and 41° 45’ W), covering a total area of 144,5 km². The collections were made three times per year and included five different vegetable formations of the park from February 2001 to November 2002. Mammals were collected in seven transects located in different plant formations. In each transects we placed 20 live traps, distant 40 m apart from each other. Traps were opened during six nights and after the third night the traps were moved 20 m (Bergallo et al., 2004; Martins-Hatano et al., 2001). The total trapping effort in each year was 6360 trap-nights (Bergallo et al., 2004). At Praia de Neves (21° 15’ 296’’ S and 40° 57’ 991’’ W) small mammals were captured and ectoparasites collected for three consecutive days in November 1999. Mammals were collected in live traps during two nights in transects located in different plant formations. The total trapping effort was 200 trap-nights (Rocha et al., 2005).

Mites from the Cerrado Province were collected in Distrito Federal and in Chapada Diamantina. In Distrito Federal (DF) the collections were accomplished in July 1983 to November 1984 in two adjacent reserves, 12 km from Brasília (15° 57’ S and 47° 54’ W) (Gettinger, 1992a). Mites from Chapada Diamantina in northeastern Brazil were collected in a municipal district of Itatê, BA (13° 09’ 46” S and 42° 10’ 58” W), during May 2004. Cerrado is savanna-like habitat that covers 12% of Brazilian territory. Mite voucher specimens from Bahia: Chapada Diamantina; Espírito Santo: Praia das Neves; and Rio de Janeiro: Restinga de Jurubatiba were deposited in the Coleção de Artrópodes Vetores Ápteros em Saúde das Comunidades (CAVAISC), FIOCRUZ, Rio de Janeiro, Brazil; voucher mite specimens from the DF collection were deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska-Lincoln, U.S.A. Host voucher material is deposited in Oklahoma Museum of Natural History (OMNH) of the University of Oklahoma, Norman, Oklahoma, USA; in the Department of Animal Biology of the University of Brasília, Brazil; the Instituto Brasileiro de Geografia e Estatística (IBGE), Brasília, Brazil and in the Museu Nacional do Rio de Janeiro, Rio de Janeiro. In all areas the mammals were brushed, and ectoparasites were preserved in 70% ethyl alcohol (for more details of arthropods collection and preservation technique see Martins-Hatano et al., 2002, 2011).

The mites were mounted on slides in Hoyer’s medium and measured with an ocular-micrometer of the compound light microscope. Thirty-seven morphological characters distributed across the whole body of each mite individual were measured, as described by Gettinger (1992a): 1) Dorsal shield length (DSL); 2) dorsal shield width at midlevel (DSW); 3) distance between j5 setae (j5-j5); 4) distance between z5 setae (z5-z5); 5) length of j5 (j5L); 6) length of z5 (z5L); 7) distance between J5 setae (J5-J5); 8) distance between Z5 setae (Z5-Z5); 9) length of J5 (J5L); 10) length of Z5 (Z5L); 11) distance between subcapitular setae (CAP-CAP); 12) length of subcapitular setae (CAPL); 13) length of inner hypostomal setae (INNL); 14) distance between subcapitular and inner hypostomal setae (CAP-INN); 15) length of sternal shield (SSL); 16) width of sternal shield at level at second sternal setae (SSW); 17) distance between first sternal setae (S1-S1); 18) distance between third sternal setae (S3-S3); 19) length of anterior sternal setae (S1L); 20) length of posterior sternal setae (S3L); 21) length of epigynial shield (ESL); 22) distance between epigynial setae (ES-E5); 23) greatest width of epigynial shield (ESW); 24) length of poststernal setae (S4L); 25) length of epigynial setae (ESL); 26) length of paranal setae (PARAL); 27) length of postanal setae (POSTL); 28) distance from postanal setae to anterior midline of anal shield (POST-EDGE); 29) distance between paranal setae (PARA-PARA); 30) greatest width of anal shield (ASW); 31) length of proximal seta, coxa I, (PROXCOX); 32) length of distal seta, coxa I (DISTCOX); 33) length of posterior seta, coxa II (POSTCOX2); 34) length of posterior seta, coxa III (POSTCOX3); 35) length of posterior seta, coxa IV (POSTCOX4); 36) length of anterior dorsal seta, femur I (DFEM1L); 37) length of posterior dorsal seta, genu I (DGEN1L). Examples of measurements can be seen in Figure 1. In some analyses, measures were excluded when the structure being measured was not visible or had been damaged during the mounting process.

2.1. Statistical treatment

Ordination techniques consist of projecting objects graphically along axes representing a relational ordinate, or forming a dispersion diagram with two or more axes (Legendre and Legendre, 1988). The graphical axes are chosen to represent the greatest fraction of variability of given multidimensional data, in a space with reduced dimensionality related to the set of original data (Legendre and Legendre, 1988).

To reduce dimensionality of the morphometric data we use the Analysis of the Principal Components, extracting the three first axes and projecting each mite on these axes (Legendre and Legendre, 1988). To accomplish this analysis, we used the Systat 11.0 program.

3. Results

3.1. Studied species

3.1.1. Gigantolaelaps vitzthumi Fonseca, 1939

The occurrence of Gigantolaelaps vitzthumi in association with the species of the former Cerradomys subflavus (Wagner 1842) group has been reported from several geographic localities in Brazil (Lizaso, 1968; Gettinger, 1987). Since Weksler et al. (2006) formally described the genus Cerradomys, it has been revised considerably. In this study, mite populations were collected from three distinct species of hosts, C. scotti (Langguth and Bonvicino, 2002), near Brasilia, DF, C. vivoi Percequillo, Hingst-Zaher and Bonvicino, 2008 from the Chapada Diamantina, BA, and C. goytaca Tavares, Pessoa and Gonçalves, 2011, from Praia das Neves, ES, and Restinga de Jurubatiba, RJ. Twenty mites collected from the fur of the mammals were randomly chosen and measured (4 from Restinga de
For *Gigantolaelaps vitzthumi*, the mites from *C. vivoi* (BA) formed a distinct cluster, well separate from specimens from *C. goytaca* (RJ) and *C. scotti* (DF) (Figure 2). The three first eigenvalues were, respectively, 12.00, 6.98 and 4.61, being the total variation explained of 63.6% (32.4%, 18.9% and 12.5%) (Table 2).

3.1.2. *Laelaps differens* Fonseca 1936 and *Laelaps manguinhosi* Fonseca 1936

The genus *Laelaps* Koch 1836 is a large and diverse group of mites of cosmopolitan distribution and generally associated with myomorph rodents, particularly the families Muridae and Cricetidae (Radovsky, 1985; Tipton, 1960). *Laelaps manguinhosi* frequently infests semi-aquatic rodents, like *Nectomys* and *Holochilus* (Furman, 1972). Furman (1972) described two subspecies for *L. manguinhosi*, indicating that this author was aware that variations could occur in this species.

The genus *Nectomys* has a wide geographical distribution in South America, from Guianas to Paraguay and northeastern Argentina (Musser and Carleton, 1993; Eisenberg and Redford, 1999). Genetic and morphologic studies of the Brazilian populations of this rodent demonstrate that there are two nominal species: *Nectomys squamipes* and *N. rattus* (Bonvicino et al., 2008). Twenty-one mites were measured (10 from the Restinga of Jurubatiba and 4 from Praia de Neves associated with *N. squamipes*, and 7 from Distrito Federal associated with *N. rattus*) (Table 1). For *Laelaps manguinhosi* specimens collected from *N. squamipes* in RJ and ES clustered together, while the Distrito Federal specimens from *N. rattus* showed a strong tendency to be separate on the third axis (Figure 3).

| Mite species              | Host species                  | Location              | Number of mites |
|---------------------------|-------------------------------|-----------------------|-----------------|
| *Gigantolaelaps vitzthumi*| *Cerradomys vivoi*            | Chapada Diamantina, BA| 09              |
|                           | *Cerradomys goytaca*          | Restinga de Jurubatiba, RJ | 04            |
|                           | *Cerradomys scotti*           | Fazenda Água Limpa, DF | 07              |
| *Laelaps differens*       | *Cerradomys goytaca*          | Restinga de Jurubatiba, RJ | 14            |
|                           | *Cerradomys goytaca*          | Praia das Neves, ES    | 10              |
|                           | *Cerradomys scotti*           | Fazenda Água Limpa, DF | 06              |
| *Laelaps manguinhosi*     | *Nectomys squamipes*          | Restinga de Jurubatiba, RJ | 10            |
|                           | *Nectomys squamipes*          | Praia das Neves, ES    | 04              |
|                           | *Nectomys rattus*             | Fazenda Água Limpa, DF | 07              |
Eigenvalues of the three first axes were 11.54; 5.53 and 3.6 and the total variation explained was 64.6% (36.1%, 27.0% and 11.2%) (Table 2).

*Laelaps differens* is a closely related species of *L. manguinhosi* and its occurrence on the species of the former “*Cerradomyys subflavus*” group has been recorded in several geographic localities (Gettinger, 1992a). Thirty individuals of this mite species were measured, including 14 from Restinga de Jurubatiba, 10 from Praia de Neves, on *C. goytaca* and 6 from Distrito Federal, associated with *C. scotti* (Table 1). *Laelaps differens* specimens from different areas formed three distinct groups, with the restinga localities (Jurubatiba and Neves) forming closer groups (Figure 4). However, the mites are distinctly grouped, implying that *C. goytaca* populations from restingas in Espírito Santo and Rio de Janeiro may be more isolated than is presently known. Eigenvalues of the first three axes were 11.29, 4.23 and 3.27, respectively. The total variation

| Morphometric Character | Laelaps differens | Gigantolaelaps vitzthumi | Laelaps manguinhosi |
|------------------------|------------------|--------------------------|---------------------|
| LDS                    | 0.65             | 0.66                     | 0.64                |
| WDS                    | 0.72             | 0.79                     | 0.89                |
| j5_j5                  | 0.72             | -0.18                    | 0.47                |
| z5_z5                  | 0.45             | 0.76                     | 0.03                |
| j5L                    | 0.37             | -0.26                    | -0.14               |
| z5L                    | 0.28             | 0.22                     | 0.09                |
| J5_J5                  | -0.01            | 0.13                     | -0.18               |
| Z5_Z5                  | 0.20             | -0.01                    | 0.71                |
| J5L                    | 0.70             | 0.26                     | 0.03                |
| Z5L                    | 0.56             | 0.63                     | 0.63                |
| CAP_CAP                | 0.39             | -0.09                    | 0.55                |
| CAPL                   | 0.02             | 0.31                     | -0.67               |
| INNL                   | -0.01            | 0.09                     | -0.67               |
| CAP_INN                | 0.69             | 0.57                     | 0.04                |
| SSL                    | 0.56             | 0.88                     | 0.80                |
| SSW                    | 0.70             | 0.85                     | 0.79                |
| S1_S1                  | -0.47            | 0.51                     | 0.81                |
| S3_S3                  | 0.31             | 0.86                     | 0.84                |
| S1L                    | 0.63             | 0.21                     | 0.61                |
| S3L                    | 0.75             | 0.45                     | 0.84                |
| ESL                    | 0.54             | 0.51                     | 0.83                |
| E5_E5                  | 0.68             | 0.72                     | 0.65                |
| ESW                    | 0.70             | 0.41                     | 0.88                |
| S4L                    | 0.47             | 0.81                     | 0.59                |
| ESL                    | 0.61             | 0.56                     | 0.55                |
| PARAL                  | 0.64             | 0.88                     | 0.13                |
| POSTL                  | 0.78             | 0.75                     | -0.07               |
| POST_EDGE              | 0.55             | 0.09                     | 0.14                |
| PARA_PARA              | 0.26             | 0.07                     | 0.14                |
| ASW                    | 0.71             | 0.93                     | 0.70                |
| PROXCOX                | 0.69             | 0.60                     | 0.36                |
| DISTCOX                | 0.57             | 0.72                     | 0.09                |
| POSTCOX2               | 0.61             | 0.61                     | 0.70                |
| POSTCOX3               | 0.80             | 0.93                     | 0.72                |
| POSTCOX4               | 0.38             | 0.40                     | -0.48               |
| DFEM1L                 | 0.77             | 0.40                     | -0.48               |
| DGEN1L                 | 0.24             | 0.53                     | -0.53               |
explained by the first three axes was 50.7% (30.5%, 11.4% and 8.8%, respectively). The restinga localities separated regarding the first axis, while those from Distrito Federal separated mostly by second axis (Table 2).

4. Discussion

The analysis of the morphometric characteristics of *L. differens* indicates that mite populations in the Distrito Federal are different from those in restingas of the Atlantic coast of Brazil. These variations may be related to environmental differences among areas, resulting in different selective pressures on populations. Both restinga populations also showed such separation, but on a smaller scale. The restinga areas, in consequence of the high degradation level of this environment, have been fragmented and isolated (Rocha et al., 2003). The lack of contact between host and, consequently, of the mites populations, may have led to a differentiation among these populations. The importance in geographic factors is poorly understood, and often difficult to evaluate because of the lack of knowledge of the species boundaries among the host mammals (Furman, 1972; Gettinger and Owen, 2000).

The population of *Gigantolelaeps vitzthumi* from Chapada Diamantina formed a distinct group. This population is associated with *C. vivoi*, and karyotypically distinct from specific populations associated with *C. scotti* from the Distrito Federal and *C. goytaca* from Restinga de Jurubatiba. This indicates that the mite physical characteristics are strongly dependent of the particular host species with which it is associated. This result illustrates the potential of this study as a tool in elucidating taxonomic relationships of the host mammals (Furman, 1972; Gettinger and Owen, 2000).

The population of *Gigantolelaeps vitzthumi* from Feira de Santana, BA. Only one individual mite of the new species was collected and, typical females of *G. vitzthumi* were found on the same host individual. Furman (1971, 1972) pointed out that morphologic variations are commonly observed in the genus *Gigantolelaeps*, especially related to body size, and could be associated with differences in the age or food quantity; Furman placed Lizaso’s species in synonymy with *G. vitzthumi*. Our studies indicate that further morphometric analysis and karyotypes of both, mammals and mites, may elucidate whether this mite represents a species distinct from *G. vitzthumi* and if the host is the recently described species *Cerradomys vivoi*. It is possible that when carefully examined and measured, Lizaso’s *G. bahiensis* is a distinct species infesting the different host species, *C. vivoi*.

The morphometric variations between populations of *G. vitzthumi* from Restinga de Jurubatiba and from Distrito Federal are not clear in contrast with that observed for *L. differens*. However, the characters used by Gettinger (1992a) as basic for the morphometric studies of laelapine mites are better applied with *Laelaps* the smaller species. *Gigantolelaeps* species have very long setae, which are broken easily during the processing for slide mounting. For this reason, a new set of morphological characters may be necessary with these large mites, to detect subtle variations in morphology among closely related hosts.

In Paraguay, Gettinger et al. (2011) found *L. manguinhosi* associated with five species of rodents in palustrine habitats, including *Nectomys*, *Holochilus*, *Pseudoryzomys*, and *Sooretamys*. Morphometric studies support the view that this mite was not a single monomorphic species capable of infesting a wide range of host species that share habitats, but is a complex of morphologically similar mite species, each infesting a distinct host species. Our studies in Brazil further verify these relationships, and show that morphometric variation can be measured between species of congeneric *Nectomys*.

The results obtained at present study indicate that laelapine mite populations can vary among geographic areas and, especially, among phylogenetically related host species. Clearly, the study of these mites at the population level is fundamental to help solving problems in evolutionary biology, especially related to the specificity relations with their hosts (Gettinger, 1992a). The morphometric analysis of laelapines associated with different species of *Cerradomys* can assist in the differentiation of both the host and mite species, and in the delimitation of their geographical distributions.

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![Figure 2](image-url)
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Figure 3. Morphometric variations in three populations of *Laelaps manguinhosi* from different localities and hosts: Distrito Federal, DF (circles) on *N. rattus*; Restinga de Jurubatiba, Macaé, RJ (crosses) and Praia das Neves, ES (triangles) on *N. squamipes*.

Figure 4. Morphometric variations in three populations of *Laelaps differens* from different species of the genera *Cerradomys*: Distrito Federal, DF (circle) associated with *C. scotti*, Restinga de Jurubatiba, Macaé, RJ (crosses) and Praia das Neves, ES (triangles) associated with *C. goytaca*.
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