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ORIGINAL RESEARCH ARTICLE

Morphometric correlation between *Apis mellifera* morphotypes (Hymenoptera) and *Varroa destructor* (Acari) from Uruguay

Pablo Giménez Martínez[a,b,c*], Yamandu Mendoza[d], Ciro Invenizzi[e], Sandra Fuselli[a,b,f], Rosa Alonso Salces[a,b,h], Pedro Fernández Iriarte[g,h] and Matias Maggi[a,g]

[a]*Centro de Investigación en Abejas Sociales, FCEyN – UNMdP, Mar del Plata, Argentina;* [b]*Grupo de Investigación Microbiología Aplicada (GIMA), Mar del Plata, Argentina;* [c]*Agencia Nacional de Promoción Científica y Tecnológica, Mar del Plata, Argentina;* [d]*Laboratorio de Apicultura, INIA La Estanzuela, Colonia del Sacramento, Uruguay;* [e]*Sección Etología, Facultad de Ciencias, Montevideo, Uruguay;* [f]*Comisión Investigaciones Científicas de la Provincia de Buenos Aires (CIC), Mar del Plata, Argentina;* [g]*Laboratorio de Genética, FCEyN – UNMdP, Mar del Plata, Argentina;* [h]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Mar del Plata, Argentina*

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Uruguay is characterized by having an Africanization gradient in its honey bee (*Apis mellifera*) populations from areas bordering Brazil to the most remote locations. Morphological differences have also been found among *Varroa destructor* populations from different regions. A possible explanation for these morphological changes in mites could be explained by morphological correlations in the host/parasite *A. mellifera/V. destructor* system. The objective of the present study was to identify *A. mellifera* ecotypes in Uruguay and their correlation with *V. destructor* populations, using geometric morphometrics analysis. The hypothesis states that there is an Africanization gradient of *A. mellifera* in the north-southeastern direction in Uruguay that correlates with morphological variations in *V. destructor* populations. A morphometrics analysis of six honey bee populations and their respective phoretic mites was carried out by sampling zones that show the gradient in the Uruguayan region. The main results obtained were a morphometric correlation between honey bees and mites. The use of landmarks could be adjusted for the intraspecific differentiation of *V. destructor* populations, which had not been considered previously. These studies are of major importance to further understand the parasitism caused by *V. destructor* in honey bees.

Correlación morfométrica entre morfotipos de *Apis mellifera* (Hymenoptera) y *Varroa destructor* (Acari) en Uruguay

Uruguay se caracteriza por tener un gradiente de africanización en sus poblaciones de abeja de la miel (*Apis mellifera*), desde las áreas que bordean Brasil hasta las regiones más remotas. Estas diferencias morfológicas también han sido encontradas en las poblaciones de *Varroa destructor* en diferentes regiones. Una explicación posible para estos cambios morfológicos en los ácaros podría ser explicada por la correlación morfológica entre hospedador y parásito, *A. mellifera* y *V. destructor*. El objetivo del presente estudio es identificar los ecotipos de *A. mellifera* en Uruguay y su correlación con las poblaciones de *V. destructor* usando análisis de morfometría geométrica. Nuestra hipótesis de partida es que hay un gradiente de africanización en *A. mellifera* en dirección norte-sureste en Uruguay y que se correlaciona con variaciones morfológicas en las poblaciones de *V. destructor*. Un análisis morfométrico de seis poblaciones de abeja de la miel y sus respectivos ácaros fóreticos fue llevado a cabo muestreando en zonas de la región uruguaya en las que se apreciaba el gradiente. Como resultado principal se obtuvo una correlación morfométrica entre abejas y ácaros. El uso de landmarks puede ser ajustado para la diferenciación intraespecífica de las poblaciones de *V. destructor*, lo que no había sido considerado previamente. Estos estudios son de gran importancia para entender el parasitismo causado por *V. destructor* en las abejas.

Keywords: bee ecotype; Africanized bees; *Varroa destructor*; morphotypes

Introduction

Morphological differences have been found in populations of *Apis mellifera* (Buco et al., 1987; Radloff & Hepburn, 2000) in Uruguay. Most bees are hybrids, crosses between European and African bees (*A. m. mellifera* × *A. m. scutellata*), and nearly 100% of African haplotypes are found on the border with Brazil, following a decreasing gradient towards the south (Dinitz, Egea Soares, Sheppard, & Del Lama, 2003). Recently, (Branchiccela et al., 2014) reported that Africanized bees are completely established in Uruguay, representing 82% of the analyzed colonies, distributed throughout the whole country. However, in recent years, beekeepers have introduced many Italian *A. m. ligustica* queens, mainly in the western coastal provinces, taking advantage of the gentleness of this bee subspecies (Branchiccela et al., 2014).

Comparative studies on the genus *Varroa* have indicated the existence of morphological and genetic

*Corresponding author. Email: beedarian@gmail.com

differences between *Varroa jacobsoni* and *Varroa destructor* populations. The *V. destructor* population is larger than that of *V. jacobsoni* (Anderson & Trueman, 2000; Delfinado-Baker & Houck, 1989). Also (Anderson & Trueman, 2000) showed clear differences between their DNA sequences; and reported 18 different mite haplotypes, but only two of them (the Korean and Japanese haplotypes) are capable of infesting *A. mellifera* populations. *V. destructor* is now considered the main bee health problem in Uruguay and beekeepers have to apply acaricides systematically at the end of the summer to avoid bee deaths in their colonies (Maggi et al., 2016). The increase in the damage caused by this mite from late 1990s up to the present day in Uruguay (mainly in the regions with higher concentration of hives) could be explained by the increase in its virulence (Maggi et al., 2016). To date, little is known about the cause of the increase in the *V. destructor* virulence observed over the past few years. Recently studies detected the presence of the Korean haplotype in Uruguay (Guerra, Issa, Carneiro, Strapazzon, & Moretto, 2010). This Korean haplotype represents the major honey bee pest infesting and destroying millions of commercial bee colonies around the world (Sammataro, Gerson, & Needham, 2000). In this sense, the different varroa virulence observed in Uruguay should be explained by other factors such as bee ecotypes, climatic conditions, morphological variation of varroa mites or their combination (Maggi, Sardella, Ruffinengo, & Eguaras, 2009). Moreover Dinitz et al. (2003), reported that the northern groups of bee populations in Uruguay are represented by Africanized bees. Africanized bees are well known for their ability to tolerate higher varroa infestation compared with European bees (Martin & Medina, 2004).

Following this premise, a particular morphotype of *V. destructor* could be related to a particular morphotype/ecotype of *A. mellifera* and the resulting interaction between both parts could partly explain the variations observed in the virulence of the parasite on its host over time (Maggi et al., 2009). In parasite populations, adjustments of life history strategies, such as body size, to changing environmental conditions can take place as a response to changing habitats in short time periods (Rose & Mueller, 1993). Given that the parasite habitat is not uniform and that they have to affront imposed selective pressures established by the environment where they live, they must adjust their biology to ensure the continuity of their lives (Poulin, Wise, & Moore, 2003; Vizoso & Ebert, 2005).

The main objective of this research was to identify the presence of *A. mellifera* morphotypes in Uruguay and to detect morphometric correlations with *V. destructor*

Figure 1. Map of Uruguay showing the six sampling sites: (1) Artigas; (2) Salto; (3) Cerro Largo; (4) Colonia; (5) Ombues; (6) Atlántida.

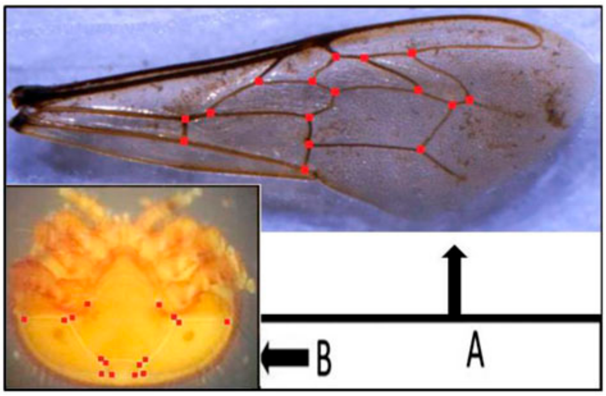

Figure 2. (a) Landmarks on the right hind wing of *A. mellifera*; and (b) on the internal plates of *V. destructor* mite.

populations infesting them, by using geometric morphometric analysis.

Materials and methods

Sampling

Samples of bees were collected from six localities in Southern and Northern Uruguay: Atlántida, Colonia, Ombues, Artigas, Cerro Largo and Salto of the Uruguay (Figure 1). Thirty worker bees and thirty phoretic mites were collected from each locality and stored in 70% alcohol until further analysis.

Mounting material and image recording

In the laboratory, the bees' right hind wings were collected and placed between microscope slides. Likewise, mites were immersed in lactic acid for two days to remove their inner tissues and visualize the outer layers, thus obtaining clearer images. The mites were then placed between microscope slides. Both materials were photographed using a Canon PowerShot SX120 IS and a magnifier model Lancet Instrument with 4x magnification was used for to record mites. The images were saved in JPG format for further analysis.

Geometric morphometrics analysis of A. mellifera

Using the tpsUtil Version 1.33 (Adams, Rohlf, & Slice, 2004) program, we obtain a file for the analysis of the images recorded. Sixteen homologous landmarks were manually digitized on the wing veins (Figure 2 (a)) with the tpsDIG2 program. The landmarks used for the bees were proposed by Francoy et al. (2008).

Geometric morphometrics analysis of *V. destructor*

Using the tpsUtil Version 1.33 (Adams et al., 2004) program, we obtain a file for the analysis of the images recorded. Sixteen homologous landmarks were manually digitized on the vertexes of the inner plates of the mites (Figure 2 (b)) with the tpsDIG2 program. No previous study has assessed the use of geometric morphometrics analysis for *V. destructor* population classification as of now. As a consequence, the landmarks used in this study were selected based on the following criteria:

(1) Homology: Homologous structures are those whose underlying similarities are the result of being derived from a common ancestral structure. It is also required that under this definition of homology, the landmarks are discrete; they must be clearly distinguishable from the surrounding structures. These features allow so any interpretation of the observed variation may have biological sense.
(2) Consistency in the relative position: refers to the ways in study should not differ radically in the position of landmarks, what happens in cases where there may be overlap, transposition or disappearance of the same, which ultimately alters completely configuration, creating problems with algebra analysis.
(3) Repeatability: this criterion is applied to avoid a significant effect of the observer error, it is important that the landmark is easy to find and clearly defined (Oxnard & O'Higgins, 2009).

To evaluate if the landmark used in this study are suitable to establish morphological variation among varroa populations we tested their stability measuring the landmarks in 30 images of the same individual of *V. destructor.*

Statistical analysis

The statistical analyses of bee and mite populations were carried out by Procrustes ANOVA and Canonical Variate Analysis (CVA) using MORPHOJ package (Klingenberg, 2011). CVA was used to calculate Mahalanobis distance and to obtain a scatter plot of the specimens along the first two canonical axes, producing the maximum separation among all the groups (multigroup discriminant analysis).

Using Mahalanobis distances obtained, we carried out a cluster analysis using the Ward's method with PAST 2.17 (Hammer, Harper, & Ryan, 2001), relating honey bees populations with their respective mites phoretic. Likewise, an analysis of correlation between bee populations from northern and southern locations with their respective mites was conducted by analyzing the covariance matrix of both populations.

Results

Population analysis of A. mellifera *from Uruguay*

Procrustes ANOVA detected significant differences between the shapes of *A. mellifera* among all populations

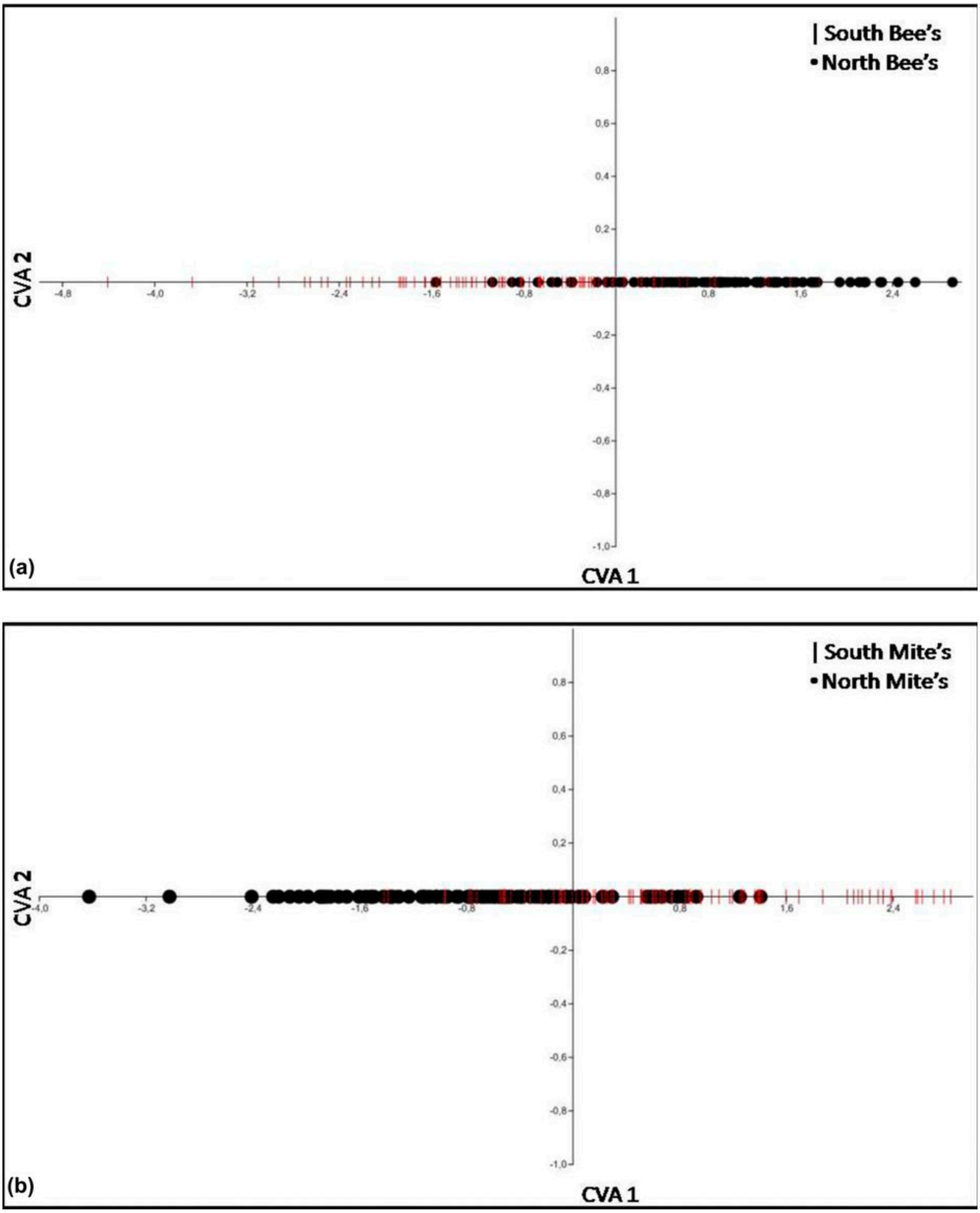

Figure 3. Canonical variates analysis (CVA) of populations of: (a) bees; and (b) mites from Uruguay.

from Uruguay ($p < 0.001$). Based on these results, a CVA was carried out and a scatter plot showing the maximum separation among the groups under study was obtained (Figure 3 (a)). Two groups were determined: the first one includes the southern localities of Atlántida-Colonia-Ombues; the second one comprises Cerro Largo, Artigas and Salto.

Population analysis of V. destructor

The analysis of stability of each *Varroa* landmark used showed that variance in each one is lower (<0,0001), which indicates that they are good markers for performing a morphometric analysis; in turn, a Principal Components analysis and analysis of procustes was performed to observe the dispersion obtained (Figure 4).

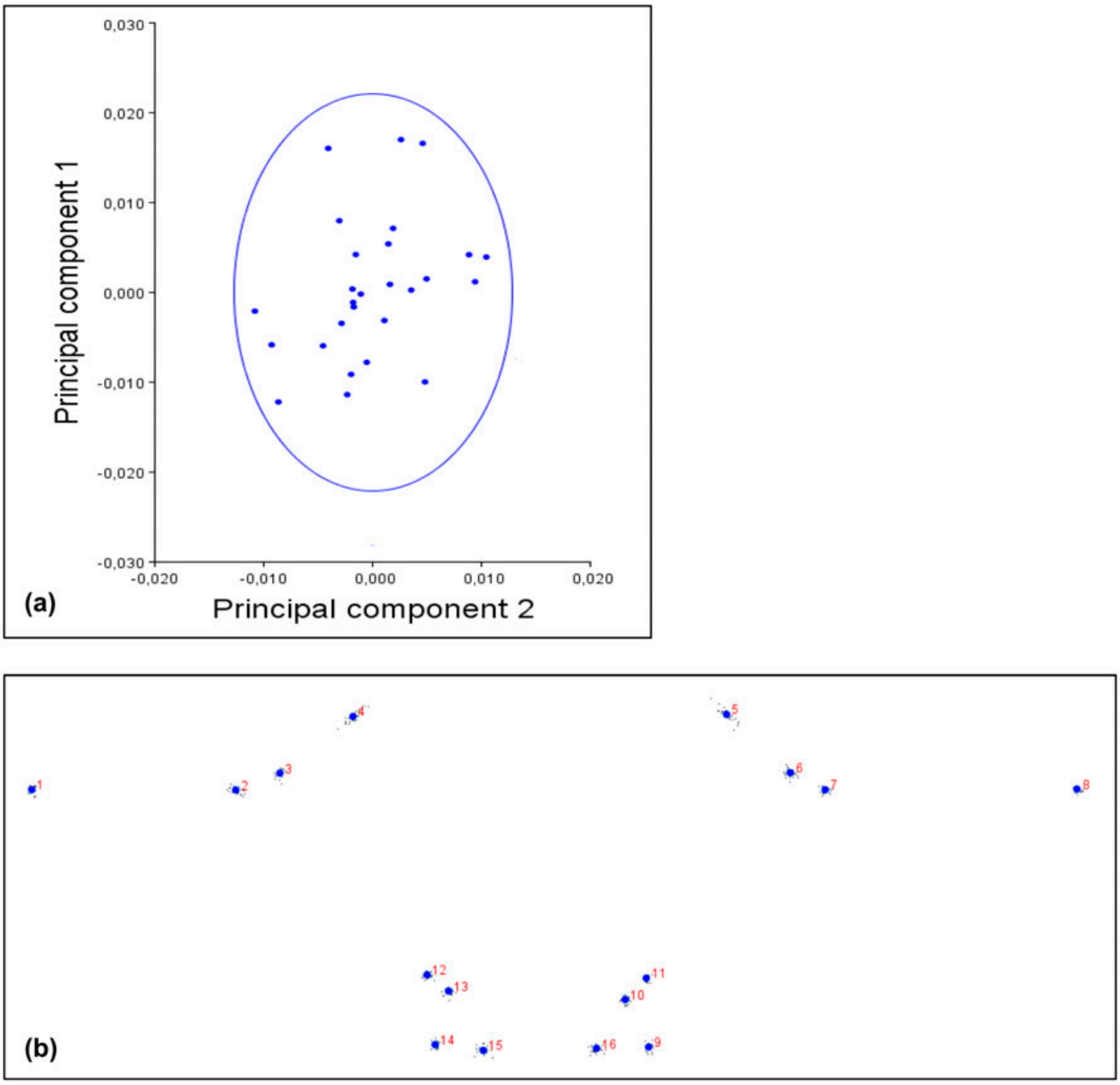

Figure 4. Analysis of repeatability of landmarks used in the morphometric analysis of populations of *V. destructor*: (a) Principal components 1 vs. 2; (b) Analysis of procrustes showing the variability of each landmark used.

Procrustes ANOVA detected significant differences between the shapes of *V. destructor* among all populations from Uruguay ($p < 0.001$). A CVA was conducted with the distances obtained. The analysis revealed separations among the populations under study (Figure 3 (b)). As it was detected on bees, two groups were determined among *V. destructor* populations: the first one includes the southern localities of Atlántida-Colonia-Ombues; the second one comprises Cerro Largo, Artigas and Salto ($p < 0.001$).

Correlation analysis

CVA analyses were performed from covariance matrices obtained for northern and southern populations of both bees to mites. Using Mahalanobis distances obtained for bees and their phoretics mites, we performed cluster analyses using the Ward's method (Figure 5). The Figure shows how mites and bees from a same location are paired.

A correlation analysis between the populations of mites and bees from southern and northern localities of Uruguay was performed. For northern populations of mites and bees an R of 0.63 ($p < 0.0001$) was estimated. The same pattern of correlation was detected for the southern populations of mites and bees ($R = 0.69$; $p < 0.0001$). When a correlation analysis was performed including all mites and bees populations (northern and southern), the R value estimated was 0.71 ($p < 0.0001$) (Figure 6).

Discussion

Morphometric studies based on multivariate analysis have been previously used to characterize the subspecies of *A. mellifera* genus and their parasites

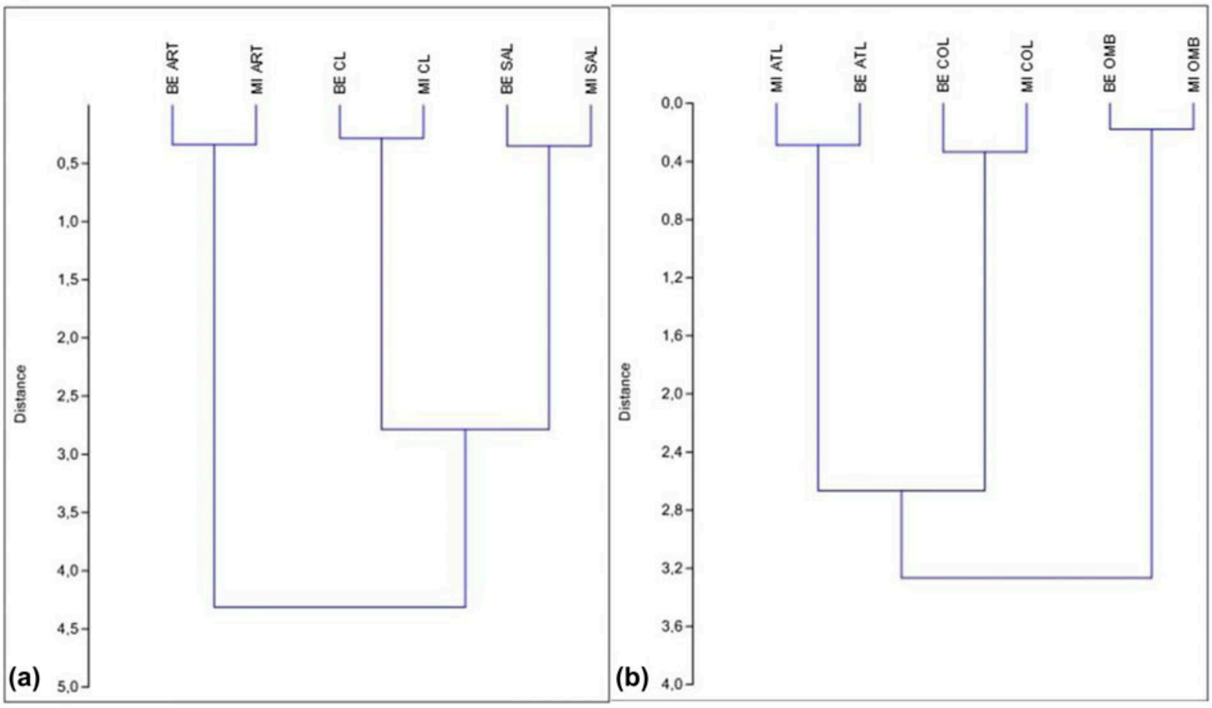

Figure 5. Cluster analysis by Ward's method for populations of bees and mites Uruguay: (a) North; (b) South. MI: Mite/BE: Bee—ART: Artigas/ATL: Atlantida/CL: Cerro Largo/COL: Colonia/OMB: Ombues/SAL: Salto.

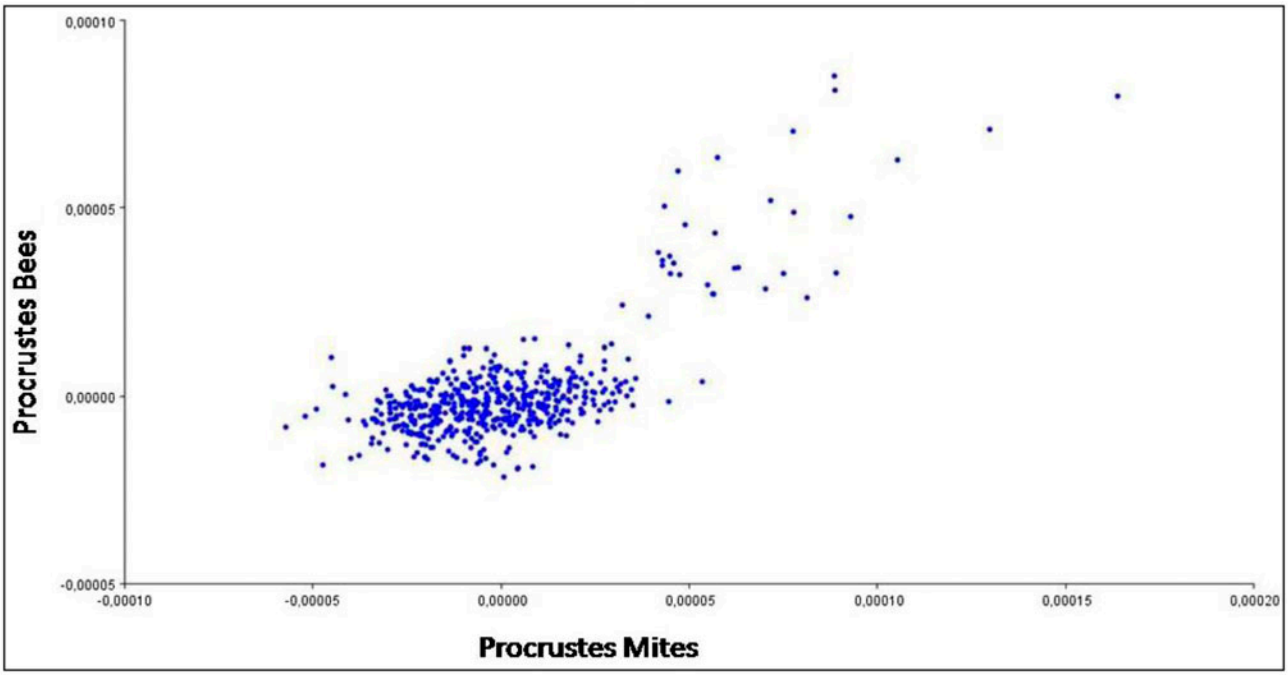

Figure 6. Correlation analysis of Procrustes matrix of populations of bees and mites from Uruguay.

(Anderson & Trueman, 2000; Buco et al., 1987; Delfinado-Baker & Houck, 1989; Maggi et al., 2009; Meixner et al., 2013; Radloff & Hepburn, 2000). This study analyses the morphometric correlation within the host/parasite *A. mellifera*/*V. destructor* system in Uruguay, as a possible explanation to the morphological variations found in *V. destructor* in previous studies (Maggi et al., 2009).

Canonical Variable Analysis (CVA) in the analyzed bee population allowed us to identify a grouping pattern with two clearly defined groups: the first one includes the southern localities of Atlántida-Colonia-Ombúes and the second one comprises Cerro Largo, Artigas and Salto. Accordingly with (Dinitz et al., 2003), the northern group of bee populations is represented by Africanized bees. The morphometrics analysis of the phoretic mite populations from Uruguay revealed (as in the case of bees) two large groups with slight differences. The first group included the southern populations (Colonia-Atlántida-Ombúes); the second group comprised the northern populations (Cerro Largo-Artigas-Salto). This pattern could be explained due to the recent introduction of *V. destructor* in Uruguay (approximately in 1978, (Invernizzi et al., 2011)). It is possible that these bees have established the morphological variation of the mites that currently prevails in Uruguay. When analyzing the Cluster, we observed that groups of bees and mites are paired by each locality.

Previous studies conducted on the genus *Varroa* have demonstrated a wide morphological plasticity in the parasite; (Akimov, Zaloznaya, Efimov, & Galaktionov, 1991) recorded a morphological plasticity between males and females of *V. destructor* within the same population analyzed in different seasons of the year. Such plasticity was reflected when analyzing the different populations of *V. destructor*. The variations observed after performing the correlation analysis showed a significant positive correlation between northern and southern bee populations with their phoretic mites. This finding is of major importance for further progress in studies of the parasite/host *V. destructor*/*A. mellifera* system, and especially the discovery of a new and useful economic tool to discriminate between mite populations present in different regions.

Recently, parasitological studies have interpreted these parasite-host interactions in terms of flux energy, demonstrating that the biomass of the parasite is controlled by food resources, the metabolic rate and the size of the host (George-Nascimento, Muñoz, Marquet, & Poulin, 2004). Further studies should be conducted to analyze if the morphotype observed in *V. destructor* is a consequence of adjustments in the morphology of the parasite to avoid for example, the hygienic behavior of its host which could be explained by the extended phenotype theory proposed by (Lambrechts, Fellous, & Koella, 2006).

Another possible explanation for the results is that different morphotypes registered mites, and their respective correlations with honey bee ecotypes would be the result of local and current conditions (bee body-size variability, selective pressures of acaricides, interactions between genotypes of host and parasite) through small changes in body size, resulting in the life-history strategy best suited to those conditions.

Finally, it should be pointed out that in this investigation the use of landmarks in the morphological analysis of *V. destructor* could be adjusted. To date, there has been no investigation on mite populations. The present results are the first indication of intraspecific differentiation, which allows discriminating different mite populations. Thus, this study reports a new statistical tool for the study of varroa populations and their implications on the *A. mellifera* populations.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Yamandu Mendoza http://orcid.org/0000-0003-0533-2787

References

Adams, D.C., Rohlf, F.J., & Slice, D.E. (2004). Geometric morphometrics: Ten years of progress following the 'revolution'. *Italian Journal of Zoology, 71*, 5–16.

Akimov, I.A., Zaloznaya, L.M., Efimov, V.M., & Galaktionov, Y.K. (1991). Seasonal and geographical variation of morphological characters of *Varroa jacobsoni* (Parasitiformes, Varrodidae): variation of mean values, standard deviations, and coefficients of fluctuating asymmetry. *Entomological Review, 70*, 106–117.

Anderson, D.L., & Trueman, J.W.H. (2000). *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental and Applied Acarology, 24*, 165–189. doi:10.1023/a:1006456720416

Branchiccela, B., Aguirre, C., Parra, G., Estay, P., Zunino, P., & Antúnez, K. (2014). Genetic changes in *Apis mellifera* after 40 years of Africanization. *Apidologie, 45*(6), 752–756. doi:10.1007/s13592-014-0293-2.

Buco, S.M. Rinderer, T.E., Sylvester, H.A., Collins, A.M., Lancaster, V.A., Crewe, R.M., … Winfrey, D. (1987). Morphometric differences between South American Africanized and South African (*Apis mellifera scutellata*) honey bees. *Apidologie, 18*, 217–222.

Delfinado-Baker, M., & Houck, M.A. (1989). Geographic variation in *Varroa jacobsoni* (Acari, Varroidae): application of multivariate morphometric techniques. *Apidologie, 20*, 345–358.

Dinitz, N.M., Egea Soares, A.E., Sheppard, W.S., & Del Lama, M.A. (2003). Genetic structure of honey bee populations from southern Brazil and Uruguay. *Genetics and Molecular Biology, 26*, 47–52.

Francoy, T.M., Wittmann, D., Drauschke, M., Müller, S., Steinhage, V., Bezerra-Laure, M.A.F., … Gonçalves, L. S. (2008). Identification of Africanized honey bees through wing morphometrics: Two fast and efficient procedures. *Apidologie, 39*, 488–494. doi:10.1051/apido:2008028

George-Nascimento, M., Muñoz, G., Marquet, P.A., & Poulin, R. (2004). Testing the energetic equivalence rule with helminth endoparasites of vertebrates. *Ecology Letters, 7*, 527–531. doi:10.1111/j.1461-0248.2004.00609.x

Guerra, J.C.V., Issa, M.R.C., Carneiro, F.E., Strapazzon, R., & Moretto, G. (2010). RAPD identification of *Varroa destructor* genotypes in Brazil and other regions of the Americas. *Genetics and Molecular Research, 9*, 303–308. doi:10.4238/vol9-1gmr696

Hammer, Ø., Harper, D.A.T., & Ryan, P.D. (2001). Past: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica, 4*, XIX–XX.

Invernizzi, C., Antúnez, K., Campa, J.P., Harriet, J., Mendoza, Y., Santos, E., & Zunino, P. (2011). Situación sanitaria de las abejas melíferas en Uruguay [Health situation of honeybees in Uruguay]. *Veterinaria, 47*, 15–27.

Klingenberg, C.P. (2011). MorphoJ: An integrated software package for geometric morphometrics. [Article]. *Molecular Ecology Resources, 11*, 353–357. doi:10.1111/j.1755-0998.2010.02924.x

Lambrechts, L., Fellous, S., & Koella, J.C. (2006). Coevolutionary interactions between host and parasite genotypes. *Trends in Parasitology, 22*, 12–16. doi:10.1016/j.pt.2005.11.008

Maggi, M., Antúnez, K., Invernizzi, C., Aldea, P., Vargas, M., Negri, P., … Eguaras, M. (2016). Honey bee health in South America. *Apidologie, 47*, 835–854. doi:10.1007/s13592-016-0445-7

Maggi, M.D., Sardella, N.H., Ruffinengo, S.R., & Eguaras, M.J. (2009). Morphotypes of *Varroa destructor* collected in *Apis mellifera* colonies from different geographic locations of Argentina. *Parasitology Research, 105*, 1629–1636. doi:10.1007/s00436-009-1605-8

Martin, S.J., & Medina, L.M. (2004). Africanized honey bees have unique tolerance to *Varroa mites*. *Trends in Parasitology, 20*, 112–114. doi:10.1016/j.pt.2004.01.001

Meixner, M.D., Pinto, M.A., Bouga, M., Kryger, P., Ivanova, E., & Fuchs, S. (2013). Standard methods for characterising subspecies and ecotypes of *Apis mellifera*. In V. Dietemann, J.D. Ellis, & P. Neumann (Eds.), *The COLOSS BEEBOOK, Volume I: standard methods for* Apis mellifera *research. Journal of Apicultural Research, 52*(4), doi:10.3896/IBRA.1.52.4.05

Oxnard, C., & O'Higgins, P. (2009). Biology clearly needs morphometrics. Does morphometrics need biology? *Biological Theory, 4*, 84–97. doi:10.1162/biot.2009.4.1.84

Poulin, R., Wise, M., & Moore, J. (2003). A comparative analysis of adult body size and its correlates in acanthocephalan parasites. *International Journal for Parasitology, 33*, 799–805. doi:10.1016/s0020-7519(03)00108-5

Radloff, S., & Hepburn, R. (2000). Population structure and morphometric variance of the *Apis mellifera scutellata* group of honey bees in Africa. *Genetics and Molecular Biology, 23*, 305–316.

Rose, M.R., & Mueller, L.D. (1993). Stearns, Stephen C., 1992. The Evolution of Life Histories. Oxford University Press, London xii + 249 pp., £16.95. *Journal of Evolutionary Biology, 6*, 304–306. doi:10.1046/j.1420-9101.1993.6020304.x

Sammataro, D., Gerson, U., & Needham, G. (2000). Parasitic mites of honey bees: Life history, implications, and impact. *Annual Review of Entomology, 45*, 519–548.

Vizoso, D.B., & Ebert, D. (2005). Phenotypic plasticity of host-parasite interactions in response to the route of infection. *Journal of Evolutionary Biology, 18*, 911–921. doi:10.1111/j.1420-9101.2005.00920.x