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ANÁLISE DA VARIAÇÃO DE FORMA E TAMANHO DO ÚMERO DAS ESPÉCIES DE *CTENOMYS* OCORRENTES NO ESTADO DO RIO GRANDE DO SUL - BRASIL. (CTENOMYIDAE - RODENTIA)

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ABSTRACT

The Ctenomyidae family is the most diverse group in number of species of underground mammals, including only one genus (*Ctenomys*) and 60 living species described.

This genus is endemic of South America, and is distributed from the Andes in southern Peru and Bolivia and at sea level in Chile, covering also Argentina to the southwest of Brazil and Uruguay. Living in excavated burrows, the genus has different morphological adaptations, represented by the robust and cylindrical body, small eyes, reduced hearing flag, robust forelimbs and modifications in the incisors and skull for excavation. In general, the genus *Ctenomys* is a scratch-digger and secondarily a chisel-tooth digger, but the predominant digging behavior for most of the *Ctenomys* species is not known. Among post-cranial bones it possibly contains the larger amount of functional adaptive modifications and in scratch-diggers, the forelimb myoskeletal system composed of the scapula, humeri, radius and ulna forms a mechanical system which has great importance in the activity of excavation. In this work we analyze the humerus (n=156) of four species of *Ctenomys* that occur in southern Brazil. The species and their areas of occurrence range from the first lines of costal dunes (*C. flamarioni*, *C. minutus*) to the sandy fields of the coastal plains (*C. minutus, C. lami*) and in the fields of southern pampas gaúchos (*C. torquatus*). Although a robust published phylogeny of the genus is not available, *C. flamarioni* belongs to the monophyletic “mendocinus-group” and C. *torquatus*, *C. minutus* and *C. lami* are part of another monophyletic group (unpublished data). *C. minutus* and *C. lami* are recently diverged sister species. To analyze the difference in the form (size + shape) of the *Ctenomys* humerus we used a geometric morphometrics assessment and myological descriptions. Geometric morphometrics represented a “revolution” in morphometric methods, because independently of size, it quantifies and distinguishes variations of geometric deformations. Nevertheless, geometric morphometrics is not safeguarded from some usual problems of traditional morphometrics, as measurement errors during data acquisition due to the operator and equipments. In our work we demonstrate in a study of the humerus (a structure prone to measurement error) the landmark error assessments by the operator (repeatability), to verify there was no significant variation among the digitizations, proving the capacity of the operator to repeat the experiment without a significant measurement error. And between 20 operators (reproducibility) we demonstrate the necessity of experience in digitalization of landmark configurations. “Pinocchio effect” is phenomenon where the greater variation of few landmarks can be distributed after Procrustes superimposition among other landmarks which have small variances. In the interspecific analysis *C. flamarioni* has the largest humerus, *C. minutus* and *C. lami* an intermediate size and without significant difference among them. And finally *C. torquatus*, had the smallest humerus and differently from the other three species, no sexual dimorphism was observed. In general *C. flamarioni* had the most different shape among the analyzed species, however when we compared the two obtained extreme shape of the linear discriminant analyzes (LDA), (*C. lami, C. minutus* and *C. torquatus)* demonstrates a more robust humerus (independently of the size) in some areas when compared with *C. flamarioni*. And by crossing the osteolgy data with the qualitative observations of the musculature, it was possible to detect a larger difference in the proximal portion of the humerus that could be related with the insertion of important extension muscles of the pectoral-shoulder joints, which could increase force. Besides, an increase in the area of fixation of the humerus with scapula promotes a possible advantage in the excavation, which could determine the capacity of exploration of different soils. However, the three related species (*C. lami, C. minutus* and *C. torquatus)* the shape differences. It was expected that *C. torquatus* would have the more robust and with the more pronounced condyles and deltopectoral crest, however or the more pronounced osteological features belonged *C. lami* and *C. minutus* (more friable soils than compared with *C. torquatus*). Corroborating that hypothesis that soil hardness is not different enough to produce strong selection. Or maybe the divergence is to recent to establish differences in shape of the humerus.

RESUMO

A família Ctenomyidae é o grupo mais diverso em número de espécies de mamíferos subterrâneos, compreendendo apenas um gênero (*Ctenomys*) e um total de 60 espécies viventes. Sendo endêmico da América do Sul, o gênero é distribuído desde os Andes no sul do Peru e Bolívia e no nível do mar no Chile, abrangendo também a Argentina até o sudoeste do Brasil e Uruguai. Vivendo em tocas escavadas no solo, suas principais adaptações morfológicas ao modo de vida são o corpo cilíndrico, órgãos de visão pequenos, pavilhão auditivo reduzidos e modificações para a escavação no crânio e incisivos, assim como membros locomotores curtos e robustos. O gênero *Ctenomys* é em geral um garra-escavador e secundariamente um dente-escavador, porém ocupam uma vasta área na América do Sul, sendo que as diferentes espécies acabam apresentando diferentes comportamentos em resposta a diversos habitats e a diversos tipos de solos. Neste trabalho foram analisados o úmero (n=156) de quatro espécies de *Ctenomys* que ocorrem no sul do Brasil, *C. flamarioni, C. minutus, C. lami* e C. *torquatus*. As áreas de ocorrência destas espécies vão desde as primeiras linhas de dunas do litoral (*C. flamarioni, C. minutus*) aos campos arenosos das planícies costeiras (*C. minutus, C. lami*) e os campos sulinos dos pampas gaúchos (*C. torquatus*). E mesmo não havendo uma filogenia robusta para o gênero, sabe-se que *C. flamarioni* pertence ao grupo monofilético “mendocinus” e C. *torquatus*, *C. minutus* e *C. lami* fazem parte de outro grupo monofilético (dados inéditos). Para analisar a diferença da forma e tamanho do úmero nos utilizamos a morfometria geométrica e observações quantitativas dos pontos de origem e inserção de músculos relacionados ao úmero. A morfometria geométrica foi uma revolução nos estudos de morfometria, porém não esta salva de problemas como a introdução de erros devido ao operador ou instrumentos de medição. E como o úmero é uma estrutura pequena e propensa a introdução de erros externo, nos desenvolvemos um protocolo funcional para o estudo da forma e medimos o erro devido ao operador (repetibilidade) e entre operadores (reprodutibilidade) e o efeitos da variação de poucos marcos anatômicos (“efeito pinóquio”) na interpretação dos resultados. Após a aplicação do protocolo foi possível confirmar a capacidade do operador em repetir as configurações de marcos anatômicos (m.a.) sem uma significativa variação, também foi constatado a necessidade de haver experiência na digitalização de m.a. e que existe um “efeito pinóquio” que pode interferir na correta visualização das deformações de marcos anatômicos. Na análise interespecífica *C. flamarioni* demonstrou ter o maior úmero, *C. minutus* e *C. lami* um tamanho intermediário e sem diferença significativa entre elas. E por último *C. torquatus*, com o menor úmero e sem dimorfismo sexual aparentes entre machos e fêmeas. Em geral *C. flamarioni* demonstrou ter a forma mais diferente entre as espécies analisadas, o que era esperado em relação as distâncias filogenéticas com as outras três espécies. Porém quando observamos as duas formas mais diferentes (*C. flamarioni* e *C. torquatus*) obtidas da análise discriminante linear (ADL), *C. torquatus* demonstra úmero mais robusto (independentemente do tamanho) em algumas regiões quando comparado com *C. flamarioni*. E cruzando os dados osteológicos com as observações qualitativas da musculatura, foi possível detectar uma maior diferença na porção proximal do úmero, que relacionada com inserção de importantes músculos extensores do ombro e peitorais podem acrescentar uma vantagem no aumento da força e que podem determinar a capacidade da exploração de diferentes durezas solos. Porém entre as três espécies (*C. lami*, *C. minutus* e *C. torquatus*) mais relacionadas filogeneticamente as diferenças de forma não são tão aparentes quando comparados com *C. flamarioni-torquatus*. Era esperado que *C. torquatus* tivesse o úmero mais robusto e com uma crista deltopectoral e côndilos mais pronunciados, porém as características osteológicas mais pronunciadas pertencem a *C. lami* e *C. minutus* (que vivem em solos mais macios quando comparados com *C. torquatus*). Corroborando com a hipótese de que dureza pode não ser diferente o bastante

para produzir uma seleção forte. Ou talvez a divergência entre essas espécies seja tão recente que ainda não foi possível estabelecer “diferenças” na forma do úmero.

I. INTRODUÇÃO

Dentre os mamíferos, os roedores são o grupo que desenvolveram a maior variedade de formas de locomoção (Hildebrand, 1985; Cubo et al., 2006). Sendo as mais conhecidas as cursoriais, saltadoras, planadoras, nadadoras e escavadoras. Diversos trabalhos têm correlacionado estas diversas atividades a particulares adaptações esqueléticas e/ou musculares (Bou & Casinos, 1985; Bou et al., 1987; Bou et al., 1990; Castiella & Casinos, 1990; Eilam, 1997; Cubo et al., 2006), sendo a escavação uma das atividades que demonstram o maior numero de modificações em roedores (Lessa, 1990). A família Ctenomyidae é o grupo mais diverso em número de espécies de mamíferos subterrâneos (Nevo, 1979), compreendendo apenas um gênero (*Ctenomys*) e um total de 60 espécies viventes (Reig et al., 1990; Lacey et al., 2000). Sendo endêmico da América do Sul, o gênero é distribuído desde os Andes no sul do Peru e Bolívia e no nível do mar no Chile, abrangendo também a Argentina até o sudoeste do Brasil e Uruguai (Lacey et al., 2000). Vivendo em tocas escavadas no solo, o gênero *Ctenomys* possui diversas adaptações ecológicas, fisiológicas e morfológicas ao modo de vida subterrâneo. Suas principais adaptações morfológicas são o corpo cilíndrico, órgãos de visão pequenos, pavilhão auditivo reduzido e modificações para a escavação no crânio e incisivos (os lábios que se fecham posteriormente aos incisivos), assim como membros locomotores curtos e robustos (Pearson, 1959; Reig et al., 1990; Casinos et al., 1993; Vassalo, 1998; Lacey et al., 2000; Verzi, 2002 e Morgan & Verzi, 2006). As espécies alimentam-se basicamente de raízes e folhas de pequenas gramíneas (Lacey et al., 2000). Em geral as espécies são solitárias (com exceção de *C. sociabilis*) e territorialistas (Reig et al., 1990), sendo que a maior parte das atividades destes roedores é feita dentro das tocas, atividades externas são reduzidas, limitando-se apenas a buscas próximas de alimentos, eventuais dispersões e limpeza (Lacey et al., 2000).

Além dos membros locomotores anteriores e posteriores, o gênero *Ctenomys* e outros grupos de roedores subterrâneos utilizam o crânio e principalmente os incisivos para a atividade de escavação (Hildebrand, 1985; Bou et al., 1987; Lessa, 1990). Esta escavação pode ser feita de três maneiras distintas; com as garras dos membros anteriores e posteriores, com o crânio (dentes incisivos) ou ainda uma combinação das duas formas (ver Lessa, 1990; Vassalo, 1998; Mora et al., 2003). Segundo Lessa (1990), para solos mais arenosos e “macios” a estratégia mais utilizada é a garra-escavadora e para solos com uma textura mais “dura” ou com maior quantidade de rochas é utilizada a estratégia dente-escavadora. O gênero *Ctenomys* é em geral um garra-escavador e secundariamente um dente-escavador (Stein, 2000; Ubilla & Altuna, 1990; Vassalo, 1998; Rebelato, 2006). Porém ocupam uma vasta área na América do Sul, sendo que as diferentes espécies acabam apresentando diferentes comportamentos em resposta a diversos habitats e a diversos tipos de solos, conseqüentemente apresentando diferentes desenhos estruturais de crânios e incisivos (Mora et al., 2003). No sul do Brasil ocorrem quatro espécies, *Ctenomys minutus* nos Estados de Santa Catarina e Rio Grande do Sul e *C. flamarioni, C. lami e C. torquatus* no Rio Grande do Sul (Figura 1). Sendo que as áreas de ocorrência destas espécies vão desde as primeiras linhas de dunas do litoral (*C. flamarioni, C. minutus*) aos campos arenosos das planícies costeiras (*C. minutus*, *C. lami*) e os campos sulinos dos pampas gaúchos (*C. torquatus*) (Freitas, 1995; Fernandes et al., 2007).

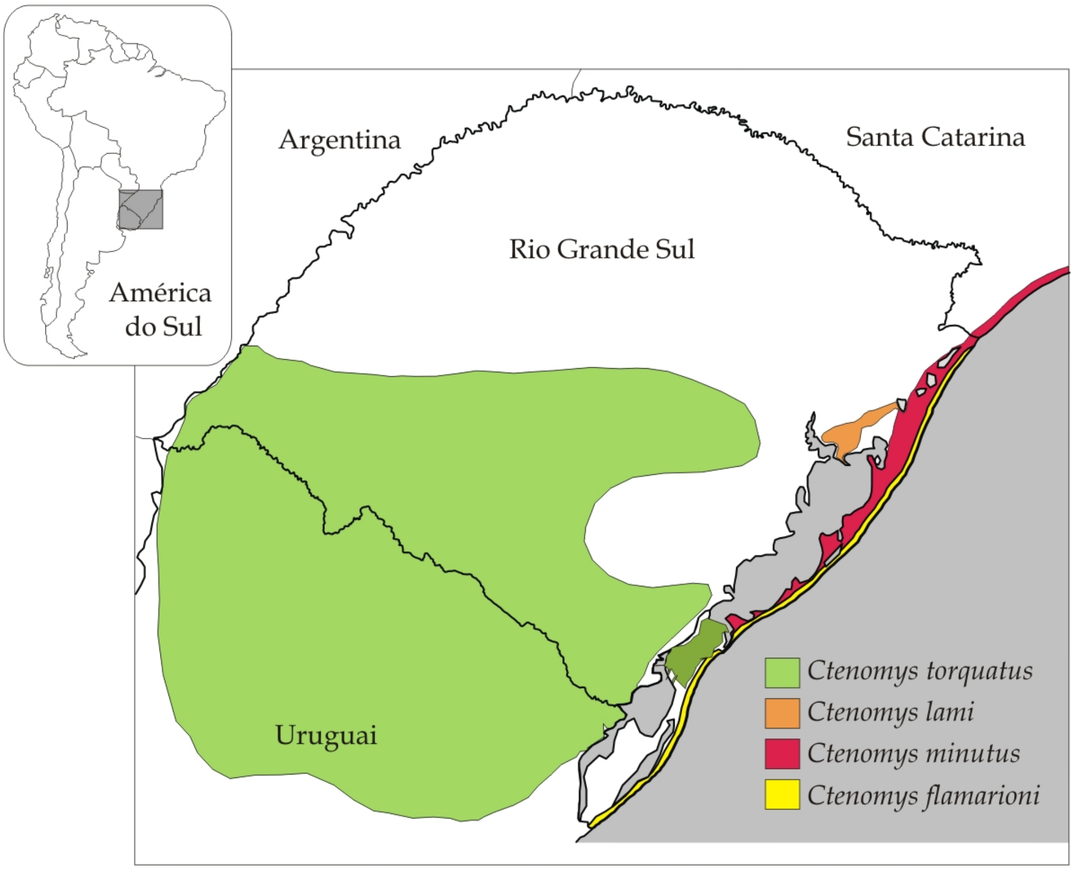

Figura 1: Mapa da distribuição de *C. torquatus, C. lami, C. minutus* e *C. flamarioni* para a América do Sul.

Diversos autores (Freitas, 1990; Vassalo, 1998; Marinho & Freitas, 2000; Mora et al., 2003; Azurduy, 2005; Freitas, 2005; Fornel, 2005; Massarini & Freitas, 2005; D’Anatro & Lessa, 2006; Rebelato, 2006) analisaram diferenças nas formas e variações no tamanho dos crânios das espécies de *Ctenomys* viventes e extintas. Porém o conhecimento das estruturas pós-cranianas, como os membros locomotores e suas possíveis variações em resposta aos diferentes tipos de solos ou parentescos filogenéticos ainda são obscuras (Reig et al., 1990; Cassinos et al., 1993; Vassalo, 1998; Lacey et al., 2000; Szalay & Sargis, 2001; Morgan & Verzi, 2006; Vassalo & Mora, 2007). Mesmo não havendo uma filogenia robusta para o gênero, sabe-se que *C. flamarioni* pertence ao grupo monofilético “mendocinus” (*C. australis*, *C. flamarioni*, *C. rionegrensis, C. porteousi*, *C. azarae*, *C. mendocinus* e *C. chasiquensis*) (Massarini et al., 1991; Massarini & Freitas, 2005). C. *torquatus*, *C. minutus* e *C. lami* fazem parte de outro grupo monofilético (dados inéditos), sendo que de acordo com Freitas (2001), *C. minutus* e *C. lami* são espécies irmãs com pouco tempo de divergência.

Segundo Lessa et al. (2008) e suas referências, a atividade de escavação requer uma capacidade de produzir e transmitir uma força considerável durante longos períodos. Ocasionando essa demanda funcional profundas mudanças nos atributos fisiológicos (McNab, 1979; Antinuchi & Luna, 2007), bem como toda a arquitetura do sistema mioesqueletal que são envolvidos na atividade na escavação (Lehman, 1963). Em espécies com outros hábitos de locomoção (cursoriais, escaladoras e saltadoras) há uma maior necessidade de ossos longos e leves e uma musculatura para movimentos ágeis. (Hildebrand, 1985; Bou et al., 1987; Hildebrand, 1988; Casinos et al., 1993; Vassalo, 1998). Nas espécies subterrâneas, para uma maior resistência à inserção muscular (aumento da força), os ossos longos (úmero e fêmur) são reduzidos e robustos, os músculos são largos, e suas origens e inserções são relativamente longe das articulações (Hildebrand, 1985; Lessa, 1990; Fernández et al., 2000; Lessa et al., 2008). Nas espécies que utilizam garra-escavação como no gênero *Ctenomys*, os membros locomotores anteriores (escápula, úmero, radio/ulna e dedos, incluindo a unha) são os que têm maior papel na escavação das tocas, sendo o úmero associado com os seus músculos o sistema mecânico de maior importância para a atividade de escavação, e com maior número de informações funcional-adaptativas do pós-crânio (Szalay & Sargis, 2001; Morgan & Verzi, 2006). Segundo Reig et al. (1990), as espécies viventes de *Ctenomys* têm adaptações similares para escavação dos membros locomotores e crânio. Porém o conhecimento das adaptações e diferenças no úmero das espécies viventes e extintas de *Ctenomys* é reduzido. Nos poucos trabalhos que analisaram estruturas pós-cranianas o número de amostras é reduzido (ver Morgan & Verzi, 2006) ou são analisadas apenas duas espécies (ver Vassalo, 1998) o que torna as resultados destes trabalhos pouco conclusivas estatística e evolutivamente. Além disso, todos os trabalhos analisaram as estruturas com métodos de morfologia clássica (medidas lineares) que não restituem a relação geométrica entre os pontos de medida (Rohlf & Marcus, 1993). O gênero *Ctenomys* tem se demonstrado um grupo modelo para estudos genéticos, evolutivos e morfológicos, porém há algumas lacunas de conhecimento a serem preenchidas, sendo o estudo do pós-craniano e suas adaptações uma delas.

II. OBJETIVOS

Objetivo Geral

- Analisar se há variação interespecífica na forma e tamanho do úmero das espécies de *Ctenomys* ocorrentes no Estado do Rio Grande do Sul, utilizando a morfometria geométrica e dados miológicos qualitativos.

Objetivos Específicos

Capítulo 1 - Artigo I

- Definir uma metodologia funcional para análise de morfometria geométrica do úmero;

Capítulo 2 - Artigo II

- Quantificar, comparar e interpretar a variação na forma e tamanho do úmero;

- Descrever a musculatura definindo os pontos de origem e inserção dos músculos dos membros anteriores que estão relacionados ao úmero;

- A partir dos dados obtidos com a morfometria geométrica, comparar e interpretar as diferenças da forma do úmero que estão relacionados com os pontos de origem e inserção dos músculos dos membros anteriores de *C. flamarioni*, *C. lami*, *C. minutus* e *C. torquatus*. ocorrentes no Estado do Rio Grande do Sul

III. CAPÍTULO 1

REPEATABILITY, REPRODUCIBILITY AND THE "PINOCCHIO EFFECT" IN TWO DIMENSIONAL GEOMETRIC MORPHOMETRICS USING DIGITAL PICTURES – A PROTOCOL TO ERROR MEASUREMENT.

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Introduction

To classify the diversity of life forms from morphological characteristics is one of the oldest procedures of taxonomy and systematic (Adams *et al.* 2004). Morphometrics designates all studies that search to describe, analyze and interpret quantitatively the variation in the form of organisms (Rohlf 1990). Geometric morphometrics (Bookstein 1991; Rohlf & Marcus 1993) represented a "revolution" in this field as a new class of methods for the acquisition and analysis of shapes, because independently of size, it quantifies and distinguishes local and global variations of geometric deformations, making it possible to study patterns of form (size + shape) and shape using homologous descriptors. Nevertheless, geometric morphometrics is not safeguarded from some usual problems of traditional morphometrics, as measurement errors during data acquisition due to the operator (Heathcote 1981; Corner *et al.* 1992; Valeri *et al.* 1998) and equipments (Corner *et al.* 1992).

In the 90's, a great number of works sought to determine the effect of measurement error (i.e. the variation of repeated measures of the same particular character, Bailey & Byrnes 1990; Merila & Bjorklund 1995) in the results and in the interpretation of the obtained data (Bailey & Byrnes 1990; Lee 1990; Lougheesd *et al.* 1991; Coorner *et al.* 1992; Yezerinac *et al.* 1992; Jamison & Ward 1993). In comparative studies that intend to analyze differences either in the form of small objects or objects with a very similar shapes, the effect of the measurement error (ME) should be analyzed with caution (Cramon-Taubadel *et al.* 2007). Therefore, the data obtained could be the result of an erroneous observation, because if the variations of ME are larger than the variations among the shapes of the analyzed objects the results will have an important component of variation attributable to ME and not to the real forms analyzed (Grubbs 1973; Lee 1990). In this work, we used the concepts repeatability and reproducibility to measure and interpret the sources of intra and inter-observer error. According to Inmetro (Brazilian institute of metrology) (2007), repeatability is defined as the degree of agreement between the results of successive measurements of the same magnitude, made under the same conditions by only one operator. Whereas reproducibility is defined as degree of agreement between the results of measurements of the same magnitude, where the individual measurements are made ranging up one or more conditions: operator, the measuring instrument, location, conditions of use and time. Therefore, reproducibility is how much a study can be reproduced or what conditions are critical to the repeatability of the study.

In studies that use two-dimensional homologous landmarks, some unwanted effects on the positioning of specimens such as translation, rotation and size (i.e. nuisance parameters) must be removed before the analysis of shape (Dryden & Mardia 1998). The generalized least squares (GLS) Procrustes superimposition of landmarks is the most commonly used procedure for this purpose. However, this method can introduce biases in the quantification and visualization of shape changes if only a few landmarks have exceedingly large variances compared to the others. This phenomenon has been termed "Pinocchio effect" (Siegel & Benson 1982; Chapman 1990; Rohlf & Slice 1990; Walker 2000; Cramon-Taubadel *et al.* 2007; Gill *et al.* 2007) and can generate visual images improperly interpreted, because an important variation of a few landmarks is distributed among other landmarks which have small variances. Walker (2000), simulated several landmark configurations with varying number of landmarks and quantified the difference between the variance/covariance (VAR/COV) matrices of two different superimposition methods, GLS and resistant fit. The conclusion of Walker's work is that the "Pinocchio effect" is intimately correlated with the number of landmarks and with the superimposition method used. In the present work we demonstrate in a study of the humerus (a structure prone to measurement error) in four species of the subterranean rodent genus *Ctenomys*, (i) the landmark error assessments of the repeatability (intra-observer error of one operator – FS) and of the reproducibility (inter-observer error between 20 operators) and (ii) the relationship of a qualitative "Pinocchio effect" with the landmark position in configuration in 2 dimensional geometric morphometrics using digital pictures.

Material and Methods

Samples

In this work we used 16 adult humerus of the four species *Ctenomys flamarioni*, *C. lami*, *C. minutus* and *C. torquatus*. For each species two males and two females were selected. All specimens are deposited in the Laboratório de Citogenética e Evolução de Vertebrados, Departamento de Genética, Universidade Federal do Rio Grande do Sul, Brazil. The genus

Ctenomys (Ctenomyidae) is the most diverse group, in number of species, of underground mammals (Nevo 1979) with a total of 56 species. Living in excavated burrows in the soil, the genus *Ctenomys* possesses different ecological, physiological and morphological adaptations to the life underground. The excavation activity can be done in three different ways, with the claws of forelimbs, with the skull and incisor teeth, or in a combination of the two forms (see Lessa, 1990). According Lessa (1990), subterranean mammals use claw-digging for sandy soils and tooth-digging in harder soils with a greater quantity of rocks, the genus *Ctenomys* is primary a claw-digger and secondarily a tooth-digger (Vassalo 1998).

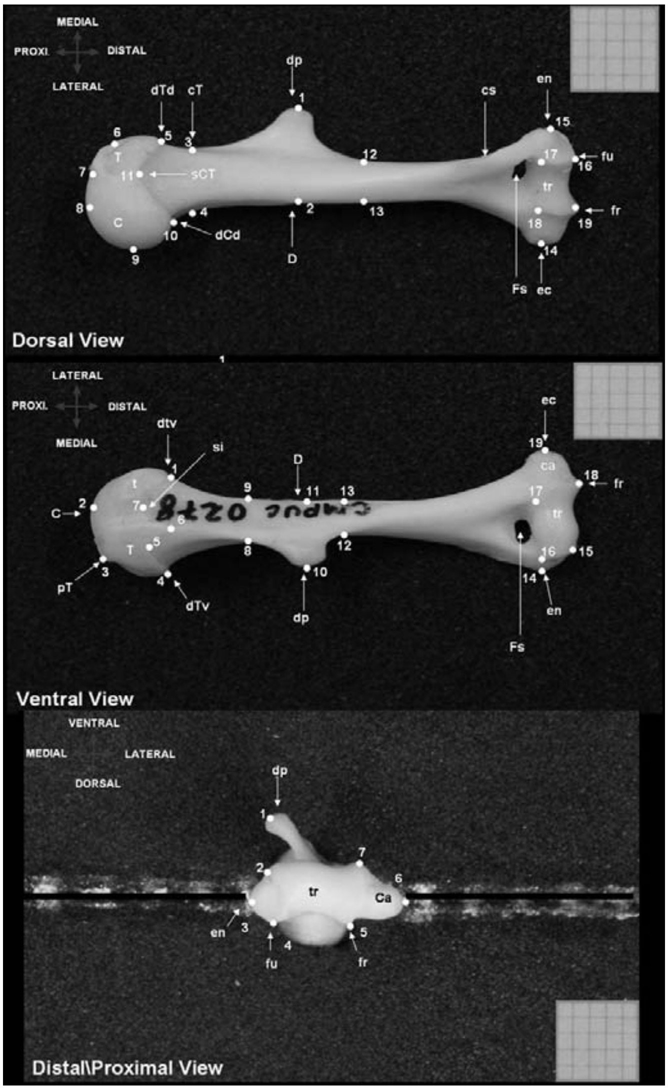
Data Acquisition

The humerus was photographed in 3 different views: Dorsal, Ventral and Distal\Proximal. In each view we defined 19, 19 and 7 landmarks respectively (Figure 1 – Table 1). For the Dorsal view the standard positioning of the humerus was made by laying it on its ventral side with the crest of the deltoid in contact with a polyethylene base. For the Ventral view the humerus was laid on the polyethylene base on its “dorsal” side with ulnar and radial facet of the trochlea in contact with the plane. For the Distal\Proximal view the condyle of the humerus was placed in a hole in the polyethylene basis and the major axis of the bone perpendicular to the basis and to the photographic plane, the proximal part of the humerus is next to the plane and the distal part next to focus.

All photos were obtained with a digital camera *Canon EOS 400 D* with 10 mega pixels of resolution, mode = A-DEP, Iso100, One-Shot, AWA with MACRO Lens an opening of -2. All images were taken from a standard distance (15cm) with the aid of a portable support for the camera. A basis of polyethylene foam served as a background to the images. The pictures were organized in lists with the program TPSUtil 1.26 (Rohlf 2004) and the landmarks were digitized with the program TPSDig 1.40 (Rohlf 2004).

Figure 1. The right humerus of *C. flamarioni***.** In the upper panel - Dorsal View, on the midle - Ventral View and in the lower panel - Distal/Proximal View. The signf. codes are: **dtd** – distal part of great tuberosity; **cT**– lateral crest of great tuberosity; **dp** – deltopectoral crest; **cs** – supinatory crest; **en** – medial epicondyle; **ec** – epicondyle; **fr** – radial facet; **fu** – ulnar facet; **Fs** – supratrochlea foramen; **D** – diaphysis; **dCd** – distal part of condyle; **C** – condyle ; **T** – great tuberosity; **sCT** – groove between condyle and great tuberosity; **tr** –trochlea; **dTv** – distal part of great tuberosity; **pT** – great tuberosity process; **dtv** – distal part of small tuberosity; **si** – intertubercular groove; **ca** – capitulum. The signify codes, definition and numbering of landmarks are: **dtd** – distal part of great tuberosity; **cT**– lateral crest of great tuberosity; **dp** – deltopectoral crest; **cs** – supinatory crest; **en** – medial epicondyle; **ec** – epicondyle; **fr** – radial facet; **fu** – ulnar facet; **Fs** – supratrochlea foramen; **D** – diaphysis; **dCd** – distal part of condyle; **C** – condyle ; **T** – great tuberosity; **sCT** – groove between condyle and great tuberosity; **tr** –trochlea; **dTv** – distal part of great tuberosity; **pT** – great tuberosity process; **dtv** – distal part of small tuberosity; **si** – intertubercular groove; **ca** – capitulum. ***Dorsal View:*** **1.** End of medial deltopectoral crest. - **2**. End of lateral diaphysis, parallel to *landmark 1*.- **3**. Encounter of the lateral crest of the great tuberosity with the deltopectoral crest.- **4.** End of the lateral diaphysis, parallel to *landmark 3*.- 5. Meeting of the distal extremity of the great tuberosity with the lateral crest of the great tuberosity.- **6.** Groove in the great tuberosity,

most lateral part.- **7.**Groove in the meeting of the great tuberosity with the condyle, most lateral part.- **8.** Proximal end of the condyle.- **9.** Lateral extremity of the condyle.- **10.** Meeting of the distal part of the condyle with the diaphysis.- **11.** Groove between condyle and the great tuberosity, most proximal part.- **12.** Meeting of the most distal part of the deltopectoral crest and supinatory crest with the diaphysis.- **13.** Extremity of the lateral diaphysis, parallel to *landmark 12*.- **14.** Lateral extremity of the epicondyle.- **15.** Medial extremity of the medial epicondyle.- **16.** Distal extremity of the ulnar facet.- **17.** Proximal extremity of the articulated part of the ulnar facet.- **18.** Proximal extremity of the articulated part of the radial facet.- **19.** Distal extremity of the radial facet. ***Ventral View:*** **1.** Meeting of the distal part of the great tuberosity with the diaphysis.- **2.** Proximal extremity of the condyle.- **3.** Proximal extremity of the distal process of the great tuberosity.- **4.** Meeting of the distal extremity of the small tuberosity with the diaphysis.- **5.** Proximal extremity of the encounter of the great tuberosity with the diaphysis.- **6.** Distal extremity of the encounter of the great tuberosity with the small tuberosity.- **7.** Meeting of the proximal extremity of the sulcus intertubercular with the small tuberosity.- **8.** Meeting of the proximal part of the deltopectoral crest with the medial diaphysis.- **9.** Extremity of the lateral diaphysis, parallel to *landmark 8*.- **10.** Medial extremity of the deltopectoral crest.- **11.** Extremity of the lateral diaphysis, parallel to *landmark 10*.- **12.** Meeting of the distal part of deltopectoral crest with the medial diaphysis.- **13.** Lateral extremity of the diaphysis, parallel to *landmark 12*.- **14.** Medial extremity of the medial epicondyle.- **15.** Distal extremity of the ulna facet.- **16.** Proximal extremity of the articular part of the ulna facet.- **17.** Proximal extremity of the articular part of the radial facet.- **18.** Distal extremity of the radial facet.- **19.** Lateral extremity of the epicondyle. ***Distal/Proximal View:*** **1.** Medial-ventral extremity of the deltopectoral crest.- **2.** Ventral extremity of the ulna facet.- **3.** Medial extremity of the medial epicondyle.- **4.** Ventral extremity of the ulnar facet.- **5.** Ventral extremity of the radial facet.- **6.** Lateral extremity of the epicondyle (capitulum). - **7.** Ventral extremity of the radial facet.



Inter and Intra-Observer Error

For the quantification of measurement error we used two different data sets to access intra-observer (repeatability) and inter-observer (reproducibility) error components of each landmark. The intra-observer set consisted of 16 adult humerus (two males and two females of each of the four species chosen randomly). Landmarks in the three views were digitized 10 times in non-consecutive sessions (total sample size per view of 160) by one of us (FS) with experience in digitization of anatomical landmarks. The inter-observer set consisted of 3 successive digitizations of landmarks in one humerus of *C. flamarioni* (CMPUC 0278) by a group of 20 operators, all post-graduation students, most of them without any experience in digitization of anatomical landmarks, but monitored by one of us (FS) (total sample size per view=60).

Morphometric and statistical analysis

Data used

In this work we used two different types of data collected from the humerus, the standard deviation of raw data – **S** and the residuals after GLS superimposition – $\mathbf{S}^{\mathbf{gls}}$ (residuals are the difference between each landmark of each individual and the landmarks of the mean configuration after scaling, translation and rotation). Henceforth each landmark is represented by two coordinates in the plane (*x* and *y* axis of the picture), the raw data - **S** are the raw coordinate of each landmark without any other transformation. In intra-observer analysis (repeatability), 16 different samples were used and the two types of data (**S**, $\mathbf{S}^{\mathbf{gls}}$). However, as there are 16 different pictures, **S** cannot be computed directly as the raw standard deviation of the data because of nuisance parameters. Therefore **S** was computed as the mean of standard errors of each of the 10 repetitions of the 16 humerus. Since repetitions are done on the same pictures there are no nuisance parameters. In inter-observer analysis (reproducibility) since there is a unique sample, only the standard deviation of the raw data (**S**) was used to quantify the landmark variation without any source of bias by superimposition methods. The raw data was used by axis $\mathbf{S}^{\mathbf{x}}$ and $\mathbf{S}^{\mathbf{y}}$ (standard deviations on the *x* and *y* axis) and sum of standard deviations on each axis $\mathbf{S}^{\mathbf{x+y}}$ (standard deviation of *x* and standard deviations of *y*). For the residuals obtained after GPS we used only the sum of axes $\mathbf{S}^{\mathbf{gls}}$ (standard deviation of *x* and standard deviations of *y*).

Repeatability

To test the repeatability of the digitalization of landmarks, first we used the raw data $\mathbf{S}^{\mathbf{x+y}}$ of each landmark from the 10 repetitions of digitalization to determine the deviation of each landmark in each of the three views from the mean. With this dataset we carried a two-way model II analysis of variance – ANOVA, with equal number of samples (Sokal & Rohlf, 1995) to test the effects of factors repetition, sex, species variations and their interactions for each landmark in the three views. This analysis estimates the variance between the observed means in each landmark between the 10 repetitions of digitalization of the 16 specimens (n=160). A bar plot was made with the sum of standard deviations (for the two axes $\mathbf{S}^{x+y}$**)** and with the GPA data ($\mathbf{S}^{gls}$).

Reproducibility

For the analysis of reproducibility (three repetitions made by the 20 operators) in the three views we used only the raw data **S** because only one humerus was used. In this data we applied two-away ANOVAs model II with equal number of samples (Sokal & Rohlf, 1995) for each landmark in the three views. A bar plot was made with the standard deviation of raw data by axis $\mathbf{S}^{x}$, $\mathbf{S}^{y}$ and the sum of axis $\mathbf{S}^{x+y}$.

Pinocchio effect

It is widely accepted that not all landmarks are equally recognizable. However no typology has been proposed to classify how well a landmark can be recognized. The only typology available (Type I, II and III) is based on the confidence on the hypothesis of homology of a landmark (Bookstein 1991). Often, but not always, a landmark constituting a good homology hypothesis will be located with more precision than one with a poor one. For example, Type I landmarks which are based on strong homology hypotheses (ex: intersection of three bony sutures) and can be located more precisely than Type II (ex: maximum curvature of a bone) and Type III (ex: center of a foramen) which uses geometric properties to postulate homology and not histological evidence like Type I landmark. Even though the "Pinocchio Effect" is a rare phenomenon (Hallgrímsson *et al.* 2004), but the humerus is a structure prone to be affected by this phenomenon, because it has few Type I landmarks. Thus, it is necessary to test for the existence disparity of variance of a few landmarks and describe its consequence in the Generalized Procrustes Analysis – GPA (Gower 1975; Rohlf & Slice 1990). For the dorsal view, we used 19 landmarks, six of them type I, ten of type II and three of type III, in the ventral view – there are also 19 landmarks, six of them type I, ten of type II and three of type III and from the distal/proximal view – there are seven landmarks, all of type II. To test the “Pinocchio effect” in the landmark configurations we used the raw data $\mathbf{S}^{x+y}$ and the data $\mathbf{S}^{gls}$ after GPA analysis obtained for repeatability. With each residual obtained after GPA of each landmark we made one-way ANOVA of 3 factors, species (4) x sex (2) x individuals (16). To visualize the difference between the results obtained we made a bar plot with sum of total standard deviations of residuals $\mathbf{S_{gpa}}$ and the sum of total standard deviations of raw data $\mathbf{S^{x+y}}$ to visualize the existence of the “Pinocchio effect” in GPA method.

Results

Repeatability and Pinocchio Effect

None of the ANOVAs of the raw data $\mathbf{S^{xy}}$ for every landmark for the 10 repetitions had significant F values. Figure 2 shows the comparison in the three views between the sum of x and y coordinates standard deviations of each landmark starting from the raw data $\mathbf{S^{x+y}}$ (left) and GPA data $\mathbf{S^{gls}}$ (right), making possible the visualization of the variations of a few landmarks and their distribution in proximal landmarks, and finally how they standard deviation is decreased after GPA pinocchio effect. The standard deviations in the three views of each landmarks ranged from 0-25 for the raw data and 0-150 for the GPA data.

The results of the ANOVAs with the residuals from the GPA (Table III, IV and V - Annex) demonstrated a significant variation among species, sex and their interaction for most of the landmarks in the 3 views (Table II). No significant variation was observed between the ten repetitions in any of the 3 views. The ANOVAs for centroid size (Table VI - Enclose), indicates highly significant variation among species and the interaction between sex and species in the dorsal, ventral and distal-proximal views. Significant variation between sexes was only detected for the dorsal-proximal view. Any significant variation between the repetitions made by the operator (F.S.) was observed.

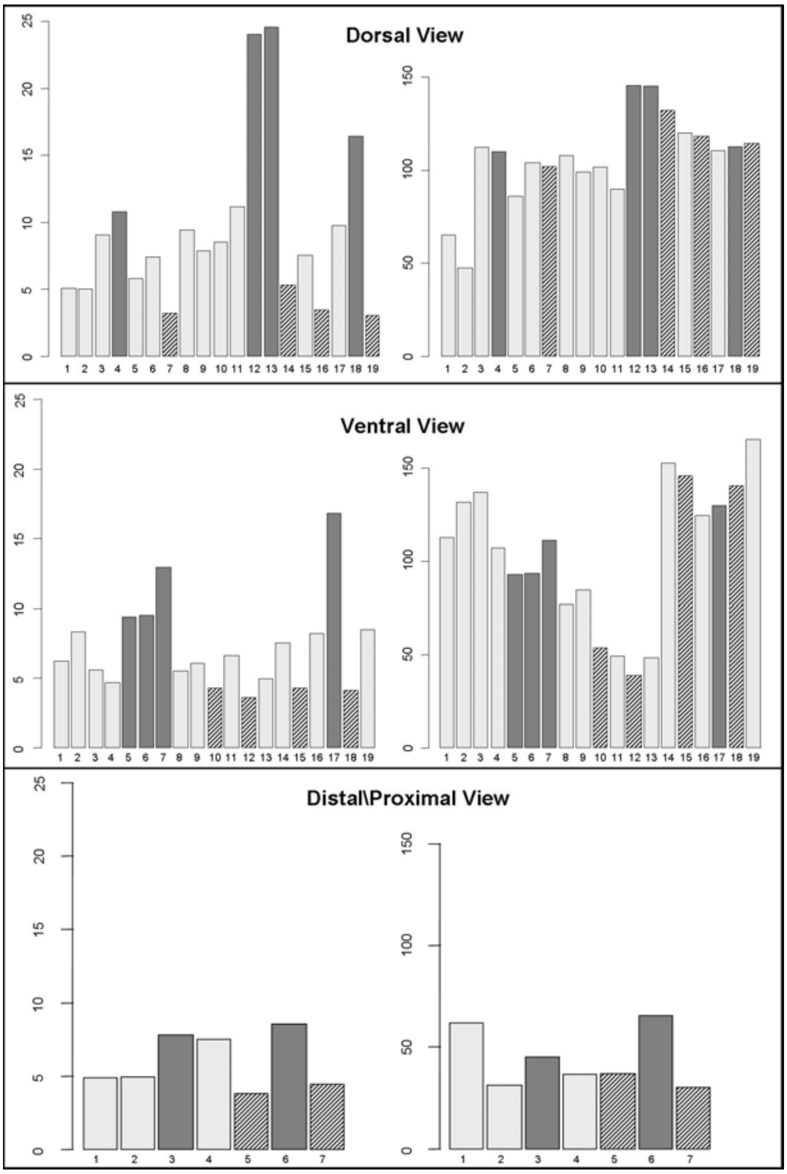


Figure 2. Repeatability of operator (FS) or intra-observer error, in left column the sum of the standard deviations of raw data $\mathbf{S}^{x+y}$ and in right the sum of the standard deviation of procrustes residuals obtained after GPA $\mathbf{S}^{gls}$. In dark gray the landmark of high variation and in medium gray the smaller. On top - Dorsal View, in the middle - Ventral View and on the bottom - Distal/Proximal View.

Table 2: Table with the results of significances of ANOVAs for repeatability (intra-observer error) with the residuals from the GPA for the factors sex, species and sex/species interaction for the 19 landmarks for Dorsal and Ventral views and 7 landmarks for Distal/Proximal View. Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '1 .

Dorsal View							**Lm**	**Sex**		**Species**		**Sex/Species**	
Lm	**Sex**		**Species**		**Sex/Species**		**-**	*X*	*Y*	*X*	*Y*	*X*	*Y*
-	*X*	*Y*	*X*	*Y*	*X*	*Y*	**10**			**	**	***	***
1	*	***		**		***	**11**	*		***		***	***
2				**	**		**12**	*	***				***
3			***	***		***	**13**	*			*		
4			***	**		***	**14**	***				*	*
5	**		***	*	*	***	**15**				**		
6	***		***				**16**					*	

7	*		**	***	**	*	**17**	*		*		**	
8			***	***	*	***	**18**	***			***		
9	**	***	***	***	***	***	**19**	*	**		*	*	***

Ventral View							**Lm**	**Sex**		**Species**		**Sex/Species**	
Lm	**Sex**		**Species**		**Sex/Species**		**-**	*X*	*Y*	*X*	*Y*	*X*	*Y*
-	*X*	*Y*	*X*	*Y*	*X*	*Y*	**10**		***		***		***
1	**			*	***	***	**11**					*	
2	***	**	***	***	**	***	**12**					*	***
3							**13**					**	*
4			**	*		**	**14**	*	**		*	**	
5		**	***	***	***	**	**15**			**	***	***	***
6	***	***	*	***	***	***	**16**				*		***
7		***	**	***		**	**17**			***	***	***	***
8			***			***	**18**		**		**	***	***
9			***	**		*	**19**			***	***	***	**

Distal/ Proximal View						
Lm	**Sex**		**Species**		**Sex/Species**	
-	*X*	*Y*	*X*	*Y*	*X*	*Y*
1		**	*	***	*	***
2	***		**	*	***	
3			***	***	***	
4	***	**	***	**	**	***
5	*	***	***	***	***	***
6	*	**	***		***	***
7			***	*	***	***

Reproducibility

The majority of ANOVAs results (Table VII, VIII and IX – Annex) of the raw data $\mathbf{S^{xy}}$ were significant for the factor "operators". In Figure 3 is demonstrated the landmark of high and smaller variation for raw data made by the operator, in the left graphic the standard deviations ($\mathbf{S^{xy}}$) and right the sum of total standard deviations ($\mathbf{S^{x+y}}$). In the Dorsal View, the reproducibility is more critical in the landmarks (8-4-3) and the landmarks (16-19-5) are the more reproducible. For the Ventral View, the critical landmark is (2) and the more reproducible landmarks are (18). The Distal/proximal View doesn't demonstrate any significant critical landmark when compared with the other views.

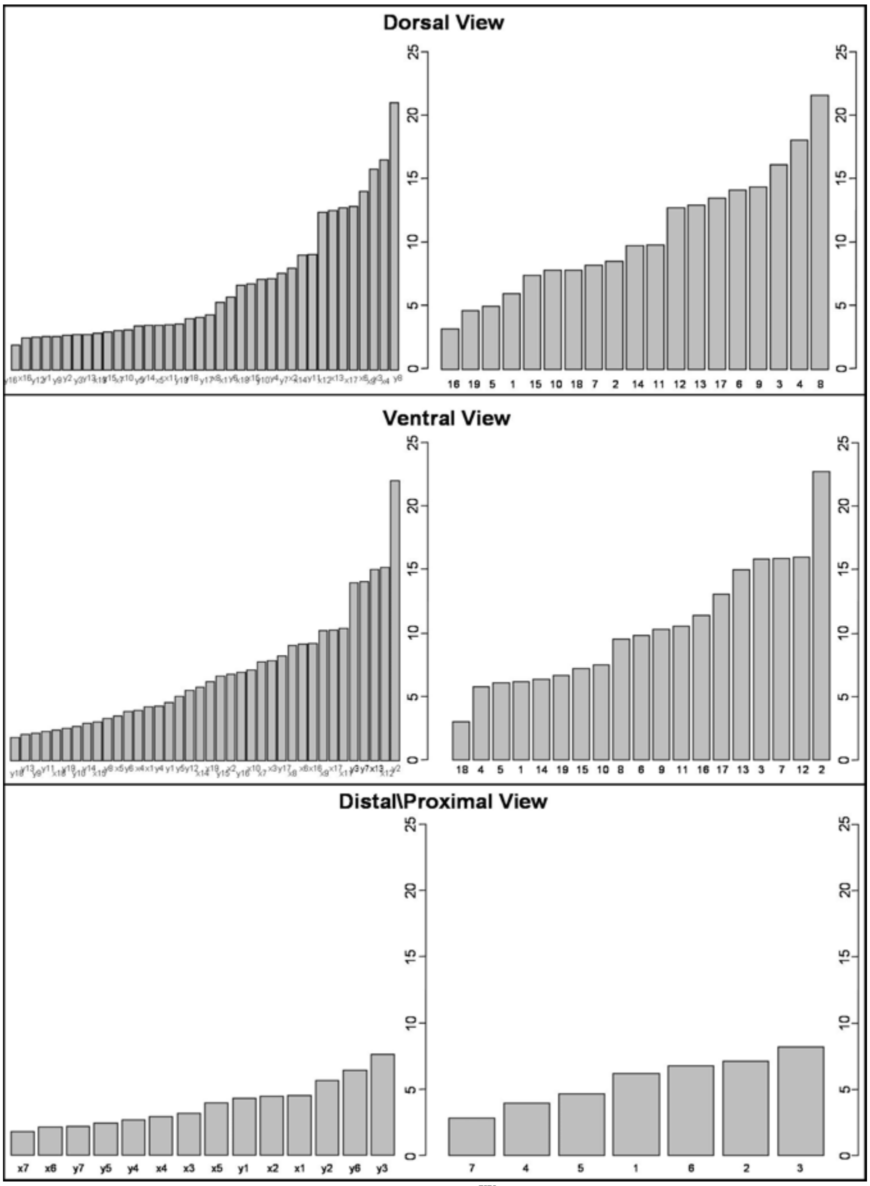


Figure 3. Reproducibility, in left the standard deviations $\mathbf{S^{xy}}$ and right the sum of total standard deviations of each landmark of high and smaller variation for the 3 views. On top - Dorsal View, in the middle - Ventral View and the above - Distal/Proximal View. In left standard deviations $\mathbf{S^{xy}}$ and right the sum of total standard deviations $\mathbf{S^{x+y}}$ of each landmark.

Discussion

Repeatability

The operator error is measured only in few works (Alibert et al., 2001; Haney et al., 2001; Lockwood et al., 2002; Viarsdóttir et al., 2002; Claude et al., 2003; Bailey et al., 2004; Hallgrímsson et al., 2004; Dvorak et al., 2006; Harmon, 2007). The results of ANOVAs raw data $\mathbf{S^{xy}}$ in the 10 repetitions made by the operator, it was verified that there was no significant difference among the means of digitizations in the 3 views, proving the capacity of the operator to repeat the experiment without a significant measurement error. However a great variation difference was observed among the landmarks, as for instance, in Dorsal view (Figure 2) the landmarks 12 and 13 possess a standard deviation six times higher than the landmarks of smaller variation 19 and 7, in Ventral view (Figure 3) the landmarks of larger variation (17) have a standard deviation four times higher than the landmarks of smaller variation (12). Among the 3 views the one that demonstrated the smallest difference among the standard deviations was the distal/proximal view (Figure 4). In the repeatability analysis we used a total of 16 samples of four different species and two sexes. We observed a great difference among the variations of the landmarks, and this variation can be explained by the natural variation among species and sexes in the sample, which can be observed in the results of ANOVAS (table II III, IV, V - Annex). For the 3 views it is possible to see that in most of the landmarks there is a significant variation for among sexes and species. In the case of the Distal-Proximal view the landmarks 3 and 7 there were no significance among sexes, tends all the others have a significance for sexes, species and interaction sex/specie.

Pinocchio effect

Walker (2000), simulated several landmark configurations varying the number of landmarks and quantified the difference between the variance/covariance (VAR/COV) matrices of two different superimposition methods, GLS and resistant fit. The conclusion of Walker's work is that the "Pinocchio effect" is rare and intimately correlated with the number of landmarks and with the superimposition method used. Comparing the results of the sum of standard deviations of the raw data $\mathbf{S}^{\mathbf{x+y}}$ and of the superimposition residuals obtained after GPA $\mathbf{S}^{\mathbf{gpa}}$ (Figure 2), it was possible to observe the high variation of a few landmarks being distributed among other landmarks that had small variances before GPA. In the analysis of the raw data $\mathbf{S}^{\mathbf{x+y}}$ of Dorsal view the landmarks 12, 13, 18 and 4 are the ones that have highest standard deviations. After GPA, the landmarks of larger variation are 12, 13, 14 and 15. In figure 1 it is possible to observe that the points 12, 13 and 14, 15 are intimately related and it is possible to observe their variances are inflated after GPA. Conversely the landmarks 16 and 19, in the same dorsal view, vary little in the raw data but after GPA they are ranked as the fifth and the sixth landmark of larger variation. The landmarks 16, 17, 18 and 19 are intimately related to 14 and 15, so consequently inflated by the landmarks 12 and 13, the source of the greater variation. In the Ventral view (Figure 2) we have another pattern of variation, the four landmarks of larger variation 17, 7, 6 and 5 are not closely and after GPA the dilution of the two larger variations is less perceptible than in the Dorsal view. After GPA the most variable landmarks are 19, 15, 14 and 18 which are close to the landmark 17, followed by 3, 2, 17, 16, 1, 7, 4 and 6, which are close landmarks to the landmark 7 and to 17. For the Distal/Proximal view, besides a small number of landmarks (19 in the Dorsal and Ventral views and 7 for Distal/Proximal view), it is possible to observe that the values of standard deviations are much smaller than the ones in other views. It is also possible to observe that in the Distal/Proximal view the distribution of the landmark variation is smaller, and just the landmarks 5 and 1 change position inside of the landmarks variations. Our results agree with the simulations made by Walker (2000), really the “Pinocchio effect” is rare and intimately correlated with the superimposition method, but in our work the number of landmarks didn't influence in the variation of landmarks. For instance, in the Distal/Proximal view we have only seven landmarks against nineteen in Dorsal and Ventral view, but even so the values of standard deviations of Distal/Proximal view are much smaller than observed in the other two views.

Reproducibility

In the analysis of the reproducibility only one sample was used, what turned possible just the use the raw data ($\mathbf{S}^{xy}$ and $\mathbf{S}^{x+y}$). The 20 operators had in their majority a small if any experience in landmarks digitization and this inexperience was observed in the results of ANOVA between the 3 repetitions among the operators. In the tables VI, VII and VIII - Annex showed a highly significant variation among all the landmarks in the 3 views, except for landmark 3 in the ventral view. These results corroborate the necessity of training before the digitalization for acquisition of definitive data for analysis. Another important point of the analysis of the reproducibility for operators is the use of graphs of the standard deviations for coordinate $\mathbf{S}^{xy}$ (Figure 3) to observe the direction of the variation of the digitizations. For example, in the figure 3 for the Dorsal view the landmark 8 is the one with the larger standard deviation and is located in a great curvature, in the ventral view the landmark 2 demonstrates the same magnitude of location problem and I located in a great curvature. This example demonstrates that located landmarks in great curvatures are more difficultly digitized and need a better definition of their location.

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IV. CAPÍTULO 2

HUMERUS ADAPTIVE SHAPE VARIATION TO EXCAVATION: GEOMETRIC MORPHOMETRICS ASSESSMENT AND QUALITATIVE MYOLOGICAL DATA IN FOUR SPECIES OF CTENOMYS (RODENTIA - CTENOMYIDAE)

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Abstract

In subterranean rodents, the excavation activity can be done in three different ways, with the claws and forelimbs (scratch-digging) and with the skull and incisor teeth (chiesel-digging), or a combination of the two forms. The genus *Ctenomys* is in general considered a scratch-digger and secondarily a chisel-tooth digger, but the predominant digging behavior among the sixty living *Ctenomys* species is not known. In scratch-diggers, the forelimb myoskeletal system composed by the scapula, humerus, radius and ulna more hand and claws forms a mechanical system which has great importance in the activity of excavation. In this work we analyze the humerus (n=156) of four species of *Ctenomys* that occur in southern Brazil, in areas ranging from the first lines of costal dunes (*C. flamarioni, C. minutus*) to the sandy fields of the coastal plains (*C. minutus, C. lami*) and in the hard soils of the fields of southern "pampas gauchos" (*C. torquatus*). Although a robust published phylogeny of the genus is not available, but *C. flamarioni* belongs to the monophyletic "mendocinus-group" and C. *torquatus*, *C. minutus* and *C. lami* are part of another monophyletic group "torquatus". *C. minutus* and *C. lami* are recently diverged sister species. To analyze the difference in the form (size + shape) of the *Ctenomys* humerus we used a geometric morphometrics assessment and myological descriptions. In the interspecific analysis *C. flamarioni* demonstrate the most different shape among the analyzed species, however when we compared the two obtained extreme shape of the linear discriminant analyzes (LDA), *C. lami, C. minutus* and *C. torquatus* demonstrates a more robust humerus, mainly in the "head" of the humerus when compared with *C. flamarioni*. Crossing the osteolgy data with the qualitative observations of the musculature, it was possible to detect a larger difference in the proximal portion of the humerus that could be related with the insertion of important extension muscles of the pectoral-shoulder joints, which could increase force. But when we compared the three related species (*C. lami, C. minutus* and *C. torquatus)* the shape differences don't demonstrate the expected. It was expected that *C. torquatus* would have the more robust and with the more pronounced differences, however the more different osteological features belonged to *C. lami* and *C. minutus* (more friable soils than compared with *C. torquatus*). Corroborating that hypothesis that soil hardness is not different enough to produce strong selection. Or maybe the divergence is to recent to establish differences in shape of the humerus.

Introduction

Living in excavated burrows, the genus *Ctenomys* has different ecological, physiological and morphological adaptations to the underground life. The major morphological adaptations are represented by the robust and cylindrical body, small eyes, reduced hearing flag, robust forelimbs and modifications in the incisors, mouth and skull for excavation (Pearson, 1959; Reig *et al.*, 1990; Casinos *et al.*, 1993; Vassalo, 1998; Lacey *et al.,* 2000; Verzi, 2002 in Morgan & Verzi, 2006). In subterranean rodents, the excavation activity can be done in three different ways, with the claws and forelimbs (scratch-digging) and with the skull and incisor teeth (chiesel-digging), or a combination of the two forms (see Lehmann, 1963; Dubost, 1968; Hildebrand, 1985; Lessa, 1990; Stein, 2000; Mora *et al.*, 2003). In the literature, the genus *Ctenomys* is considered a scratch-digger (Lehmann, 1963) and secondarily a chiesel-tooth digger (Dubost, 1968; Ubilla & Altuna, 1990; Vassallo 1998; Stein, 2000), but the predominant digging behavior for most of the *Ctenomys* (±60ssp) species is unknown (Ubilla & Altuna, 1990; Vassalo, 1998; Stein, 2000; Lessa *et al.,* 2008). Among the post-cranial bones, the humerus is possibly the bone that contains the larger amount of functional adaptive modifications (Szalay & Sargis, 2001; Morgan & Verzi, 2006) and in scratch-diggers the forelimb myoskeletal system of the scapula, humerus, radius, ulna and the manus and claws forms a mechanical system of great importance in excavation. However, the knowledge about this system (i.e. relationship between muscles, tendons and bones, or candidate adaptive structures) in living species of *Ctenomys* is insufficient. In the few studies of humerus structure, the sample size is reduced (Morgan & Verzi, 2006) or only two species are analyzed (Vassalo, 1998), which makes the results of these works, although pioneering, with little conclusive power statistically and evolutionarily. In addition, all studies analyzed the structures with classical morphometric methods (linear measurements) which do not analyze the shape *per se* independently from size (Rohlf & Marcus, 1993).

The four species included in the present study, occur in southern Brazil. The first, *C. minutus* in the states of Santa Catarina and Rio Grande do Sul and *C. flamarioni*, *C. lami* and *C. torquatus* in Rio Grande do Sul. The occurrence areas of these species ranges from the first lines of costal dunes (*C. flamarioni, C. minutus*) to the sandy fields of the coastal plains (*C. minutus, C. lami*) as well as in the harder soils of the pampas (*C. torquatus*). Although a robust published phylogeny of the genus is not available yet, many studies allow us to establish a synthetic phylogenetic hypothesis for the species here studied. The species *C. flamarioni* belongs to the monophyletic “mendocinus-group” (*C. australis, C. flamarioni, C. rionegrensis, C. porteousi*, *C. azarae*, *C. mendocinus* and *C. chasiquensis*) (Massarini *et al.*, 1991; Massarini & Freitas, 2005). The three remaining species, *C. torquatus, C. minutus* and *C. lami* are part of another monophyletic group (“torquatus-group”, unpublished data), where according Freitas (2001), *C. minutus* and *C. lami* are recently diverged sister species.

Hypotheses regarding the evolution of the humerus of these four species can be stated as follow. Firstly, assuming neutral phylogenetic divergence we expect *C. flamarioni* to be phenetically the more distinct and the sister species *C. lami* and *C. minutus* the more similar. Second, assuming that the humerus shape is selected for optimal excavating performance, *C. flamarioni* and *C. torquatus* which lie at each extreme of the soil hardness gradient should have very divergent shapes, whereas the two sister species should again be similar to each other and have intermediate shapes between these extremes. To provide additional support to the adpative hypotheses based on shape configurations we provide a detailed qualitative myological analysis. In fact, selection for particular shapes should be acompanied and/or modified by the muscular system. Finally, the intrepretation of shape differences conjointly with the muscular groups implied in these differences allows us to put forward hypotheses about the forces and movements which are selected for during excavation.

Material and Methods

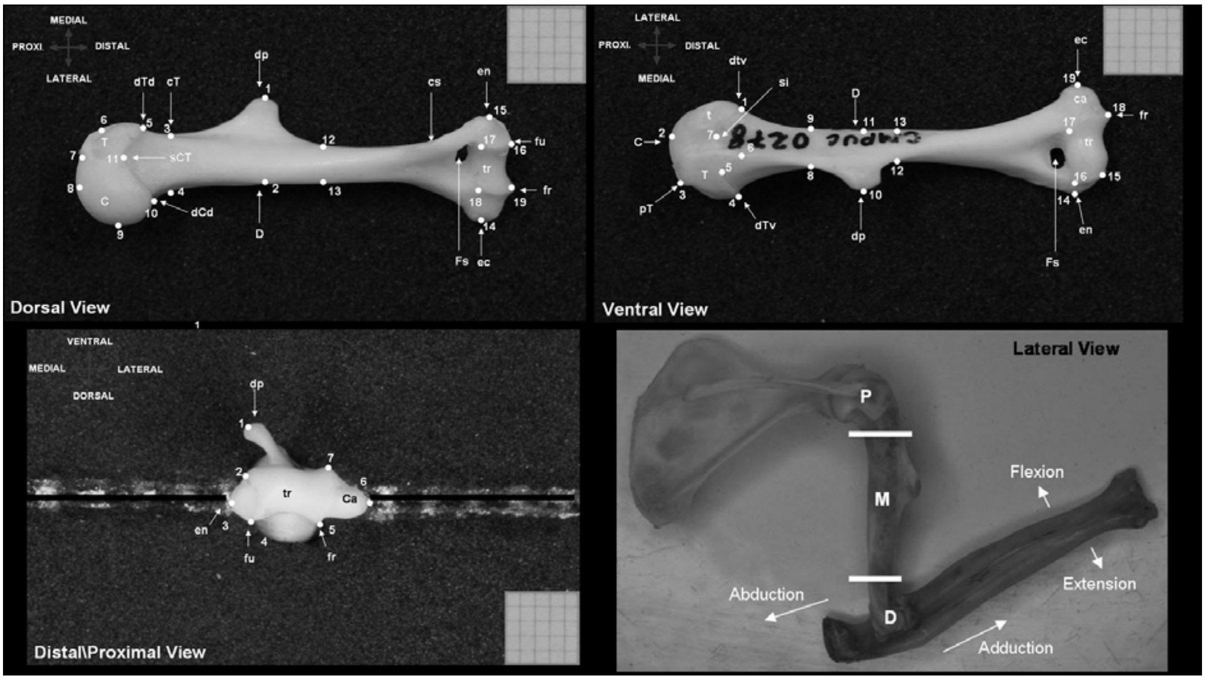
Samples and data acquisition

We digitized data on 165 samples from four species of the genus *Ctenomys*: *C. minutus* (n = 75), *C. torquatus* (n = 54), *C. flamarioni* (n = 14) and *C. lami* (n = 22) of different localities in the state of Rio Grande do Sul. For each sample we recorded information about sex and collection locality, only adult specimens were used. All specimens are deposited in the Laboratório de Citogenética e Evolução, Departamento de Genética, Universidade Federal do Rio Grande do Sul, Brazil.

All photos were obtained with a digital camera *Canon EOS 400 D* with 10 mega pixels of resolution, mode = A-DEP, Iso100, One-Shot, AWA with MACRO Lens ??? (opening of -2). All images were taken from a standard distance (15 cm) with the aid of a tripod. A basis of polyethylene foam with millimeter paper served as background image and scale factor. The photos were organized in lists with the program TPSUtil 1.26 (Rohlf, 2004) and the digitization of landmarks was made with the program TPSDig 1.40 (Rohlf, 2004). We photographed the humerus in Dorsal, Ventral and Distal/Proximal view where 19, 19 and 7 landmarks were defined respectively (Figure 1). Textual definition of landmarks can be found in the legend of Figure 1. In the Dorsal view the humerus was naturally laid upon the photographic plane in such a way that its dorsal part faced the camera and was equilibrated laterally by the deltopectoral crest. Likewise, in the Ventral view the ventral part of the humerus faced the camera and was naturally laid on the support and equilibrated naturally by the trochlea. In the Distal\Proximal view the proximal part of the humerus (condyle) was inserted in a hole in a polyethylene base in such a way that its major axis was perpendicular to the photographic plane, and the distal part (trochlea) faced the lens.

Figure 1. The right humerus of *C. flamarioni***.** In the upper left panel - ***Dorsal view***, on the upper right - ***Ventral view***, on the lower left - ***Distal/Proximal view*** and on the lower right the position of the scapula, humerus, radius and ulna in the skeletal system of right forelimb of *C. torquatus*. The abreviations, definitions and numbering of landmarks are: **dtd** – distal part of the great tuberosity; **cT**– lateral crest of the great tuberosity; **dp** – deltopectoral crest; **cs** – supinatory crest; **en** – medial epicondyle; **ec** – epicondyle; **fr** – radial facet; **fu** – ulnar facet; **Fs** – supratrochlea foramen; **D** – diaphysis; **dCd** – distal part of condyle; **C** – condyle ; **T** – great tuberosity; **sCT** – groove between condyle and great tuberosity; **tr** –trochlea; **dTv** – distal part of great tuberosity; **pT** – great tuberosity process; **dtv** – distal part of small tuberosity; **si** – intertubercular groove; **ca** –

capitulum. ***Dorsal View:*** **1.** End of medial deltopectoral crest. - **2**. End of lateral diaphysis, parallel to *landmark 1*.- **3**. Encounter of the lateral crest of the great tuberosity with the deltopectoral crest.- **4.** End of the lateral diaphysis, parallel to *landmark 3*.- **5.** Meeting of the distal extremity of the great tuberosity with the lateral crest of the great tuberosity.- **6.** Groove in the great tuberosity, most lateral part.- **7.** Groove in the meeting of the great tuberosity with the condyle, most lateral part.- **8.** Proximal end of the condyle.- **9.** Lateral extremity of the condyle.- **10.** Meeting of the distal part of the condyle with the diaphysis.- **11.** Groove between condyle and the great tuberosity, most proximal part.- **12.** Meeting of the most distal part of the deltopectoral crest and supinatory crest with the diaphysis.- **13.** Extremity of the lateral diaphysis, parallel to *landmark 12*.- **14.** Lateral extremity of the epicondyle.- **15.** Medial extremity of the medial epicondyle.- **16.** Distal extremity of the ulnar facet.- **17.** Proximal extremity of the articulated part of the ulnar facet.- **18.** Proximal extremity of the articulated part of the radial facet.- **19.** Distal extremity of the radial facet. ***Ventral View:*** **1.** Meeting of the distal part of the great tuberosity with the diaphysis.- **2.** Proximal extremity of the condyle.- **3.** Proximal extremity of the distal process of the great tuberosity.- **4.** Meeting of the distal extremity of the small tuberosity with the diaphysis.- **5.** Proximal extremity of the encounter of the great tuberosity with the diaphysis.- **6.** Distal extremity of the encounter of the great tuberosity with the small tuberosity.- **7.** Meeting of the proximal extremity of the intertubercular sulcus with the small tuberosity.- **8.** Meeting of the proximal part of the deltopectoral crest with the medial diaphysis.- **9.** Extremity of the lateral diaphysis, parallel to *landmark 8*.- **10.** Medial extremity of the deltopectoral crest.- **11.** Extremity of the lateral diaphysis, parallel to *landmark 10*.- **12.** Meeting of the distal part of deltopectoral crest with the medial diaphysis.- **13.** Lateral extremity of the diaphysis, parallel to *landmark 12*.- **14.** Medial extremity of the medial epicondyle.- **15.** Distal extremity of the ulnar facet.- **16.** Proximal extremity of the articular part of the ulnar facet.- **17.** Proximal extremity of the articular part of the radial facet.- **18.** Distal extremity of the radial facet.- **19.** Lateral extremity of the epicondyle. ***Distal/Proximal View**:* **1.** Medial-ventral extremity of the deltopectoral crest.- **2.** Ventral extremity of the ulna facet.- **3.** Medial extremity of the medial epicondyle.- **4.** Ventral extremity of the ulnar facet.- **5.** Ventral extremity of the radial facet.- **6.** Lateral extremity of the epicondyle (capitulum). - **7.** Ventral extremity of the radial facet.



Mophometrical and statistical procedures (Size and Shape)

For the analysis of the form of the humerus we used the Generalized Procrustes Analysis – GPA (Gower, 1975; Dryden & Mardia, 1998). The GPA allows the study of centroid size and shape (superimposition residuals) as different sets of variables. First the configuration of landmarks is translated, then scaled to the same size and in a last step rotated to minimize the partial Procrustes distance between configurations, by a least squares criterion (Rohlf & Slice, 1990; Bookstein, 1991).

For the analysis of size variation of the humerus we used the centroid size obtained from the GPA (Rohlf & Slice, 1990; Bookstein, 1991). Uncorrelated with shape, the centroid size is the size measure used in geometric morphometrics (Monteiro & Reis, 1999; Zelditch *et al.*, 2004) and corresponds to the square root of the sum of squared distances between each landmark and the centroid of the configuration. The presence of interspecific variation, sexual dimorphism and their interaction for the size was tested by a model II ANOVA (Sokal & Rohlf, 1995). Pairwise size differences were tested with Tukey honest significant difference test.

Shape variables (superimposition residuals) are the difference of each configuration to a mean shape after a GPA. With these shape variables a Principal Components Analysis (PCA) was computed to find the axes of major shape differences. Interspecific differences and sexual dimorphism in shape and their interaction was tested through a MANOVA on shape variables after a correction of minus 4 degrees of freedom lost in the GPA after translation, scaling and rotation. The linear discriminant analysis (LDA) is a widespread classification technique used to quantify and displaying variation among identify groups, in our case the species. We used PCs as shape variables and reduced the dimensionality of the data using the criterion of maximum classification percentage (Baylac & Friess, 2005). To evaluate the performance of classification by LDA, we used the leave-one-out cross-validation. In this procedure all the data except one individual is used in the discriminant function, then this individual is classified. This is repeated to compute a mean classification error and a probability of group reclassification for each individual. With the values of the three discriminant axes we visualized the shape differences through multivariate regression of shape variables on discriminant axes. Shape changes along axes are visualized as the shapes obtained at the positive and negative extremes of each axis. The shapes at the extreme negative values are figured in dashed lines whereas at the positive extreme in solid lines. In a last step, the squared Mahalanobis distances (D2) between the four species were computed on shape variables.

Firstly, all statistical procedures were applied on the three views separately (Dorsal, Ventral and Distal/Proximal), then the three views were pooled (Total view). In the Total view we used the natural logarithms of centroid size values of each view, calculated first for each view separately and then we summed the logs of centroid size. In the Total view, shape variables were pooled as described in Cordeiro-Estrela *et al.* (2006). Principal components of each view were pooled to calculate a pooled principal component analysis.

All statistical analyses and graphs were made under "R" language and environment for statistical computing version 2.0 (R Development Core Team, 2004; http://cran.r-project.org/, last accessed 26 November 2008). Morphometric analyses were done with the Rmorph library (Baylac, 2007).

Myology

Dissectable museum specimens of *Ctenomys* are rare, first we tried to use animals (carcasses) conserved in alcohol 70%, but these specimens were not preserved in correct anatomical position, which turned the dissection and the comparison between the specimens impossible. To make a comparative dissection we used only one forelimb (right) of one specimen of "similar size" from each of the species *C. flamarioni, C. lami* and *C. torquatus* preserved in 10% formaldehyde solution. The dissections were performed to confirm the region of origin and insertion of the muscles related with the humerus (excluding the intrinsic muscles of the manus) as reported by Lehmann (1963) for some fossorial rodents and by Woods (1972) for Hystricomorph rodents and finnaly to test for any variation in muscle insertion point between species that might affect shape. Other guides used in the observations were based on Walker & Homberger (1997), Vassalo (1998), Fernández *et al.* (2000) and Elizambru & Vizcaíno (2004).

Results

Size

The results for the centroid size between sexes and among species for the four views showed similar results (including Total view using logarithms). The ANOVAs of centroid size yielded highly significant F values (almost of the same magnitude) among species and sexes (Table 2). The interactions were also significant. In Figure 2 the mean of centroid size for species/sexes is shown in an interaction plot where sexual dimorphism is absent for *C. torquatus*. The results of Tukey test only from the Total view (Table 3) show that *C. flamarioni* was the species with the larger humerus, followed by *C. lami* and *minutus* (without significant difference among them p = 0.998). *C. torquatus* have the smallest humerus and a different pattern.

Table 2: ANOVA of centroid size (*F* and *p* values) among sex and species in four species of *Ctenomys*. Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'

	Dorsal View		Ventral View		Dist/Prox View		Total View	
	F	***p***	***F***	***p***	***F***	***p***	***F***	***p***
Sex	38.00	7e-09***	34.94	2.56e-08***	37.00	1.05e-08***	25.84	1.21e-06***
Sp	35.00	2.2e-16***	44.68	2.2e-16***	38.31	2.2e-16***	36.79	2.2e-16***
Sex:Sp	3.10	0.027*	2.82	0.041*	2.56	0.056	2.78	0.043*

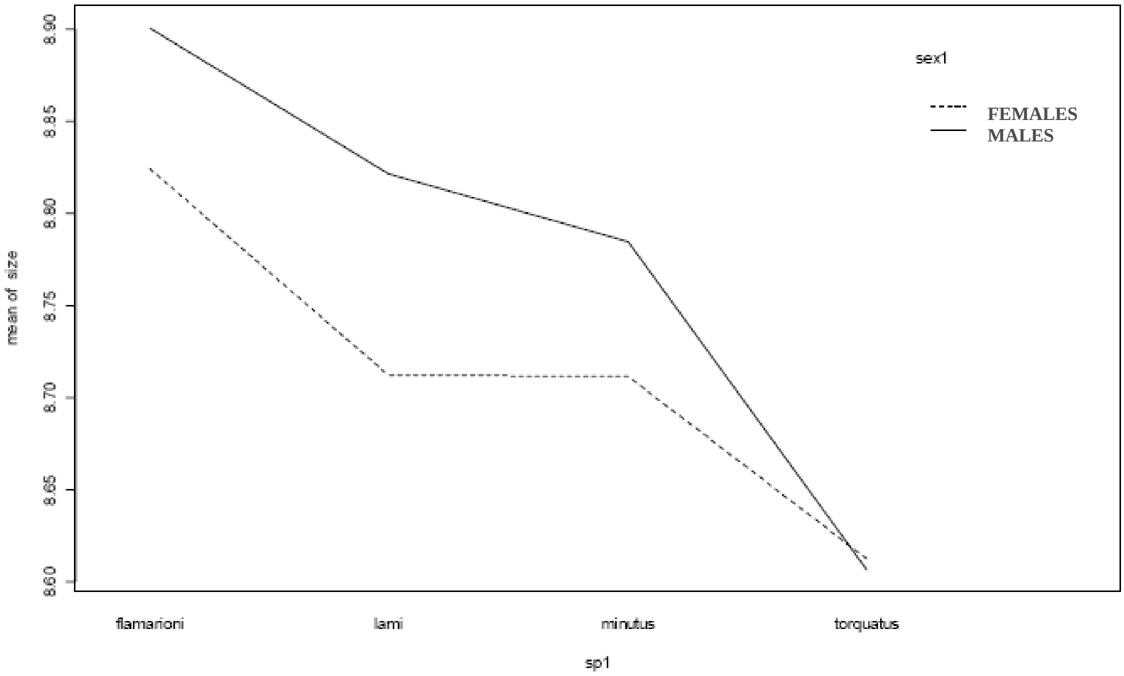


Figure 2: Interaction plot with mean centroid size by sex and species.

Table 3: Probabilities of Tukey´s pairwise honest significant test for size

	***p* adj**
lami-flamarioni	0.0053877
minutus-flamarioni	0.0003887
torquatus-flamarioni	0.0000000
minutus-lami	0.9984392
torquatus-lami	0.0000004
torquatus-minutus	0.0000000

Shape

Data from any of the four views considered shows similar results. In all cases MANOVAs of shape variable were significant for sexes and species (Table 4), except for the distal/proximal view that did not show any sexual dimorphism (p = 0.2087). For the four views the interaction term between sex and species was not significant.

Table 4. MANOVA (approx *F* and *P* values) of shape variables (residuals of superimposition) for sex and species in four species of *Ctenomys*. Signify. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'

	Dorsal View		Ventral View		Dist/Prox View		Total View	
	Approx F	***p***	***Approx F***	***p***	***Approx F***	***p***	***Approx F***	***p***
Sex	2.51	0.0001 ***	2.20	0.0011**	1.35	0.2087	2.22	0.0008 ***
Sp	8.85	2.2e-16***	7.58	2.2e-16***	7.27	2.2e-16***	5.43	2.2e-16***
Sex:Sp	1.19	0.1230	0.83	0.8554	0.93	0.5632	0.98	0.5525

For the four views in Principal Components Analysis (PCA) the main axes of variation (PC1 and 2) are not equivalent to interspecific patterns of shape differences, since no clear specific grouping was detected (results not shown). Only in the PC1 of the dorsal view did we find some clustering between *C. flamarioni* and the other three species (not illustrated). To measure the association between PC1, PC2 and size (allometry) we made a correlation test, but no significant correlation was detected.

The classification table with the percentage of reclassification for the four views by the linear discriminant analysis (LDA) using leave-one-out cross-validation is show in Table 5.

Table 5. The number of classification of each species and a percent of reclassification for the four views by the linear discriminant analysis (LDA) using leave-one-out cross-validation.

Dorsal View	*C. flamarioni*	*C. lami*	*C. minutus*	*C. torquatus*	*%*
C. flamarioni	14	0	0	0	100.0
C. lami	0	19	3	0	86.36
C. minutus	0	1	71	2	95.94
C. torquatus	0	1	1	51	96.22
Ventral View	*C. flamarioni*	*C. lami*	*C. minutus*	*C. torquatus*	*%*
C. flamarioni	14	0	0	0	100.0
C. lami	0	15	6	0	71.43
C. minutus	0	2	70	1	95.89
C. torquatus	0	0	1	53	98.15
Dist/Prox V.	*C.flamarioni*	*C. lami*	*C. minutus*	*C. torquatus*	*%*
C. flamarioni	12	0	2	0	85.71
C. lami	0	13	9	0	59.10
C. minutus	3	5	57	11	75.00
C. torquatus	1	1	13	39	72.22
Total View	*C.flamarioni*	*C. lami*	*C. minutus*	*C. torquatus*	*%*
C. flamarioni	14	0	0	0	100.0
C. lami	0	20	1	0	95.24
C. minutus	0	0	70	0	100.0
C. torquatus	1	0	1	49	96.08

Analyzed separately, the dorsal view was the one that best discriminated the species globally. *C. flamarioni* have the higher percentage of reclassification and *C. lami* the smaller, as expected all misclassified *C. lami* were considered as *C. minutus*. The ventral view gives similar results to the dorsal view. In the Distal/Proximal we obtained the view the smaller percentages, including a low percentage for *C. lami* In the Total View we obtained the highest discrimination percentages (100% for *C. flamarioni* and *C. minutus*).

The dorsal and ventral views give similar results for LDA (Figure 3-4). In dorsal view (Figure 3, A-B) the first axis (39.0% of variance) discriminates *C. torquatus* from *C. lami* and *C. minutus*. Explaining most of the variance in the discriminant analysis, the shape changes inferred from this axis demonstrates a difference in robustness of the head (namely small tuberosity and condyle), a more enlarged supinatory crest region and a small difference the "size" and direction of deltopectoral crest. *C. flamarioni* was separated from the other species by the second axis (37.9% of variance), for which inferred shape differences are a slender head and neck (proximal diaphysis) of the humerus when compared with the other three species. Other perceptive differences are detected in the small robustness of medial diaphysis and a slim and elongated supinatory crest. Finally we observed a difference in the distal part in the region of the epicondyles, which consequently affects the orientation of the trochlea. The third axis (23.1% of variance) discriminates *C. lami* from the others, and higlights a small difference in the overall robustness of the humerus and a difference in the tip of the deltopectoral crest.

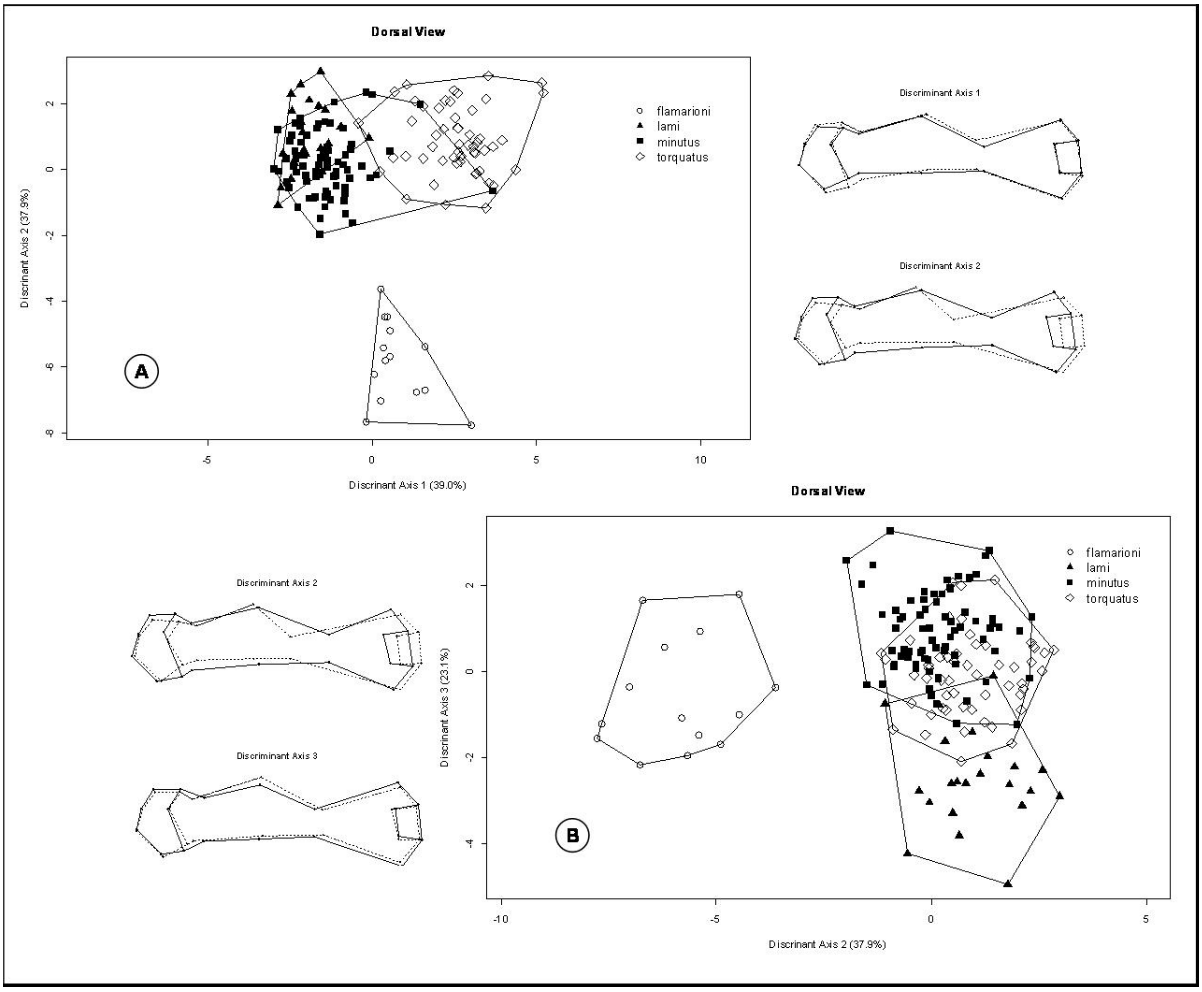


Figure 3: Dorsal view, the results (%) of the three axis of the linear discriminant analysis (LDA) for the first and second axes (A), second and third axes (B). The figures correspond to the projections of individuals on discriminant axes with convex hulls delimiting species. The shapes inferred at the extremes of the axes are figured in black and grey lines, for the positive and negative extremes respectively.

The results of first and second axis for the ventral view (Figure 4, A-B) gave similar results as the dorsal view. In the first axis (46.9%) we obtained *C. flamarioni* in most extreme negative values and *C. torquatus* in extreme positive values, *C. minutus* and C. *lami* are mixed in the same group, forming a intermediate group between *C. flamarioni* and *C. torquatus*. For this view the most perceptive differences in shape are observed again in the head of humerus, deltopectoral crest orientation and a “relative size” of trochlea and medial epicondyle. In the second discriminant axis (32.0%), as in the first axis of the dorsal view, we observed a shape differentiation between the *C. flamarioni-torquatus* group and the *C. lami-minutus* group**.** The shape difference inferred is observed in the region between the distal part of great tuberosity and the deltopectoral crest, other small differences occurs in proximal part of the condyle and greater tuberosity, medial diaphysis and medial epicondyle of humerus. The third axis (21.1%) discriminates *C. lami* from the other species, and again by the orientation and more pronounced deltopectoral crest.

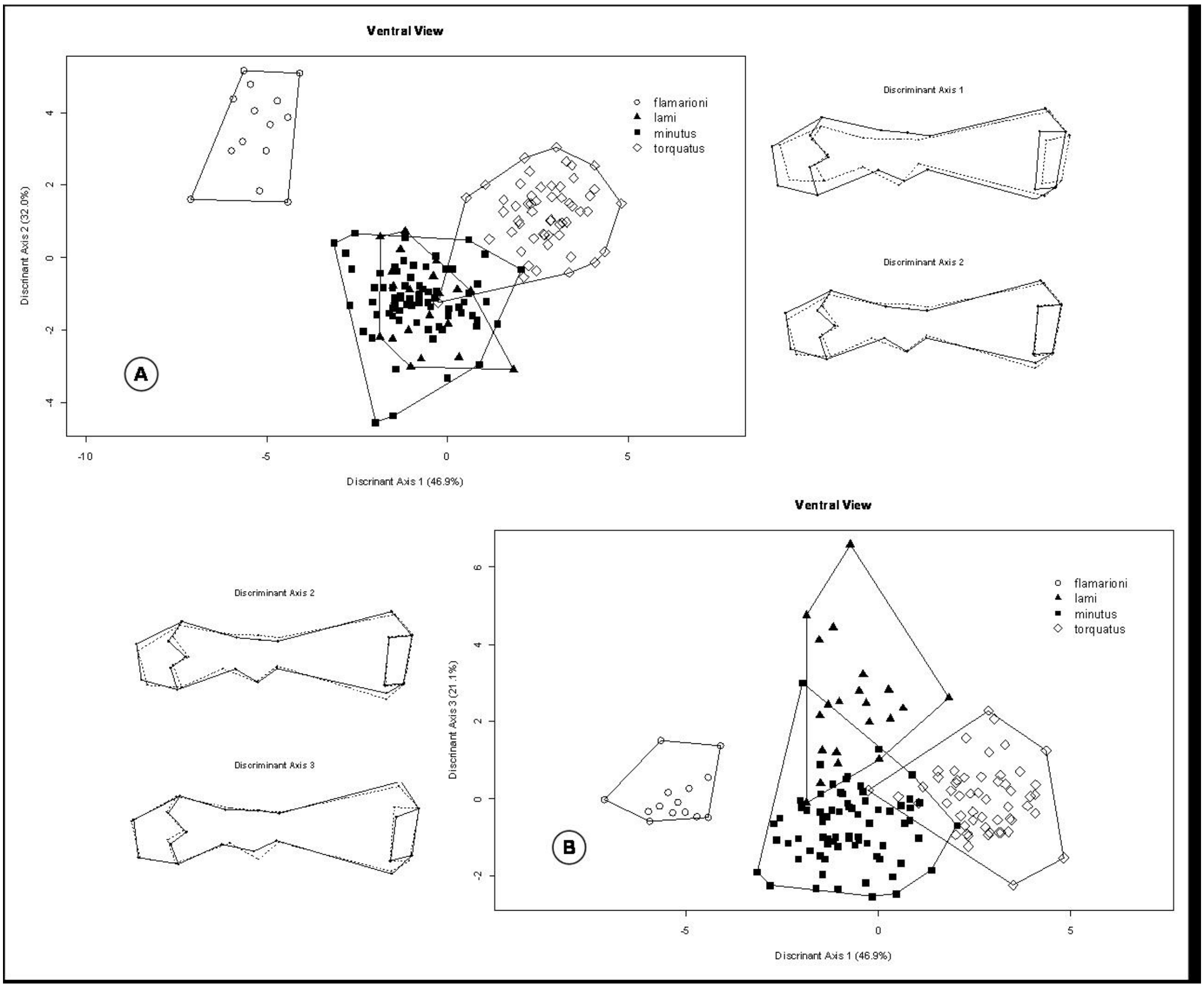


Figure 4: Ventral View, the results (%) of the three axis of the linear discriminant analysis (LDA) for the first and second axes (A), second and third axes (B). The figures correspond to the projections of individuals on discriminant axes with convex hulls delimiting species. The shapes inferred at the extremes of the axes are figured in black and grey lines, for the positive and negative extremes respectively.

The distal/proximal view doesn't show clear results by LDA for the four species (Figure 5, A-B), only in the second axis a difference was observed between *C. flamarioni* against *C. lami*. The shape differences occur in medial epicondyle and in the direction (angle) of deltopectoral crest in relation of the medial epicondyle and epicondyle. The first and third axes don't show any clear distinction between the species. Finally for the Total View (not illustrated), the first axis (39.2%) discriminates *C. flamarioni* from the other species and the second discriminant axis (35.6%) we have *C. flamarioni, C. minutus, C. lami* in the positive values and in the negative a great amount of individuals from *C. torquatus*. The shape visualization obtained from Total View gave similar results, but less pronounced shape differences when we compared with the shape visualization of the three views analyzed separately.

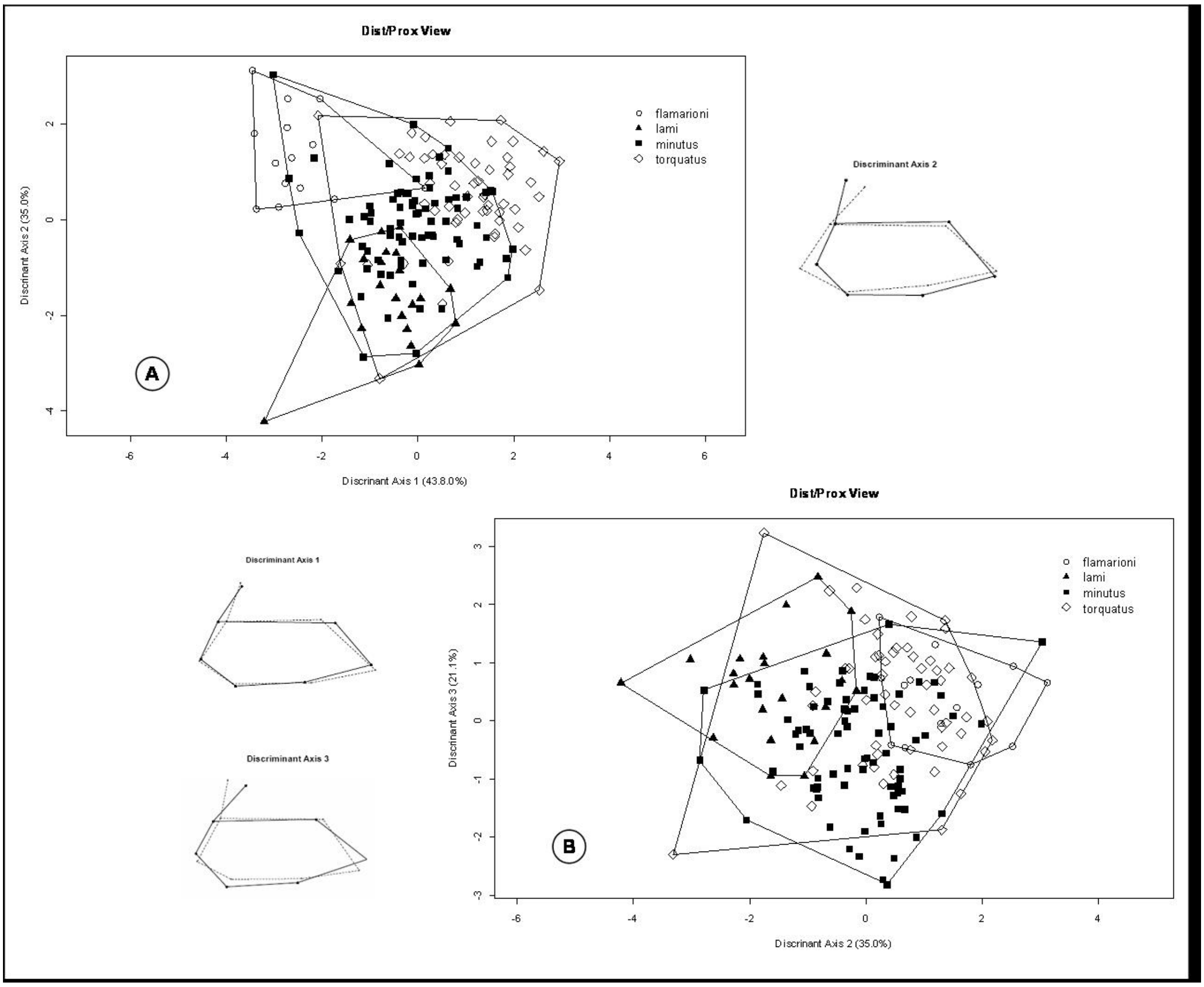


Figure 5: Distal Proximal View, the results (%) of the three axis of the linear discriminant analysis (LDA) for the first and second axes (A), second and third axes (B). The figures correspond to the projections of individuals on discriminant axes with convex hulls delimiting species. The shapes inferred at the extremes of the axes are figured in black and grey lines, for the positive and negative extremes respectively.

The quantification of shape difference of humerus between the species was examined through the squared Mahalanobis distances (D2) (Table 6). In all views, *C. flamarioni* has the most different shape when compared with three other species, *C. minutus* and *C. lami* are closer, followed by *C. torquatus* that is more adjacent to *C. minutus* than to *C. lami*. But the three views don't follow the same pattern, for example in the dorsal view, *C. flamarioni* and *C. lami* have the largest distance, however in ventral view *C. flamarioni* and *C. torquatus* are the most different. The distal/proximal view proceeds similarly to the ventral view and the Total View proceeds similarly to the dorsal view, but the two with a significant difference in the magnitude when compared with the other views.

Table 6. Mahalanobis distances (D2) between the four species calculated from shape variables using the group averages of Linear Discriminant Analysis (LDA) for the Distal, Ventral, Distal/Proximal and Total View. Dorsal and Ventral views on PCs 1-34, Distal/Proximal view PCs 1-10 and Total view PCs 1-55.

Dorsal V.	*C. flamarioni*	*C. lami*	*C. minutus*

C. lami	57.81	-	-
C. minutus	42.93	12.48	-
C. torquatus	49.09	26.14	16.95
Ventral V.	*C. flamarioni*	*C. lami*	*C. minutus*
C. lami	54.51	-	-
C. minutus	46.58	10.48	-
C. torquatus	72.08	24.41	18.67
Dist/Pro V.	*C. flamarioni*	*C. lami*	*C. minutus*
C. lami	13.89	-	-
C. minutus	9.88	3.63	-
C. torquatus	15.49	7.02	2.56
Total	*C. flamarioni*	*C. lami*	*C. minutus*
C. lami	95.72	-	-
C. minutus	88.95	27.34	-
C. torquatus	90.00	44.05	27.89

Myology

Based in bibliography and our observations, nineteen muscles (Table 7) attached to the humerus were found. Thirteen were part of the extensor system and six of flexor system. The origin and insertion of the muscles attached to the humerus are similar in all the three species examined and as Vassalo (1998) pointed out, the major difference observed in the dissection between *C. flamarioni, C. lami* and *C. torquatus* was the muscle robustness. The forelimb myologycal system (excluding the intrinsic muscles of the manus) can be divided in two groups, shoulder-pectoral and elbow-arm muscles (including *M. Coracobrachialis* and *M. Brachialis* with more than one origin and insertion in the humerus). Based in Lehmann (1963) and Woods (1972), we separated the muscles inserted in the proximal part (Condyle, lesser and greater tuberosity), medial part (medial diaphysis and deltopectoral crest region) and distal part (supinatory crest, epicondyle, medial epicondyle and trochlea) in three different groups. In the proximal part we found ten muscles, eight of them belonging to the shoulder-pectoral group and two of them to the elbow-arm group, in medial part (seven muscles, including complex muscles with more than one origin or insertion) three making part of shoulder-pectoral group and four to the elbow-arm group, finally in the distal part the six muscles are part exclusively of the elbow-arm group.

Table 7. The list of the Shoulder-Pectoral and Elbow-Arm Muscles of extensor and flexor system attached to the humerus based Lehmann 1963 and Woods 1972.

Shoulder-Pectoral Muscles	

Extensor System	*M. Latissimus Dorsi*
	M. Teres Major, Minor
	M. Subscapularis
	M. Clavodeltoideus
	M. Spinodeltoideus
	M. Acromiodeltoideus
	M. Suprasupinatus
	M. Infrasupinatus
Flexor Sytem	*M. Pectoralis Major,*
	Minor & Abdominalis
	M. Cutaneos Maximus
Elbow-Arm Muscles	
Extensor System	*M. Triceps B. Lateralis*
	M. Tríceps B. Medialis
	M. Anconeus
	M. Supinator
Flexor Sytem	*M. Coracobrachialis*
	M. Brachialis

In the proximal part, precisely in the lesser tuberosity, we have insertions of the shoulder flexor muscle *M. Pectoralis Abdominalis* and some important extensor muscles like *M. Latissimus Dorsi*, *M. Teres Major* and *M. Subscapularis*, these muscles help in arm retraction, a necessary movement during the digging movement of the scratch-diggers (Lehmann, 1963; Woods, 1972; Walker & Homberger, 1997; Lagaria & Youlatos, 2006). In the greater tuberosity we have the insertion of one part of the flexor *M. Pectoralis Menor* and shoulder extensor muscles *M. Teres Minor*, *M. Infrasupinatus* and *M. Suprasupinatus* and the origin of two important elbow muscles, one is the forearm extensor *M. Triceps Brachii* Lateralis and a "part" of the flexor *M. Brachialis* (Lehmann, 1963; Woods, 1972). The "Triceps brachii group" is involved in the dissociation of soil particles during digging (Lagaria & Youlatos, 2006). In the medial part, we find an equal concentration of shoulder-pectoral and elbow-arm muscles, for the deltoid group we have the insertion of extensor muscle *M. Clavodeltoideus* in the margin dorsal of the tip of deltopectoral crest and in the ventral margin we have the *M. Spinodeltoideus* and *M. Acromideltoideus* (Wood, 1972). *M. Clavodeltoideus* protracts the humerus and *M. Spinodeltoideus* has an important paper in arm retraction and movement of shoulder joints (Fernández *et al.*, 2000; Elissamburu & Vizcaino, 2004; Lagaria & Youlatos, 2006). Still in the shoulder-pectoral muscles of medial part, we have in the deltoid crest region the inserts of flexor *M. Pectoralis Major* and a part of the complex *M. Cutaneos Maximus*, these two muscles have as a primary function the retraction of the arm (Walker & Homberger, 1997). In the elbow muscles of the medial part we have two important muscles, first the inserts of *M. Coracobrachialis* (small adductor of the humerus) in the medial diaphysis and in the proximal part of epicondyle (Woods, 1972; Walker & Homberger, 1997) and still the origin of the second part of *M. Brachialis* in the tip of deltopectoral crest (Woods, 1972). For the distal part we have only elbow muscles, four of extensor group and two of flexor group (*M. Coracobrachialis* and *M. Brachialis*) (Lehmann, 1963; Woods, 1972). The principal extensor group is formed by *M. Triceps Medialis*, *M. Anconeus* and *M. Supinator*. The *M. T. Medialis* and *M. Anconeus* originate in the four-fifths of the distal part of humerus (supinatory crest region) and have a important role in the extension of the arm (Woods, 1972; Thorington *et al.*, 1997; Walker & Homberger, 1997). Another important extensor muscle is the *M. Supinator*, his origin is in the supinatory crest and in the epicondyle and medial epicondyle areas, and is considered a good indicator of fossoriality (Hildebrand, 1985; Lessa & Stein, 1992; Elissamburu & Vizcaino, 2004).

Synthesis between shape differences and myological system

In our work the most perceptive shape differences are observed in *C. flamarioni* when compared with the other three species, proceeding like the phylogenetic distance. Between the shape of related species *C. lami*, *C. minutus* and *C. torquatus* we didn't find great differences, but in the Dorsal view the first discriminant axis separating *C. minutus* and *C. lami* from *C. torquatus* and *C. flamarioni*, having the first group a more elongated region in the encounter of deltopectoral and supinatory crest, and in the condyle (articulation of scapula). In the same view, the third axis demonstrates a more pronounced tip of deltopectoral crest in *C. lami* when compared with the other species. In Ventral view the second axis, like the first axis of dorsal view, discriminates *C. minutus* and *C. lami* from *C. torquatus* and *C. flamarioni*, again we have a more pronounced condyle and a difference in the later crest of the greater tuberosity. The third axis discriminate *C. lami* from the other species, however differently of dorsal view *C. lami* demonstrates a lesser tip of deltopectoral crest (but in the Distal/Proximal view discussed above, *C. lami* demonstrate the major variation in the direction of deltopectoral crest (Figure 5) and in LDA the Ventral view have the lower reclassification percentage, what can be generated this misleading shape or a the structure articular (trochlea and capitulum) have a great variation between the species. The Distal/Proximal View doesn't demonstrate clear results, only in the second axis a difference was observed between majority of *C. torquatus* and *C. minutus* against *C. lami*, the shape differences is in medial epicondyle and in the direction of deltopectoral crest in relation of the medial epicondyle and epicondyle, what can be indicating a more pressure of work of the muscles in this region. Between the phylognetically closer species *C. lami*, *C. minutus* and *C. torquatus* the most perceptive differences occurs in the condyles, deltopectoral crest and supinatory crest. In the condyles is located the articulation with the scapula, in the region of deltopectoral crest the attaches of important extensor muscles of shoulder-pectoral group with great importance in humeral movements (*M. Spinodeltoideus, M. Acromideltoideus, M. Clavodeltoideus, M. Pectoralis Major*, one origin of *M. Brachialis* and some parts of complex muscle *M. Cutaneos Maximus*) and in the supinatory crest the insertion of *M. Coracobrachialis* and origin of other elbow extensor muscles like *M. Tríceps Medialis*, *M Anconeus* and *M. Supinator*. In the dorsal view *C. lami* and *C. minutus* have a more pronounced condyle and supinatory crest.

Discussion

The humerus is probably and apropriate structure to study adaptive pattern of locomotion among terrestrial vertebrates (to the except of serpentes, gimnophiona and some lizards). Understanding how natural selection acts on the phenotypic traits and understanding the relationship between phenotype and performance (the link between morphology and ecology) are the realm of functional morphology and other linked areas like evolution and ecology (Arnold, 1983; Wainwrigth, ????; Kingsolver & Huey, 2003). But a rule in the nature is that habitat (ecology) can cause changes on phenotypic traits and consequently cause differences in performance among individuals and species (Wainwrigth, ????).

Berev resumo resut geral: Study of GM, established protocol, significant differences in size and shape (up to 98.2 % of correct classification)

Insights on digging modes of *Ctenomys*

In the literature the genus *Ctenomys* is considered primarily a scratch-digger (Lehman, 1963) and secondarily a skull-tooth digger (Dubost, 1968). Vassalo (1998) observed the digging activity of two sympatric species of *Ctenomys*, and considered *C. australis* a scratch-digger and *C. talarum* both a scratch and skull-tooth digger. *C. australis* occupies a narrow area in the coastal dunes of Buenos Aires Province with extremely friable and sandy soils and *C. talarum* occupies interdune and inland soils varying from sandy to loamy (Justo *et al.*, 2003). *C. australis* is a sister species of *C. flamarioni* (both of the “mendocinus group”) and occurs only in the first line of coastal dunes (Freitas, 1995), tehrefore from a phylogenetical and ecological point of view we expected *C. flamarioni* to be primarily a scratch digger. *C. lami* inhabits a narrow space in the sandy fields in the oldest area of the Coastal Plain of southern Brazil (Freitas, 2001). *C. minutus* is a closely related species with *C. lami* and occurs inland in sandy fields and in the coastal dunes and interdunes in some regions in the southern Brazil

(Freitas, 1995; Gava & Freitas, 2002) and finally *C. torquatus* occurs in the fields of southern pampas "gaúchos" and some places in sandy fields (Fernandes *et al.*, 2007). Likewise, we expected, from a phylogenetic and ecological point of view to be a scratch and skull-tooth digger.

Size variation

The body size in *Ctenomys* has a great diversity, ranging of 100 g to 1000g (Vassalo, 1998). Of the four species, the bigger *C. flamarioni* has the larger mean size humerus, *C. lami* and *C. minutus* have a intermediate mean and *C. torquatus* have the smaller. In Fernández *et al.* (2000) and their references, the biggest living subterranean rodents live in very friable "like sand" soils, once the body size increases the energy expense during long periods of excavation (Vleck, 1979, 1981; Du Toit *et al.,* 1985; Lessa & Stein, 1992; *Lessa et al.,* 2008).

Heterogeneous sexual dimorphism

The sexual dimorphism inducing larger males than the females is recognized for *Ctenomys* in *C. minutus* (Gastal, 1994; Marinho & Freitas, 2006), *C. opinus* (Pearson, 1959; Cook *et al.*, 1990), *C. talarum* (Pearson *et al.*, 1968), *C. flamarioni* (Bretschneider, 1987) and inclusively *C. torquatus* (Travi, 1983). In our work the closely related species *C. lami* and *C. minutus* didn't demonstrate a significant difference in mean size, but the sexual dimorphism shows a different pattern, *C. lami* have larger males (while the females are of similar size) when compared with *C. minutus* and *C. flamarioni,* who demonstrated a similar pattern of dimorphism. *C. lami* have a narrow occurrence when compared with the sister species *C. minutus* and *C. flamarioni*. *C. torquatus* occupies a large area of pampas (hard soils) and demonstrated the smaller humerus and absence of a clear sexual dimorphism, what can be indicating selection for smaller size. This selection for small size could have at leat two explanations. First, be inposed by a mechanical a limit to dig in hard soils therefore selecting for smaller males or larger females. Second, the absence of sexual dimorphism could arise from differences in behavior between the sexes compared with other species. For example, sexual selection for bigger males in "narrow area" species as *C. lami* or *C. flamarioni*.

Bioemechanicis of the myoskeletal system

Compared with nondigging mammals, the humerus bone of subterranean rodents has a well developed "head", a stout diaphysis with a pronounced deltopectoral crest and elongated epicondyles. The muscles are wider and their origins and insertions are relatively farther away from the articulations (Hildebrand, 1985; Lessa & Stein, 1992; Vassallo, 1998; Fernández *et al.,* 2000; Morgan & Verzi, 2006; Lessa *et al.*, 2008). These alterations (i.e. relationships between bone structures and “origin and inserts” of the muscles) can result in an advantage in the mechanical elevation of the muscles and a resistance to the bone imposed to muscular actions (Morgan & Verzi, 2006; Lessa *et al.*, 2008). In scratch-diggers, the forelimb system is adapted to strong forces of extension of the shoulder and elbow joint (Vassalo, 1998). In almost all comparative works between diggers and nondiggers, the bone resistance is shown by robustness of the diaphysis and proximal part (shoulder joint), the deltopectoral crest is roughened and triangular for the fixation of important extensor muscles of pectoral and deltoid. Finally, a wider epicondyle process tends to increase the force in the manus in digging forms (Leahman, 1963; Cassinos *et al.,* 1993; Vassalo, 1998; Fernández *et al.*, 2000; Elissamburu & Vizcaino, 2004; Morgan & Verzi, 2006). Vassalo (1998) studying the myoskeletal system of two sympatric *Ctenomys* species found major differences in the humerus only in pronounced epicondylar processes and a relatively larger forelimb extensor muscles in *C. talarum*. Morgan & Verzi (2006) using classical morphometric methods (linear measurement) comparing only living species found specializations comprising the great robustness of the diaphysis, a distalization of the deltopectoral crest region and as Vassalo (1998), more pronounced epicondylar processes and an extensive trochlear area.

Selective and phylogentic origins of shape differences

Usually the bone reflects the influence of a dynamic functional loading (in our case burrowing) on a genetically determinated structure (Lanyon, 1981). In several other papers, the digging activity has demonstrated to cause diverse burrowing adaptations in subterranean rodents (Reig *et al.*, 1990; Quintana, 1994; Fernández *et al.*, 2000; Verzi, 2002) and shown as a important selective pressure (see, e.g. Bou *et al.*, 1987; Castiella & Casinos, 1990; Casinos *et al.*, 1993). A robust published phylogeny of the genus is not available, but it is known that *C. flamarioni* is a species more distantly related phylogenetically than the other three species, being *C. minutus* and *C. lami* recently diverged sister species (see Freitas, 2001). In our results from Mahalanobis Distances (Table 5) with the discriminant analysis, *C. flamarioni* have a more different shape of humerus when compared with *C. torquatus*, *C. lami* and *C. minutus*. The three related species demonstrated are much closer when compared with *C. flamarioni* (Table 5). The difference observed between *C. flamarioni* can be imputed either to phylogenetic divergence or to an adaptive divergence. In fact, *C. flamarioni* belongs to another monophyletic clade, the mendocinus group, and is the only species exclusively living in sand dunes in our work. Therefore, the large differences observed could have arisen through a gradual accumulation of differences or by the action of natural selection, which both effects could be cumulative and have the same direction.

However, the musculoskeletal analysis indicates an interesting point of view. In comparative works between diggers and nondigging caviomorph rodents, the clearest distinction is in the epicondyles, deltopectoral crest and muscle robustness (Hildebrand 1985; Lessa and Stein 1992; Vassallo, 1998; Fernandez *et al.*, 2000; Morgan & Verzi, 2006; Lessa *et al.*, 2008). In our work we compared only diggers and the most observed changes occur in areas of fixation of important extensor muscles of shoulder-pectoral joints, these changes can give a greater mechanical advantage to the forelimb movements and fixation of scapula-humerus joint. Still according Morgan & Verzi (2006), the two more different analyzed species of *Ctenomys* is *C. flamarioni* and *C. Lewis,* and for all morphological indexes they are similar, with the exception of index – with epicondyles divided by humeral length. *C. lewisi* (with *C. frater*) showed the most specialized humeral morphology and habits deep soil often near creeks and rivers (Morgan & Verzi, 2006 and their references). These results corroborate with the differences observed (major shape differences in the proximal and medial part of the humerus) between *C. flamarioni* and *C. torquatus.* Furthermore, *C. flamarioni* occur only in areas of sandy soils and *C. torquatus* in several types of different soils, including the hard ones. Still, in the Dorsal and Ventral View, the major difference between the species was observed in region of insertion of a major number of extensor muscles of the shoulder-pectoral joints (distal and medial part) of the humerus, having *C. torquatus* a stouter humerus when compared with *C. flamarioni*. While analyzing small mammal limb kinematics, Fischer *et al.* (2002) concluded that more than 50 % of its humeral displacement results from scapular retraction alone and only 25 % of its forearm displacement is actually achieved in the elbow joint, still indicates the predominance of scapular retraction in the forelimb. Elissamburu and Vizcaino (2004) studying limb proportion and adaptations in caviomorph rodents argues that functional specialization for speed are in the proximal part of the forelimb and for force in the medial segments, still analyzing indexes involving the shoulder, differences in the musculoskeletal system in this region can increase the force, especially in digging forms. We found a difference in the “size” of the trochlea and in the epicondyle and a great difference in condyles and in the supinatory crest. These musculoskeletal interpretations take us to argue the hypothesis that differences can originate from an adaptative components for the recognized difference of type of soils (costal dunes and fields), indicated by a more robust and “specialized” hard soil digger for *C. torquatus* and a slender humerus for soft soils for *C.*

flamarioni. But the two species are not close phylogenetically, what turn our hypothesis imprecise because such a difference could also arise with time and a formal statistical test in a phylogenetic comparative analysis is still needed.

Between the three correlated species (*C. lami, C. minutus and C. torquatus)* the shape difference was less pronounced (but with a greater probability that it originates mainly from an adaptative component) when compared with *C. flamarioni - C. torquatus* and shape difference was not the expected. It was expected that *C. torquatus* would have the more robust and with the more pronounced condyles and deltopectoral crest, however or the more pronounced osteological features belonged *C. lami* and *C. minutus* (more frialble soils than compared with *C. torquatus*). Corroborating that hypothesis that soil hardness is not different enough to produce strong selection of the humerus. Perhaps, in hard soils, the main differencesm might only arise in the skull. Or maybe the divergence is to recent to establish differences in shape of the humerus of the three related species. Finnaly a former test with more species using the phylogenetic comparative method could help to elucidate which part of the variation is of selective origin.

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VII. ANEXOS

Anexo Capítulo 1

Table III. Tables of ANOVAs of 3 factors (sex, species and repetitions) with the residuals (X, Y) obtained after GPA for the 19 landmarks used in the Dorsal View. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0000744	0.0000744	0.6072	0.43812	
sp	3	0.0009654	0.0003218	2.6275	0.05593	.
rep	9	0.0000987	0.0000110	0.0895	0.99973	
sex:sp	3	0.0010528	0.0003509	2.8653	0.04177	*
sex:rep	9	0.0000550	0.0000061	0.0499	0.99998	
sp:rep	27	0.0001545	0.0000057	0.0467	1.00000	
sex:sp:rep	27	0.0000582	0.0000022	0.0176	1.00000	
Residuals	80	0.0097984	0.0001225			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0012046	0.0012046	17.9873	5.928e-05	***
sp	3	0.0010014	0.0003338	4.9845	0.003201	**
rep	9	0.0000505	0.0000056	0.0838	0.999795	
sex:sp	3	0.0017909	0.0005970	8.9140	3.649e-05	***
sex:rep	9	0.0000005	0.0000001	0.0009	1.000000	
sp:rep	27	0.0000157	0.0000006	0.0087	1.000000	
sex:sp:rep	27	0.0000089	0.0000003	0.0049	1.000000	
Residuals	80	0.0053575	0.0000670			

2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0002156	0.0002156	1.3646	0.246208	
sp	3	0.0005932	0.0001977	1.2512	0.296828	
rep	9	0.0000304	0.0000034	0.0214	0.999999	
sex:sp	3	0.0023515	0.0007838	4.9601	0.003296	**
sex:rep	9	0.0000620	0.0000069	0.0436	0.999987	
sp:rep	27	0.0001140	0.0000042	0.0267	1.000000	
sex:sp:rep	27	0.0000806	0.0000030	0.0189	1.000000	
Residuals	80	0.0126421	0.0001580			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0000204	0.0000204	0.2586	0.61251	
sp	3	0.0009701	0.0003234	4.0944	0.00932	**
rep	9	0.0000011	0.0000001	0.0016	1.00000	
sex:sp	3	0.0002264	0.0000755	0.9556	0.41792	
sex:rep	9	0.0000016	0.0000002	0.0022	1.00000	
sp:rep	27	0.0000093	0.0000003	0.0044	1.00000	
sex:sp:rep	27	0.0000153	0.0000006	0.0072	1.00000	
Residuals	80	0.0063181	0.0000790			

3

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.000018	0.000018	0.0337	0.8548	
sp	3	0.014079	0.004693	8.8931	3.733e-05	***
rep	9	0.000093	0.000010	0.0195	1.0000	
sex:sp	3	0.001288	0.000429	0.8136	0.4901	
sex:rep	9	0.000039	0.000004	0.0083	1.0000	
sp:rep	27	0.000164	0.000006	0.0115	1.0000	
sex:sp:rep	27	0.000197	0.000007	0.0138	1.0000	
Residuals	80	0.042216	0.000528			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.00007803	0.00007803	3.2183	0.0766	.
sp	3	0.00170126	0.00056709	23.3891	5.739e-11	***
rep	9	0.00002912	0.00000324	0.1335	0.9986	
sex:sp	3	0.00075671	0.00025224	10.4033	7.455e-06	***
sex:rep	9	0.00001892	0.00000210	0.0867	0.9998	
sp:rep	27	0.00002884	0.00000107	0.0441	1.0000	
sex:sp:rep	27	0.00003242	0.00000120	0.0495	1.0000	
Residuals	80	0.00193966	0.00002425			

4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.000009	0.000009	0.0177	0.8944	
sp	3	0.015363	0.005121	9.6810	1.599e-05	***
rep	9	0.000065	0.000007	0.0136	1.0000	
sex:sp	3	0.002531	0.000844	1.5949	0.1971	
sex:rep	9	0.000026	0.000003	0.0054	1.0000	
sp:rep	27	0.000133	0.000005	0.0093	1.0000	
sex:sp:rep	27	0.000262	0.000010	0.0184	1.0000	
Residuals	80	0.042318	0.000529			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0000117	0.0000117	0.2127	0.645890	
sp	3	0.0007286	0.0002429	4.4048	0.006408	**
rep	9	0.0000161	0.0000018	0.0324	0.999996	
sex:sp	3	0.0031359	0.0010453	18.9593	2.21e-09	***
sex:rep	9	0.0000250	0.0000028	0.0505	0.999976	
sp:rep	27	0.0000574	0.0000021	0.0386	1.000000	
sex:sp:rep	27	0.0000521	0.0000019	0.0350	1.000000	
Residuals	80	0.0044107	0.0000551			

5

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.00023177	0.00023177	7.3970	0.008011	**
sp	3	0.00111368	0.00037123	11.8476	1.686e-06	***
rep	9	0.00004819	0.00000535	0.1709	0.996430	
sex:sp	3	0.00032144	0.00010715	3.4196	0.021182	*
sex:rep	9	0.00001101	0.00000122	0.0390	0.999992	
sp:rep	27	0.00006523	0.00000242	0.0771	1.000000	
sex:sp:rep	27	0.00005068	0.00000188	0.0599	1.000000	
Residuals	80	0.00250667	0.00003133			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0002009	0.0002009	2.3003	0.13329	
sp	3	0.0007134	0.0002378	2.7234	0.04972	*
rep	9	0.0000142	0.0000016	0.0181	1.00000	
sex:sp	3	0.0022673	0.0007558	8.6551	4.838e-05	***
sex:rep	9	0.0000024	0.0000003	0.0030	1.00000	
sp:rep	27	0.0000455	0.0000017	0.0193	1.00000	
sex:sp:rep	27	0.0000292	0.0000011	0.0124	1.00000	
Residuals	80	0.0069856	0.0000873			

6

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.00030160	0.00030160	14.0361	0.000337	***
sp	3	0.00113304	0.00037768	17.5769	7.407e-09	***
rep	9	0.00002773	0.00000308	0.1434	0.998192	
sex:sp	3	0.00016089	0.00005363	2.4959	0.065735	.
sex:rep	9	0.00002291	0.00000255	0.1185	0.999155	
sp:rep	27	0.00008033	0.00000298	0.1385	1.000000	
sex:sp:rep	27	0.00006492	0.00000240	0.1119	1.000000	
Residuals	80	0.00171898	0.00002149			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.00001381	0.00001381	0.3803	0.5392	
sp	3	0.00018420	0.00006140	1.6911	0.1755	
rep	9	0.00003045	0.00000338	0.0932	0.9997	
sex:sp	3	0.00020458	0.00006819	1.8782	0.1399	
sex:rep	9	0.00000345	0.00000038	0.0106	1.0000	
sp:rep	27	0.00001150	0.00000043	0.0117	1.0000	
sex:sp:rep	27	0.00003019	0.00000112	0.0308	1.0000	
Residuals	80	0.00290461	0.00003631			

7

```
           Df     Sum Sq    Mean Sq F value   Pr(>F)
sex         1 0.00004492 0.00004492  4.7093 0.032966 *
sp          3 0.00011613 0.00003871  4.0579 0.009742 **
rep         9 0.00000412 0.00000046  0.0480 0.999981
sex:sp      3 0.00016158 0.00005386  5.6460 0.001465 **
sex:rep     9 0.00001089 0.00000121  0.1269 0.998888
sp:rep     27 0.00005632 0.00000209  0.2187 0.999981
sex:sp:rep 27 0.00003775 0.00000140  0.1466 1.000000
Residuals  80 0.00076317 0.00000954
           Df     Sum Sq    Mean Sq F value   Pr(>F)
sex         1 0.00002211 0.00002211  1.7032   0.19561
sp          3 0.00145635 0.00048545 37.3969 3.301e-15 ***
rep         9 0.00004808 0.00000534  0.4115   0.92549
sex:sp      3 0.00011701 0.00003900  3.0047   0.03521 *
sex:rep     9 0.00001928 0.00000214  0.1650   0.99688
sp:rep     27 0.00002080 0.00000077  0.0593   1.00000
sex:sp:rep 27 0.00002791 0.00000103  0.0796   1.00000
Residuals  80 0.00103848 0.00001298
```

8

```
           Df     Sum Sq    Mean Sq F value    Pr(>F)
sex         1 0.00000945 0.00000945  0.3267   0.56923
sp          3 0.00093459 0.00031153 10.7687 5.093e-06 ***
rep         9 0.00000532 0.00000059  0.0204   1.00000
sex:sp      3 0.00024367 0.00008122  2.8077   0.04483 *
sex:rep     9 0.00001055 0.00000117  0.0405   0.99999
sp:rep     27 0.00005366 0.00000199  0.0687   1.00000
sex:sp:rep 27 0.00004604 0.00000171  0.0589   1.00000
Residuals  80 0.00231434 0.00002893
           Df     Sum Sq    Mean Sq F value    Pr(>F)
sex         1 0.00005293 0.00005293  1.8154    0.1817
sp          3 0.00083035 0.00027678  9.4939 1.953e-05 ***
rep         9 0.00007552 0.00000839  0.2878    0.9763
sex:sp      3 0.00081896 0.00027299  9.3637 2.245e-05 ***
sex:rep     9 0.00004536 0.00000504  0.1729    0.9963
sp:rep     27 0.00023603 0.00000874  0.2999    0.9996
sex:sp:rep 27 0.00021005 0.00000778  0.2668    0.9999
Residuals  80 0.00233231 0.00002915
```

9

```
           Df     Sum Sq    Mean Sq F value    Pr(>F)
sex         1 0.00020795 0.00020795  7.4582  0.007766 **
sp          3 0.00100161 0.00033387 11.9747 1.483e-06 ***
rep         9 0.00004837 0.00000537  0.1928  0.994365
sex:sp      3 0.00113622 0.00037874 13.5840 3.017e-07 ***
sex:rep     9 0.00002405 0.00000267  0.0958  0.999644
sp:rep     27 0.00015059 0.00000558  0.2000  0.999992
sex:sp:rep 27 0.00023495 0.00000870  0.3121  0.999431
Residuals  80 0.00223050 0.00002788
           Df     Sum Sq    Mean Sq F value    Pr(>F)
sex         1 0.00030676 0.00030676 32.4728 1.942e-07 ***
sp          3 0.00030369 0.00010123 10.7159 5.381e-06 ***
rep         9 0.00001012 0.00000112  0.1190    0.9991
sex:sp      3 0.00122041 0.00040680 43.0629 < 2.2e-16 ***
sex:rep     9 0.00000257 0.00000029  0.0302    1.0000
sp:rep     27 0.00002256 0.00000084  0.0885    1.0000
sex:sp:rep 27 0.00004204 0.00000156  0.1648    1.0000
Residuals  80 0.00075574 0.00000945
```

10

```
           Df     Sum Sq    Mean Sq F value    Pr(>F)
sex         1 0.00010736 0.00010736  3.2604  0.074735 .
sp          3 0.00044714 0.00014905  4.5264  0.005536 **
rep         9 0.00006851 0.00000761  0.2312  0.989007
sex:sp      3 0.00155187 0.00051729 15.7097 4.023e-08 ***
sex:rep     9 0.00000470 0.00000052  0.0159  1.000000
sp:rep     27 0.00006708 0.00000248  0.0754  1.000000
sex:sp:rep 27 0.00003121 0.00000116  0.0351  1.000000
Residuals  80 0.00263425 0.00003293
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0002719 0.0002719  1.9311  0.168498
sp          3 0.0019730 0.0006577  4.6709  0.004655 **
rep         9 0.0002085 0.0000232  0.1645  0.996915
sex:sp      3 0.0044337 0.0014779 10.4961 6.765e-06 ***
sex:rep     9 0.0001948 0.0000216  0.1537  0.997630
sp:rep     27 0.0009234 0.0000342  0.2429  0.999945
sex:sp:rep 27 0.0004465 0.0000165  0.1174  1.000000
Residuals  80 0.0112643 0.0001408
```

11

```
           Df     Sum Sq    Mean Sq F value    Pr(>F)
sex         1 0.00008364 0.00008364  6.6641   0.01166 *
sp          3 0.00115439 0.00038480 30.6591 2.697e-13 ***
rep         9 0.00000911 0.00000101  0.0807   0.99983
sex:sp      3 0.00059529 0.00019843 15.8101 3.667e-08 ***
sex:rep     9 0.00005670 0.00000630  0.5020   0.86896
sp:rep     27 0.00010709 0.00000397  0.3160   0.99936
sex:sp:rep 27 0.00009081 0.00000336  0.2680   0.99986
Residuals  80 0.00100407 0.00001255
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0002446 0.0002446  3.3047   0.07283 .
sp          3 0.0001224 0.0000408  0.5513   0.64871
rep         9 0.0002015 0.0000224  0.3025   0.97194
sex:sp      3 0.0021229 0.0007076  9.5623 1.815e-05 ***
sex:rep     9 0.0001605 0.0000178  0.2410   0.98724
sp:rep     27 0.0002986 0.0000111  0.1494   1.00000
sex:sp:rep 27 0.0001338 0.0000050  0.0670   1.00000
Residuals  80 0.0059202 0.0000740
```

12

```
           Df   Sum Sq  Mean Sq F value  Pr(>F)
sex         1 0.012532 0.012532  5.6862 0.01947 *
sp          3 0.007519 0.002506  1.1373 0.33914
rep         9 0.000837 0.000093  0.0422 0.99999
sex:sp      3 0.008827 0.002942  1.3350 0.26887
sex:rep     9 0.001052 0.000117  0.0530 0.99997
sp:rep     27 0.005450 0.000202  0.0916 1.00000
sex:sp:rep 27 0.003757 0.000139  0.0631 1.00000
Residuals  80 0.176310 0.002204
           Df     Sum Sq    Mean Sq F value    Pr(>F)
sex         1 0.00030167 0.00030167 20.6686 1.915e-05 ***
sp          3 0.00008784 0.00002928  2.0060    0.1197
rep         9 0.00000590 0.00000066  0.0449    1.0000
sex:sp      3 0.00054216 0.00018072 12.3820 9.854e-07 ***
sex:rep     9 0.00000151 0.00000017  0.0115    1.0000
sp:rep     27 0.00000264 0.00000010  0.0067    1.0000
sex:sp:rep 27 0.00000654 0.00000024  0.0166    1.0000
Residuals  80 0.00116764 0.00001460
```

13

```
           Df   Sum Sq  Mean Sq F value  Pr(>F)
sex         1 0.011332 0.011332  5.1008 0.02664 *
sp          3 0.007004 0.002335  1.0508 0.37482
rep         9 0.001080 0.000120  0.0540 0.99997
sex:sp      3 0.008553 0.002851  1.2833 0.28583
sex:rep     9 0.000974 0.000108  0.0487 0.99998
sp:rep     27 0.005447 0.000202  0.0908 1.00000
sex:sp:rep 27 0.003695 0.000137  0.0616 1.00000
Residuals  80 0.177732 0.002222
           Df     Sum Sq    Mean Sq F value  Pr(>F)
sex         1 0.00003291 0.00003291  1.1787 0.28087
sp          3 0.00023388 0.00007796  2.7927 0.04566 *
rep         9 0.00000396 0.00000044  0.0157 1.00000
sex:sp      3 0.00018810 0.00006270  2.2461 0.08929 .
sex:rep     9 0.00000624 0.00000069  0.0248 1.00000
sp:rep     27 0.00001144 0.00000042  0.0152 1.00000
sex:sp:rep 27 0.00000545 0.00000020  0.0072 1.00000
```

14

```
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0010247 0.0010247 13.5252 0.0004249 ***
sp          3 0.0005506 0.0001835  2.4224 0.0719361 .
rep         9 0.0002568 0.0000285  0.3766 0.9431070
sex:sp      3 0.0020987 0.0006996  9.2337 2.582e-05 ***
sex:rep     9 0.0000882 0.0000098  0.1293 0.9987986
sp:rep     27 0.0003275 0.0000121  0.1601 0.9999993
sex:sp:rep 27 0.0003514 0.0000130  0.1718 0.9999985
Residuals  80 0.0060609 0.0000758
           Df    Sum Sq   Mean Sq F value  Pr(>F)
sex         1 0.0001639 0.0001639  1.4890 0.22596
sp          3 0.0004150 0.0001383  1.2571 0.29476
rep         9 0.0000373 0.0000041  0.0377 0.99999
sex:sp      3 0.0009927 0.0003309  3.0069 0.03511 *
sex:rep     9 0.0000032 0.0000004  0.0032 1.00000
sp:rep     27 0.0000216 0.0000008  0.0073 1.00000
sex:sp:rep 27 0.0000091 0.0000003  0.0031 1.00000
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
esiduals	80	0.00223326	0.00002792			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Residuals	80	0.0088039	0.0001100			

15

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0000182	0.0000182	0.1178	0.7324	
sp	3	0.0003854	0.0001285	0.8320	0.4802	
rep	9	0.0002430	0.0000270	0.1748	0.9961	
sex:sp	3	0.0006019	0.0002006	1.2992	0.2805	
sex:rep	9	0.0000904	0.0000100	0.0650	0.9999	
sp:rep	27	0.0003734	0.0000138	0.0896	1.0000	
sex:sp:rep	27	0.0001721	0.0000064	0.0413	1.0000	
Residuals	80	0.0123545	0.0001544			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0000745	0.0000745	0.8608	0.356307	
sp	3	0.0012911	0.0004304	4.9716	0.003251	**
rep	9	0.0000139	0.0000015	0.0178	1.000000	
sex:sp	3	0.0001494	0.0000498	0.5754	0.632864	
sex:rep	9	0.0000258	0.0000029	0.0331	0.999996	
sp:rep	27	0.0000301	0.0000011	0.0129	1.000000	
sex:sp:rep	27	0.0000348	0.0000013	0.0149	1.000000	
Residuals	80	0.0069251	0.0000866			

16

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0001470	0.0001470	1.6927	0.19698	
sp	3	0.0002520	0.0000840	0.9671	0.41251	
rep	9	0.0001606	0.0000178	0.2054	0.99286	
sex:sp	3	0.0010085	0.0003362	3.8700	0.01224	*
sex:rep	9	0.0000508	0.0000056	0.0650	0.99993	
sp:rep	27	0.0002334	0.0000086	0.0995	1.00000	
sex:sp:rep	27	0.0001533	0.0000057	0.0654	1.00000	
Residuals	80	0.0069493	0.0000869			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0000370	0.0000370	0.7390	0.39254	
sp	3	0.0003840	0.0001280	2.5565	0.06102	.
rep	9	0.0000168	0.0000019	0.0372	0.99999	
sex:sp	3	0.0001075	0.0000358	0.7159	0.54534	
sex:rep	9	0.0000088	0.0000010	0.0195	1.00000	
sp:rep	27	0.0000265	0.0000010	0.0196	1.00000	
sex:sp:rep	27	0.0000227	0.0000008	0.0168	1.00000	
Residuals	80	0.0040053	0.0000501			

17

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0004582	0.0004582	6.4222	0.013221	*
sp	3	0.0006147	0.0002049	2.8723	0.041414	*
rep	9	0.0000552	0.0000061	0.0859	0.999773	
sex:sp	3	0.0010688	0.0003563	4.9942	0.003164	**
sex:rep	9	0.0000584	0.0000065	0.0910	0.999713	
sp:rep	27	0.0002540	0.0000094	0.1319	1.000000	
sex:sp:rep	27	0.0001848	0.0000068	0.0960	1.000000	
Residuals	80	0.0057071	0.0000713			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0000071	0.0000071	0.1117	0.7391	
sp	3	0.0002976	0.0000992	1.5580	0.2061	
rep	9	0.0000150	0.0000017	0.0262	1.0000	
sex:sp	3	0.0003252	0.0001084	1.7027	0.1731	
sex:rep	9	0.0000317	0.0000035	0.0554	1.0000	
sp:rep	27	0.0000446	0.0000017	0.0260	1.0000	
sex:sp:rep	27	0.0000357	0.0000013	0.0208	1.0000	
Residuals	80	0.0050935	0.0000637			

18

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0009953	0.0009953	18.8161	4.164e-05	***
sp	3	0.0001907	0.0000636	1.2016	0.3146	
rep	9	0.0004773	0.0000530	1.0026	0.4450	
sex:sp	3	0.0001685	0.0000562	1.0618	0.3701	
sex:rep	9	0.0000794	0.0000088	0.1667	0.9968	
sp:rep	27	0.0002984	0.0000111	0.2089	1.0000	
sex:sp:rep	27	0.0005422	0.0000201	0.3797	0.9970	
Residuals	80	0.0042317	0.0000529			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.00008371	0.00008371	3.3035	0.072878	.
sp	3	0.00047273	0.00015758	6.2187	0.000751	***
rep	9	0.00009878	0.00001098	0.4331	0.913351	
sex:sp	3	0.00012046	0.00004015	1.5846	0.199580	
sex:rep	9	0.00001173	0.00000130	0.0514	0.999974	
sp:rep	27	0.00015047	0.00000557	0.2199	0.999980	
sex:sp:rep	27	0.00011146	0.00000413	0.1629	0.999999	
Residuals	80	0.00202712	0.00002534			

19

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0003458	0.0003458	4.9686	0.02862	*
sp	3	0.0005404	0.0001801	2.5883	0.05869	.
rep	9	0.0001449	0.0000161	0.2314	0.98897	
sex:sp	3	0.0006979	0.0002326	3.3426	0.02327	*
sex:rep	9	0.0000295	0.0000033	0.0471	0.99998	
sp:rep	27	0.0002829	0.0000105	0.1506	1.00000	
sex:sp:rep	27	0.0001659	0.0000061	0.0883	1.00000	
Residuals	80	0.0055678	0.0000696			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.00038743	0.00038743	10.2519	0.00196	**
sp	3	0.00036356	0.00012119	3.2068	0.02748	*
rep	9	0.00001556	0.00000173	0.0457	0.99998	
sex:sp	3	0.00166670	0.00055557	14.7010	1.034e-07	***
sex:rep	9	0.00000063	0.00000007	0.0018	1.00000	
sp:rep	27	0.00001945	0.00000072	0.0191	1.00000	
sex:sp:rep	27	0.00001587	0.00000059	0.0156	1.00000	
Residuals	80	0.00302329	0.00003779			

Table IV. Tables of ANOVAs of 3 factors (sex, species and repetitions) with the residuals (X, Y) obtained before GPA for the 19 landmarks used in the Ventral View. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '1

1

```
           Df    Sum Sq   Mean Sq F value   Pr(>F)
sex         1 0.0000107 0.0000107  0.2084 0.649229
sp          3 0.0008197 0.0002732  5.3375 0.002106 **
rep         9 0.0000439 0.0000049  0.0952 0.999653
sex:sp      3 0.0024805 0.0008268 16.1511 2.68e-08 ***
sex:rep     9 0.0000220 0.0000024  0.0477 0.999981
sp:rep     27 0.0001086 0.0000040  0.0786 1.000000
sex:sp:rep 27 0.0001280 0.0000047  0.0926 1.000000
Residuals  80 0.0040954 0.0000512
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0001379 0.0001379  2.8686   0.09421 .
sp          3 0.0004873 0.0001624  3.3781   0.02229 *
rep         9 0.0000016 0.0000002  0.0037   1.00000
sex:sp      3 0.0016194 0.0005398 11.2268 3.174e-06 ***
sex:rep     9 0.0000068 0.0000008  0.0157   1.00000
sp:rep     27 0.0000437 0.0000016  0.0336   1.00000
sex:sp:rep 27 0.0000381 0.0000014  0.0294   1.00000
Residuals  80 0.0038466 0.0000481
```

2

```
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0011537 0.0011537 27.0417 1.496e-06 ***
sp          3 0.0010673 0.0003558  8.3385 6.847e-05 ***
rep         9 0.0000193 0.0000021  0.0502  0.999977
sex:sp      3 0.0005881 0.0001960  4.5950  0.005099 **
sex:rep     9 0.0000049 0.0000005  0.0127  1.000000
sp:rep     27 0.0000287 0.0000011  0.0249  1.000000
sex:sp:rep 27 0.0000251 0.0000009  0.0218  1.000000
Residuals  80 0.0034132 0.0000427
           Df     Sum Sq    Mean Sq F value    Pr(>F)
sex         1 0.00029983 0.00029983 11.5642  0.001052 **
sp          3 0.00086388 0.00028796 11.1062 3.593e-06 ***
rep         9 0.00006529 0.00000725  0.2798  0.978472
sex:sp      3 0.00112063 0.00037354 14.4072 1.367e-07 ***
sex:rep     9 0.00003518 0.00000391  0.1508  0.997800
sp:rep     27 0.00014382 0.00000533  0.2054  0.999990
sex:sp:rep 27 0.00016846 0.00000624  0.2406  0.999950
Residuals  80 0.00207422 0.00002593
```

3

```
           Df    Sum Sq   Mean Sq F value Pr(>F)
sex         1 0.0003021 0.0003021  3.1233 0.0810 .
sp          3 0.0005656 0.0001885  1.9493 0.1283
rep         9 0.0000036 0.0000004  0.0042 1.0000
sex:sp      3 0.0005969 0.0001990  2.0574 0.1125
sex:rep     9 0.0000090 0.0000010  0.0103 1.0000
sp:rep     27 0.0000215 0.0000008  0.0082 1.0000
sex:sp:rep 27 0.0000402 0.0000015  0.0154 1.0000
Residuals  80 0.0077368 0.0000967
           Df    Sum Sq   Mean Sq F value Pr(>F)
sex         1 0.0000048 0.0000048  0.0624 0.8033
sp          3 0.0000943 0.0000314  0.4058 0.7492
rep         9 0.0000595 0.0000066  0.0853 0.9998
sex:sp      3 0.0001003 0.0000334  0.4315 0.7310
sex:rep     9 0.0000297 0.0000033  0.0426 1.0000
sp:rep     27 0.0000181 0.0000007  0.0087 1.0000
sex:sp:rep 27 0.0000237 0.0000009  0.0113 1.0000
Residuals  80 0.0061965 0.0000775
```

4

```
           Df    Sum Sq   Mean Sq F value   Pr(>F)
sex         1 0.0002754 0.0002754  3.9557 0.050133 .
sp          3 0.0010007 0.0003336  4.7916 0.004029 **
rep         9 0.0000149 0.0000017  0.0238 0.999999
sex:sp      3 0.0004398 0.0001466  2.1059 0.106001
sex:rep     9 0.0000091 0.0000010  0.0146 1.000000
sp:rep     27 0.0000221 0.0000008  0.0118 1.000000
sex:sp:rep 27 0.0000371 0.0000014  0.0197 1.000000
Residuals  80 0.0055693 0.0000696
           Df    Sum Sq   Mean Sq F value   Pr(>F)
sex         1 0.0001257 0.0001257  0.7979 0.374409
sp          3 0.0016473 0.0005491  3.4856 0.019542 *
rep         9 0.0000051 0.0000006  0.0036 1.000000
sex:sp      3 0.0027540 0.0009180  5.8273 0.001184 **
sex:rep     9 0.0000190 0.0000021  0.0134 1.000000
sp:rep     27 0.0000293 0.0000011  0.0069 1.000000
sex:sp:rep 27 0.0000305 0.0000011  0.0072 1.000000
Residuals  80 0.0126027 0.0001575
```

5

```
           Df     Sum Sq    Mean Sq F value    Pr(>F)
sex         1 0.00000307 0.00000307  0.1439 0.7054225
sp          3 0.00050315 0.00016772  7.8644 0.0001158 ***
rep         9 0.00003984 0.00000443  0.2076 0.9925791
sex:sp      3 0.00071694 0.00023898 11.2059 3.243e-06 ***
sex:rep     9 0.00004608 0.00000512  0.2401 0.9874032
sp:rep     27 0.00003483 0.00000129  0.0605 1.0000000
sex:sp:rep 27 0.00002658 0.00000098  0.0462 1.0000000
Residuals  80 0.00170609 0.00002133
           Df     Sum Sq    Mean Sq F value    Pr(>F)
sex         1 0.00028841 0.00028841  8.7411  0.004088 **
sp          3 0.00092784 0.00030928  9.3736 2.222e-05 ***
rep         9 0.00023444 0.00002605  0.7895  0.626730
sex:sp      3 0.00056486 0.00018829  5.7066  0.001364 **
sex:rep     9 0.00007178 0.00000798  0.2417  0.987101
sp:rep     27 0.00008770 0.00000325  0.0984  1.000000
sex:sp:rep 27 0.00013114 0.00000486  0.1472  1.000000
Residuals  80 0.00263958 0.00003299
```

6

```
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0014318 0.0014318 12.9206 0.0005602 ***
sp          3 0.0011200 0.0003733  3.3688 0.0225396 *
rep         9 0.0000733 0.0000081  0.0735 0.9998820
sex:sp      3 0.0026553 0.0008851  7.9872 0.0001010 ***
sex:rep     9 0.0000411 0.0000046  0.0412 0.9999900
sp:rep     27 0.0000876 0.0000032  0.0293 1.0000000
sex:sp:rep 27 0.0001451 0.0000054  0.0485 1.0000000
Residuals  80 0.0088654 0.0001108
           Df     Sum Sq    Mean Sq F value    Pr(>F)
sex         1 0.00015482 0.00015482 13.4911 0.0004315 ***
sp          3 0.00125324 0.00041775 36.4032 6.136e-15 ***
rep         9 0.00001766 0.00000196  0.1710 0.9964188
sex:sp      3 0.00089923 0.00029974 26.1201 7.044e-12 ***
sex:rep     9 0.00001206 0.00000134  0.1168 0.9992033
sp:rep     27 0.00004517 0.00000167  0.1458 0.9999997
sex:sp:rep 27 0.00006177 0.00000229  0.1994 0.9999926
Residuals  80 0.00091804 0.00001148
```

7

```
           Df    Sum Sq   Mean Sq F value   Pr(>F)
sex         1 0.0001480 0.0001480  1.2391 0.268984
sp          3 0.0018746 0.0006249  5.2298 0.002392 **
rep         9 0.0001274 0.0000142  0.1185 0.999155
sex:sp      3 0.0009711 0.0003237  2.7090 0.050602 .
sex:rep     9 0.0001203 0.0000134  0.1119 0.999330
sp:rep     27 0.0002015 0.0000075  0.0625 1.000000
sex:sp:rep 27 0.0003141 0.0000116  0.0974 1.000000
Residuals  80 0.0095587 0.0001195
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0005560 0.0005560 13.1558 0.0005029 ***
sp          3 0.0014876 0.0004959 11.7339 1.892e-06 ***
rep         9 0.0001866 0.0000207  0.4905 0.8769083
sex:sp      3 0.0006692 0.0002231  5.2787 0.0022576 **
sex:rep     9 0.0001090 0.0000121  0.2865 0.9766567
sp:rep     27 0.0005290 0.0000196  0.4636 0.9866194
sex:sp:rep 27 0.0003469 0.0000128  0.3041 0.9995488
```

8

```
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0001213 0.0001213  0.3657   0.54709
sp          3 0.0112673 0.0037558 11.3239 2.873e-06 ***
rep         9 0.0000712 0.0000079  0.0239   1.00000
sex:sp      3 0.0025148 0.0008383  2.5275   0.06324 .
sex:rep     9 0.0000283 0.0000031  0.0095   1.00000
sp:rep     27 0.0000938 0.0000035  0.0105   1.00000
sex:sp:rep 27 0.0000466 0.0000017  0.0052   1.00000
Residuals  80 0.0265335 0.0003317
           Df     Sum Sq    Mean Sq F value    Pr(>F)
sex         1 0.00007224 0.00007224  3.1813   0.07828 .
sp          3 0.00008450 0.00002817  1.2403   0.30064
rep         9 0.00000573 0.00000064  0.0281   1.00000
sex:sp      3 0.00062414 0.00020805  9.1614 2.792e-05 ***
sex:rep     9 0.00000474 0.00000053  0.0232   1.00000
sp:rep     27 0.00001362 0.00000050  0.0222   1.00000
sex:sp:rep 27 0.00001318 0.00000049  0.0215   1.00000
```

Residuals 80 0.0033807 0.0000423	Residuals 80 0.00181671 0.00002271
9 Df Sum Sq Mean Sq F value Pr(>F) sex 1 0.0003391 0.0003391 0.9652 0.32885 sp 3 0.0111001 0.0037000 10.5308 6.524e-06 *** rep 9 0.0000575 0.0000064 0.0182 1.00000 sex:sp 3 0.0023983 0.0007994 2.2753 0.08615 . sex:rep 9 0.0000335 0.0000037 0.0106 1.00000 sp:rep 27 0.0001122 0.0000042 0.0118 1.00000 sex:sp:rep 27 0.0000571 0.0000021 0.0060 1.00000 Residuals 80 0.0281082 0.0003514 Df Sum Sq Mean Sq F value Pr(>F) sex 1 0.0000018 0.0000018 0.0302 0.862530 sp 3 0.0008581 0.0002860 4.6914 0.004542 ** rep 9 0.0000076 0.0000008 0.0139 1.000000 sex:sp 3 0.0005518 0.0001839 3.0169 0.034682 * sex:rep 9 0.0000017 0.0000002 0.0032 1.000000 sp:rep 27 0.0000152 0.0000006 0.0092 1.000000 sex:sp:rep 27 0.0000081 0.0000003 0.0049 1.000000 Residuals 80 0.0048778 0.0000610	**10** Df Sum Sq Mean Sq F value Pr(>F) sex 1 0.0000067 0.0000067 0.0737 0.7867 sp 3 0.0001713 0.0000571 0.6238 0.6017 rep 9 0.0000232 0.0000026 0.0281 1.0000 sex:sp 3 0.0005697 0.0001899 2.0745 0.1101 sex:rep 9 0.0000229 0.0000025 0.0279 1.0000 sp:rep 27 0.0000296 0.0000011 0.0120 1.0000 sex:sp:rep 27 0.0000236 0.0000009 0.0095 1.0000 Residuals 80 0.0073227 0.0000915 Df Sum Sq Mean Sq F value Pr(>F) sex 1 0.00133379 0.00133379 42.6330 5.602e-09 *** sp 3 0.00129362 0.00043121 13.7830 2.488e-07 *** rep 9 0.00000395 0.00000044 0.0140 1 sex:sp 3 0.00134607 0.00044869 14.3418 1.455e-07 *** sex:rep 9 0.00000245 0.00000027 0.0087 1 sp:rep 27 0.00001046 0.00000039 0.0124 1 sex:sp:rep 27 0.00001582 0.00000059 0.0187 1 Residuals 80 0.00250284 0.00003129
11 Df Sum Sq Mean Sq F value Pr(>F) sex 1 0.0001256 0.0001256 1.1416 0.28853 sp 3 0.0003358 0.0001119 1.0171 0.38963 rep 9 0.0000399 0.0000044 0.0403 0.99999 sex:sp 3 0.0009713 0.0003238 2.9421 0.03801 * sex:rep 9 0.0000263 0.0000029 0.0265 1.00000 sp:rep 27 0.0000427 0.0000016 0.0144 1.00000 sex:sp:rep 27 0.0000446 0.0000017 0.0150 1.00000 Residuals 80 0.0088033 0.0001100 Df Sum Sq Mean Sq F value Pr(>F) sex 1 0.0001150 0.0001150 1.6981 0.1963 sp 3 0.0004206 0.0001402 2.0712 0.1106 rep 9 0.0000239 0.0000027 0.0393 1.0000 sex:sp 3 0.0004543 0.0001514 2.2369 0.0903 . sex:rep 9 0.0000030 0.0000003 0.0049 1.0000 sp:rep 27 0.0000064 0.0000002 0.0035 1.0000 sex:sp:rep 27 0.0000191 0.0000007 0.0105 1.0000 Residuals 80 0.0054156 0.0000677	**12** Df Sum Sq Mean Sq F value Pr(>F) sex 1 0.0000234 0.0000234 0.2155 0.64374 sp 3 0.0002842 0.0000947 0.8732 0.45861 rep 9 0.0000066 0.0000007 0.0067 1.00000 sex:sp 3 0.0012970 0.0004323 3.9845 0.01065 * sex:rep 9 0.0000069 0.0000008 0.0070 1.00000 sp:rep 27 0.0000142 0.0000005 0.0049 1.00000 sex:sp:rep 27 0.0000165 0.0000006 0.0056 1.00000 Residuals 80 0.0086803 0.0001085 Df Sum Sq Mean Sq F value Pr(>F) sex 1 0.00000014 0.00000014 0.0066 0.9353 sp 3 0.00007948 0.00002649 1.2621 0.2931 rep 9 0.00000340 0.00000038 0.0180 1.0000 sex:sp 3 0.00061282 0.00020427 9.7304 1.517e-05 *** sex:rep 9 0.00000057 0.00000006 0.0030 1.0000 sp:rep 27 0.00000903 0.00000033 0.0159 1.0000 sex:sp:rep 27 0.00000517 0.00000019 0.0091 1.0000 Residuals 80 0.00167946 0.00002099
13 Df Sum Sq Mean Sq F value Pr(>F) sex 1 0.0000631 0.0000631 0.5304 0.468554 sp 3 0.0002197 0.0000732 0.6158 0.606740 rep 9 0.0000460 0.0000051 0.0430 0.999988 sex:sp 3 0.0017977 0.0005992 5.0386 0.003002 ** sex:rep 9 0.0000030 0.0000003 0.0028 1.000000 sp:rep 27 0.0000116 0.0000004 0.0036 1.000000 sex:sp:rep 27 0.0000279 0.0000010 0.0087 1.000000 Residuals 80 0.0095145 0.0001189 Df Sum Sq Mean Sq F value Pr(>F) sex 1 0.0000784 0.0000784 1.8734 0.17492 sp 3 0.0001652 0.0000551 1.3162 0.27493 rep 9 0.0000261 0.0000029 0.0692 0.99991 sex:sp 3 0.0004292 0.0001431 3.4192 0.02119 * sex:rep 9 0.0000012 0.0000001 0.0032 1.00000 sp:rep 27 0.0000164 0.0000006 0.0145 1.00000 sex:sp:rep 27 0.0000098 0.0000004 0.0087 1.00000 Residuals 80 0.0033476 0.0000418	**14** Df Sum Sq Mean Sq F value Pr(>F) sex 1 0.0005743 0.0005743 5.7018 0.019305 * sp 3 0.0005122 0.0001707 1.6952 0.174675 rep 9 0.0000843 0.0000094 0.0929 0.999686 sex:sp 3 0.0014084 0.0004695 4.6609 0.004711 ** sex:rep 9 0.0000195 0.0000022 0.0215 0.999999 sp:rep 27 0.0001994 0.0000074 0.0733 1.000000 sex:sp:rep 27 0.0001755 0.0000065 0.0645 1.000000 Residuals 80 0.0080578 0.0001007 Df Sum Sq Mean Sq F value Pr(>F) sex 1 0.0004702 0.0004702 8.5342 0.004528 ** sp 3 0.0005357 0.0001786 3.2407 0.026362 * rep 9 0.0000033 0.0000004 0.0066 1.000000 sex:sp 3 0.0001244 0.0000415 0.7524 0.524170 sex:rep 9 0.0000062 0.0000007 0.0124 1.000000 sp:rep 27 0.0000171 0.0000006 0.0115 1.000000 sex:sp:rep 27 0.0000071 0.0000003 0.0048 1.000000 Residuals 80 0.0044081 0.0000551

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	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0001248	0.0001248	2.4560	0.121024	
sp	3	0.0007410	0.0002470	4.8623	0.003703	**
rep	9	0.0000134	0.0000015	0.0294	0.999998	
sex:sp	3	0.0028971	0.0009657	19.0115	2.113e-09	***
sex:rep	9	0.0000077	0.0000009	0.0167	1.000000	
sp:rep	27	0.0000393	0.0000015	0.0287	1.000000	
sex:sp:rep	27	0.0000372	0.0000014	0.0271	1.000000	
Residuals	80	0.0040637	0.0000508			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0000142	0.0000142	0.2724	0.6032	
sp	3	0.0017269	0.0005756	11.0061	3.984e-06	***
rep	9	0.0000022	0.0000002	0.0047	1.0000	
sex:sp	3	0.0022741	0.0007580	14.4931	1.259e-07	***
sex:rep	9	0.0000044	0.0000005	0.0093	1.0000	
sp:rep	27	0.0000163	0.0000006	0.0115	1.0000	
sex:sp:rep	27	0.0000128	0.0000005	0.0091	1.0000	
Residuals	80	0.0041842	0.0000523			

16

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0001960	0.0001960	2.3556	0.1288	
sp	3	0.0003650	0.0001217	1.4621	0.2311	
rep	9	0.0000671	0.0000075	0.0896	0.9997	
sex:sp	3	0.0004567	0.0001522	1.8293	0.1485	
sex:rep	9	0.0000126	0.0000014	0.0168	1.0000	
sp:rep	27	0.0001034	0.0000038	0.0460	1.0000	
sex:sp:rep	27	0.0000536	0.0000020	0.0239	1.0000	
Residuals	80	0.0066572	0.0000832			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.00002963	0.00002963	0.9283	0.33820	
sp	3	0.00037731	0.00012577	3.9399	0.01124	*
rep	9	0.00004264	0.00000474	0.1484	0.99793	
sex:sp	3	0.00076842	0.00025614	8.0238	9.696e-05	***
sex:rep	9	0.00001996	0.00000222	0.0695	0.99991	
sp:rep	27	0.00004314	0.00000160	0.0501	1.00000	
sex:sp:rep	27	0.00008058	0.00000298	0.0935	1.00000	
Residuals	80	0.00255378	0.00003192			

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	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.00005786	0.00005786	2.2740	0.1354960	
sp	3	0.00060612	0.00020204	7.9407	0.0001063	***
rep	9	0.00009736	0.00001082	0.4252	0.9179384	
sex:sp	3	0.00054328	0.00018109	7.1175	0.0002681	***
sex:rep	9	0.00002911	0.00000323	0.1271	0.9988787	
sp:rep	27	0.00032900	0.00001219	0.4789	0.9832532	
sex:sp:rep	27	0.00013357	0.00000495	0.1944	0.9999943	
Residuals	80	0.00203549	0.00002544			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0000005	0.0000005	0.0045	0.9468326	
sp	3	0.0021678	0.0007226	6.5352	0.0005212	***
rep	9	0.0001482	0.0000165	0.1489	0.9979044	
sex:sp	3	0.0030223	0.0010074	9.1110	2.948e-05	***
sex:rep	9	0.0000530	0.0000059	0.0533	0.9999699	
sp:rep	27	0.0003716	0.0000138	0.1245	1.0000000	
sex:sp:rep	27	0.0000948	0.0000035	0.0317	1.0000000	
Residuals	80	0.0088457	0.0001106			

18

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.00002006	0.00002006	1.3634	0.24643	
sp	3	0.00011867	0.00003956	2.6879	0.05193	.
rep	9	0.00004711	0.00000523	0.3557	0.95243	
sex:sp	3	0.00070055	0.00023352	15.8675	3.478e-08	***
sex:rep	9	0.00000823	0.00000091	0.0621	0.99994	
sp:rep	27	0.00002633	0.00000098	0.0663	1.00000	
sex:sp:rep	27	0.00001587	0.00000059	0.0399	1.00000	
Residuals	80	0.00117734	0.00001472			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.00021306	0.00021306	7.4990	0.007607	**
sp	3	0.00037169	0.00012390	4.3608	0.006756	**
rep	9	0.00003793	0.00000421	0.1483	0.997936	
sex:sp	3	0.00091718	0.00030573	10.7608	5.135e-06	***
sex:rep	9	0.00000896	0.00000100	0.0350	0.999995	
sp:rep	27	0.00002300	0.00000085	0.0300	1.000000	
sex:sp:rep	27	0.00001409	0.00000052	0.0184	1.000000	
Residuals	80	0.00227289	0.00002841			

19

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0000892	0.0000892	2.2182	0.1403	
sp	3	0.0014634	0.0004878	12.1287	1.270e-06	***
rep	9	0.0001170	0.0000130	0.3231	0.9651	
sex:sp	3	0.0034061	0.0011354	28.2300	1.496e-12	***
sex:rep	9	0.0000418	0.0000046	0.1154	0.9992	
sp:rep	27	0.0001153	0.0000043	0.1062	1.0000	
sex:sp:rep	27	0.0001728	0.0000064	0.1591	1.0000	
Residuals	80	0.0032175	0.0000402			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0000023	0.0000023	0.0318	0.85881	
sp	3	0.0019771	0.0006590	8.9652	3.452e-05	***
rep	9	0.0000217	0.0000024	0.0328	1.00000	
sex:sp	3	0.0009089	0.0003030	4.1216	0.00902	**
sex:rep	9	0.0000052	0.0000006	0.0078	1.00000	
sp:rep	27	0.0000338	0.0000013	0.0170	1.00000	
sex:sp:rep	27	0.0000079	0.0000003	0.0040	1.00000	
Residuals	80	0.0058808	0.0000735			

Table V. Tables of ANOVAs of 3 factors (sex, species and repetitions) with the residuals (X, Y) obtained before GPA for the 7 landmarks used in the Distal/Proximal View. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '1

1

```
           Df   Sum Sq  Mean Sq F value  Pr(>F)
sex         1 0.000446 0.000446  0.2523 0.61685
sp          3 0.019440 0.006480  3.6616 0.01577 *
rep         9 0.000314 0.000035  0.0197 1.00000
sex:sp      3 0.015741 0.005247  2.9650 0.03696 *
sex:rep     9 0.000129 0.000014  0.0081 1.00000
sp:rep     27 0.000648 0.000024  0.0136 1.00000
sex:sp:rep 27 0.000364 0.000013  0.0076 1.00000
Residuals  80 0.141574 0.001770
           Df   Sum Sq  Mean Sq F value    Pr(>F)
sex         1 0.012077 0.012077 10.7814  0.001522 **
sp          3 0.038024 0.012675 11.3153 2.899e-06 ***
rep         9 0.000291 0.000032  0.0288  0.999998
sex:sp      3 0.035072 0.011691 10.4368 7.198e-06 ***
sex:rep     9 0.000109 0.000012  0.0108  1.000000
sp:rep     27 0.000470 0.000017  0.0155  1.000000
sex:sp:rep 27 0.000387 0.000014  0.0128  1.000000
Residuals  80 0.089612 0.001120
```

2

```
           Df   Sum Sq  Mean Sq F value    Pr(>F)
sex         1 0.007389 0.007389 14.1353 0.0003222 ***
sp          3 0.008366 0.002789  5.3348 0.0021128 **
rep         9 0.000413 0.000046  0.0878 0.9997520
sex:sp      3 0.009695 0.003232  6.1825 0.0007832 ***
sex:rep     9 0.000107 0.000012  0.0227 0.9999993
sp:rep     27 0.000428 0.000016  0.0303 1.0000000
sex:sp:rep 27 0.000406 0.000015  0.0288 1.0000000
Residuals  80 0.041816 0.000523
           Df    Sum Sq   Mean Sq F value  Pr(>F)
sex         1 0.0001023 0.0001023  0.5782 0.44925
sp          3 0.0018292 0.0006097  3.4468 0.02049 *
rep         9 0.0002870 0.0000319  0.1803 0.99562
sex:sp      3 0.0007328 0.0002443  1.3808 0.25466
sex:rep     9 0.0001804 0.0000200  0.1133 0.99929
sp:rep     27 0.0003844 0.0000142  0.0805 1.00000
sex:sp:rep 27 0.0002184 0.0000081  0.0457 1.00000
Residuals  80 0.0141518 0.0001769
```

3

```
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0000774 0.0000774  0.2472    0.6205
sp          3 0.0136457 0.0045486 14.5164 1.232e-07 ***
rep         9 0.0000861 0.0000096  0.0305    1.0000
sex:sp      3 0.0089821 0.0029940  9.5553 1.829e-05 ***
sex:rep     9 0.0000917 0.0000102  0.0325    1.0000
sp:rep     27 0.0006564 0.0000243  0.0776    1.0000
sex:sp:rep 27 0.0002302 0.0000085  0.0272    1.0000
Residuals  80 0.0250670 0.0003133
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0000443 0.0000443  0.1160 0.7343510
sp          3 0.0071325 0.0023775  6.2266 0.0007442 ***
rep         9 0.0023551 0.0002617  0.6853 0.7200302
sex:sp      3 0.0030813 0.0010271  2.6899 0.0518032 .
sex:rep     9 0.0002552 0.0000284  0.0743 0.9998767
sp:rep     27 0.0013412 0.0000497  0.1301 0.9999999
sex:sp:rep 27 0.0008859 0.0000328  0.0859 1.0000000
Residuals  80 0.0305463 0.0003818
```

4

```
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0058352 0.0058352 38.7324 2.103e-08 ***
sp          3 0.0075033 0.0025011 16.6015 1.777e-08 ***
rep         9 0.0010960 0.0001218  0.8083   0.60985
sex:sp      3 0.0018422 0.0006141  4.0760   0.00953 **
sex:rep     9 0.0002133 0.0000237  0.1573   0.99740
sp:rep     27 0.0014894 0.0000552  0.3661   0.99778
sex:sp:rep 27 0.0016335 0.0000605  0.4016   0.99537
Residuals  80 0.0120524 0.0001507
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0027745 0.0027745 10.9358  0.001414 **
sp          3 0.0036656 0.0012219  4.8161  0.003913 **
rep         9 0.0005216 0.0000580  0.2284  0.989469
sex:sp      3 0.0066214 0.0022071  8.6996 4.609e-05 ***
sex:rep     9 0.0001755 0.0000195  0.0768  0.999858
sp:rep     27 0.0007175 0.0000266  0.1047  1.000000
sex:sp:rep 27 0.0009896 0.0000367  0.1445  1.000000
Residuals  80 0.0202965 0.0002537
```

5

```
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0023739 0.0023739  6.0209 0.0163099 *
sp          3 0.0084257 0.0028086  7.1235 0.0002662 ***
rep         9 0.0000719 0.0000080  0.0203 0.9999996
sex:sp      3 0.0078804 0.0026268  6.6624 0.0004504 ***
sex:rep     9 0.0000928 0.0000103  0.0261 0.9999986
sp:rep     27 0.0002532 0.0000094  0.0238 1.0000000
sex:sp:rep 27 0.0003650 0.0000135  0.0343 1.0000000
Residuals  80 0.0315417 0.0003943
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0092235 0.0092235 30.1912 4.517e-07 ***
sp          3 0.0089309 0.0029770  9.7445 1.495e-05 ***
rep         9 0.0002173 0.0000241  0.0790    0.9998
sex:sp      3 0.0138966 0.0046322 15.1626 6.695e-08 ***
sex:rep     9 0.0000262 0.0000029  0.0095    1.0000
sp:rep     27 0.0003990 0.0000148  0.0484    1.0000
sex:sp:rep 27 0.0002412 0.0000089  0.0292    1.0000
Residuals  80 0.0244402 0.0003055
```

6

```
           Df   Sum Sq  Mean Sq F value    Pr(>F)
sex         1 0.003905 0.003905  5.1981   0.02527 *
sp          3 0.047946 0.015982 21.2772 3.141e-10 ***
rep         9 0.000212 0.000024  0.0314   1.00000
sex:sp      3 0.032091 0.010697 14.2408 1.602e-07 ***
sex:rep     9 0.000066 0.000007  0.0098   1.00000
sp:rep     27 0.000266 0.000010  0.0131   1.00000
sex:sp:rep 27 0.000171 0.000006  0.0084   1.00000
Residuals  80 0.060091 0.000751
           Df    Sum Sq   Mean Sq F value    Pr(>F)
sex         1 0.0020819 0.0020819  9.3761 0.0029936 **
sp          3 0.0015562 0.0005187  2.3361 0.0799631 .
rep         9 0.0013314 0.0001479  0.6662 0.7368546
sex:sp      3 0.0051362 0.0017121  7.7103 0.0001375 ***
sex:rep     9 0.0000762 0.0000085  0.0381 0.9999929
sp:rep     27 0.0013204 0.0000489  0.2202 0.9999793
sex:sp:rep 27 0.0008455 0.0000313  0.1410 0.9999998
Residuals  80 0.0177638 0.0002220
```

7

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.000450	0.000450	0.7293	0.3957	
sp	3	0.052861	0.017620	28.5729	1.169e-12	***
rep	9	0.000061	0.000007	0.0110	1.0000	
sex:sp	3	0.028296	0.009432	15.2949	5.916e-08	***
sex:rep	9	0.000156	0.000017	0.0282	1.0000	
sp:rep	27	0.000293	0.000011	0.0176	1.0000	
sex:sp:rep	27	0.000341	0.000013	0.0205	1.0000	
Residuals	80	0.049335	0.000617			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	0.0000991	0.0000991	0.5016	0.48088	
sp	3	0.0017103	0.0005701	2.8840	0.04082	*
rep	9	0.0003004	0.0000334	0.1688	0.99659	
sex:sp	3	0.0165582	0.0055194	27.9216	1.869e-12	***
sex:rep	9	0.0001260	0.0000140	0.0708	0.99990	
sp:rep	27	0.0003807	0.0000141	0.0713	1.00000	
sex:sp:rep	27	0.0003864	0.0000143	0.0724	1.00000	
Residuals	80	0.0158140	0.0001977			

Table VI. Tables of ANOVAs of 3 factors (sex, species and repetitions) with the centroid obtained before GPA for the 3 view used. Upper left Dorsal View, Upper right Ventral View and left below Distal/Proximal View. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	50085	50085	0.4734	0.4934	
sp	3	3101988	1033996	9.7736	1.449e-05	***
rep	9	3880	431	0.0041	1.0000	
sex:sp	3	7071373	2357124	22.2802	1.389e-10	***
sex:rep	9	672	75	0.0007	1.0000	
sp:rep	27	3490	129	0.0012	1.0000	
sex:sp:rep	27	3418	127	0.0012	1.0000	
Residuals	80	8463574	105795			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	18624	18624	0.2294	0.6333	
sp	3	2034647	678216	8.3533	6.736e-05	***
rep	9	525	58	0.0007	1.0000	
sex:sp	3	5506272	1835424	22.6063	1.069e-10	***
sex:rep	9	79	9	0.0001	1.0000	
sp:rep	27	1056	39	0.0005	1.0000	
sex:sp:rep	27	462	17	0.0002	1.0000	
Residuals	80	6495272	81191			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
sex	1	44104	44104	9.4781	0.0028485	**
sp	3	296894	98965	21.2678	3.165e-10	***
rep	9	100	11	0.0024	1.0000000	
sex:sp	3	100028	33343	7.1655	0.0002539	***
sex:rep	9	76	8	0.0018	1.0000000	
sp:rep	27	354	13	0.0028	1.0000000	
sex:sp:rep	27	307	11	0.0024	1.0000000	
Residuals	80	372260	4653			

Table VII. Tables of ANOVAs of raw data (X, Y) for the 20 operators with 3 repetitions of 19 landmarks used in the Dorsal View. Signif. codes 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
op	19	851.07	44.79	2.6557	0.00461	**
Residuals	40	674.67	16.87			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
op	19	240.983	12.683	4.0265	0.0001027	***
Residuals	40	126.000	3.150			

2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
op	19	2328.98	122.58	4.3212	4.83e-05	***
Residuals	40	1134.67	28.37			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
op	19	270.733	14.249	4.5235	2.916e-05	***
Residuals	40	126.000	3.150			

3

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
op	19	8280.7	435.8	3.3354	0.0006588	***
Residuals	40	5226.7	130.7			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
op	19	303.067	15.951	5.9816	1.031e-06	***
Residuals	40	106.667	2.667			

4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
op	19	10433.5	549.1	5.1361	6.74e-06	***
Residuals	40	4276.7	106.9			

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
op	19	2143.40	112.81	7.1702	9.41e-08	***
Residuals	40	629.33	15.73			

5 Df Sum Sq Mean Sq F value Pr(>F) op 19 569.65 29.98 12.073 4.191e-11 *** Residuals 40 99.33 2.48 Df Sum Sq Mean Sq F value Pr(>F) op 19 475.25 25.01 5.817 1.467e-06 *** Residuals 40 172.00 4.30	**6** Df Sum Sq Mean Sq F value Pr(>F) op 19 4846.9 255.1 2.5121 0.00704 ** Residuals 40 4062.0 101.6 Df Sum Sq Mean Sq F value Pr(>F) op 19 1011.93 53.26 2.8557 0.002573 ** Residuals 40 746.00 18.65
7 Df Sum Sq Mean Sq F value Pr(>F) op 19 403.52 21.24 7.4957 5.107e-08 *** Residuals 40 113.33 2.83 Df Sum Sq Mean Sq F value Pr(>F) op 19 1831.33 96.39 3.0438 0.001498 ** Residuals 40 1266.67 31.67	**8** Df Sum Sq Mean Sq F value Pr(>F) op 19 840.07 44.21 9.6819 1.264e-09 *** Residuals 40 182.67 4.57 Df Sum Sq Mean Sq F value Pr(>F) op 19 17067.1 898.3 5.2581 5.089e-06 *** Residuals 40 6833.3 170.8
9 Df Sum Sq Mean Sq F value Pr(>F) op 19 6824.2 359.2 3.72 0.0002305 *** Residuals 40 3862.0 96.5 Df Sum Sq Mean Sq F value Pr(>F) op 19 272.933 14.365 5.9854 1.022e-06 *** Residuals 40 96.000 2.400	**10** Df Sum Sq Mean Sq F value Pr(>F) op 19 425.92 22.42 8.7338 5.803e-09 *** Residuals 40 102.67 2.57 Df Sum Sq Mean Sq F value Pr(>F) op 19 1782.40 93.81 3.9666 0.00012 *** Residuals 40 946.00 23.65
11 Df Sum Sq Mean Sq F value Pr(>F) op 19 373.25 19.64 2.5078 0.007129 ** Residuals 40 313.33 7.83 Df Sum Sq Mean Sq F value Pr(>F) op 19 3347.0 176.2 6.3366 4.902e-07 *** Residuals 40 1112.0 27.8	**12** Df Sum Sq Mean Sq F value Pr(>F) op 19 6230.8 327.9 6.3801 4.482e-07 *** Residuals 40 2056.0 51.4 Df Sum Sq Mean Sq F value Pr(>F) op 19 242.933 12.786 4.6214 2.293e-05 *** Residuals 40 110.667 2.767
13 Df Sum Sq Mean Sq F value Pr(>F) op 19 6071.9 319.6 5.2332 5.388e-06 *** Residuals 40 2442.7 61.1 Df Sum Sq Mean Sq F value Pr(>F) op 19 332.98 17.53 8.1514 1.569e-08 *** Residuals 40 86.00 2.15	**14** Df Sum Sq Mean Sq F value Pr(>F) op 19 2352.67 123.82 2.4153 0.00938 ** Residuals 40 2050.67 51.27 Df Sum Sq Mean Sq F value Pr(>F) op 19 580.32 30.54 14.779 1.589e-12 *** Residuals 40 82.67 2.07
15 Df Sum Sq Mean Sq F value Pr(>F) op 19 1852.00 97.47 6.52 3.372e-07 *** Residuals 40 598.00 14.95 Df Sum Sq Mean Sq F value Pr(>F) op 19 416.27 21.91 13.984 3.938e-12 *** Residuals 40 62.67 1.57	**16** Df Sum Sq Mean Sq F value Pr(>F) op 19 252.983 13.315 6.193 6.6e-07 *** Residuals 40 86.000 2.150 Df Sum Sq Mean Sq F value Pr(>F) op 19 97.650 5.139 1.9035 0.04306 * Residuals 40 108.000 2.700
17 Df Sum Sq Mean Sq F value Pr(>F) op 19 7039.9 370.5 8.5145 8.393e-09 *** Residuals 40 1740.7 43.5 Df Sum Sq Mean Sq F value Pr(>F) op 19 473.07 24.90 2.3018 0.01315 * Residuals 40 432.67 10.82	**18** Df Sum Sq Mean Sq F value Pr(>F) op 19 1612.60 84.87 4.3787 4.181e-05 *** Residuals 40 775.33 19.38 Df Sum Sq Mean Sq F value Pr(>F) op 19 457.92 24.10 2.2701 0.01445 * Residuals 40 424.67 10.62
19 Df Sum Sq Mean Sq F value Pr(>F) op 19 338.98 17.84 6.4877 3.599e-07 *** Residuals 40 110.00 2.75 Df Sum Sq Mean Sq F value Pr(>F) op 19 443.73 23.35 3.7567 0.0002089 *** Residuals 40 248.67 6.22	

Table VIII. Tables of ANOVAs of raw data (X, Y) for the 20 operators with 3 repetitions of 19 landmarks used in the Ventral View. Signif. codes 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

1 Df Sum Sq Mean Sq F value Pr(>F) op 19 804.32 42.33 7.6736 3.684e-08 *** Residuals 40 220.67 5.52 Df Sum Sq Mean Sq F value Pr(>F) op 19 892.85 46.99 5.9234 1.167e-06 *** Residuals 40 317.33 7.93	**2** Df Sum Sq Mean Sq F value Pr(>F) op 19 2070.85 108.99 7.1942 8.99e-08 *** Residuals 40 606.00 15.15 Df Sum Sq Mean Sq F value Pr(>F) op 19 21763.1 1145.4 7.5118 4.957e-08 *** Residuals 40 6099.3 152.5
3 Df Sum Sq Mean Sq F value Pr(>F) op 19 1588.85 83.62 1.6928 0.07986 . Residuals 40 1976.00 49.40 Df Sum Sq Mean Sq F value Pr(>F) op 19 3655.5 192.4 1.0154 0.466 Residuals 40 7579.3 189.5	**4** Df Sum Sq Mean Sq F value Pr(>F) op 19 602.60 31.72 4.2956 5.153e-05 *** Residuals 40 295.33 7.38 Df Sum Sq Mean Sq F value Pr(>F) op 19 494.32 26.02 1.8628 0.04856 * Residuals 40 558.67 13.97
5 Df Sum Sq Mean Sq F value Pr(>F) op 19 460.18 24.22 3.8343 0.0001700 *** Residuals 40 252.67 6.32 Df Sum Sq Mean Sq F value Pr(>F) op 19 674.85 35.52 1.7939 0.05948 . Residuals 40 792.00 19.80	**6** Df Sum Sq Mean Sq F value Pr(>F) op 19 3262.3 171.7 4.3894 4.07e-05 *** Residuals 40 1564.7 39.1 Df Sum Sq Mean Sq F value Pr(>F) op 19 591.33 31.12 4.8629 1.281e-05 *** Residuals 40 256.00 6.40
7 Df Sum Sq Mean Sq F value Pr(>F) op 19 1976.07 104.00 2.7796 0.003209 ** Residuals 40 1496.67 37.42 Df Sum Sq Mean Sq F value Pr(>F) op 19 9014.2 474.4 8.014 1.999e-08 *** Residuals 40 2368.0 59.2	**8** Df Sum Sq Mean Sq F value Pr(>F) op 19 3844.7 202.4 9.191 2.744e-09 *** Residuals 40 880.7 22.0 Df Sum Sq Mean Sq F value Pr(>F) op 19 574.32 30.23 19.091 2.115e-14 *** Residuals 40 63.33 1.58
9 Df Sum Sq Mean Sq F value Pr(>F) op 19 4470.0 235.3 6.0583 8.758e-07 *** Residuals 40 1553.3 38.8 Df Sum Sq Mean Sq F value Pr(>F) op 19 181.650 9.561 4.1568 7.336e-05 *** Residuals 40 92.000 2.300	**10** Df Sum Sq Mean Sq F value Pr(>F) op 19 1903.7 100.2 3.9447 0.0001271 *** Residuals 40 1016.0 25.4 Df Sum Sq Mean Sq F value Pr(>F) op 19 310.000 16.316 6.1569 7.117e-07 *** Residuals 40 106.000 2.650
11 Df Sum Sq Mean Sq F value Pr(>F) op 19 4619.3 243.1 5.842 1.390e-06 *** Residuals 40 1664.7 41.6 Df Sum Sq Mean Sq F value Pr(>F) op 19 219.400 11.547 5.4128 3.582e-06 *** Residuals 40 85.333 2.133	**12** Df Sum Sq Mean Sq F value Pr(>F) op 19 12343.7 649.7 29.801 < 2.2e-16 *** Residuals 40 872.0 21.8 Df Sum Sq Mean Sq F value Pr(>F) op 19 1677.93 88.31 34.632 < 2.2e-16 *** Residuals 40 102.00 2.55
13 Df Sum Sq Mean Sq F value Pr(>F) op 19 12218.6 643.1 32.021 < 2.2e-16 *** Residuals 40 803.3 20.1 Df Sum Sq Mean Sq F value Pr(>F) op 19 211.600 11.137 11.932 5.046e-11 *** Residuals 40 37.333 0.933	**14** Df Sum Sq Mean Sq F value Pr(>F) op 19 628.18 33.06 1.0268 0.4548 Residuals 40 1288.00 32.20 Df Sum Sq Mean Sq F value Pr(>F) op 19 347.65 18.30 5.0826 7.632e-06 *** Residuals 40 144.00 3.60

15 Df Sum Sq Mean Sq F value Pr(>F) op 19 394.98 20.79 6.1143 7.783e-07 *** Residuals 40 136.00 3.40 Df Sum Sq Mean Sq F value Pr(>F) op 19 1881.52 99.03 6.0321 9.257e-07 *** Residuals 40 656.67 16.42	**16** Df Sum Sq Mean Sq F value Pr(>F) op 19 4322.3 227.5 15.599 6.487e-13 *** Residuals 40 583.3 14.6 Df Sum Sq Mean Sq F value Pr(>F) op 19 2377.40 125.13 12.534 2.311e-11 *** Residuals 40 399.33 9.98
17 Df Sum Sq Mean Sq F value Pr(>F) op 19 2498.6 131.5 1.4571 0.1556 Residuals 40 3610.0 90.2 Df Sum Sq Mean Sq F value Pr(>F) op 19 1864.98 98.16 1.9115 0.04205 * Residuals 40 2054.00 51.35	**18** Df Sum Sq Mean Sq F value Pr(>F) op 19 246.983 12.999 5.9087 1.204e-06 *** Residuals 40 88.000 2.200 Df Sum Sq Mean Sq F value Pr(>F) op 19 132.317 6.964 4.7482 1.686e-05 *** Residuals 40 58.667 1.467
19 Df Sum Sq Mean Sq F value Pr(>F) op 19 1550.98 81.63 4.7414 1.714e-05 *** Residuals 40 688.67 17.22 Df Sum Sq Mean Sq F value Pr(>F) op 19 303.517 15.975 9.4898 1.706e-09 *** Residuals 40 67.333 1.683	

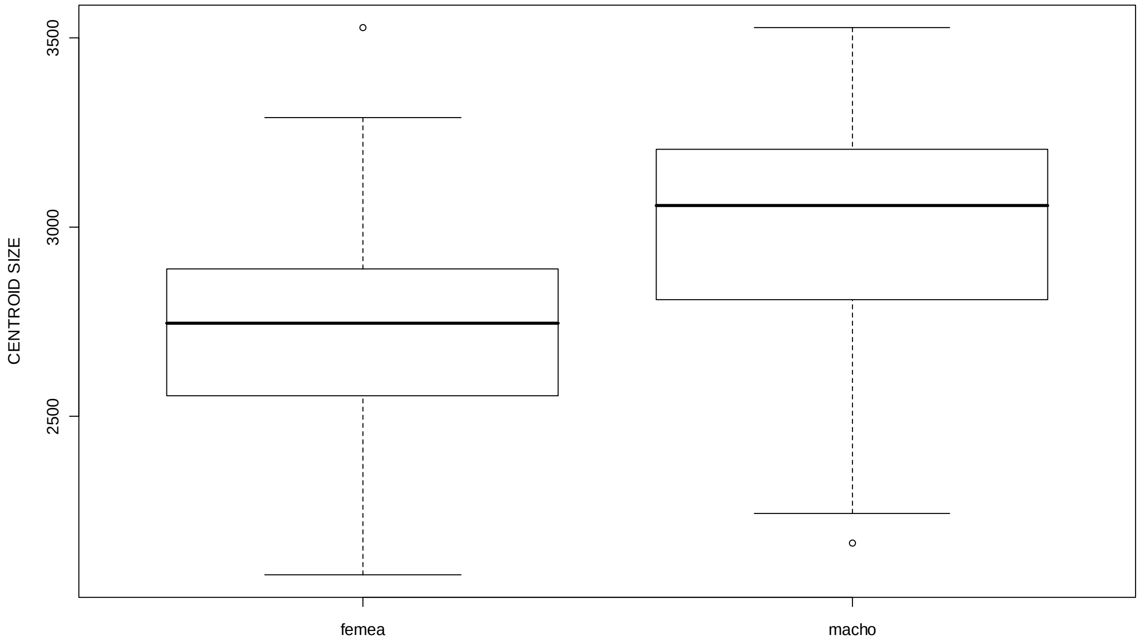
Table IX. Tables of ANOVAs of raw data (X, Y) for the 20 operators with 3 repetitions of 19 landmarks used in the Distal/Proximal View. Signif. codes 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

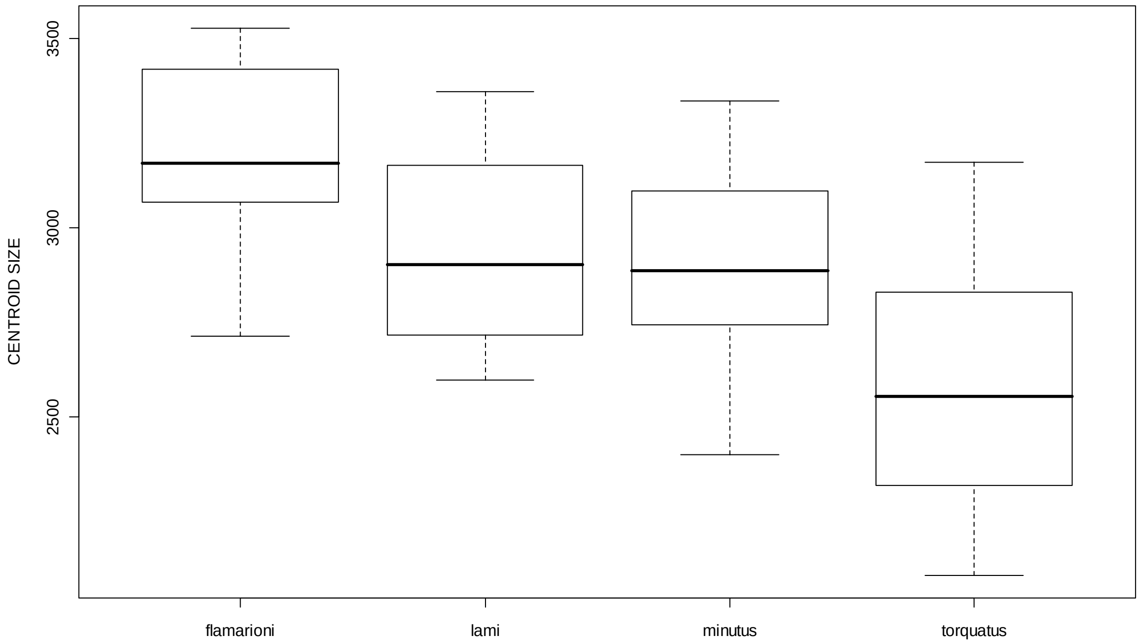
1 Df Sum Sq Mean Sq F value Pr(>F) op 19 955.65 50.30 8.7728 5.438e-09 *** Residuals 40 229.33 5.73 Df Sum Sq Mean Sq F value Pr(>F) op 19 844.58 44.45 7.7758 3.06e-08 *** Residuals 40 228.67 5.72	**2** Df Sum Sq Mean Sq F value Pr(>F) op 19 824.98 43.42 5.3276 4.343e-06 *** Residuals 40 326.00 8.15 Df Sum Sq Mean Sq F value Pr(>F) op 19 1076.07 56.64 2.9244 0.00211 ** Residuals 40 774.67 19.37
3 Df Sum Sq Mean Sq F value Pr(>F) op 19 487.52 25.66 11.32 1.154e-10 *** Residuals 40 90.67 2.27 Df Sum Sq Mean Sq F value Pr(>F) op 19 2374.18 124.96 4.8433 1.342e-05 *** Residuals 40 1032.00 25.80	**4** Df Sum Sq Mean Sq F value Pr(>F) op 19 250.183 13.168 2.0736 0.02597 * Residuals 40 254.000 6.350 Df Sum Sq Mean Sq F value Pr(>F) op 19 354.27 18.65 12.713 1.841e-11 *** Residuals 40 58.67 1.47
5 Df Sum Sq Mean Sq F value Pr(>F) op 19 754.00 39.68 9.1228 3.063e-09 *** Residuals 40 174.00 4.35 Df Sum Sq Mean Sq F value Pr(>F) op 19 280.600 14.768 9.6316 1.367e-09 *** Residuals 40 61.333 1.533	**6** Df Sum Sq Mean Sq F value Pr(>F) op 19 170.317 8.964 4.0439 9.813e-05 *** Residuals 40 88.667 2.217 Df Sum Sq Mean Sq F value Pr(>F) op 19 1826.85 96.15 6.23 6.11e-07 *** Residuals 40 617.33 15.43
7 Df Sum Sq Mean Sq F value Pr(>F) op 19 102.933 5.418 2.8513 0.002606 ** Residuals 40 76.000 1.900 Df Sum Sq Mean Sq F value Pr(>F) op 19 248.733 13.091 17.455 9.785e-14 *** Residuals 40 30.000 0.750	

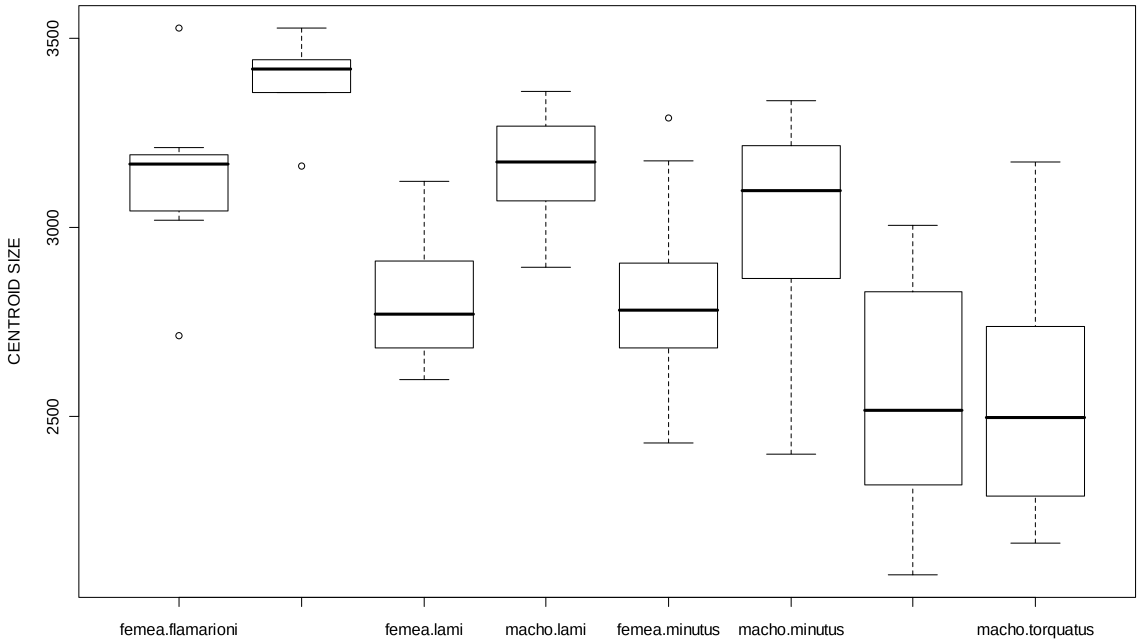
Anexo Capítulo II

<u>Vista Dorsal</u>

Análise Tamanho







Anova 2 Fatores - Sexo e Espécie

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sex1	1	1973368	1973368	38.0094	7.192e-09 ***
sp1	3	5545802	1848601	35.6062	< 2.2e-16 ***
sex1:sp1	3	487440	162480	3.1296	0.02777 *
Residuals	139	7216589	51918		

Sexo

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sex1	1	1970024	1970024	21.596	7.494e-06 ***
Residuals	145	13253941	90780		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
16 observations deleted due to missingness

Espécie

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sp1	3	6276230	2092077	34.193	< 2.2e-16 ***
Residuals	159	9759252	60995		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

$sp1

	diff	lwr	upr	p adj
lami-flamarioni	-280.30248	-500.0751	-60.52989	0.0062556
minutus-flamarioni	-305.16251	-492.5148	-117.81018	0.0002294

torquatus-flamarioni	-641.97460	-835.1417	-448.80753	0.0000000
minutus-lami	-24.86003	-180.9612	131.24111	0.9760893
torquatus-lami	-361.67212	-524.7064	-198.63781	0.0000003
torquatus-minutus	-336.81209	-452.4887	-221.13544	0.0000000

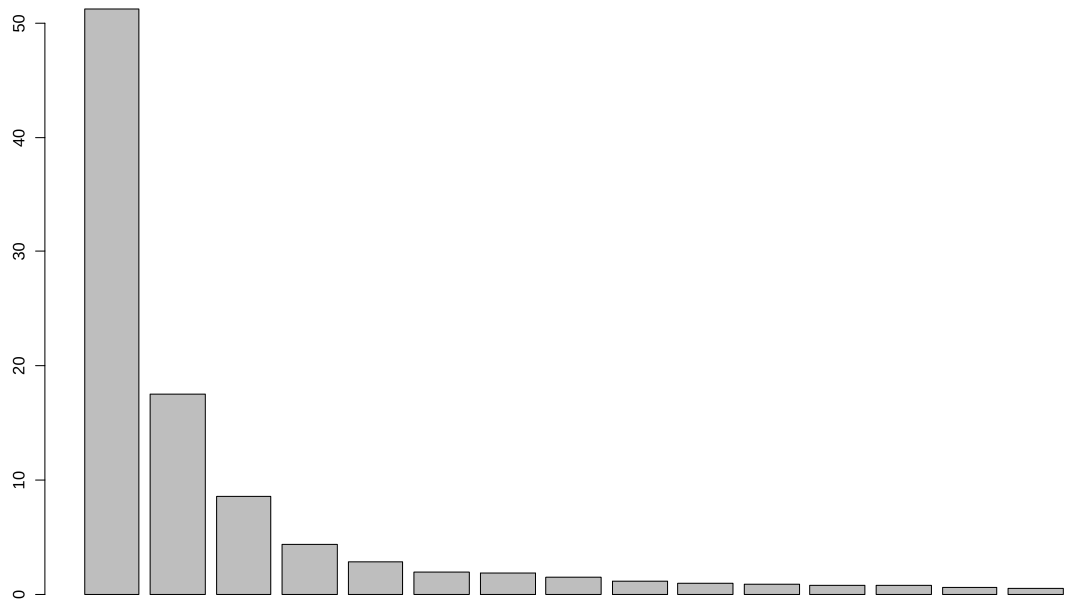
Analise De Forma

MANOVA

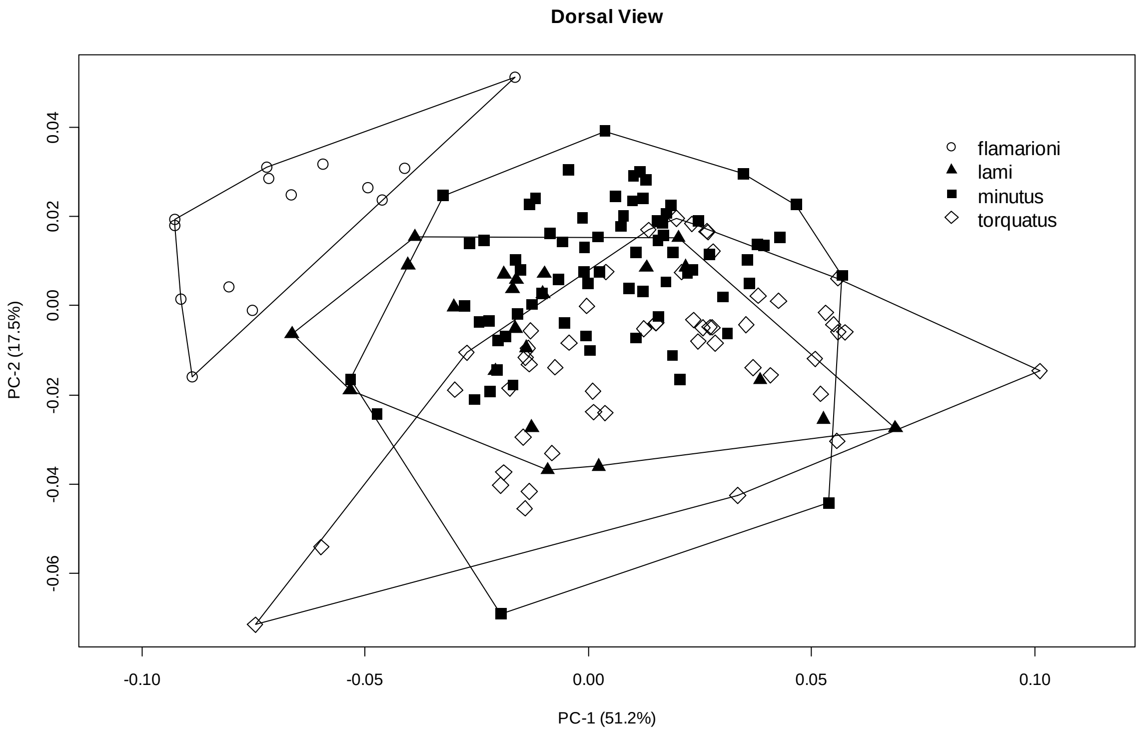
	Df	Pillai	approx F	num Df	den Df	Pr(>F)
sex1	1	0.4463	2.5133	34	106	0.0001809 ***
sp1	3	2.2082	8.8584	102	324	< 2.2e-16 ***
sex1:sp1	3	0.8208	1.1964	102	324	0.1230045
Residuals	139					

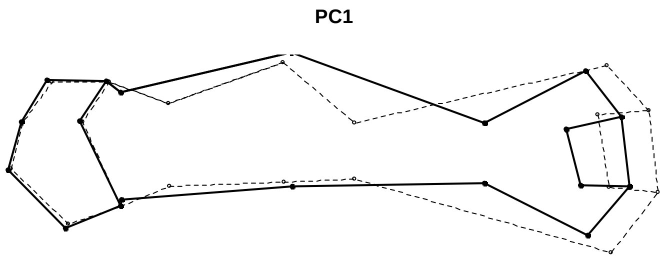
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

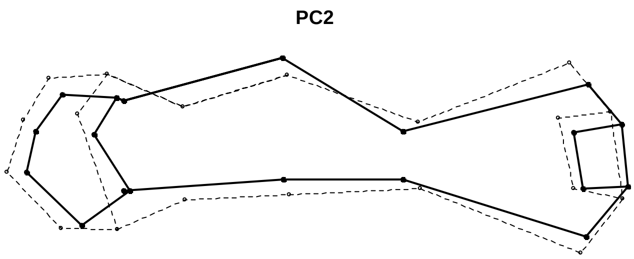
Componentes Principais



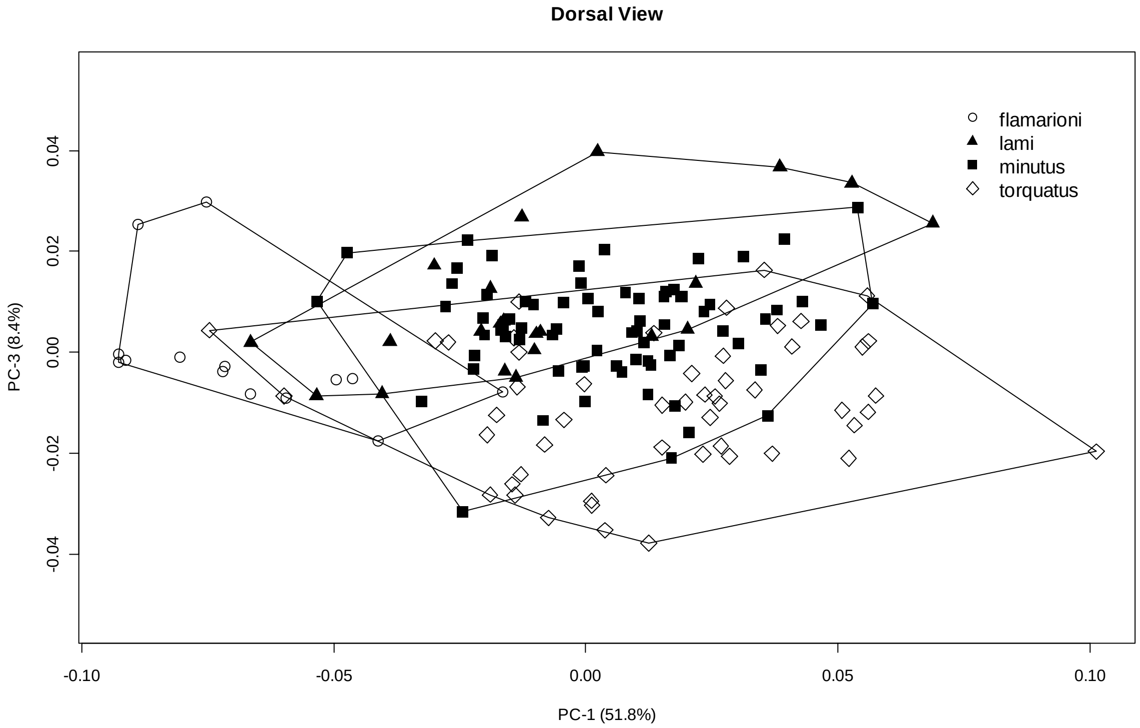
1 e 2 Componentes Principais

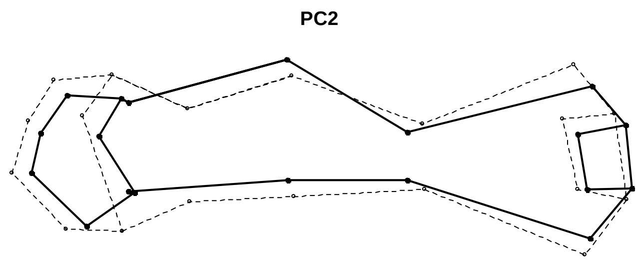


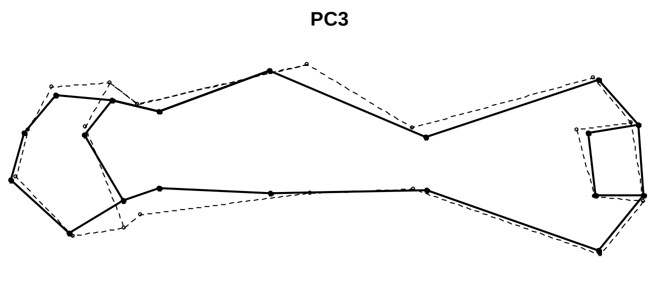




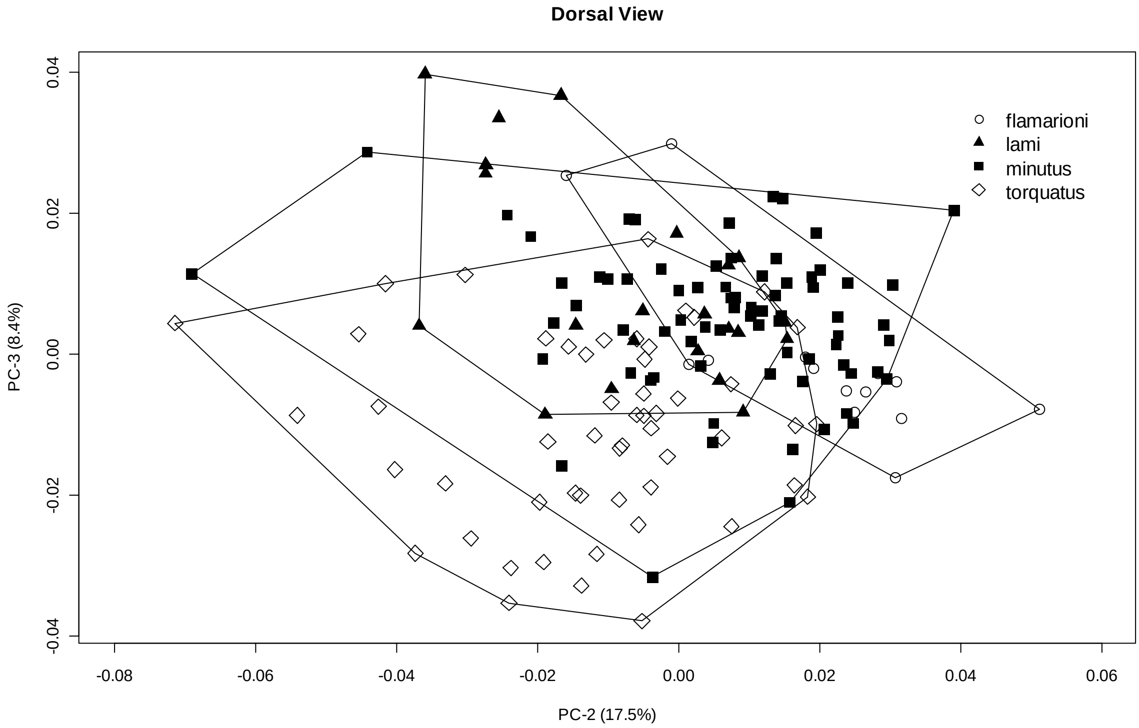
1 e 3 Componentes Principais

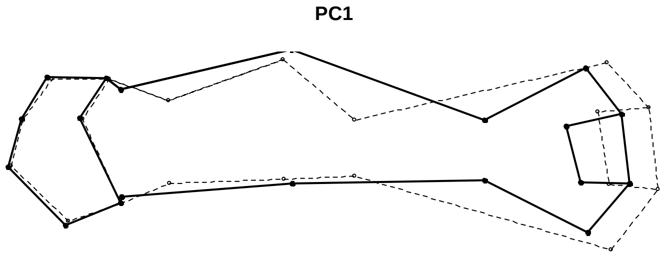


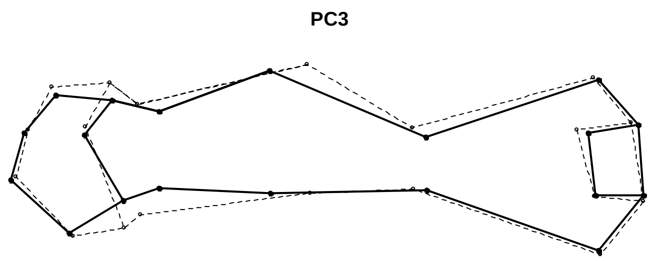




2 e 3 Componentes Principais







Análise Discriminante

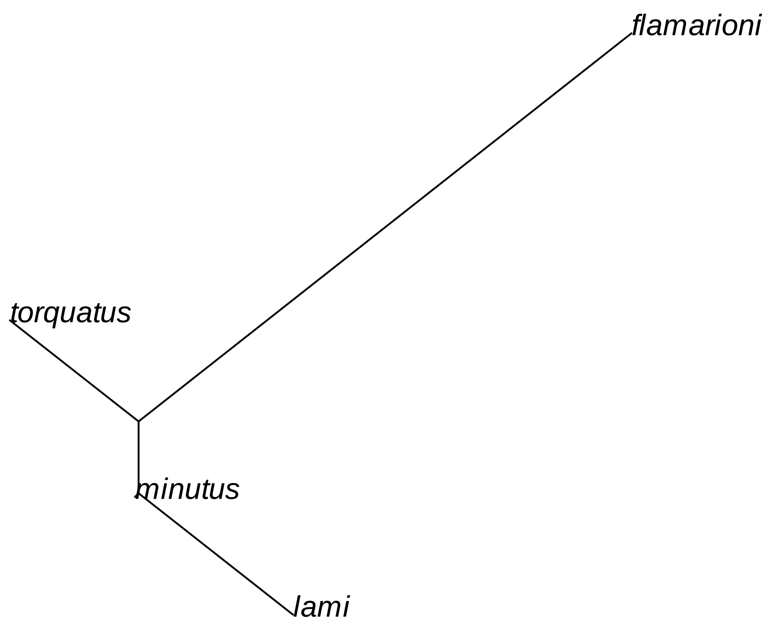
Espécie

sp1	flamarioni	lami	minutus	torquatus
flamarioni	14	0	0	0
lami	0	19	3	0
minutus	0	1	71	2
torquatus	0	1	1	51

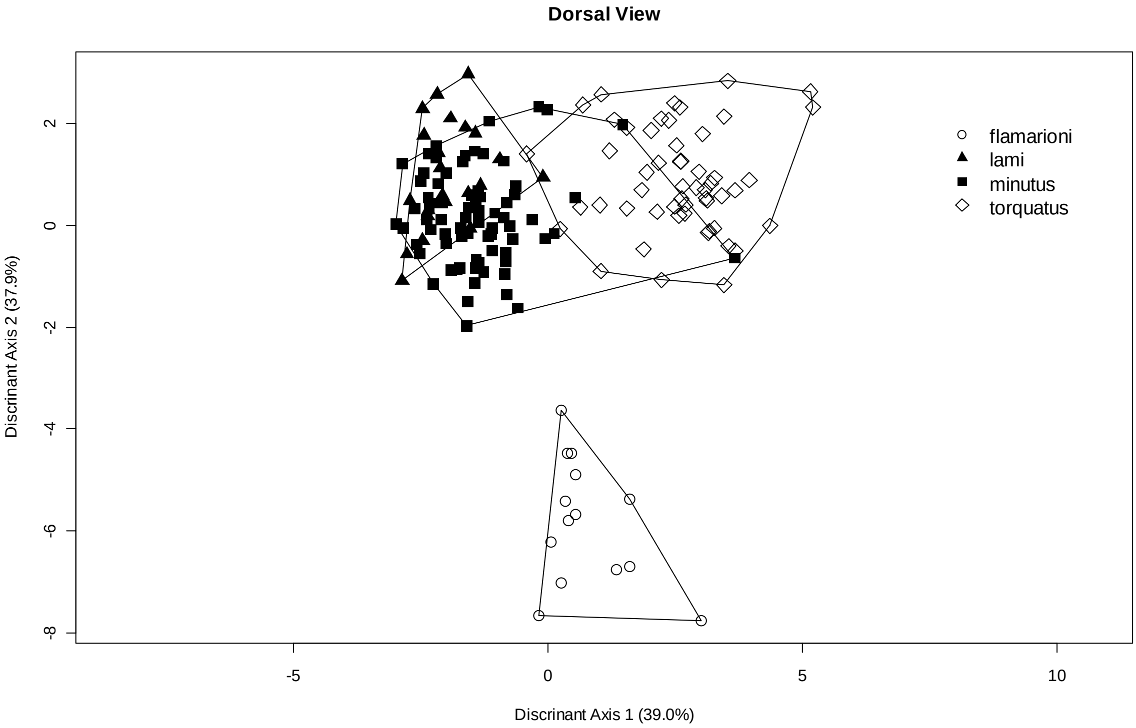
Tabela traduzida

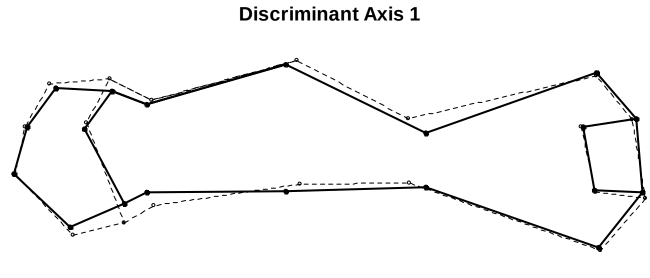
flamarioni	lami	minutus	torquatus
100.00000	86.36364	95.94595	96.22642

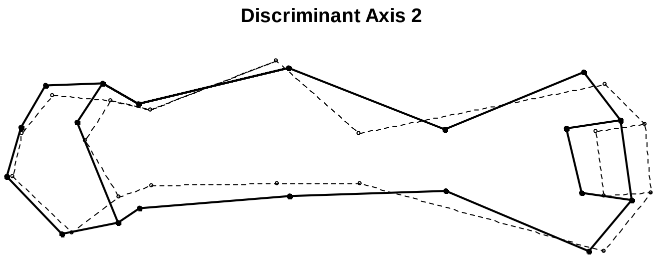
Arvore de Distâncias de Mahalanobis



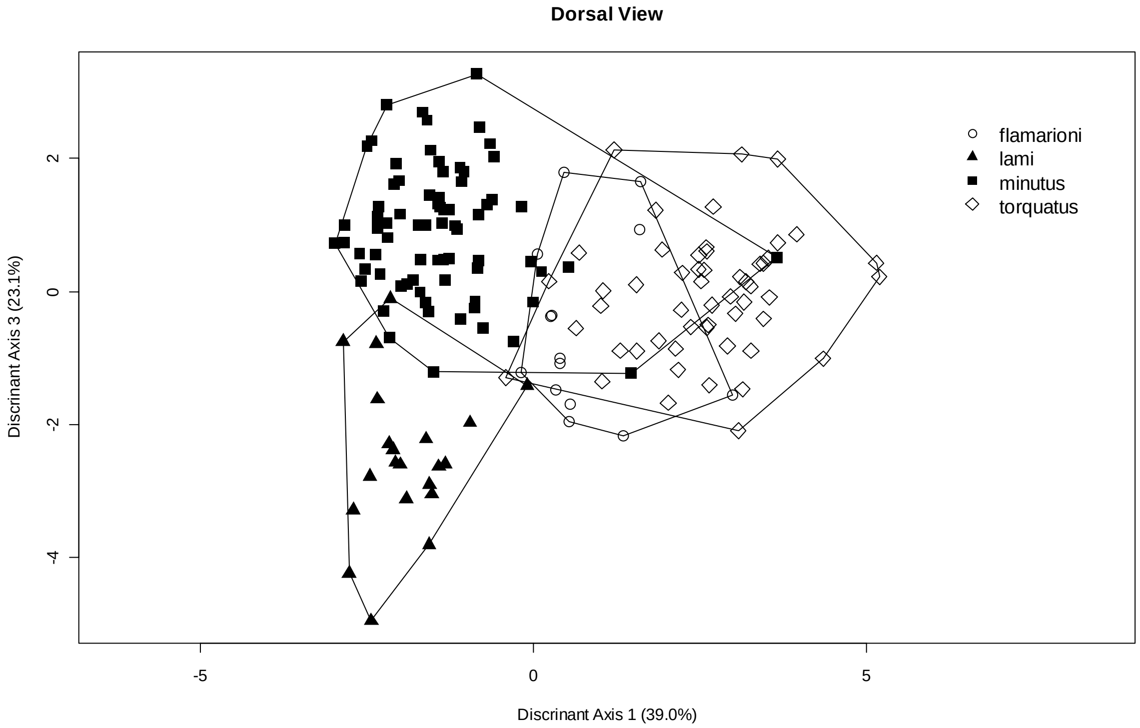
1 e 2 Eixo Discriminante

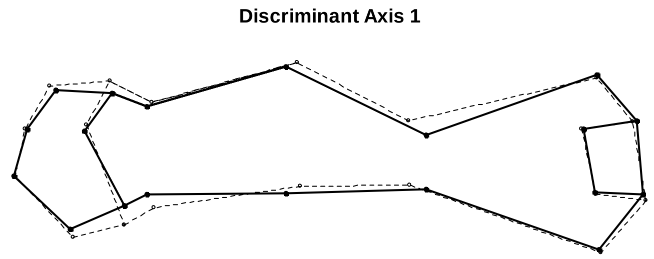


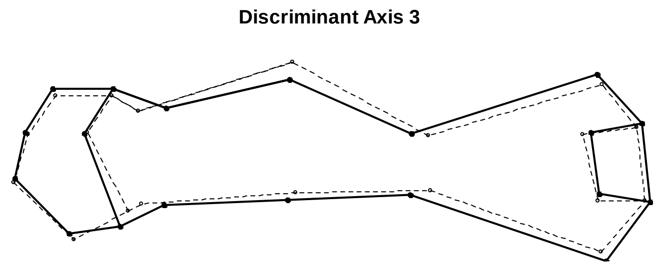




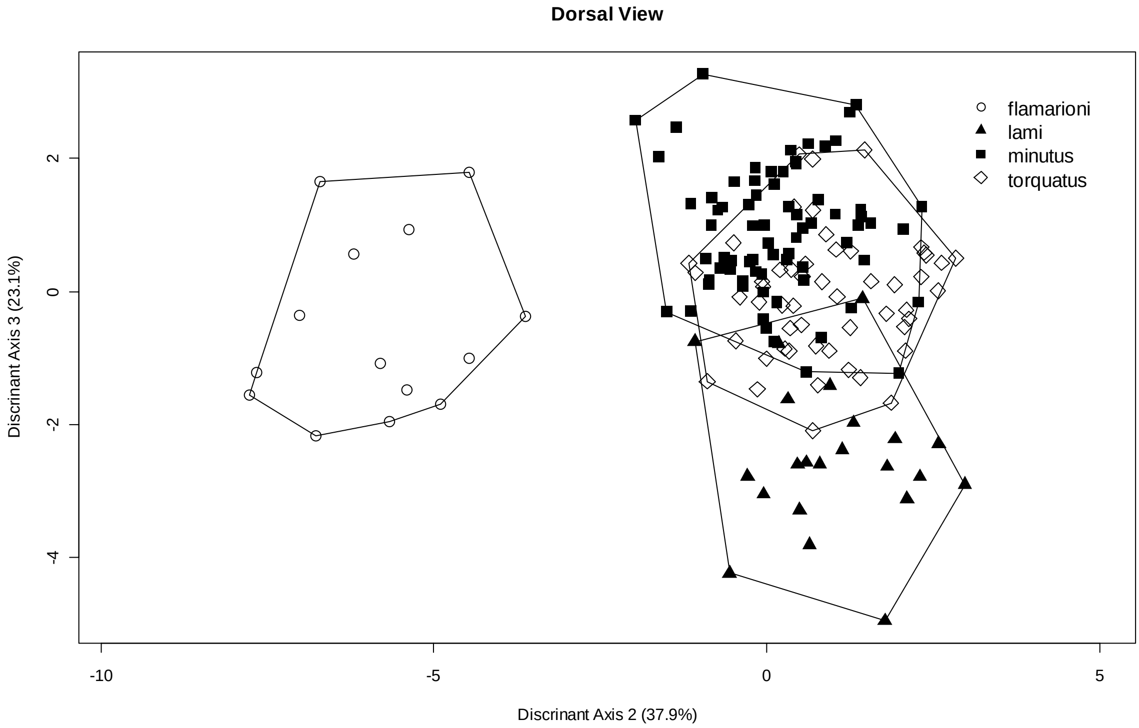
1 e 3 Eixo Discriminante

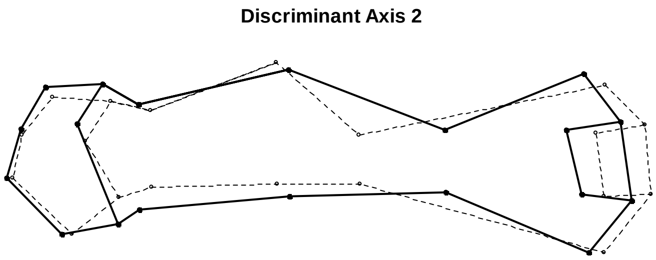


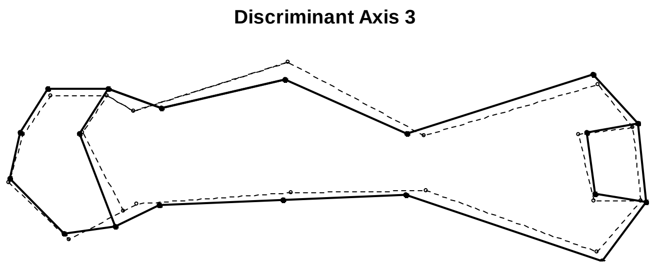




2 e 3 Eixo Discriminante

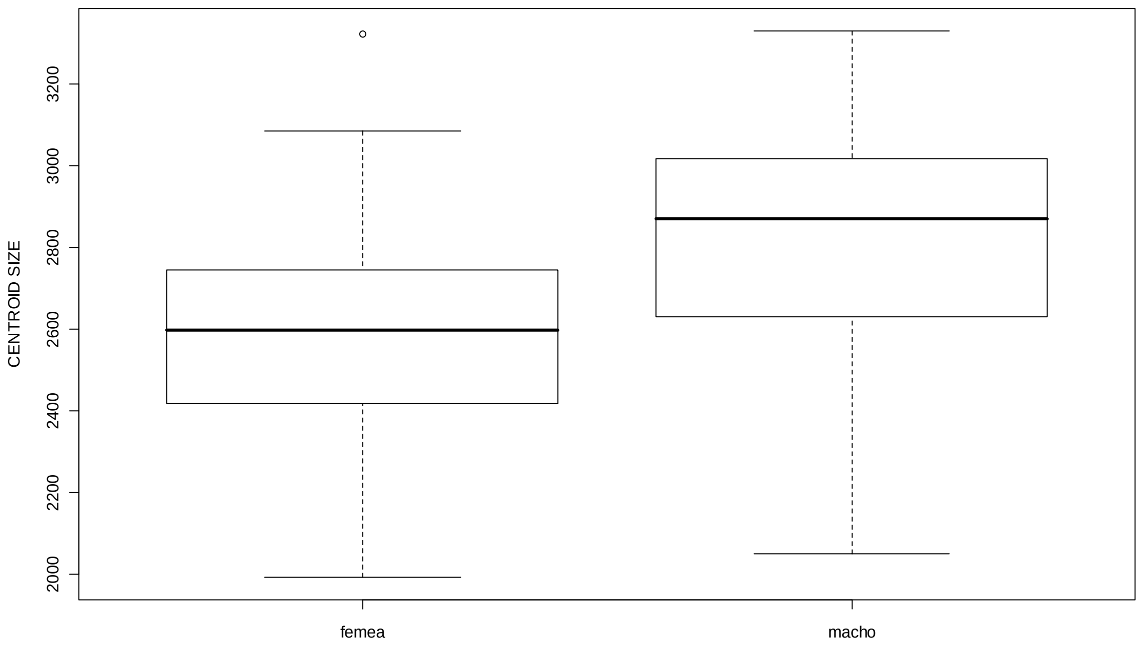


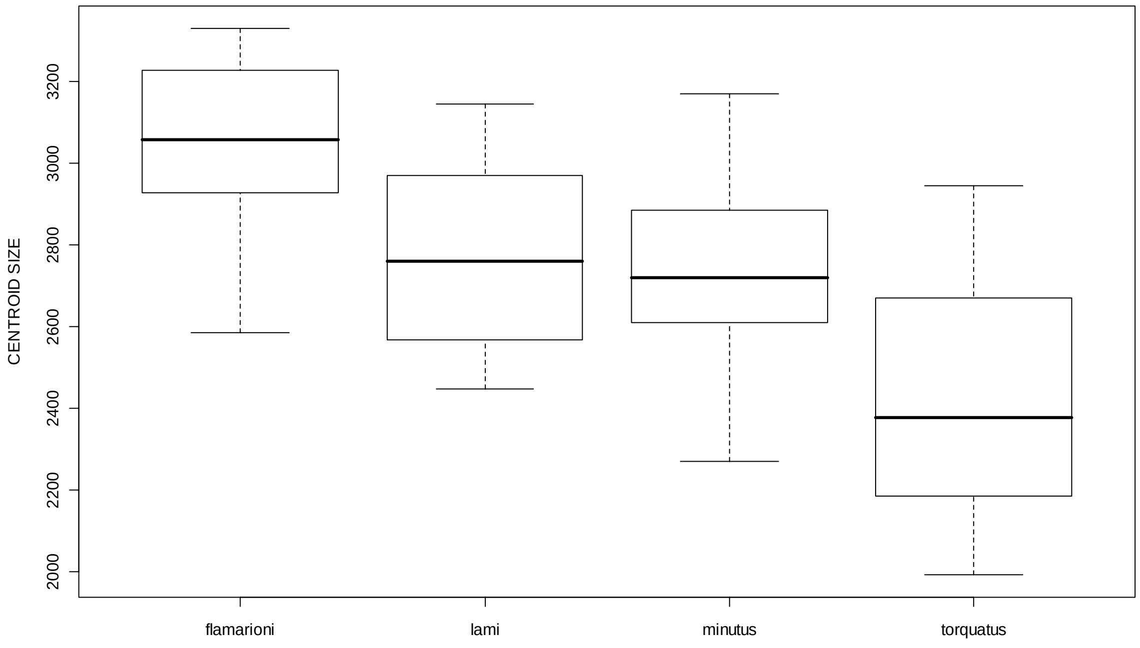


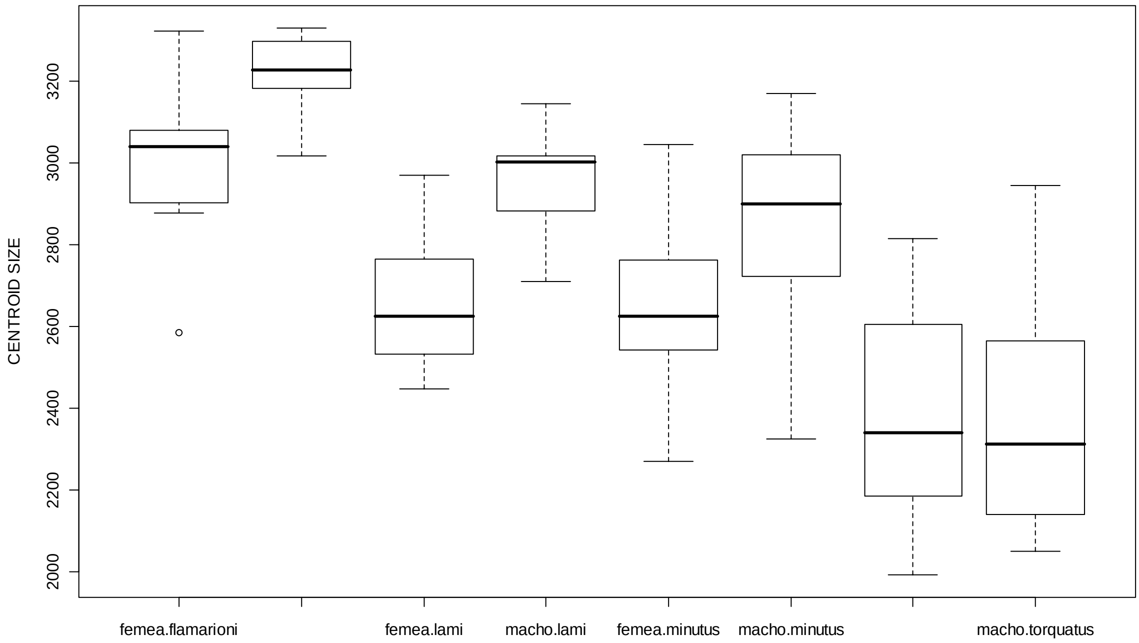


Vista Ventral

Análise Tamanho







Anova 2 Fatores - Sexo e Espécie

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sex2	1	1514086	1514086	34.9401	2.567e-08 ***
sp2	3	5808677	1936226	44.6817	< 2.2e-16 ***
sex2:sp2	3	366719	122240	2.8209	0.04128 *
Residuals	137	5936729	43334		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
17 observations deleted due to missingness

Sexo

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sex2	1	1514086	1514086	17.876	4.185e-05 ***
Residuals	143	12112125	84700		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
17 observations deleted due to missingness

Espécie

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
sp2	3	6273829	2091276	40.839	< 2.2e-16 ***
Residuals	159	8142146	51208		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

$sp2

	diff	lwr	upr	**p adj**
lami-flamarioni	-309.87906	-513.2073	-106.5508	0.0006554
minutus-flamarioni	-324.17895	-496.1168	-152.2411	0.0000142
torquatus-flamarioni	-652.75112	-829.4895	-476.0127	0.0000000
minutus-lami	-14.29990	-160.2251	131.6253	0.9941996
torquatus-lami	-342.87207	-494.4240	-191.3202	0.0000001
torquatus-minutus	-328.57217	-434.3466	-222.7977	0.0000000

Analise de Forma

MANOVA

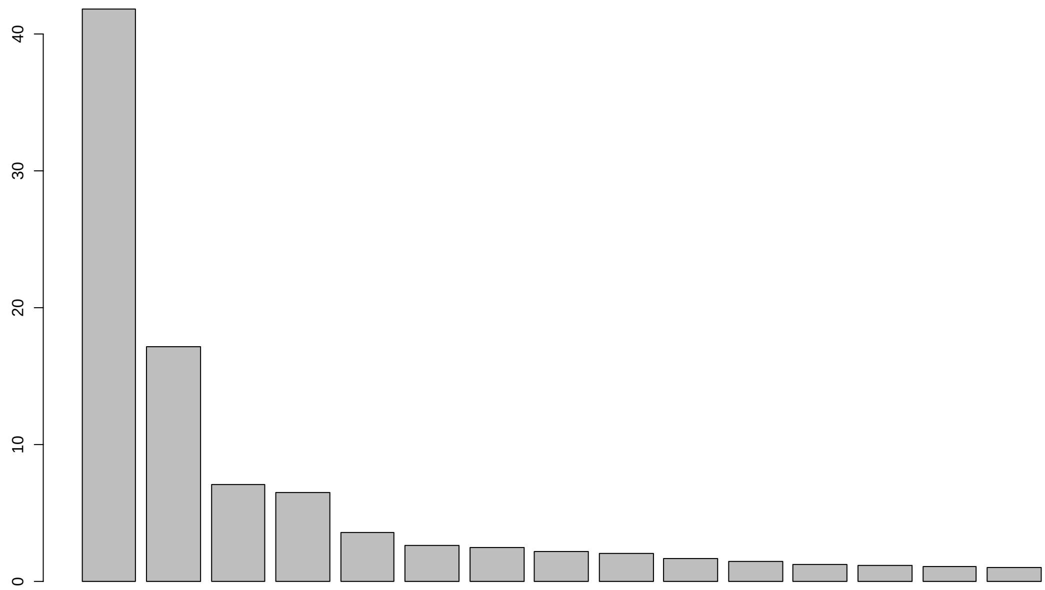
	Df	**Pillai**	**approx F**	**num Df**	**den Df**	**Pr(>F)**
sex2	1	0.4193	2.2085	34	104	0.001185 **
sp2	3	2.1263	7.5874	102	318	< 2.2e-16 ***
sex2:sp2	3	0.6349	0.8369	102	318	0.855410
Residuals	137					

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

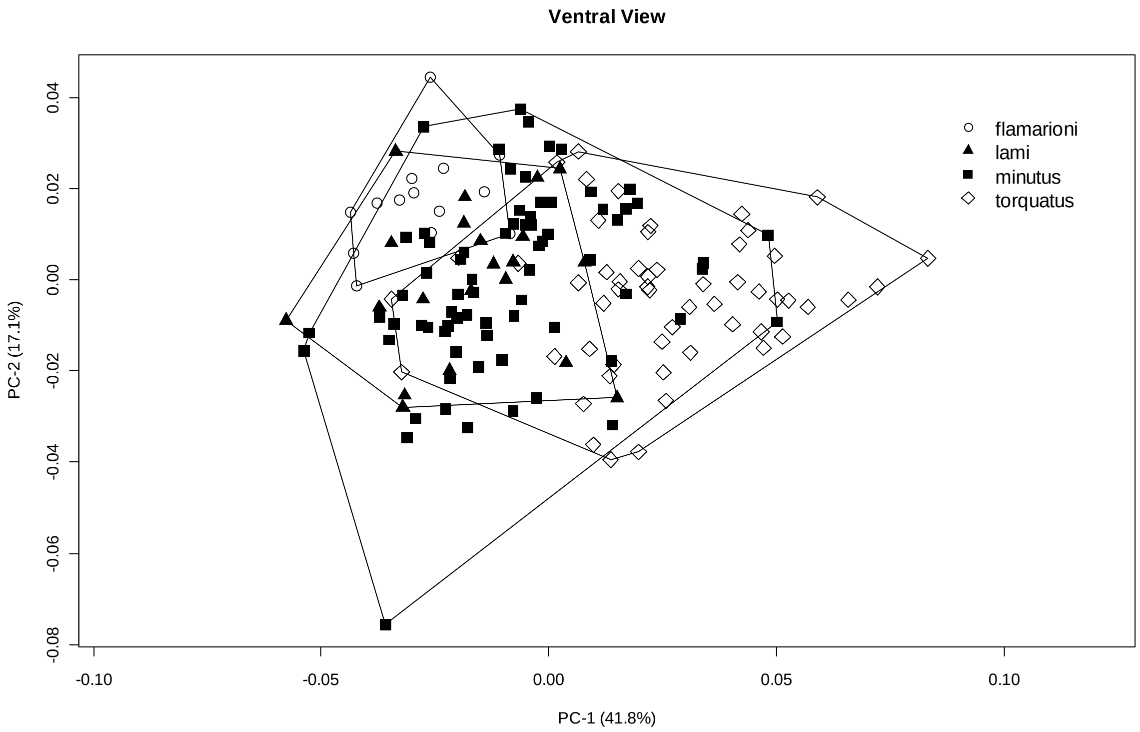
Warning message:

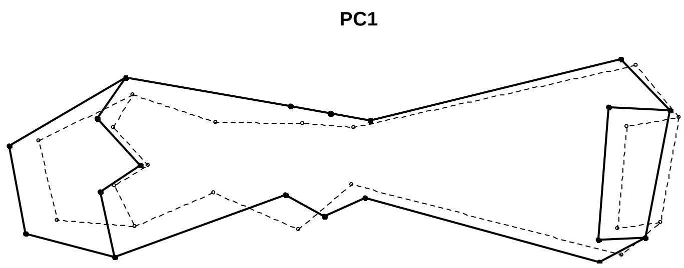
In model.matrix.default(mt, mf, contrasts) : variable 'sex2' converted to a factor

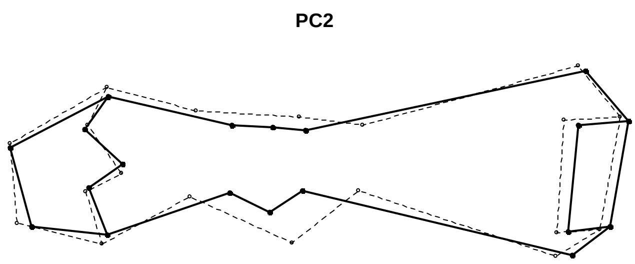
Componentes Principais



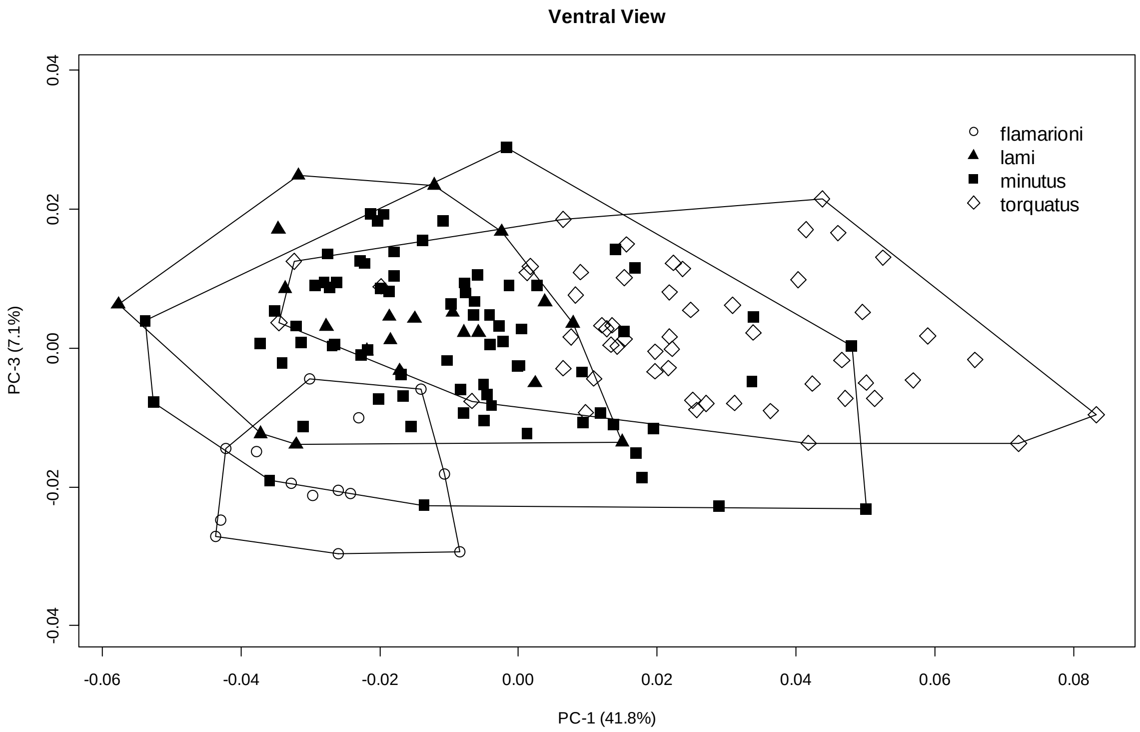
1 e 2 Componentes Principais

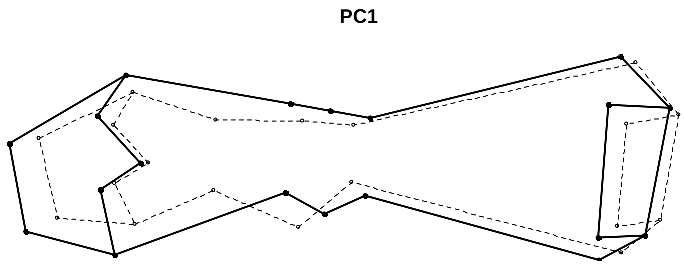


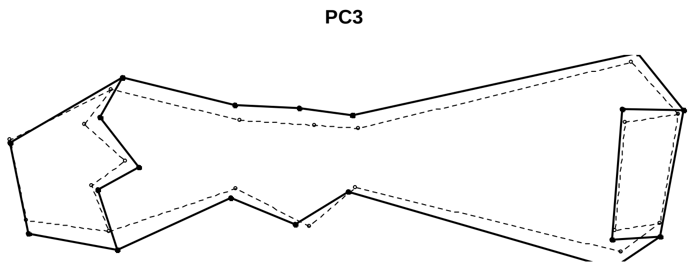




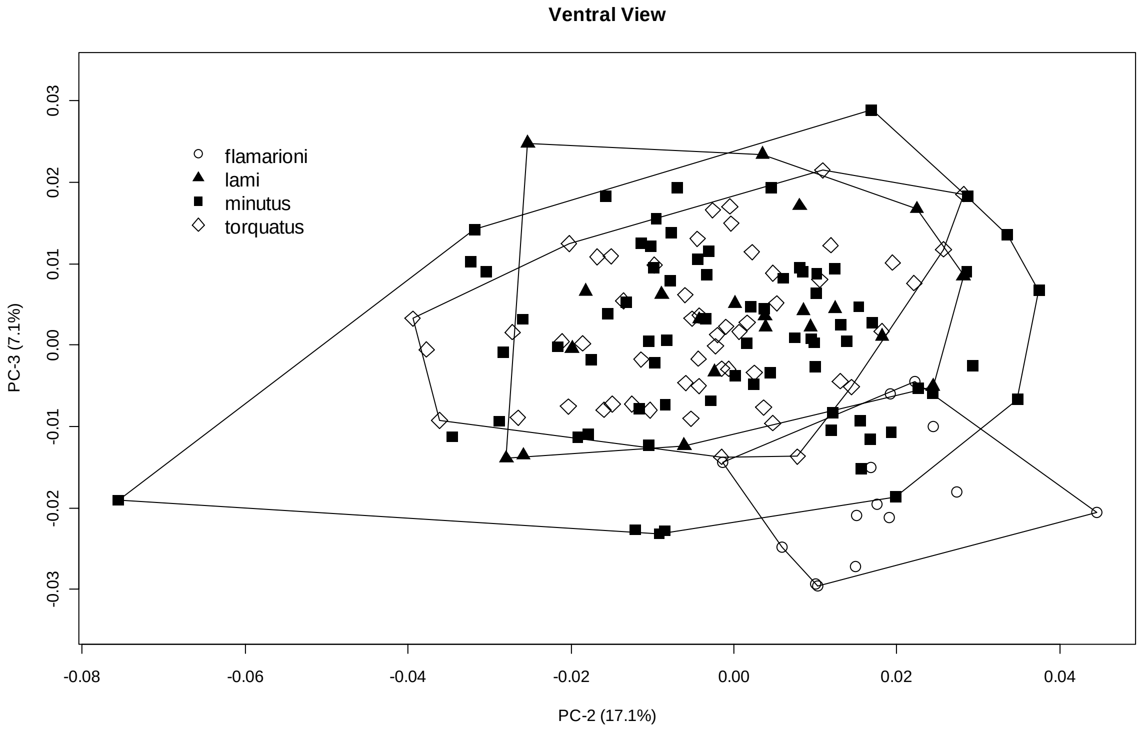
1 e 3 Componentes Principais

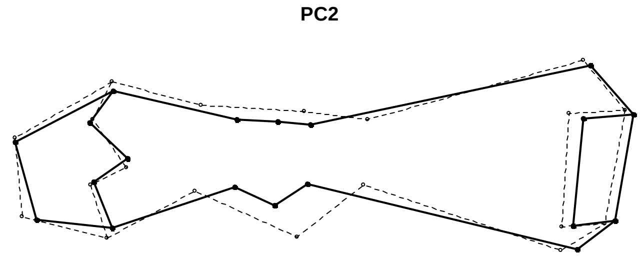


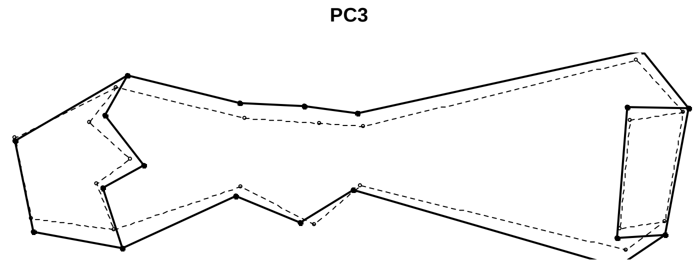




2 e 3 Componentes Principais







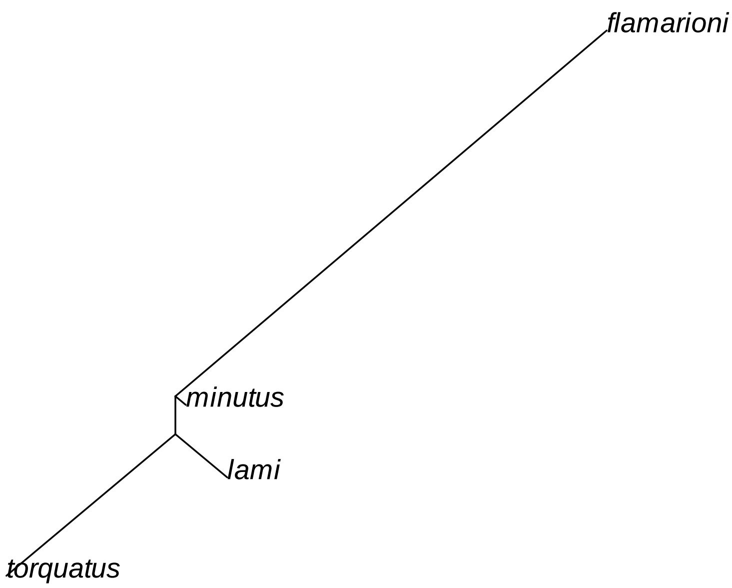
Análise Discrimintate

Espécie				
sp2	**flamarioni**	**lami**	**minutus**	**torquatus**
flamarioni	14	0	0	0
lami	0	15	6	0
minutus	0	2	70	1
torquatus	0	0	1	53

Tabela Traduzida

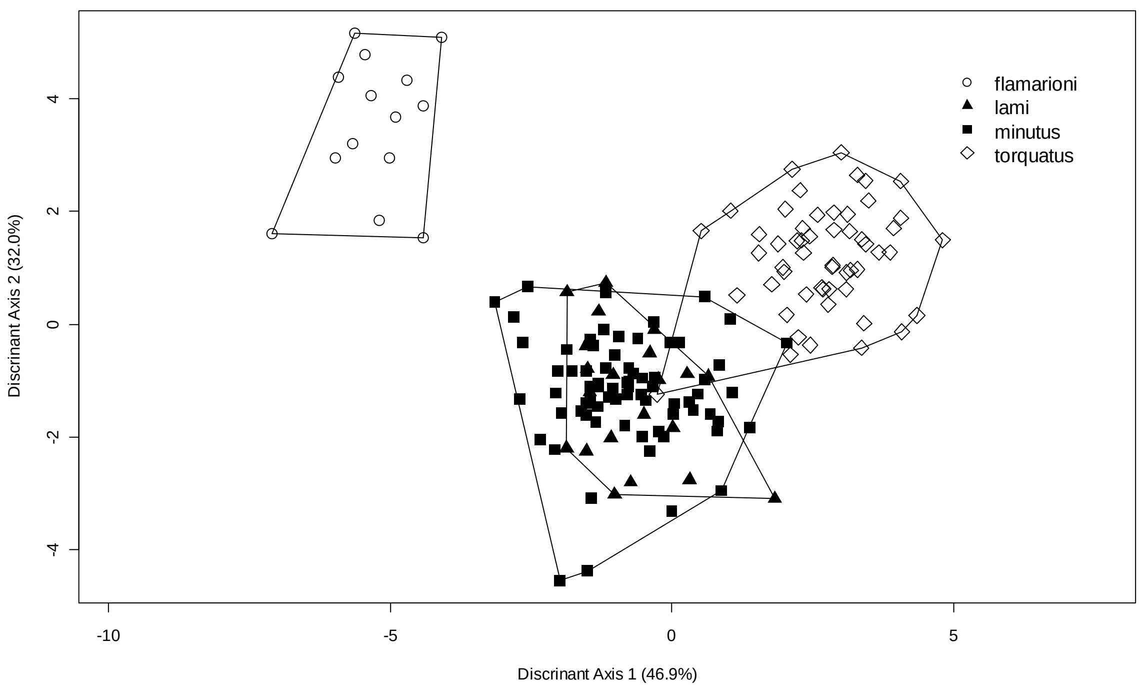
flamarioni	lami	minutus	torquatus
100.00000	71.42857	95.89041	98.14815

Arvore de Distancia de Mahalanobis

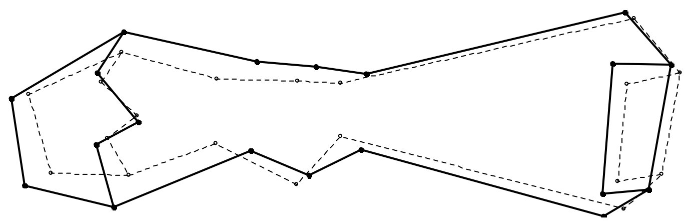


1 e 2 Eixo Discriminante

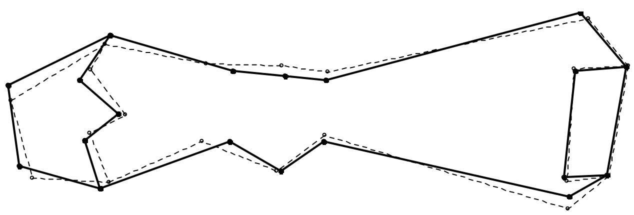
Ventral View



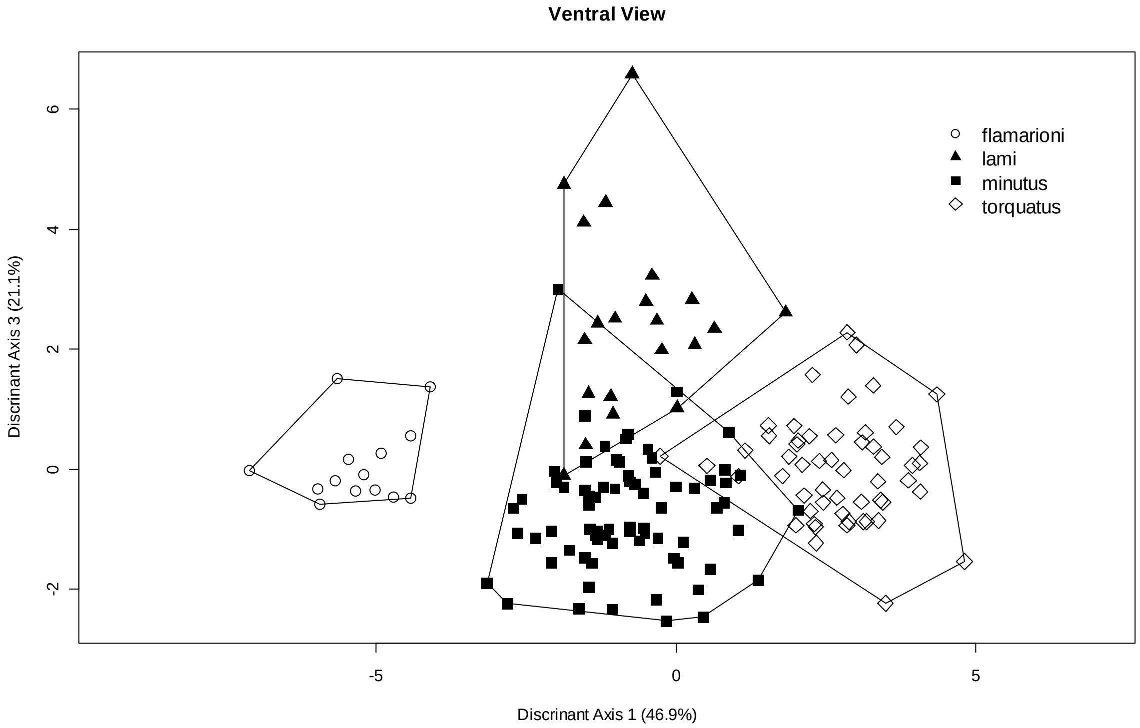
Discriminant Axis 1

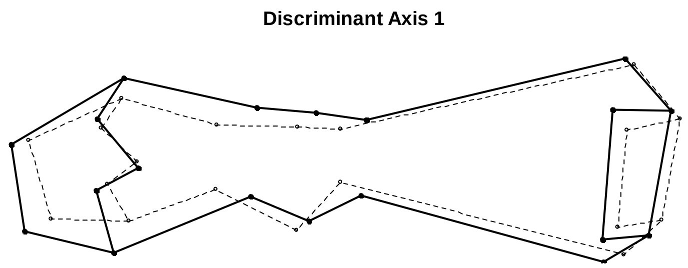


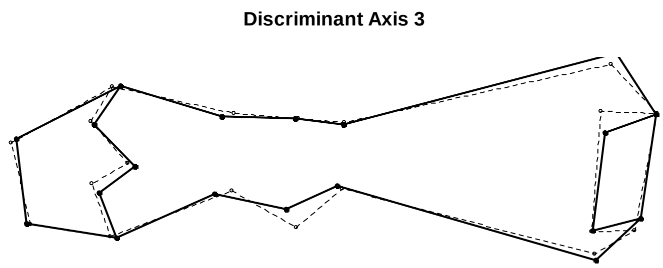
Discriminant Axis 2



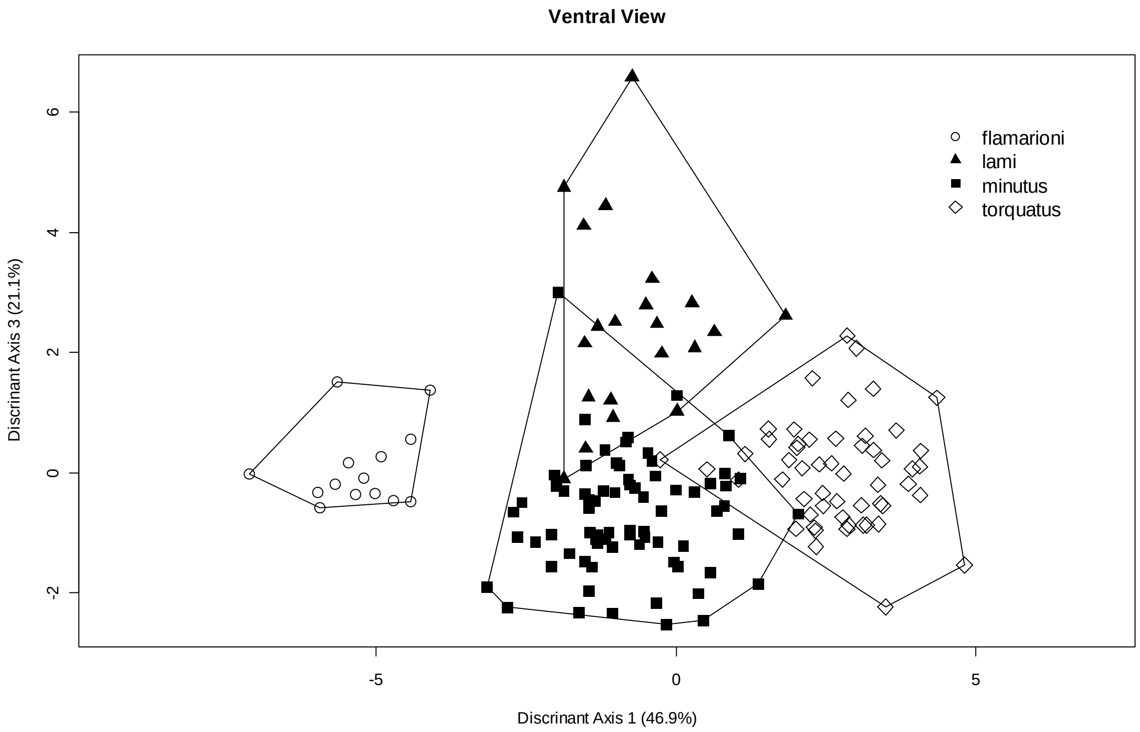
1 e 3 Eixo Discriminante



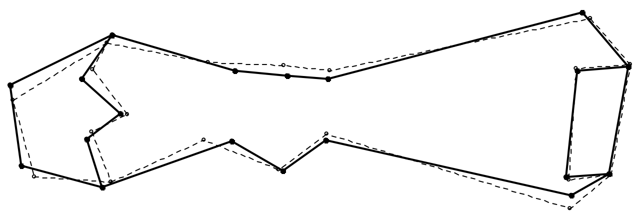




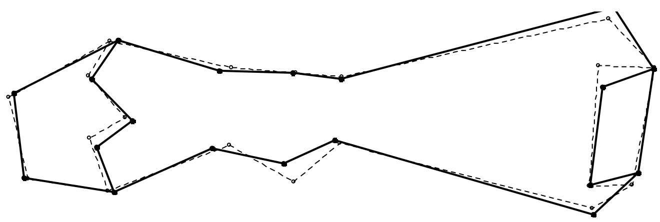
2 e 3 Eixo Discriminante



Discriminant Axis 2

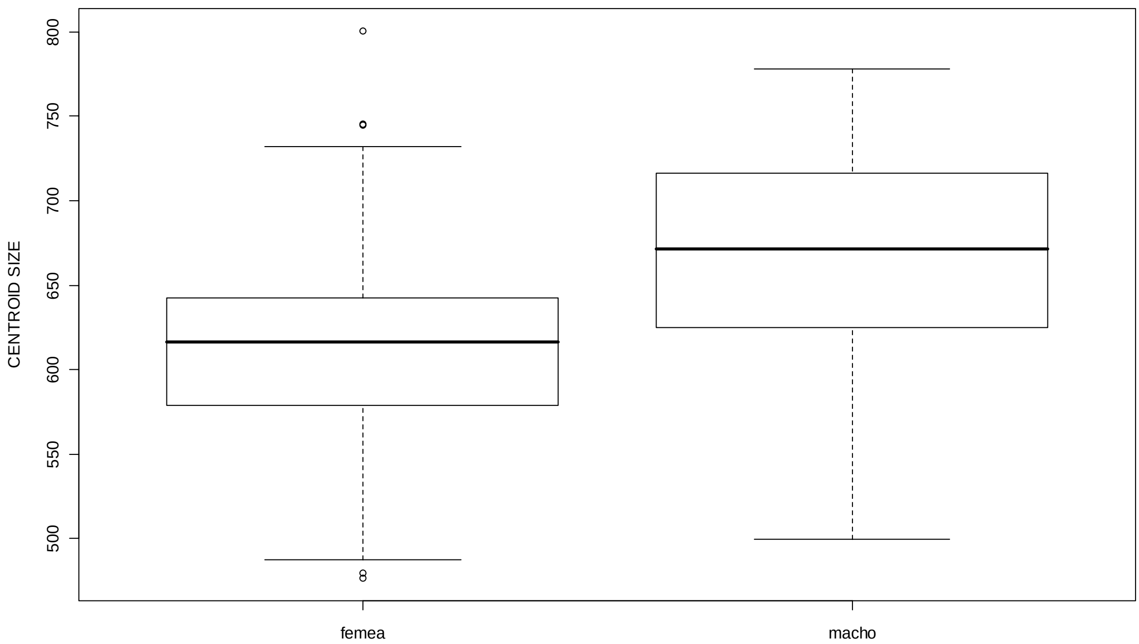


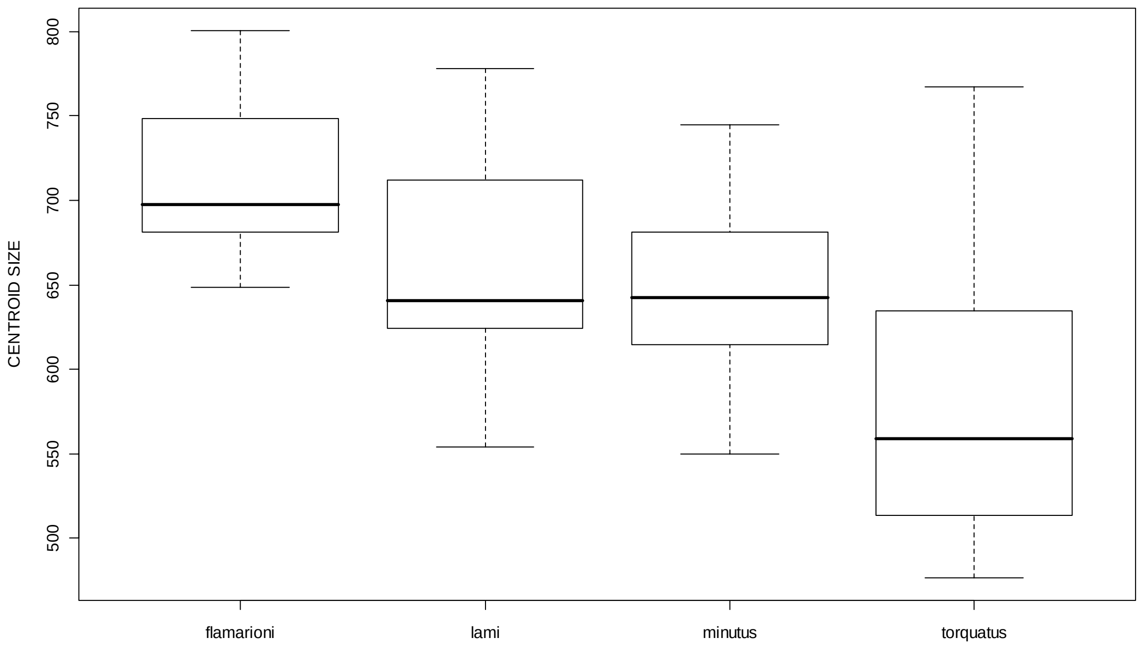
Discriminant Axis 3

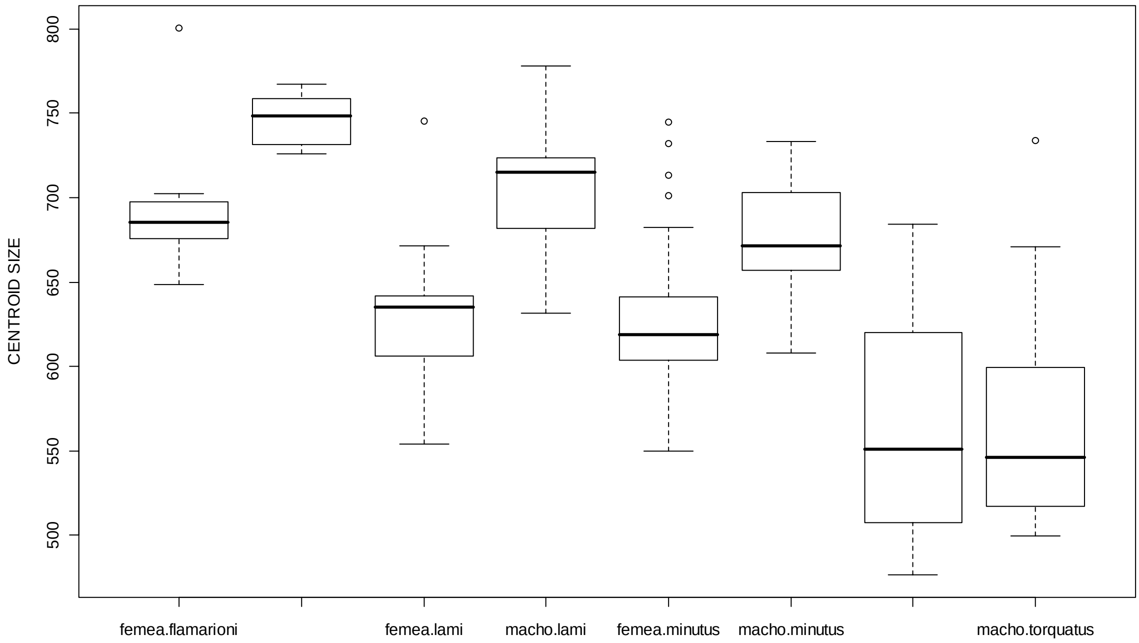


Vista Distal-Proximal

Análise Tamanho







Anova 2 Fatores - Sexo e Espécie

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
sex3	1	91563	91563	37.0030	1.051e-08 ***
sp3	3	284450	94817	38.3180	< 2.2e-16 ***
sex3:sp3	3	19119	6373	2.5755	0.05632 .
Residuals	141	348899	2474		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
17 observations deleted due to missingness

Sexo

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
sex3	1	91563	91563	20.629	1.153e-05 ***
Residuals	147	652468	4439		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Espécie

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
sp3	3	295572	98524	30.806	8.213e-16 ***
Residuals	162	518101	3198		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

$sp3

	diff	**lwr**	**upr**	**p adj**
lami-flamarioni	-53.655647	-103.84516	-3.466137	0.0310525

minutus-flamarioni	-63.540924	-106.23696	-20.844893	0.0009216
torquatus-flamarioni	-136.133754	-180.16192	-92.105585	0.0000000
minutus-lami	-9.885277	-45.42647	25.655913	0.8882014
torquatus-lami	-82.478107	-119.60902	-45.347193	0.0000002
torquatus-minutus	-72.592830	-98.72076	-46.464896	0.0000000

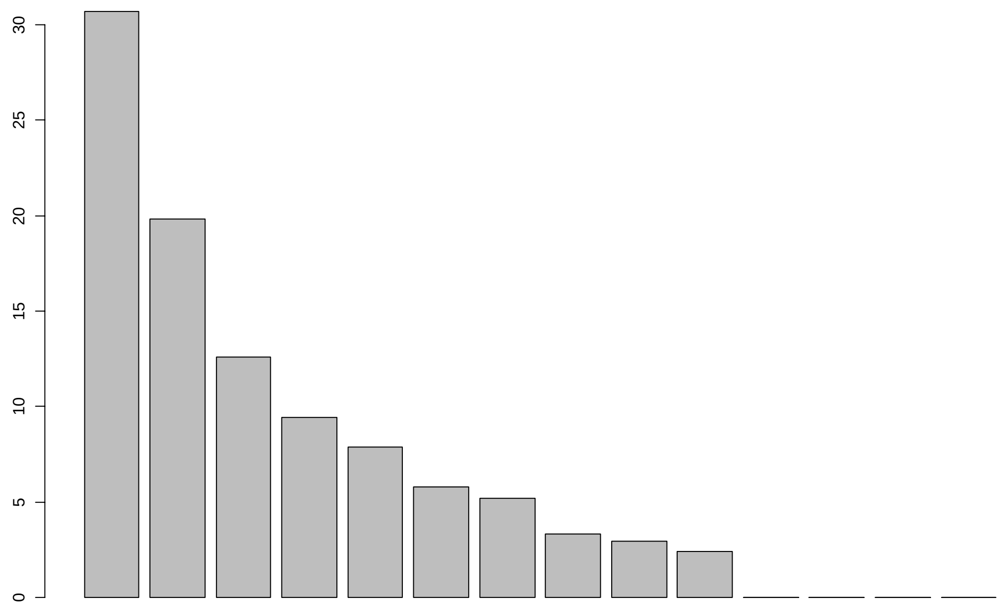
Analise de Forma

MANOVA

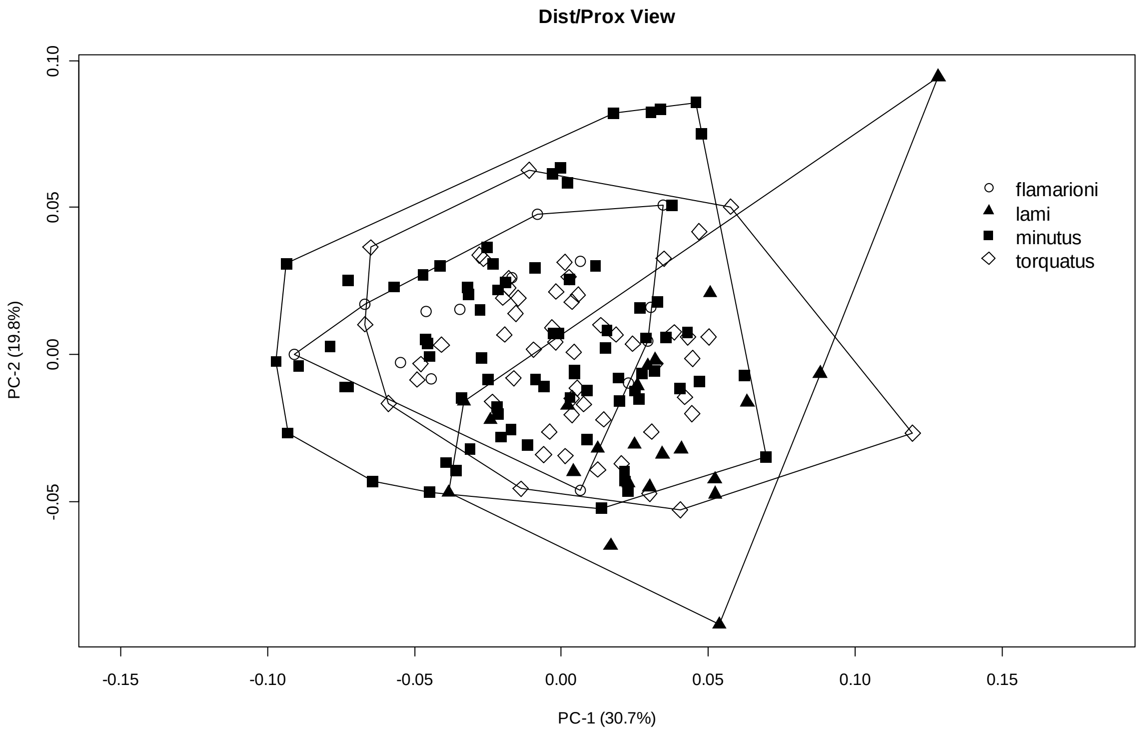
	Df	**Pillai**	**approx F**	**num Df**	**den Df**	**Pr(>F)**
sex3	1	0.0930	1.3541	10	132	0.2087
sp3	3	1.0560	7.2785	30	402	<2e-16 ***
sex3:sp3	3	0.1963	0.9381	30	402	0.5632
Residuals	141					

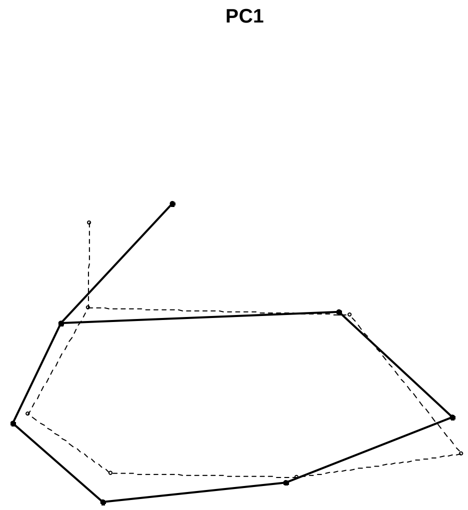
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Warning message:
In model.matrix.default(mt, mf, contrasts) :variable 'sex3' converted to a factor

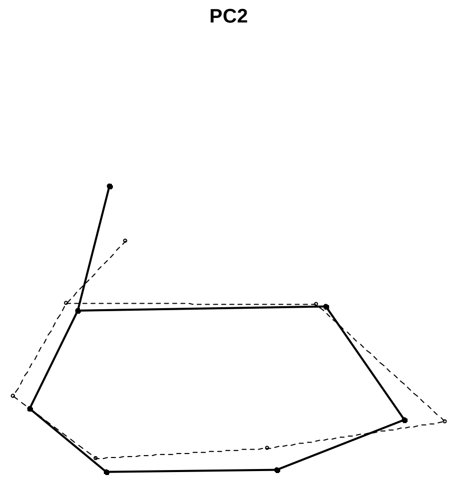
Componentes Principais



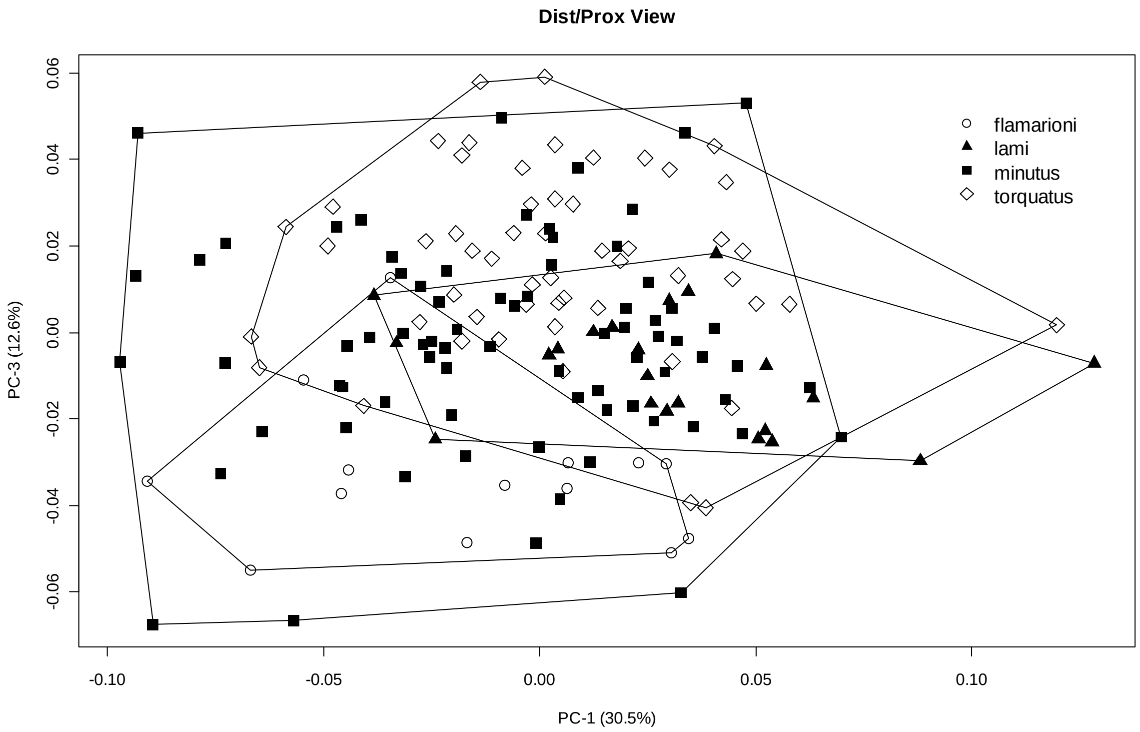
1 e 2 Componentes Principais

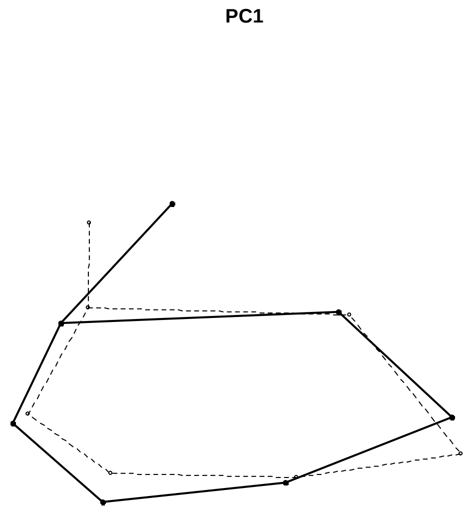


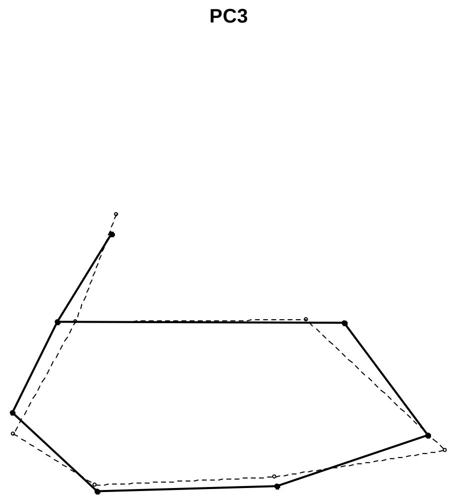




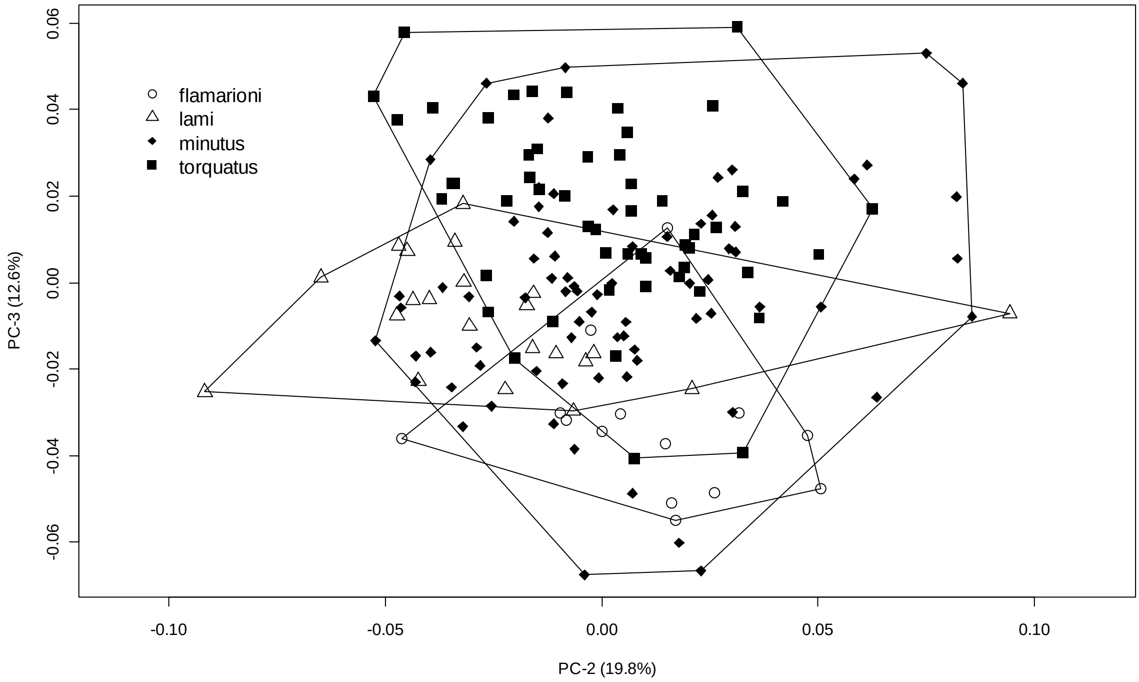
1 e 3 Componentes Principais



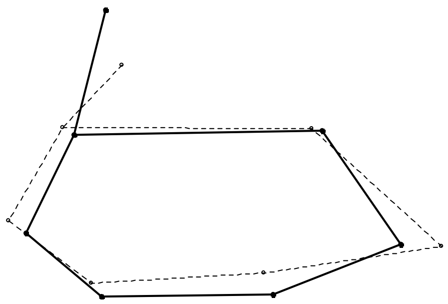




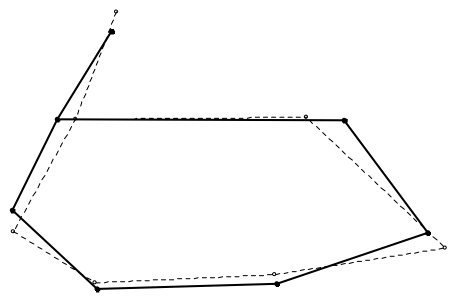
2 e 3 Componentes Principais



PC2



PC3



Análise Discrimintate

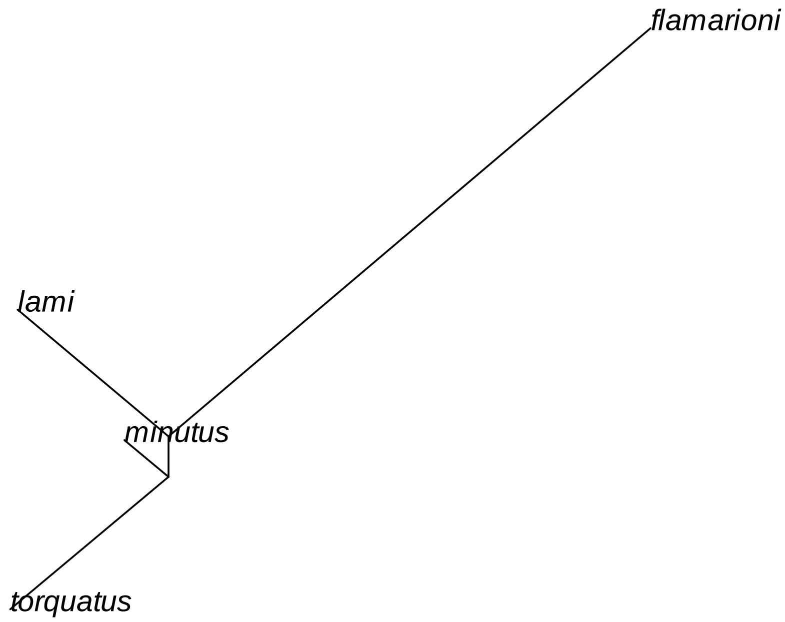
Espécie

sp3	flamarioni	lami	minutus	torquatus
flamarioni	12	0	2	0
lami	0	13	9	0
minutus	3	5	57	11
torquatus	1	1	13	39

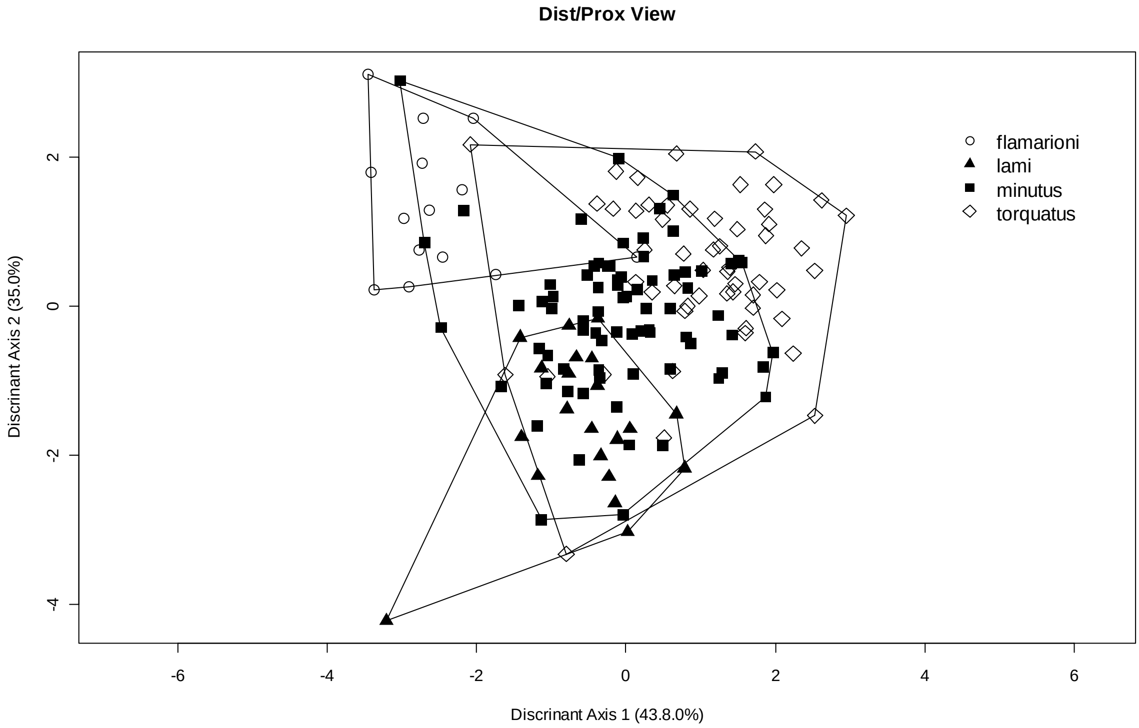
Tabela traduzida

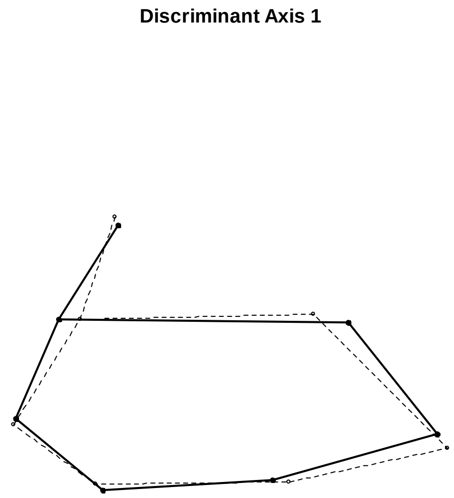
flamarioni	lami	minutus	torquatus
85.71429	59.09091	75.00000	72.22222

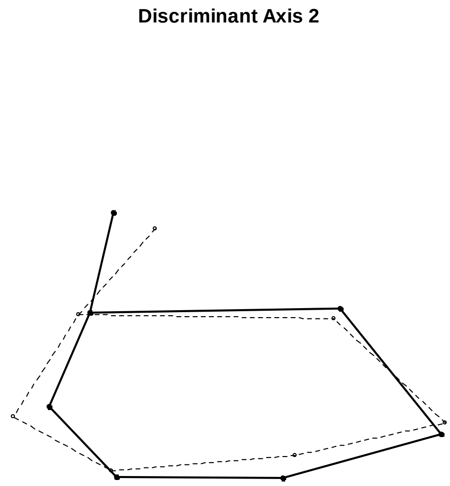
Arvore de Distâncias de Mahalanobis



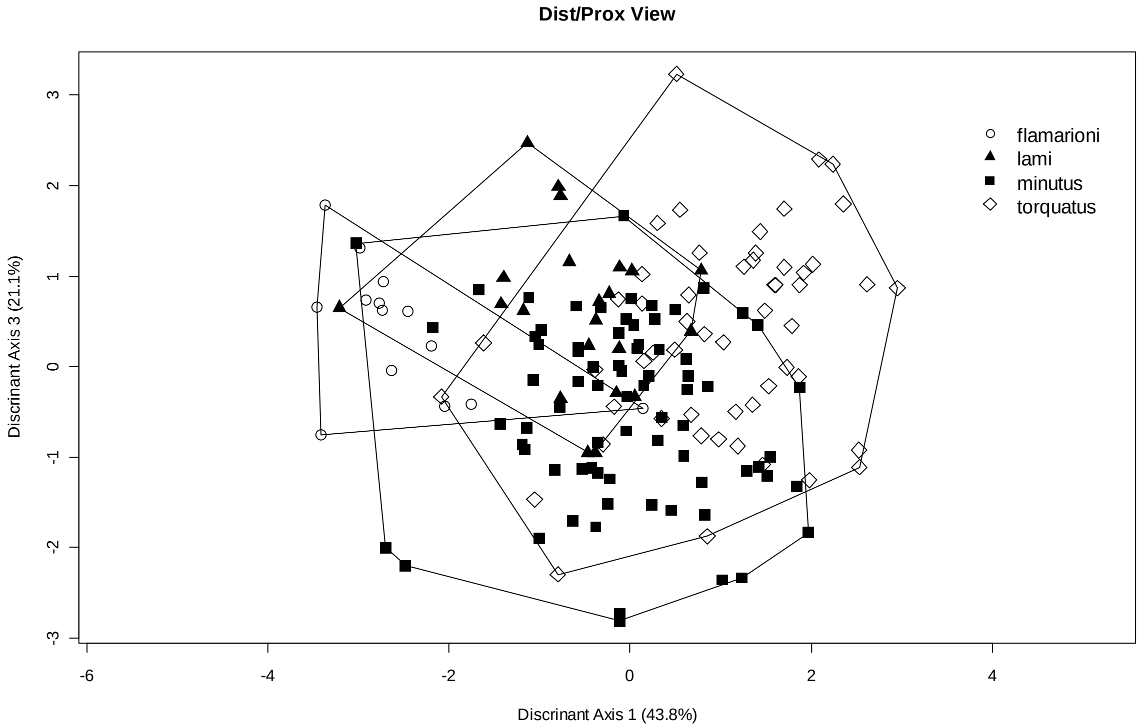
1 e 2 Eixo Discriminante

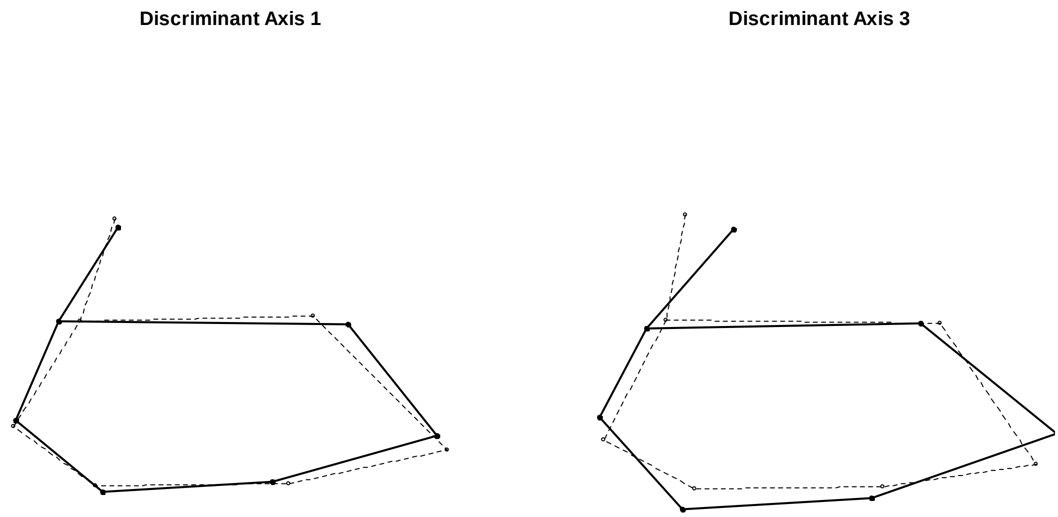




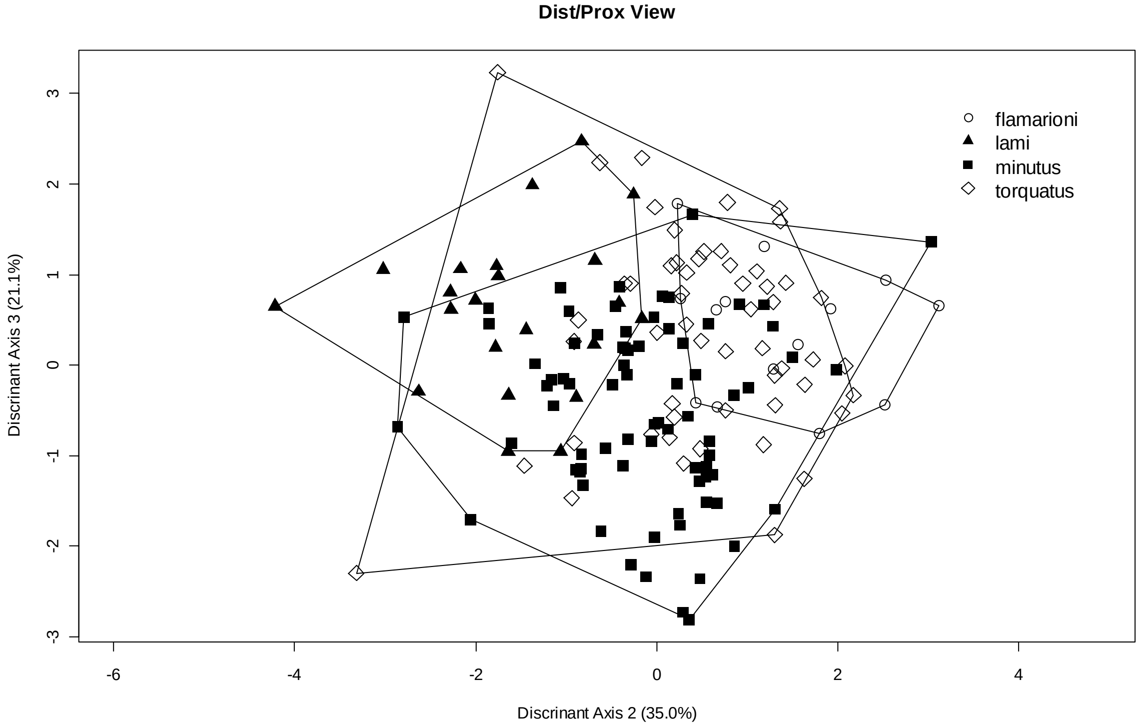


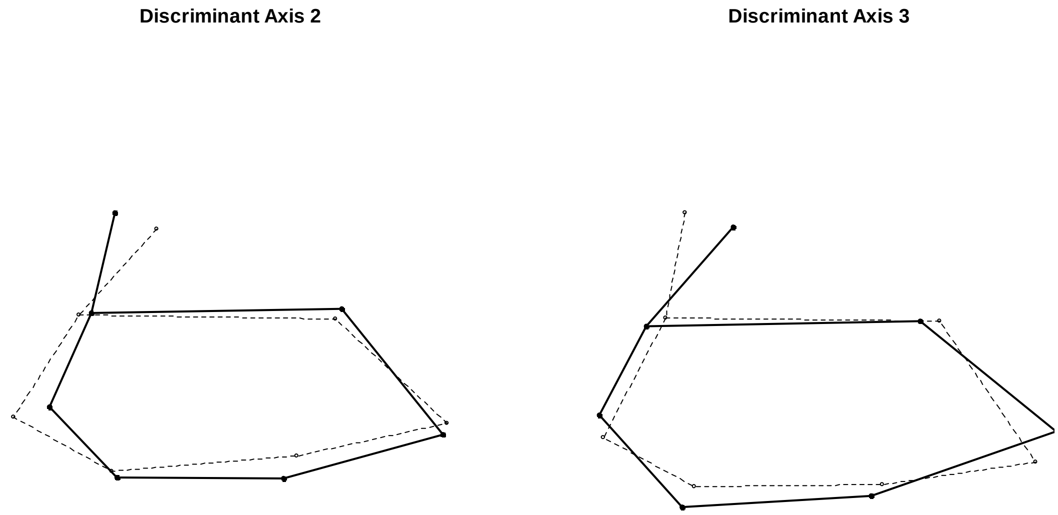
1 e 3 Eixo Discriminante





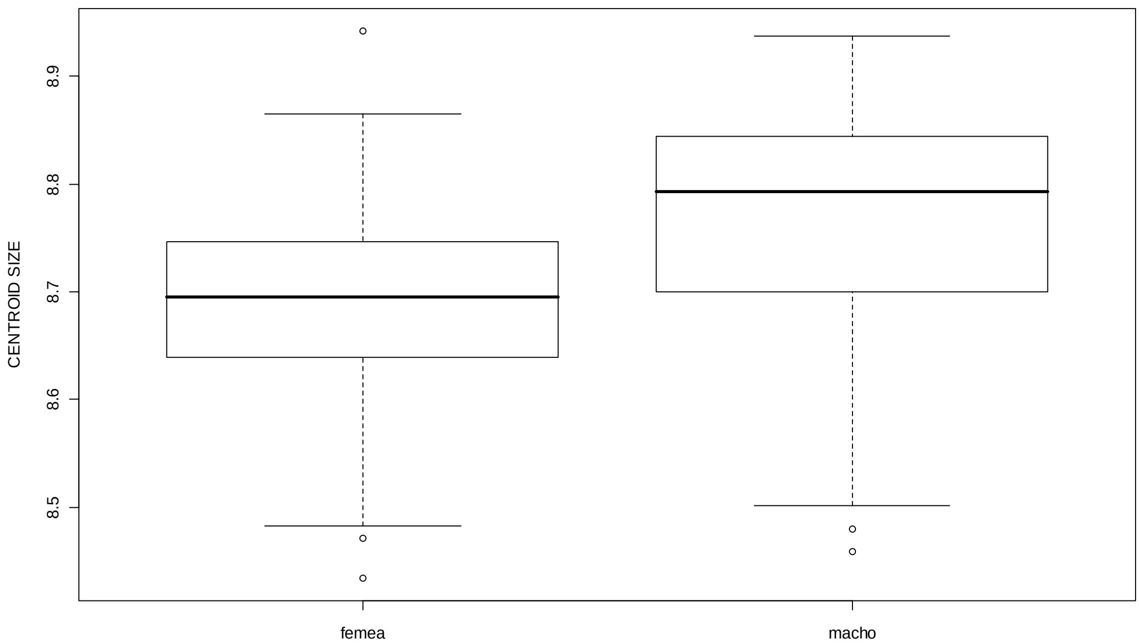
2 e 3 Eixo Discriminante

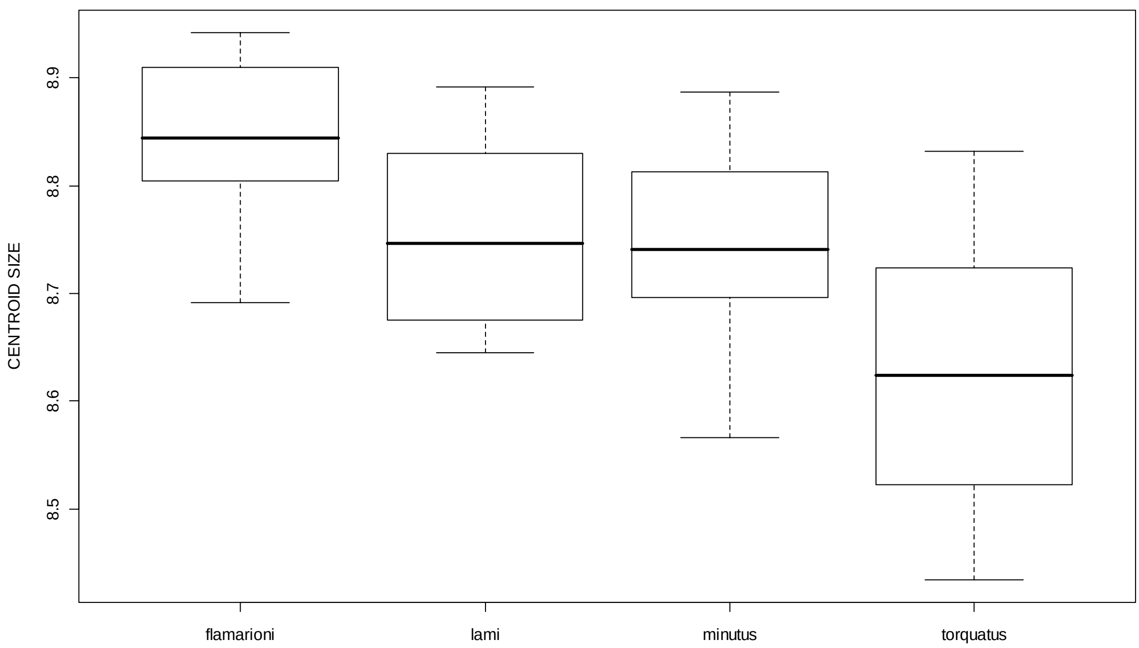


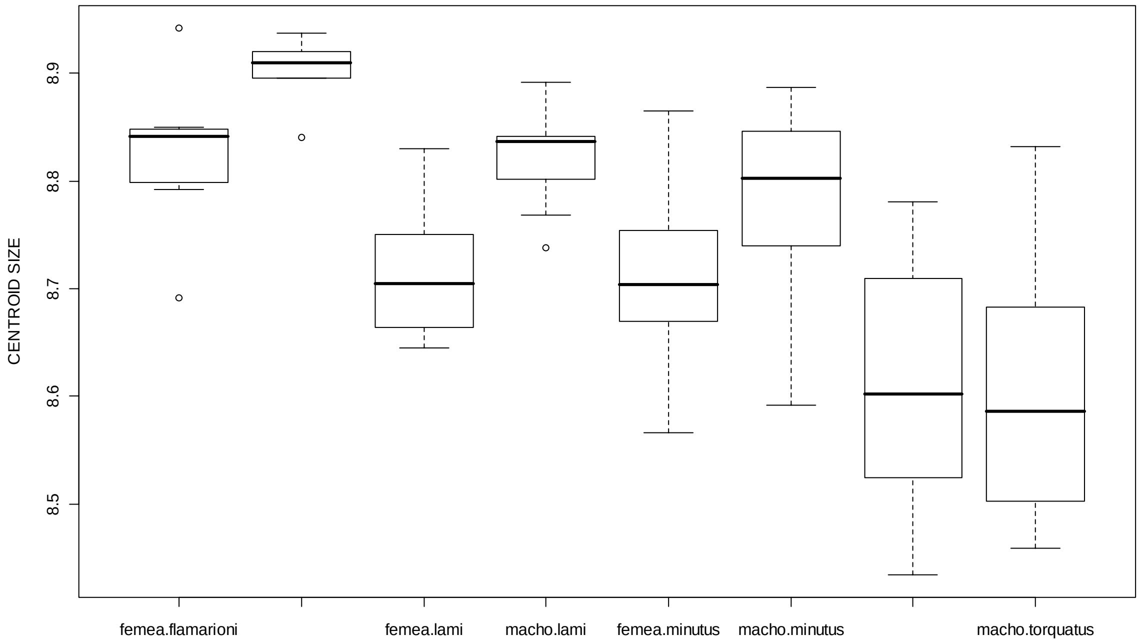


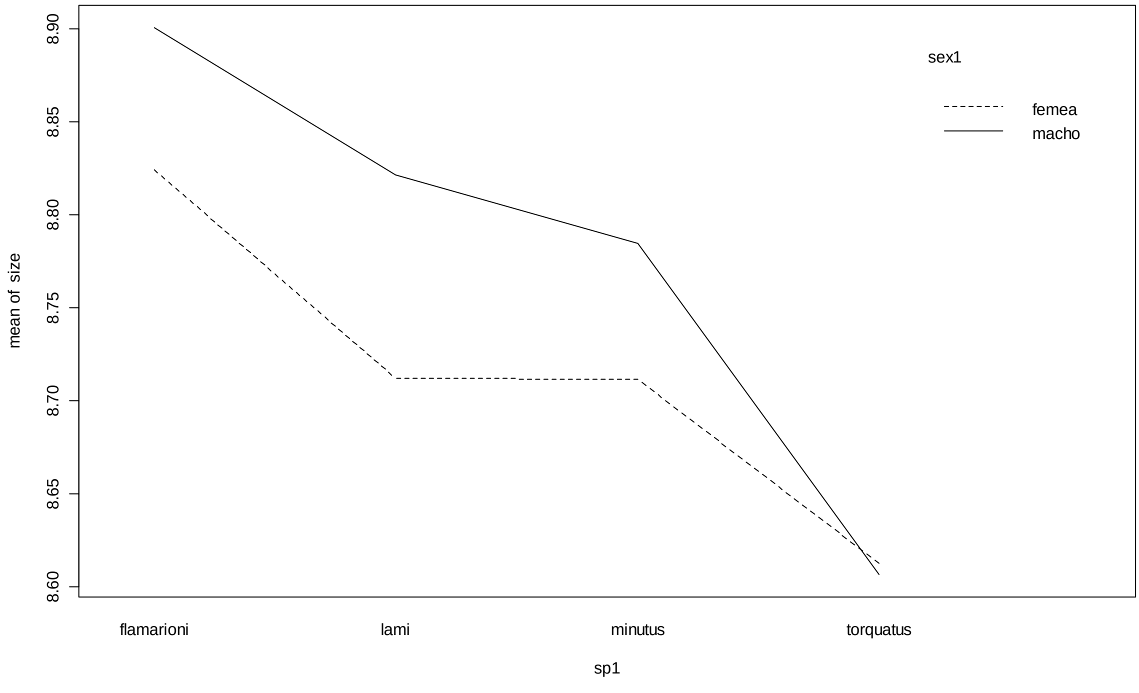
Vista Total

Análise Tamanho






CENTROID SIZE
8.5
8.6
8.7
8.8
8.9
femea.flamarioni
femea.lami
macho.lami
femea.minutus
macho.minutus
macho.torquatus


mean of size
8.60
8.65
8.70
8.75
8.80
8.85
8.90
sex1
femea
macho
flamarioni
lami
minutus
torquatus
sp1

Anova 2 Fatores - Sexo e Espécie

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
sex1	1	0.17161	0.17161	25.8424	1.221e-06 ***
sp1	3	0.73297	0.24432	36.7923	< 2.2e-16 ***
sex1:sp1	3	0.05553	0.01851	2.7875	0.04317 *
Residuals	134	0.88984	0.00664		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
14 observations deleted due to missingness

Sexo

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
sex1	1	0.17161	0.17161	14.315	0.0002285 ***
Residuals	140	1.67833	0.01199		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
14 observations deleted due to missingness

Espécie

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
sp1	3	0.79886	0.26629	35.147	< 2.2e-16 ***
Residuals	152	1.15159	0.00758		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

$sp1

	diff	lwr	upr	**p adj**
lami-flamarioni	-0.100916210	-0.17893034	-0.02290208	0.0053877
minutus-flamarioni	-0.104456512	-0.17065369	-0.03825933	0.0003887
torquatus-flamarioni	-0.229354054	-0.29757543	-0.16113268	0.0000000
minutus-lami	-0.003540303	-0.05979709	0.05271648	0.9984392
torquatus-lami	-0.128437844	-0.18706305	-0.06981264	0.0000004
torquatus-minutus	-0.124897542	-0.16652419	-0.08327089	0.0000000

Analise De Forma

MANOVA

	Df	**Pillai**	**approx F**	**num Df**	**den Df**	**Pr(>F)**
sex1	1	0.7531	2.2291	78	57	0.0008912 ***
sp1	3	2.6336	5.4370	234	177	< 2.2e-16 ***
sex1:sp1	3	1.6950	0.9825	234	177	0.5525203
Residuals	134					

Análise Discrimintate

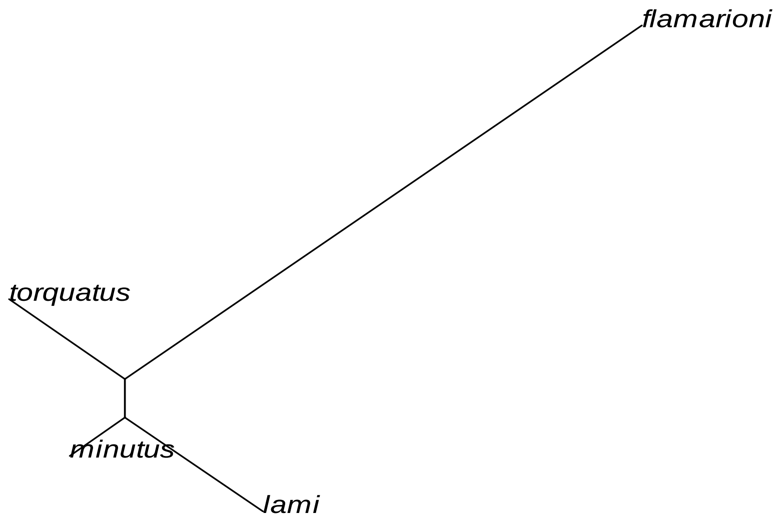
Espécie

sp1	flamarioni	lami	minutus	torquatus
flamarioni	14	0	0	0
lami	0	20	1	0
minutus	0	0	70	0
torquatus	1	0	1	49

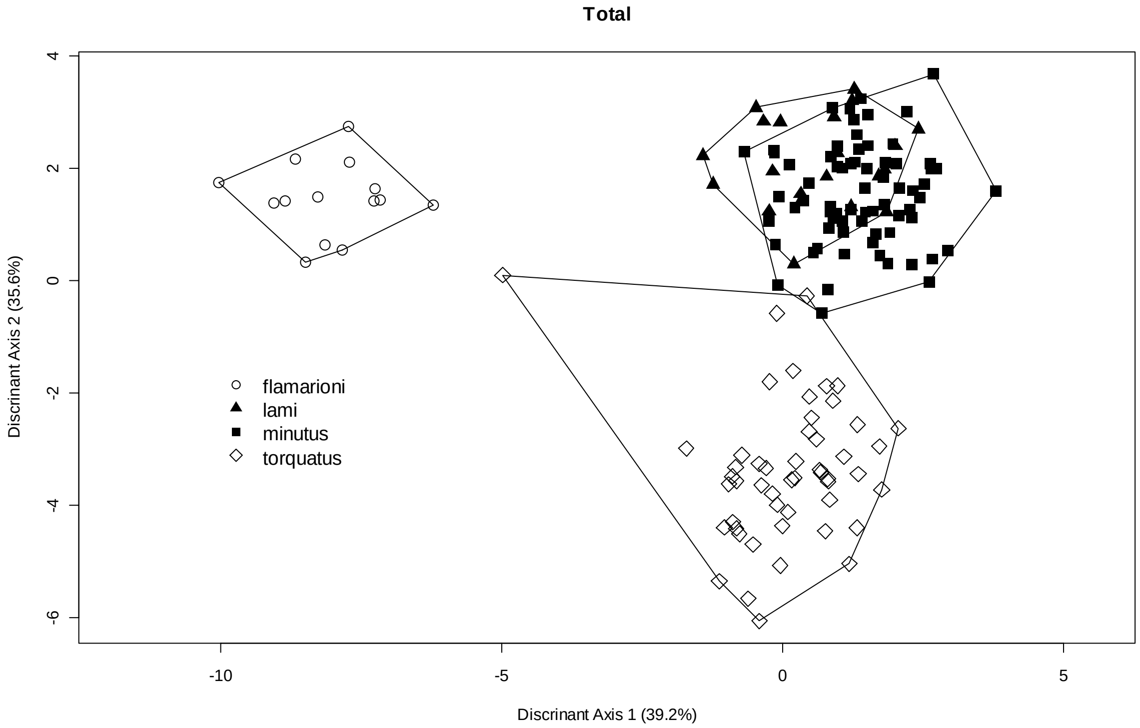
Tabela traduzida

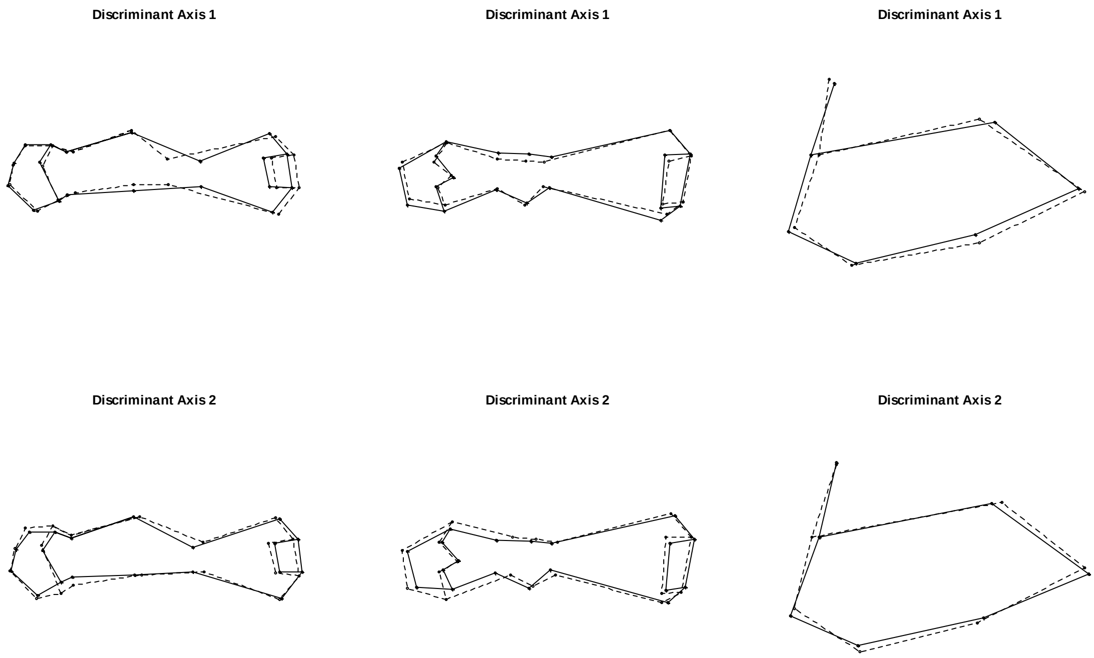
flamarioni	lami	minutus	torquatus
100.00000	95.23810	100.00000	96.07843

Arvore de Distâncias de Mahalanobis

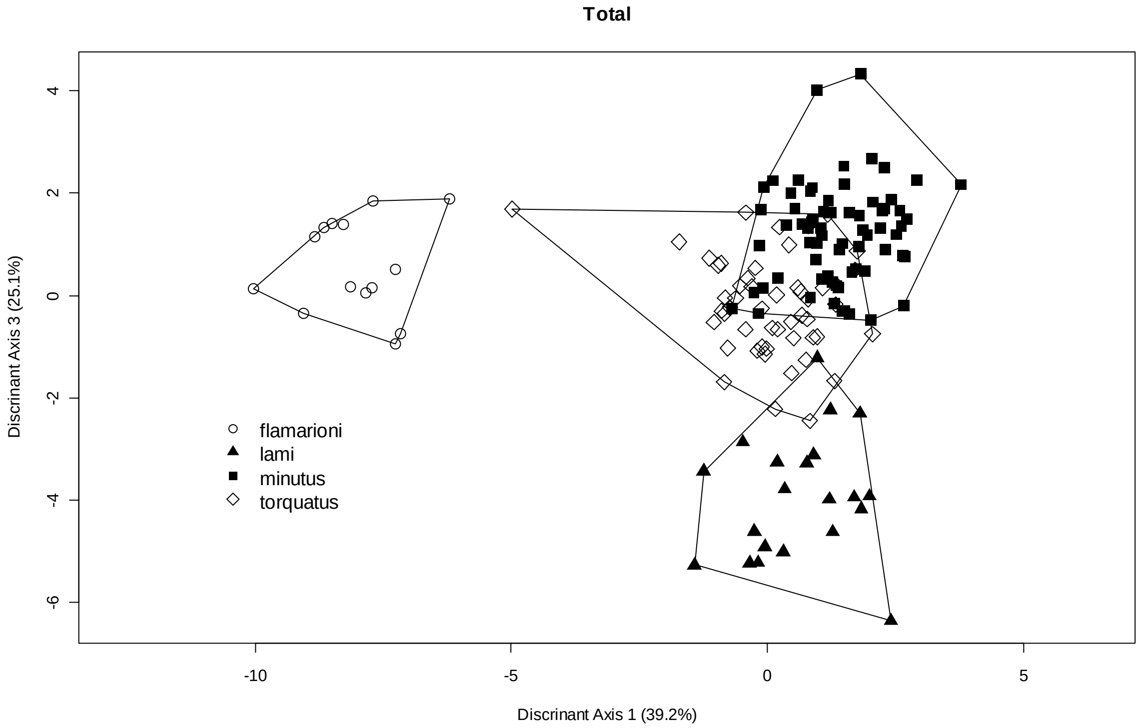


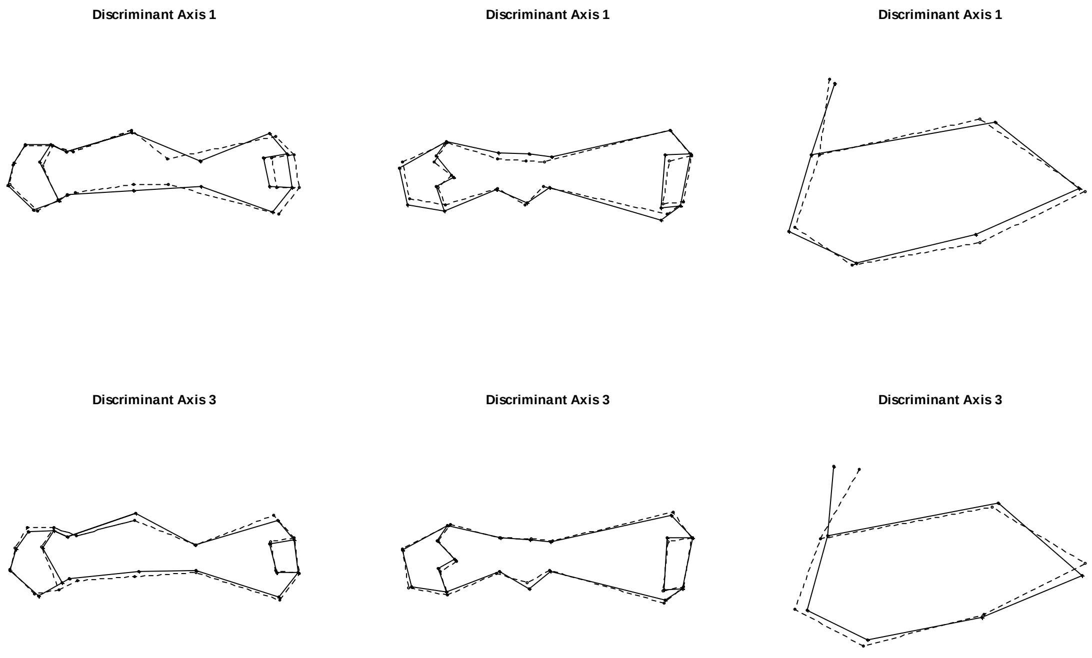
1 e 2 Eixo Discriminante





1 e 3 Eixo Discriminante





2 e 3 Eixo Discriminante