

Geometric Morphometric Differences between *Panstrongylus geniculatus* from Field and Laboratory

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The finding of Panstrongylus geniculatus nymphs inside a house in northeastern Antioquia, Colombia, and the reports related to their increasing presence in homes suggest the need for surveillance methods for monitoring the invasion processes. We analyzed the morphological differences between a wild population and its laboratory descendants, using the techniques of geometric morphometry, with the idea that such differences might parallel those between sylvatic and synanthropic populations. The analyses over five generations showed differences in size but not in shape. Head size and wing size were both reduced from sylvatic to laboratory populations, but the decrease in head size occurred only up to the second generation while the decrease in wing size proceeded up to the fifth generation. In contrast, although a decrease in sexual size dimorphism has been proposed as a marker of colonization in human dwellings, we did not detect any significant loss of dimorphism between sexes of P. geniculatus over the five generations studied. We conclude that size changes may have a physiological origin in response to a change of ecotopes, but more than five generations may be required for the expression of permanent morphological markers of human dwellings colonization.

Key words: geometrical morphometry - isometric size - shape - Triatominae - *Panstrongylus geniculatus* - Chagas disease

Panstrongylus geniculatus (Latreille, 1911) is one of the most widely distributed species of Triatominae (Hemiptera, Reduviidae) on the American continent, with a range known to extend from southern Mexico to northern Argentina, and including several of the Caribbean islands (Carcavallo et al. 1999). Its main habitats are the burrows and nesting places of marsupials, bats, rodents and birds, but adult specimens have also been collected from human peridomicile and homes – presumably attracted by light (Carcavallo et al. 1998). It has even been found in urban areas of Caracas, Venezuela (Pifano 1986) and in the city of Corrientes, Argentina (Carcavallo et al. 1998).

Although Miles et al. (1981) reported that Amazon populations of *P. geniculatus* were difficult to rear in the laboratory, requiring 100% rh in their hands, peridomicile and synanthropic colonies of this species have since been reported from parts of southern Venezuela and the Brazilian Amazon (Valente et al. 1998) with evidence that it has been feeding on domestic pigs and on people. In the Ecuadorian Amazon *P. geniculatus* has also shown a tendency to establish peridomicile colonies in which they have exhibited the capacity of flying from their breeding places, in order to feed inside the dwellings during the night (Chico et al. 1997, Aguilar et al. 1999, Abad-Franch et al. 2001). In the northeast of the Department of

Antioquia, Colombia, *P. geniculatus* has recently been found colonizing human dwellings (Wolff & Castillo 2000) and we were interested to study these populations in order to assess possible morphometric changes associated with the apparently recent trend to synanthropic behavior (Dujardin et al. 1999b, Schofield et al. 1999). Accordingly, we report here a comparison between natural populations of *P. geniculatus* and their laboratory descendants, using geometric morphometry to assess changes in size and shape of the adult bugs.

MATERIALS AND METHODS

The insects - Eight females and 21 males of *P. geniculatus* were collected in homes of the locality of Montebello in the Municipality of Amalfi, Department of Antioquia, Colombia (6° 55' 58" N; 75° 05' 30" W). These insects were reared in the laboratory under environmental conditions regulated at 28 ± 2°C, 90 ± 5% rh, and fed weekly on hens. The original parents were used for morphometric analysis, together with 26 males and 30 females taken randomly from the F2 generation, and 32 males and 32 females taken randomly from the F5 generation.

Morphometric measurements - The head of each insect was adhered to the sharp end of a small cardboard triangle, sustained by a pin. The wings were mounted between microscope slides and coverslips using a commercial adhesive. Using a camera lucida connected to a stereomicroscope, 10 landmarks of the heads and five of the wings were drawn on paper (Fig. 1), and afterwards landmarks were input into the computer by scanning drawings. The geometric coordinates of each landmark were then deduced using TPSDIG version 1.18, a free access computer software available on the Internet (Rohlf 1999a). This was repeated three times for each specimen in order to obtain average coordinate matrices. For the morphometric analyses of changes between generations the average coordinates of heads and wings were prepared by

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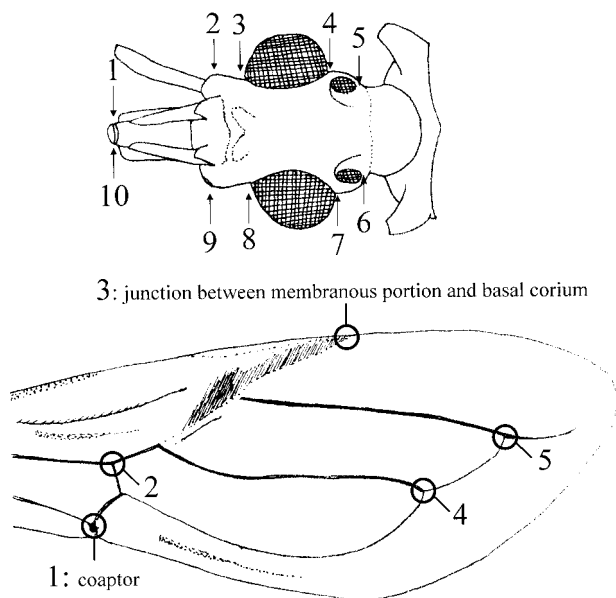


Fig. 1: points of reference measured as coordinates of heads and wings of *Panstrongylus geniculatus*. Numbering on the points indicated denotes the arrangement followed in order to obtain the coordinates by means of computer software TPSDIG version 1.18 (Rohlf 1999a).

sex for each generation. Joint matrices of males and females were used for the analyses of sexual dimorphism. Centroid size was extracted from each matrix as an isometric estimator of size (Bookstein 1990) using TPSREGR software, version 1.18 (Rohlf 1999b). Shape variables were computed utilizing TPSRELW software, version 1.18 (Rohlf 1999c).

Statistical analysis - We performed Principal Component Analysis (PCA) based on variance-covariance matrices of the shape variables. Individuals of each generation and/or sex were projected onto the first two components of the PCA (PC-1 and PC-2) to detect shape differences in factorial maps. For comparisons of isometric size and of shape among generations and between sexes, we used the non-parametric Kruskal-Wallis (1952) analysis of variance, with Bonferroni correction (Sokal & Rohlf 1995). PCA was done using NTSYSpc® software version 2.02j (Applied Biostatistics Inc. 1998). Non-parametric comparisons and graphs were done using JMP® version 3.2.6 (SAS Institute Inc. 1999) and Intercooled STATA 6.0 for Windows® (Stata Corporation 2000) software.

RESULTS

Analysis of isometric size - The isometric head size diminished significantly between the parental (wild) males and females and their laboratory offspring, but this decrease in isometric size was quickly stabilised since the second and fifth laboratory generations showed no significant differences (Figs 2A, B). Similarly, the isometric wing size also diminished significantly from wild parents to laboratory offspring, but this reduction continued from the second to fifth generations (Figs 2C, D) (Table).

Sexual dimorphism was significant in all generations, as shown by comparing head and wing isometric size between sexes for each generation (Fig. 3). Such dimorphism was also confirmed when sexes were compared by generation using Kruskal Wallis non-parametric test (Table).

Analysis of shape - Inspection of specimens of each generation projected onto the first two Principal Components obtained from shape variables did not show significant detection of changes either in heads or wings of males and females (Fig. 4). However, regression analyses of the first two Principal Components that represent shape against the isometric size estimator were significant in all cases ($p < 0.01$) showing that, on average, 13% of the individual shape distribution can be predicted by their size.

Regarding sexual dimorphism, none of the generations exhibited large differences in shape between males and females. This can be verified by the quantile box plots that represent distributions of individuals on the Principal Components which were obtained from head and wing shape variables (Fig. 5). Comparison between sexes over the three generations using the Kruskal Wallis test confirmed a lack of significant differences between sexes in the shape of heads and wings of any generation ($p > 0.05$).

DISCUSSION

This is the first attempt to use geometric morphometry to assess the changes associated with laboratory colonization of *P. geniculatus* up to the fifth generation. Previous Triatominae morphometrics work have been based in traditional multivariate techniques which do not allow to recover the shape of the original form from the usual data of distance measurements, even as an abstract representation (Rohlf & Marcus 1993). The value of geometric morphometry for such studies lies in its preservation of information on spatial arrangement of the organism, independent of differences in growth (allometry) that generally have environmental causes. Present computational methods extract isometric size from geometric configuration but not the influence of allometric growth on shape, though the mathematical possibility of doing this does exist (Dujardin 2001). Organisms do not grow in an isometric way, but the elimination of this component can produce a reduction, important at times, of the allometric changes in such a way that shape variables can come very close to the true form variables which would be obtained if every allometric effect were eliminated (Dujardin 2001). In order to verify the independence of growth with respect to shape we performed linear regression analysis of the variables representing shape against the isometric size variable. In all cases allometric size significantly contributed to shape, though this contribution was only 13% on average, suggesting that the shape variables mainly reflect genetic rather than environmental influences on the actual shape of the organisms.

All species of Triatominae grown under controlled laboratory conditions or colonizing human dwellings tend to show a reduction in sizes relative to their original sylvatic populations (Szumlewics 1976, Zeledón 1981, Schofield 1999, Dujardin et al. 1997a, b, 1998a, 1999a, b). It would appear that natural selection favors larger phenotypes in the sylvatic habitats (possibly due to a greater

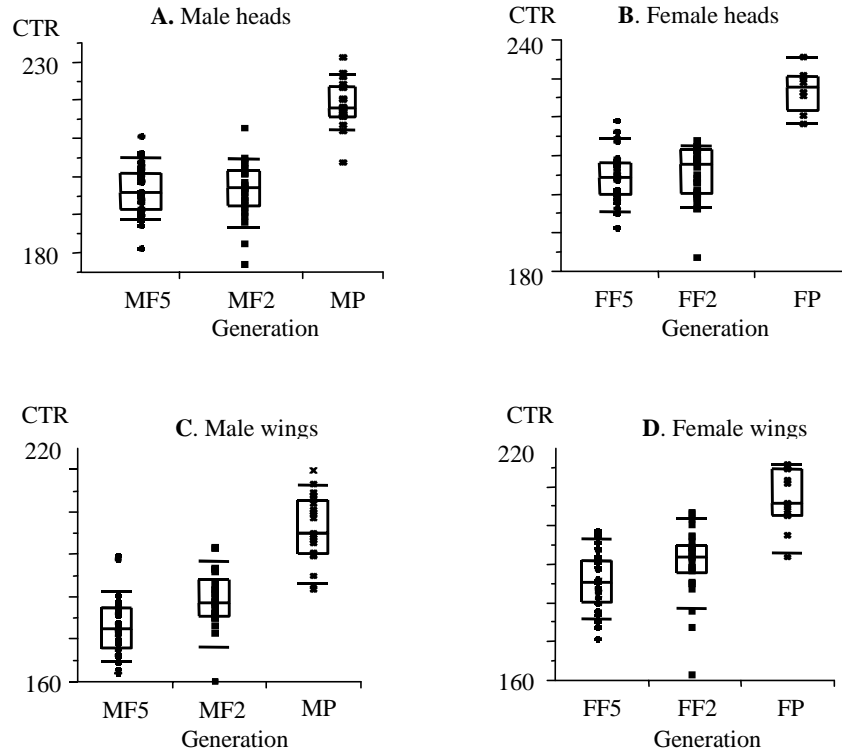


Fig. 2: quantile box plots showing the isometric size differences of heads and wings between parental, F2 and F5 generations of *Panstrongylus geniculatus*. The boxes represent the distribution of males and females of parental generation (MP and FP, respectively), second generation (MF2 and FF2, respectively), and fifth generation (MF5 and FF5, respectively) along the isometric estimator of size, centroid-size (CTR). Each box shows the median of the group, as a line in the middle of the quartiles (25th and 10th percentiles on one side and 75th and 90th on the other). Besides, distribution of individuals on the CTR is shown.

TABLE

Comparison of isometric size differences between males and females and between field and laboratory populations of *Panstrongylus geniculatus* using the Kruskal-Wallis test with Bonferroni correction

Comparison between sexes (sexual dimorphism)						
Generation	Heads			Wings		
	F5	F2	P	F5	F2	P
Chi-square	17.8890	16.6000	8.8600	27.2040	23.3300	9.4550
<i>p</i>	0.0001	0.0001	0.0029	0.0001	0.0001	0.0021
Bonferroni	0.0170	0.0253	0.0500	0.0170	0.0253	0.0500
Interpretation	S	S	S	S	S	S
Comparison of the size of the heads between ecosystems						
Generation	Males			Females		
	P vs F5	P vs F2	F2 vs F5	P vs F2	P vs F5	F2 vs F5
Chi-square	36.4500	32.8860	0.1290	18.4620	18.4400	1.4000
<i>p</i>	0.0001	0.0001	0.7191	0.0001	0.0001	0.2367
Bonferroni	0.0085	0.0102	0.0500	0.0127	0.0170	0.0253
Interpretation	S	S	NS	S	S	NS
Comparison of the size of wings between ecosystems						
Comparison	Males			Females		
	P vs F5	P vs F2	F2 vs F5	P vs F5	P vs F2	F2 vs F5
Chi-square	33.3860	27.7090	9.7100	21.6080	17.2130	7.9370
<i>p</i>	0.0001	0.0001	0.0018	0.0001	0.0001	0.0048
Bonferroni	0.0085	0.0102	0.0253	0.0127	0.0170	0.0500
Interpretation	S	S	S	S	S	S

p: level of significance; P: population captured in the field; F2 and F5: second and fifth generation respectively, grown in the laboratory and descending of P.

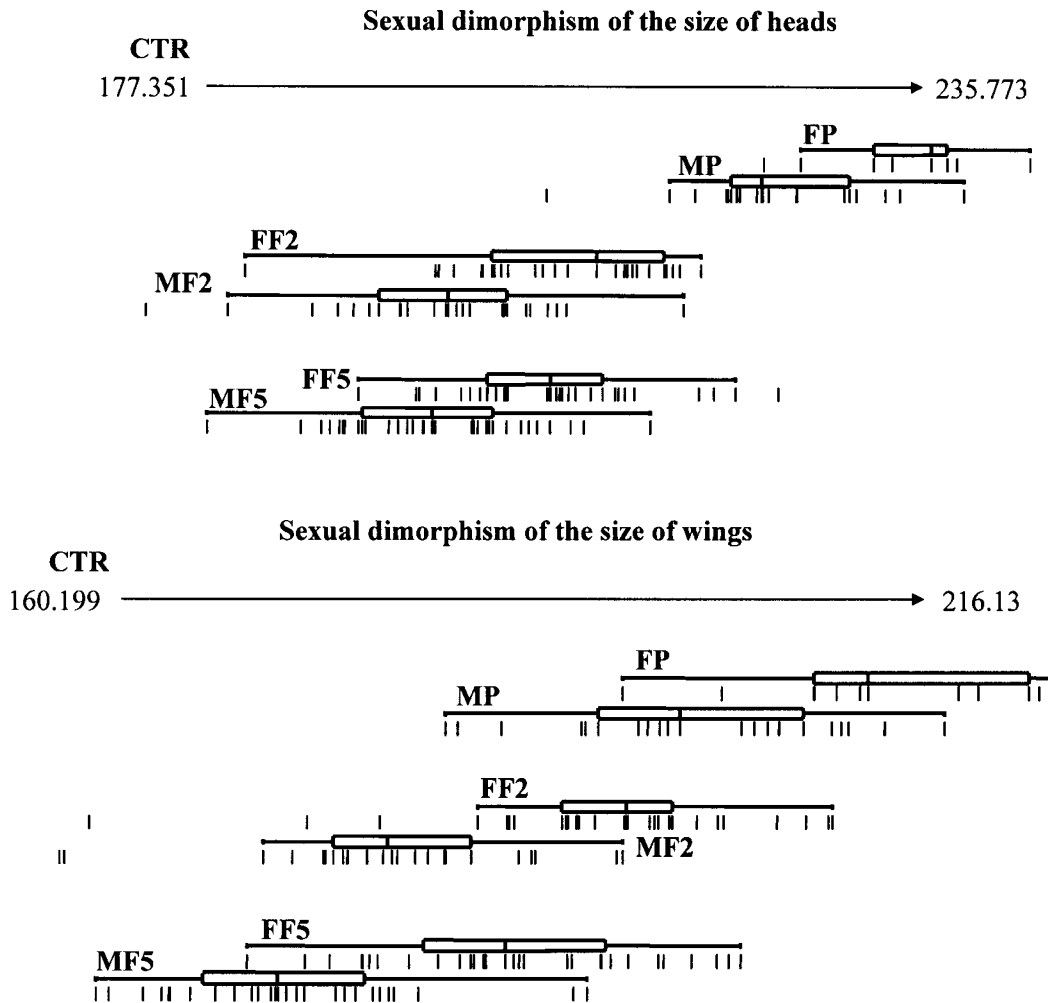


Fig. 3: quantile box plots showing the magnitude of the sexual dimorphism in the head and wing size of parental, F2 and F5 generations of *Panstrongylus geniculatus*. The boxes represent the distribution of males and females of the parental generation (MP and FP, respectively), of second generation (MF2 and FF2, respectively), and of fifth generation (MF5 and FF5, respectively) along the isometric estimator of size, centroid-size (CTR). Each box shows the median of the group, as a line in the middle of the quartiles (25th and 10th percentiles on one side and 75th and 90th on the other). Under each box the position of individuals is indicated on the CTR.

capacity to resist temporary food shortages) while smaller individuals apparently survive better in laboratory or synanthropic conditions where there is less restriction on the availability of food. Dujardin et al. (1999a) have suggested that the morphological changes observed between sylvatic and laboratory populations may parallel those existing between sylvatic and synanthropic populations. Moreover, synanthropic process of Triatominae seems to involve a one-way process of specialization with phenotypic and genetic simplification (Schofield et al. 1999) from which the reduction in size and sexual dimorphism are just two of the more noticeable manifestations (Dujardin et al. 1997a, b, 1998b, 1999a, b).

Dujardin et al. (1999a) showed a reduction in head size in synanthropic and laboratory colonies of *Triatoma infestans*, *Rhodnius domesticus* and *R. prolixus*, but made no similar comparison of wings. In the present study of *P. geniculatus* we observed a significant reduction in the

size of head and of wings when the sylvatic population was compared with its laboratory descendants, but while the reduction of head size stopped after the second generation the reduction in wing size continued at least to the fifth generation. This may be explained by constraints for change coming from the presence in the head of vital organs, whereas the wings are not vital for laboratory Triatominae.

The larger size of females, compared with that of males, is another characteristic of sylvatic populations, the loss of which in synanthropic generations is assumed to be a visible marker of the synanthropic process (Dujardin et al. 1999a, b). In *P. geniculatus* studied here up to the fifth generation we did not detect any significant changes in the magnitude of the sexual dimorphism of heads and wings in any of the generations analyzed. In similar way strong sexual dimorphism of domiciliary *P. rufotuberculatus* was shown by means of traditional multi-

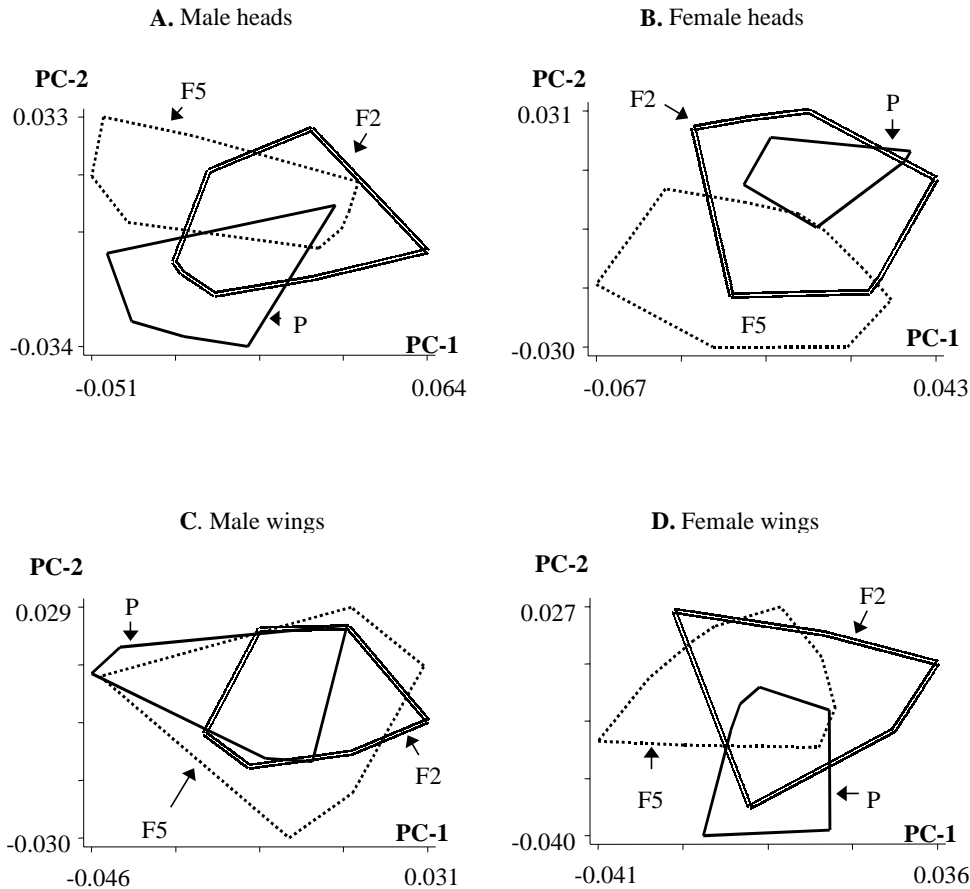


Fig. 4: projection of the heads and wings of individuals of each generation on the Principal Components PC-1 and PC-2. These axes (PC-1 and PC-2) were computed from shape variables by Principal Components Analysis. The polygons include individuals of each group. P, population captured in the field; F2 and F5, second and fifth laboratory generation respectively.

variate analysis which was interpreted as evidence for recent domiciliation (Dujardin et al. 1998a). By contrast, Dujardin et al. (1999a) found a significant reduction in the dimorphism of heads in *R. domesticus*, due to a decrease in size that was greater in females than in males, although this work probably involved a colony that had spent more than five generations in the laboratory. In a previous work, one of us (Jaramillo 2000) also found a significant reduction of the sexual dimorphism of head and wing sizes in *R. pallescens*, but this phenomenon was present in colonies with more than 10 generations in the laboratory.

The present research has extended knowledge of the biology of *P. geniculatus* in Colombia. This species seems to be beginning a synanthropic process, so that a future process of stable colonization with serious consequences for public health cannot be discarded. Our results suggest that if we detect significant reduction in the sexual dimorphism of populations found in houses, this could signify the development of more than five generations within dwellings and probably an active synanthropic process. In the meantime it will be necessary to go on with entomological surveillance and to try to put barriers to the access of adults

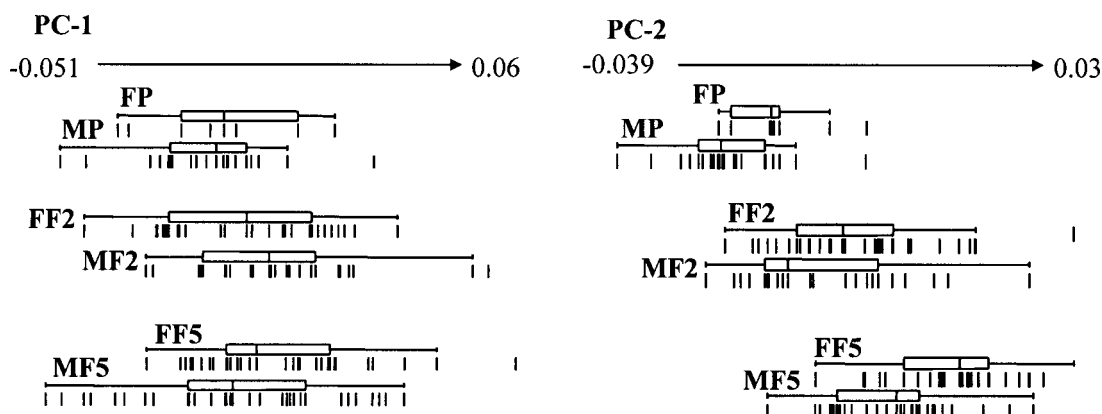
to dwellings; for example, by preserving forest zones in which the local wild fauna keeps acting as a source of nourishment for the sylvatic vector; in addition to undertaking educational campaigns so the communities turn their houses into places that give this species scarce shelter or no shelter at all for its proliferation.

In conclusion, we observe changes in the size of heads and wings of *P. geniculatus* grown in the laboratory, when they were compared with their parents from the field; but correspondent shape changes were not observed. Then, those size changes can have a physiologic origin in response to an ecosystem change. On the other hand, the reduction in the sexual dimorphism, frequently used as a marker of synanthropic behavior, was not detected in five generations, suggesting that more than those are necessary to colonize in a stable way the human dwellings.

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Sexual dimorphism of the shape of heads



Sexual dimorphism of the shape of wings

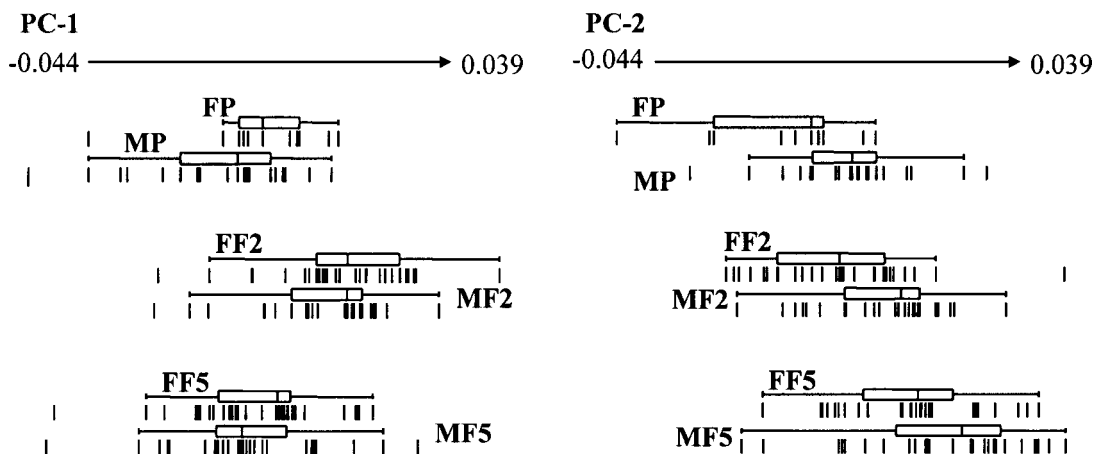


Fig. 5: quantile box plots showing magnitude of sexual dimorphism in the shape of heads and wings along three generations of *Panstrongylus geniculatus*. The boxes represent the distribution of males and females of parental generation (MP and FP, respectively), second generation (MF2 and FF2, respectively), and fifth generation (MF5 and FF5, respectively) along shape estimators, PC-1 and PC-2. Each box shows the median of the group separating percentiles 25th and 75th, whereas the lines to the left and right correspond to percentiles 10th and 90th, respectively. Under each box, the position of individuals on the respective shape component is indicated.

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