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Phenotypic divergences in a subpopulation of Mangalarga Marchador equine: May we improve the breed's definitive register?

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ABSTRACT - The objective of this study was to evaluate which morphometric variables have the greatest influence on the conformation differences observed in a subpopulation of adult Mangalarga Marchador (MM) horses and to summarize these traits through calculated scores (PCA eigenvectors) to later compare with the scores obtained by these animals during the act of registration. Forty-six morphometric traits were used, which included the 12 mandatory measures of the definitive genealogical registration and two of these new ones: "triangle" and the "Z" of the gait. We used 273 adult MM horses with known genealogy. In addition, their weight and 11 indexes used for functional analysis were calculated. Gait and morphology scores were provided by the Associação Brasileira dos Criadores do Cavalo Mangalarga Marchador (ABCCMM). Initially, the correlations between linear morphometry, angular morphometry, live weight, and functional indexes were analyzed separately. There was a greater proportional reduction of variables among linear measurements (from 32 to 15) than angular traits (from 12 to 7), which did not occur for weight and all the indexes. The low association between PCA scores and the subjective scores for morphology and gait at the registration, coupled with the fact that only six of the 12 mandatory linear measurements were related to the variability of the studied animals, suggests a review of the current registration system to improve sensitivity in the search for phenotypic traits of interest to maintain the current breed standard.

Keywords: gait, morphology, morpho-function, PCA, scores

1. Introduction

Conformation traits are part of the genealogical registration system for animals of different species bred for zootechnical purposes (Alderson et al., 1999; Janssens et al., 2004; Khmelnychyi and Karpenko, 2021; Bonow et al., 2024). In equines, studies on morphometry have direct or indirect economic value for breed enhancement, as they provide useful information for performance improvement (Ricard et al., 2020; Ripollés-Lobo et al., 2024), reduction of injury susceptibility (Mostafa and Elemmawy, 2020), and animal development. These characteristics are heritable and linked to the genetic base, allowing for genetic progress (Ricard et al., 2020; Ripollés-Lobo et al., 2024).

The Mangalarga Marchador (MM) is a Brazilian equine breed (Costa et al., 2004; Casiuch, 2016). In 2024, the studbook of the Associação Brasileira dos Criadores do Cavalo Mangalarga Marchador (ABCCMM; "Brazilian Association of Mangalarga Marchador Horse Breeders") surpassed 700,000 registered animals (ABCCMM, 2024). A scientific study (Baena et al., 2020) identified 163 individuals as the main ancestors responsible for the genetic variability observed from 1906 to 2015.

The MM breed standard defines morphofunctional traits for breed expression, conformation, and gait, detailing the head, neck, trunk, limbs, and gait qualities. Horses over three years old are inspected and scored on morphology and gait for classification into books MM3 and MM4 (unknown genealogy) or books MM5 and MM6 (known genealogy), using twelve body measurements (e.g., heights of the withers and croup, thoracic and shin perimeters, and head and croup widths) with standards for specific values (ABCCMM, 2024).

However, the current system for genealogical registration and judging in official events for the MM breed faces limitations that impact the necessary efficiency and reliability for identifying and selecting animals according to breeders' desired criteria. The use of only twelve body measurements, for instance, introduces redundancy, as some are highly correlated with each other or may be insufficient for analyzing the most influential data for the defined breed standard. Furthermore, the subjective assessment of conformation and gait, even with ongoing training to standardize inspectors and judges, introduces unwanted biases or variability, affecting the consistency and analysis of this data.

Objective methods such as morphometry and zootechnical indices help optimize selection (Misk et al., 2015; Bussiman, 2021), and Principal Component Analysis (PCA) offers a solution to simplify data. The PCA reduces complexity by consolidating correlated traits into fewer, independent components, focusing evaluations on critical variables and enhancing objectivity. This approach reduces redundant data and aids a scientifically sound, efficient selection aligned with the morphofunctional profile of the breed.

While few studies apply PCA to analyze MM body proportions and functionality, existing research supports its potential to improve selection accuracy and efficiency (Pinto et al., 2005; Meira et al., 2013; Maruch, 2018; Sellani et al., 2020; Bussiman et al., 2022).

The objective of this study was to examine the correlations between linear morphometry, which includes direct measurements such as lengths and heights of body parts, and angular morphometry, which refers to measurements of body angles, live weight, and zootechnical indices for the functional evaluation of a subpopulation of adult MM horses. Additionally, the study aimed to summarize these parameters through PCA, identifying new scores that highlight which variables contribute most to variability and conformation differences. Finally, the PCA-derived scores were compared with the traditional morphology and gait scores used in genealogical registration.

The hypotheses were: PCA scores could represent conformation differences in the studied animals, and PCA eigenvectors could create new indices more representative than current morphology and gait scores in MM breed control.

2. Material and methods

The data used in this study are part of the sampling obtained during the execution of the research project "Genetic and phenotypic characterization of Mangalarga Marchador horses" (protocol no. CEUA-UFLA: 009/15).

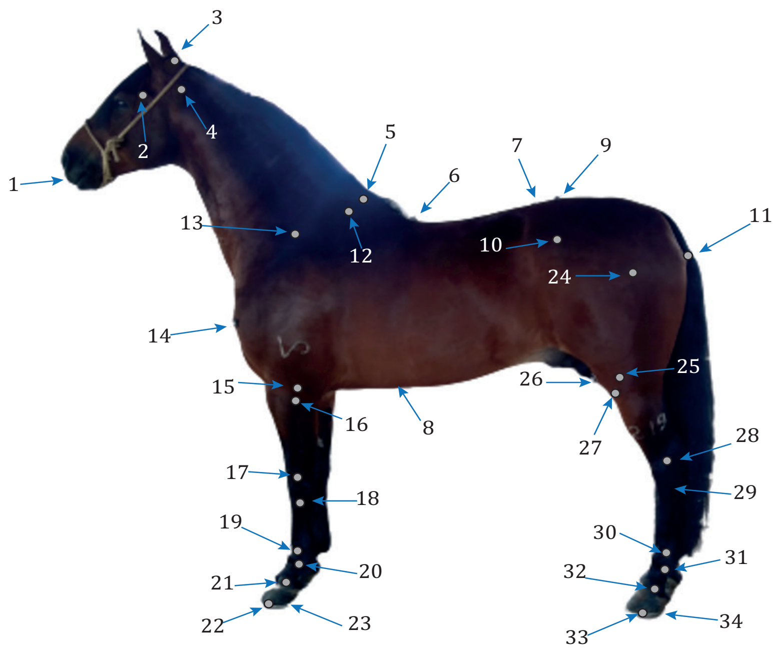
2.1. General data

We evaluated 273 adult MM horses (71 males and 202 females) with a mean age of 11.7 ± 5.1 years, from 52 properties located in the states of Minas Gerais (n = 215), São Paulo (n = 22), Rio de Janeiro (n = 20), Rio Grande do Sul (n = 8), and Bahia (n = 8). All animals had definitive registrations in the

MM5 and MM6 books (with known genealogy) controlled by the Breeders' Association accredited to the Ministry of Agriculture and Livestock in Brazil, descending (sons/daughters, grandsons/ granddaughters, granddaughters or great-grandsons/daughters) from 49 ancestors (30% of the total identified by Baena et al., 2020).

2.2. Morphometric data collection

Initially, the horses were positioned in a forced or controlled stationary position, under a firm and flat floor, for anatomical identification through palpation and subsequent marking of 34 anatomical points with circular adhesive tapes (Figure 1). Then, 46 body measurements (34 linear and 12 angular), cited in the literature (Lage, 2001; Santiago, 2013; Moura et al., 2020), were taken (Tables 1 and 2). Measurements were always taken from the left side of the animals, by a single previously trained evaluator, using a hipometer, measuring tape, universal goniometer, and hoof angulator.



Head-tip of the snout (1); free portion of the left and right temporomandibular joint (2); nuchal crest (3); neck - cranial portion of the lateral aspect of the left atlas wing (4); trunk - highest point of the withers, between the spinous processes of the thoracic vertebrae T5-T6 (5); dorsal portion of the eighth thoracic vertebra T8, after withers (6); space between lumbar vertebra L6 and cranial potion of the sacral vertebra S1 (7); xiphoid process of the external bone (8); highest point of sacral tuberosity (9); cranial portion of the thigh tuberosity of the left and right ileum (10); caudal portion of the left and right ischial tuberosity (11); dorsal edge of the scapula cartilage (12); middle third of the cranial edge of the scapula (13); cranial portion of the greater tuberosity of the left and right humerus (14); lateral epicondyle of the left humerus (15); lateral epicondyle of the left radius (16); lateral styloid process of the left radius, just above the left carpal accessory bone (17); proximal end of the fourth left metacarpal bone (18); lateral of the third left metacarpal bone (19); proximal lateral epiphysis of the left proximal phalanx (20); lateral epiphysis of the left middle phalanx (21); latero-caudal face of the left distal phalanx (22); palmar face of the left distal phalanx (23); pelvic limb-cranial part of the greater trochanter of the left femur (24); lateral epicondyle of the left femur (25); cranial face of the left patella (26); lateral border (27) and lateral malleolus (28) of the left tibia; proximal end of the fourth left metatarsal bone (29); lateral epiphysis of the third left metatarsal bone (30); lateral proximal epiphysis of the left middle phalanx (31); lateral epiphysis of the left middle phalanx (32); latero-caudal face of the left distal phalanx (33); palmar face of the left distal phalanx (34).

Figure 1 - Location of anatomical points used for linear and angular morphometry of Mangalarga Marchador horses.

In addition to the measurements described above, two other body measurements, popularly called "march-gait triangle" and "march-gait Z", were estimated by summing linear measurements (Figure 2), which are empirically associated with gait quality by traditional MM breeders and have not yet been scientifically validated.

Table 1 - Methodology for linear morphometry in Mangalarga Marchador horses, performed from the anatomical points defined in Figure 1

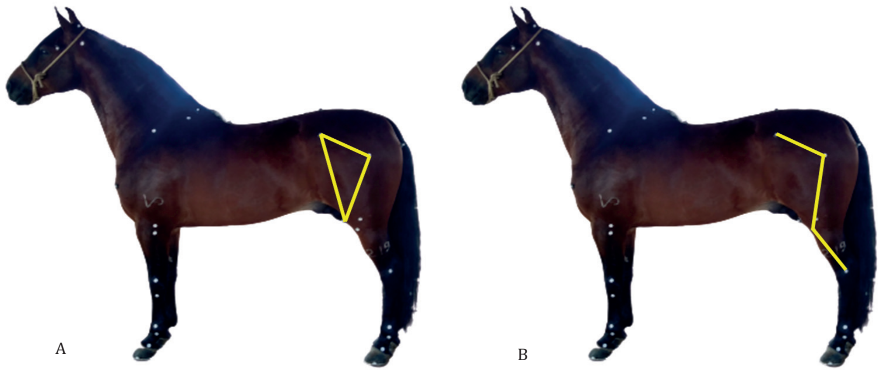
Hipometer measurement (cm)		
Linear measurement	Acronym	Measurement definition
Withers height	WH	Perpendicular distance between point 5 to ground
Back height	BH	Perpendicular distance from point 6 to the ground
Rump height	RH	Perpendicular distance from point 9 to the ground
Side height	SH	Distance between points 6 and 8
Head width	HW	Distance from points 2a and 2b
Rump width	RW	Distance between right and left sides of point 10
Chest width	CW	Distance between the right and left sides of point 14
Head length	HL	Distance between points 1 and 3
Neck length	NL	Distance between points 4 and 13
Length of back-loin	LBL	Distance between points 6 and 7
Croup length	CL	Distance between points 10 and 11
Body length	BL	Distance between points 14 and 11
Metric tape measurement (cm)		
Shoulder length	SL	Distance between points 12 and 14
Arm length	AL	Distance between points 14 and 15
Forearm length	FL	Distance between points e 17 and 15
Cannon bone length	CBL	Distance between points 18 and 19
Cranial gaskin length	CGL	Distance between points 27 and 28
Front hoof-toe length	FHTL	Distance between points 22 and 23
Front hoof-heel length	FHHL	Distance between points 21 and 22
Hip-femur length	HFL	Distance between points 10 and 24
Hip-stifle length	HSL	Distance between points 10 and 26
Femur-stifle length	FSL	Distance between points 24 and 26
Thigh length	TL	Distance between points 24 and 25
Thigh-hock length	THL	Distance between points 24 and 28
Leg length	LL	Distance between points 27 and 28
Posterior cannon bone length	PCBL	Distance between points 29 and 30
Pastern length	PL	Distance between points 31 and 32
Posterior hoof-toe length	PHTL	Distance between points 33 and 34
Posterior hoof-heel length	PHHL	Distance between points 31 and 32
Forearm perimeter	FP	Circumference at the midpoint between the points 16 and 17
Knee perimeter	KP	Circumference at the midpoint between the points 17 and 18
Anterior cannon bone perimeter	ACP	Circumference at the midpoint between the points 18 and 19
Posterior cannon bone perimeter	PCBP	Circumference at the midpoint between the points 29 and 30
Thoracic perimeter	TP	Circumference between points 6 and 8

Adapted from Lage (2001), Santiago (2013), and Moura et al. (2020).

Table 2 - Angular morphometry of Mangalarga Marchador horses

	Left thoracic limb	
Angular measument	Acronym	Measurement definition
Shoulder-ground angle	SGA	Center of the universal goniometer positioned near the scapular-umeral joint (point 14), where the rod containing the protractor will be placed in the horizontal plane, parallel to the ground, while the movable rod will be positioned in the direction of the dorsal edge of the scapula cartilage (point 12).
Humerus-scapula angle	HSA	The center of the universal goniometer positioned near the scapular-umeral joint (point 14); the rod containing the protractor will be placed in the direction of the bone radius of the humerus (point 15), while the movable rod will be positioned in the direction of the dorsal edge of the cartilage of the scapula (point 12).
Radius-humerus angle	RHuA	The center of the universal goniometer positioned near the humerus-radius joint (point 15); the movable rod will be positioned parallel to the lateral face of the forearm to follow its bone radius, while the rod with protractor will be positioned parallel to the arm towards the scapulohumeral joint (point 14).
Metacarpophalangeal angle	MCPA	Placed in the metacarpophalangeal joint (point 20), the rod with the protractor will be placed parallel to the lateral face of the anterior shin to follow its bone radius (point 18), while the small movable rod will be placed parallel to the lateral face of the barracks, following the bone axis of the phalanges (point 21).
Anterior hoof angle	AHA	Hoof angulator placed on the palmar face of the hoof by shifting the movable rod to the cranial face of the hoof clamp.
	Left pelvic member	
Pelvis-ground angle	PGA	The center of the universal goniometer placed on the coxofemoral joint (anatomical point 24), where the rod containing the protractor is positioned in the horizontal plane, parallel to the ground, while the movable rod is positioned towards the center of the iliac tuberosity (anatomical point 10).
Pelvis-femoral angle	PFA	The center of the universal goniometer placed on the coxofemoral joint (anatomical point 24); the rod containing the protractor being placed in the direction of the center of the iliac tuberosity (anatomical point 10), while the movable rod is in the direction of the patella (anatomical point 26), following the bone axis of the femur.
Femur-tibio-patellar angle	FTPA	Universal goniometer used on the reverse side with the marking of the angles facing the horse. Equipment center positioned near the femur-tibio-patellar joint (anatomical point 26); the rod with the protractor is placed in the direction of the coxofemoral joint (anatomical point 24), while the movable rod is in the direction of the center of the tibio-tarsus-metatarsal joint (anatomical point 28).
Femur-tibial angle	FTA	The center of the universal goniometer positioned near the femur-tibial joint (anatomical point 25); the rod with the protractor being placed in the direction of the center of the iliac tuberosity (anatomical point 10), while the movable rod is in the direction of the leg, following the bone axis of the tibia (anatomical point 28).
Tibia-tarsus-metatarsal angle	TTMA	The center of the universal goniometer positioned near the tibio-tarsal-metatarsal joint (anatomical point 28); the rod with the protractor being placed parallel to the lateral face of the leg following its bone axis (anatomical point 27), while the movable rod is on the lateral face of the posterior shin, following its bone axis (anatomical point 30).
Metatarsal-phalangeal angle	MTPA	The center of the universal goniometer positioned in the metatarsophalangeal joint (anatomical point 30); the rod with the protractor being placed parallel to the lateral face of the posterior shin to follow its bone radius (anatomical point 29), while the small movable rod is parallel to the lateral face of the quarter, following the bone axis of the phalanges (anatomical point 32).
Rear hoof angle	RHA	Hoof angulator placed on the plantar face of the hoof by shifting the movable rod to the cranial face of the hoof clamp.

Adapted from Lage (2001), Santiago (2013), and Moura et al. (2020).



A: Linear summation of the hip-femur (HFL), femur-stifle (FSL), and hip-stifle (HSL) lengths; B: linear summation of the hip-femur (HFL), thigh (TL), and leg (LL) lengths.

Figure 2 - Methodology for quantification (in cm) of the "march-gait triangle" (A) and "march-gait Z" (B) empirically cited by Mangalarga Marchador breeders.

2.3. Estimation of live weight and calculation of zootechnical indices used for functional analysis

Live weight was estimated using a formula specifically developed for MM horses (Souza et al., 2017). This formula uses measurements such as thoracic perimeter and specific constants adjusted to accurately reflect the weight of MM, taking into account the morphological traits of the breed. The method was validated by comparing estimated values with actual weights measured on a scale, demonstrating its effectiveness under practical conditions. The following zootechnical indices cited in the literature for the analysis of the functional fitness of equines were also calculated (Bortoni, 1990; Cabral et al., 2004; Rezende et al., 2016):

Withers-rump ratio index (WRR): withers height (WH; cm) divided by rump height RH, cm). A value equal to 1 describes an animal with thoracic and pelvic limbs of the same heights (balance).

Dactylo-thoracic index (DTI): anterior cannon bone perimeter (ACP, cm) divided by the thoracic perimeter (TP, cm), in which DTI > 11.5 (hypermetric animals intended for traction); 10.5 < DTI < 10.8 (eumetric animals intended for saddle); and DTI < 10.5 (hypometric animals considered to have a weak saddle structure).

Body index (BI): body length (BL, cm) divided by TP (cm), in which BL ≥ 90 (longilinear animals); 86 < BL < 88 (mesomorphic animals); and BL < 85 (brevilinear animals). The longilinear animal is more suitable for speed sports activities (e.g., equines of the English thoroughbred breed). The mesomorphic equine has balanced body proportions, being used for saddle activities (e.g., horses of the MM breed); and the brevilinear animal is robust and intended for traction activities (e.g., horses of the Breton breed).

Thoracic index (TI): chest width (CW, cm) divided by TP (cm), in which TI < 0.105 (longilinear animals); 0.110 < TI < 0.108 (mesomorphic animals); and TI > 0.115 (brevilinear animals).

Conformation index (CI): TP squared divided by WH, in which CI < 2.1125 (animals selected for saddle) and CI > 2.1125 (animals aimed at traction).

Load index 1 (LI1): TP (cm) squared and multiplied by the constant 56, divided by WH. A zootechnical index used to estimate the weight that the animal can withstand without excessive effort on the back, working at a trot or gallop (in kg).

Load index 2 (LI2): TP (cm) squared and multiplied by constant 95, divided by WH (cm). A zootechnical index used to estimate the weight that the animal can withstand without excessive effort on the back, working at a pace (in kg).

Relative body index (RBI): BL (cm) multiplied by constant 100 and divided by WH (cm).

Compactness index 1 (CI1): estimated live weight divided by WH (cm), dividing this ratio by 100 in which CI1 > 3.15 (animals for heavy traction): CI1 ≈ 2.75 (animals for light traction); and CI1 ≈ 2.60 (animals intended for saddle).

Compactness index 2 (CI2): estimated live weight divided by WH (cm), subtracted from the value 1, and dividing this ratio by 100 in which CI2 > 9.5 (heavy-draft animals); 8.0 < CI2 < 9.5 (light-draft animals); and 6.0 < CI2 < 7.75 (saddle animals).

Shin load index (SLI): ACP (cm), divided by live weight, the result being multiplied by 100.

2.4. Morphology and march-gait scores

The morphology and gait scores of the animals used in the present study were provided by the genealogical registry sector of the ABCCMM. In the breed standard of the MM available in <breed standard of the Mangalarga Marchador>, the criteria and rules carried out for final approval of the MM horses considered fit for inclusion in the definitive genealogical register/stud book are described in detail.

2.5. Statistical analysis

One of the requirements for dimensionality reduction through PCA is the detection of correlations, at least close to moderate (0.5 or more; −0.5 or less), between variables. Pearson's correlation coefficient has been widely used for the selection of variables, due to its simplicity and for assisting in the recognition of the degree of correlation between input and output variables (Jayaweera and Aziz, 2018).

Then, the main components were analyzed for each subset of selected phenotypes (linear, angular, and zootechnical indices), retaining the components that, together, explained at least 70% of the total variance (Rencher, 2005; Jolliffe and Cadima, 2016; Salem and Hussein, 2019).

At the end of the present study, another PCA was performed to compare whether the scores obtained in the first dimension of each parameter studied were consistent with the qualification made through the subjective notes regarding the score of morphology and gait obtained in the practice of genealogical registration of the equines studied.

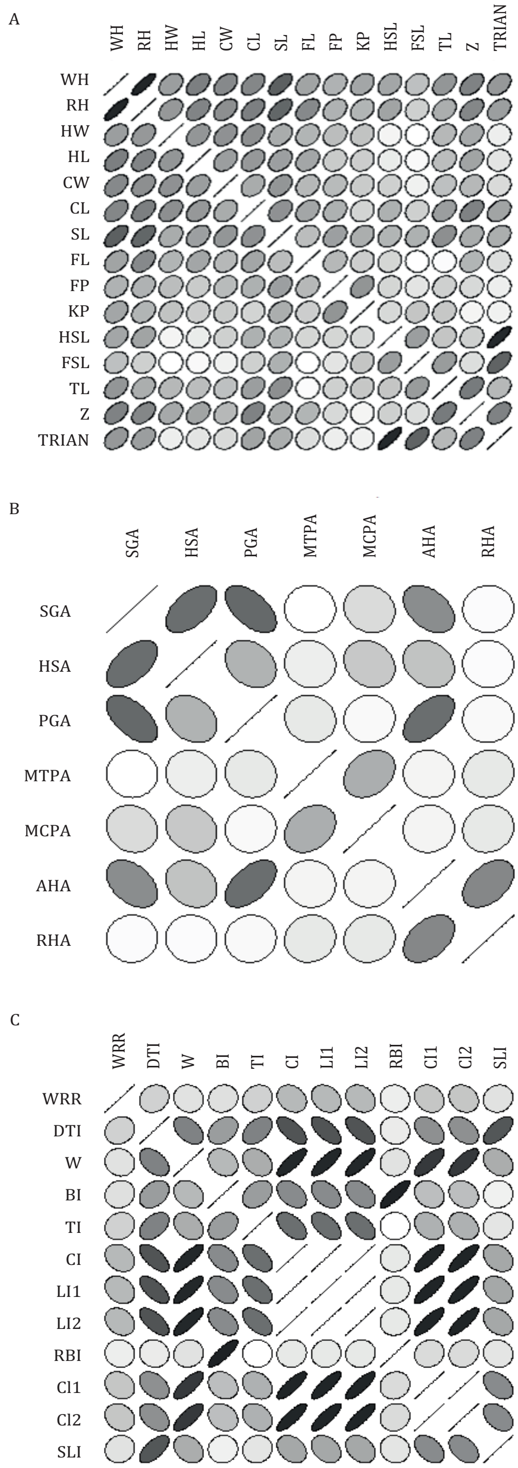
Statistical analyses were performed using R software (R Core Team, 2022), version 4.4.1.

3. Results

3.1. Tracking the variables for PCA (correlation matrices)

Of the total linear measurements, 44.12% (15) showed positive correlations of moderate to high magnitude (Figure 3A). The highest values observed for withers height (WH) and rump height (RH) = 0.83; WH and shoulder length (SL) = 0.61; SL and RH = 0.58; hip-stifle length (HSL) and triangle of the gait (TRIAN) = 0.82; femur-stifle length (FSL) and TRIAN = 0.60; thigh length (TL) and march-gait Z (Z) = 0.60.

In the case of angular measurements, 58.33% (seven) were selected (Figure 3B), with the highest values (0.55 to −0.57) observed between shoulder-ground angle (SGA) and humerus-scapula angle (HSA), SGA and pelvis-ground angle (PGA), and PGA and anterior hoof angle (AHA). All were positive, except for SGA and PGA.



A: WH - withers height; RH - rump height; HW - head width; HL - head length; CW - chest width; CL - croup length; SL - shoulder length; FL - forearm length; FP - forearm perimeter; KP - knee perimeter; HSL - hip-stifle length; FSL - femur-stifle length; TL - thigh length; Z - march-gait Z; TRIAN - march-gait triangle.
B: SGA - shoulder-ground angle; HSA - humerus-scapula angle; PGA - pelvis-ground angle; MTPA - metatarsal-phalangeal angle; MCPA - metacarpophalangeal angle; AHA - anterior-hoof angle; RHA - rear hoof angle.
C: WRR - withers-rump ratio index; DTI - dactyl-thoracic index; W - weight; BI - body index; TI - thoracic index; CI - conformation index; LI1 - load index 1; LI2 - load index 2; RBI - relative body index; CI1 - compactness index 1; CI2 - compactness index 2; SLI - shin load index.

Figure 3 - Graphical representation of significant correlation matrices among linear measurements (A), angular measurements (B), and weight and zootechnical indices for Functional Analysis (C) observed in a Mangalarga Marchador equine subpopulation.

All zootechnical indices calculated showed significant correlations (Figure 3C), with the highest values observed for load index 1 (LI1) and conformation index (CI) = 1; load index 2 (LI2) with LI1, CI = 1; compactness index 1 (CI1) and compactness index 2 (CI2) = 1; CI1, CI2 with CI, LI1, LI2 = 0.83; weight with CI, LI1, LI2 = 0.82.

3.2. Summary of phenotypic data studied through statistical modelling by PCA

For linear measurements (Figure 4A), the retention of the first five components was sufficient to describe 69.2% of the total variability. With regard to angular measurements (Figure 4B), the retention of three dimensions was sufficient to describe 70.7% of the total variability of the data. Regarding the zootechnical indices (Figure 4C), 68.4% of the variability of these variables was explained by only two principal components (PC).

3.3. Eigenvectors of the parameters studied through statistical modelling by PCA

The projection of each vector on the axes illustrates the strength of the association between each variable and the respective main component for linear (Figure 5A) and angular (Figure 5B) measurements, and zootechnical indices (Figure 5C).

There was no relationship between the scores of each trait of the PC1 and the score given subjectively to the MM horses for the score of morphology and gait (Figure 6) for the linear (6A) and angular (6B) measurements and zootechnical indices (6C).

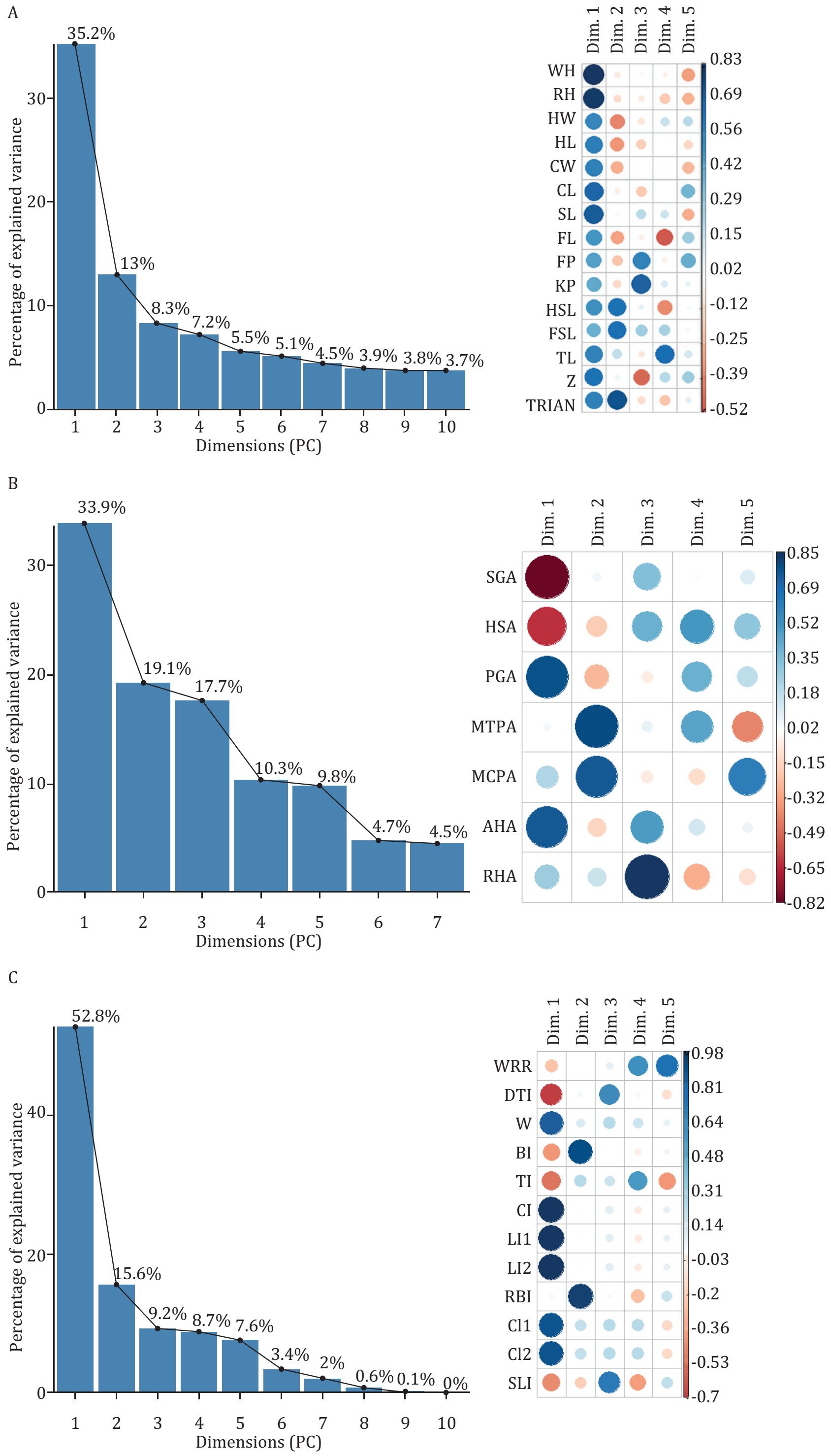
4. Discussion

Various breeders' associations aim to identify phenotypic differences within their animal breeds to select those that meet desired functions while establishing a breed standard. However, focusing on a specific breed standard preserves unique traits but may reduce phenotypic variability within the population (Brooks et al., 2010; Sellani et al., 2020).

Most of the studies related to characterization and/or phenotypic evaluation of the MM breed did not include as many body measurements and other zootechnical indices (for example: 18 linear measurements (Sellani et al., 2020), 20 linear and nine angular measurements (Fonseca et al., 2018), 24 linear and nine angular measurements (Barcelos, 2016), 12 linear measurements (Santiago et al., 2016), 12 linear measurements (Santiago et al., 2014), 22 linear and nine angular measurements (Santiago et al., 2013), 14 linear measurements (Maruch, 2018), 13 linear measurements (Gonçalves et al., 2012), 12 linear and 11 angular measurements (Lage et al., 2009), 25 linear and seven angular measurements (Pinto et al., 2005; Pinto et al., 2008), 12 linear measurements (Zamborlini et al., 1996), and 12 linear measurements (Barbosa, 1993). All of these studies included the 12 measurements obtained compulsorily in the act of definitive genealogical registration of the ABCCMM.

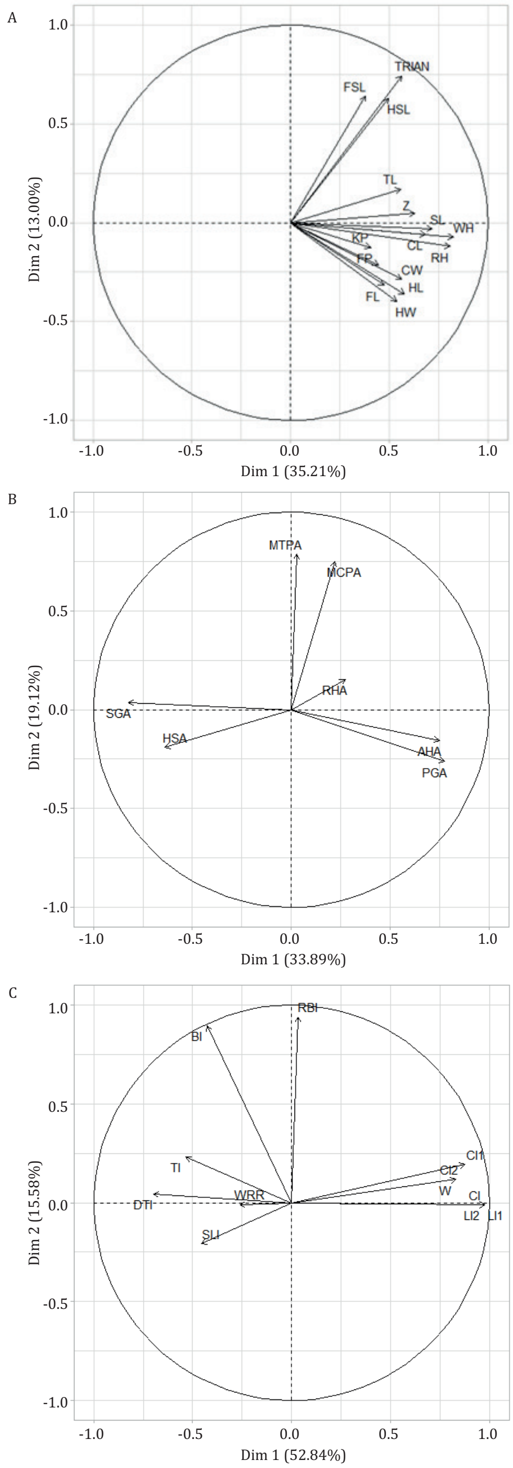
According to Catalano et al. (2017), conformational differences in horses do not seem sufficiently specified for different types of horse use within the same population, such as body shape (rectangular or not), body lines, harmonious proportions, neck length, and height, which can vary widely among individuals of the same breed (Cardoso et al., 2020).

The number of measurements evaluated in this study allowed a more detailed and complete analysis of the MM animals. Therefore, all 15 linear measurements including the two unpublished (Z, TRIAN) summarized in PC1 had a positive contribution. This indicates an increase in variation in the body size of equines as well as traits related to the ability to support the weight and body size of animals. Among them, only six (withers height [WH], rump height [RH], head width [HW], head length [HL], shoulder length [SL], and croup length [CL]) are of a tax nature for the purpose of definitive genealogical registration in the MM. The inclusion of these additional measurements may change the way future studies will be conducted, enabling a better understanding of the morphofunctional traits of equines.



A: WH - withers height; RH - rump height; HW - head width; HL - head length; CW - chest width; CL - croup length; SL - shoulder length; FL - forearm length; FP - forearm perimeter; KP - knee perimeter; HSL - hip-stifle length; FSL - femur-stifle length; TL - thigh length; Z - march-gait Z; TRIAN - march-gait triangle.
B: SGA - shoulder-ground angle; HSA - humerus-scapula angle; PGA - pelvis-ground angle; MTPA - metatarsal-phalangeal angle; MCPA - metacarpophalangeal angle; AHA - anterior-hoof angle; RHA - rear hoof angle.
C: WRR - withers-rump ratio index; DTI - dactyl-thoracic index; W - weight; BI - body index; TI - thoracic index; CI - conformation index; LI1 - load index 1; LI2 - load index 2; RBI - relative body index; CI1 - compactness index 1; CI2 - compactness index 2; SLI - shin load index.

Figure 4 - Percentage (bars) and loads (circles) of the variables of each main component retained and which explained approximately 70% of the accumulated variance for linear (A) and angular (B) measurements and weight and zootechnical indices (C) observed in adult horses Mangalarga Marchador.



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B: SGA - shoulder-ground angle; HSA - humerus-scapula angle; PGA - pelvis-ground angle; MTPA - metatarsal-phalangeal angle; MCPA - metacarpophalangeal angle; AHA - anterior-hoof angle; RHA - rear hoof angle.
C: WRR - withers-rump ratio index; DTI - dactyl-thoracic index; W - weight; BI - body index; TI - thoracic index; CI - conformation index; LI1 - load index 1; LI2 - load index 2; RBI - relative body index; CI1 - compactness index 1; CI2 - compactness index 2; SLI - shin load index.

Figure 5 - Eigenvectors of morphometric characteristics for linear (A) and angular (B) measurements and weight and zootechnical indices (C) with the largest contributions to each dimension of the main components observed in adult Mangalarga Marchador horses.

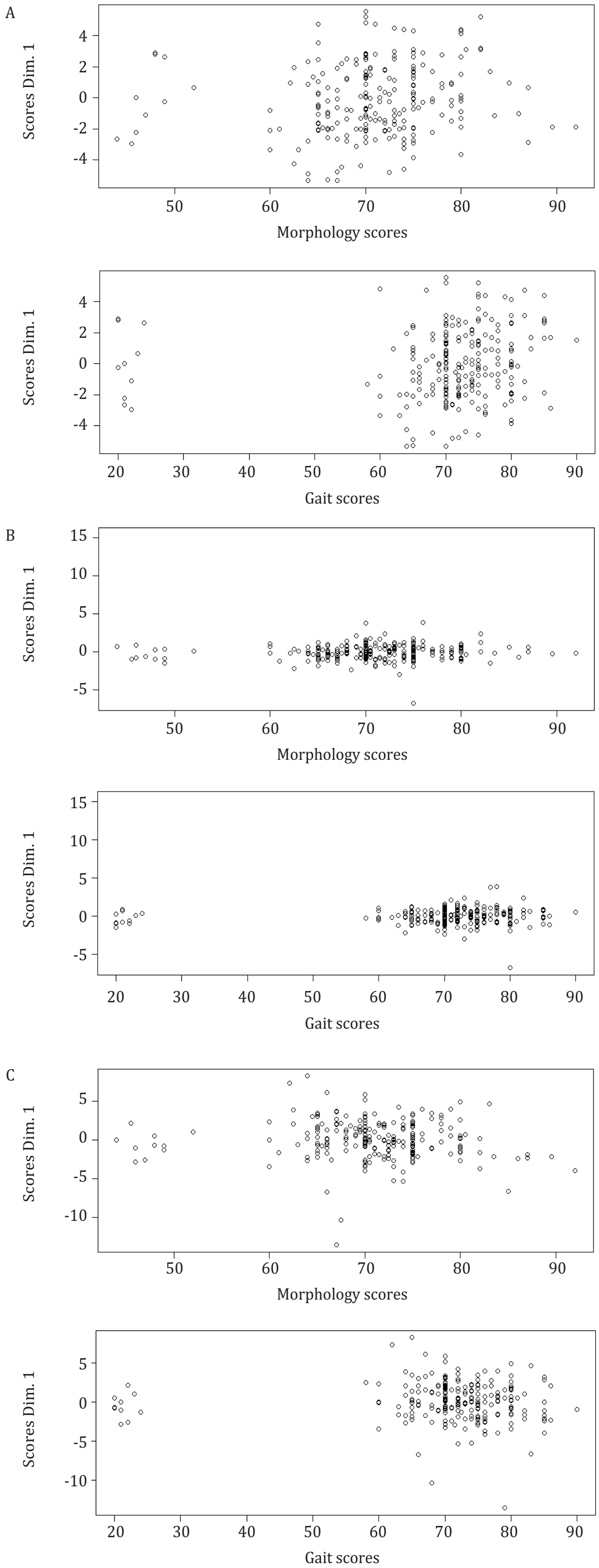

Figure 6 - Scores of PC1 morphometric characteristics for linear (A) and angular (B) measurements and weight and zootechnical indices (C) associated with morphology and gait scores observed in adult Mangalarga Marchador horses.

Application of PCA in sets of morphofunctional traits in MM is scarce. We identified that the studies of Sellani et al. (2020), Meira et al. (2013), and Pinto et al. (2005) used PCA in phenotypic data of MM foals. The authors reported that PCA helped in the identification of measurements that did not contribute significantly to the total variation or that were strongly correlated with other variables of greater relevance. This, in practical terms, could result in significant savings of time and resources without substantial loss of information.

The variability observed in these traits in this study across PC1 and PC2 supports findings reported by Brooks et al. (2010) and Staiger et al. (2016). Brooks et al. (2010) employed PCA to understand morphological variation across 65 horse breeds, resulting in two key dimensions for authors' comprehension. The PC1 showed a positive correlation of all morphometric measures with animal body size, while PC2 captured variation in bone width versus length, quantifying bone thickness variation in animals. Similarly, Staiger et al. (2016) evaluated 35 morphometric measurements and four subjective trait scores of horses with different gaits using PCA. The PC1 summarized positive variations related to overall body size and PC2 highlighted bone thicknesses.

The variations observed in the linear and angular measurements of this study can be attributed to the historical process of MM development, as suggested by Santiago et al. (2016). Costa et al. (2004) reported that, at first, breeders chose animals based on their preferences and interests to set specific traits that would affect their breeding, which would thus directly imply the quality of gait. This resulted in a poorly defined animal population, both phenotypically and genetically.

Phenotypic variations can also be attributed to the different functions in which MM animals are used, such as field work, leisure, and sports (Costa et al., 2004). Additionally, MM has also adapted to different environments in the Brazilian territory where it is raised. This was reflected in the conformation of the animals, since it is often shaped to adapt to different climatic and geographical conditions, as observed by Lage et al. (2009) and Zamborlini et al. (1996).

For a possible improvement of the genealogical record in the ABCCMM, it would be interesting to include measures such as chest width, forearm length, knee circumference, femur-stifle length, hip-stifle length, and thigh length, whose values suffered relevant variations in the present study.

The angular measurements, at the moment, are not measured by technicians accredited by ABCCMM for the definitive genealogical record and are only evaluated indirectly and subjectively through the morphology and gait scores. The lengths of the anatomical bases as well as their directions are observed. The lower accuracy of this form of evaluation when compared with the adoption of objective methods possibly influenced the lack of standardization in these segments.

Zootechnical indices for functional fitness analysis help breeders select horses with morphological and biomechanical traits suited for specific functions like riding, work, sports, and leisure (Rezende et al., 2016). However, there is a lack of scientific literature validating these indices for adult horses. This study calculated indices using withers height (WH), chest width (CW), and thoracic perimeter (TP). Withers height and CW were key components in the PCA. Notably, CW is not included in the genealogical registration of horses in the ABCCMM, indicating a need to review which morphometric variables aid in phenotypic standardization and improve selection criteria for horses with four-stroke progress.

In the analysis of linear and angular measurements, weight, and zootechnical indices, it was expected to find an association between the indices calculated in PC1 and the scores assigned by a specialized evaluator to the animals. However, animals with almost identical conformations, according to objective criteria, received discrepant evaluations. The divergences between the scores of the summarized morphometric traits in PC1 and the gait and morphology scores of the definitive genealogical record suggest that the current practice is little sensitive to distinguish the conformation and gait of the animals selected as breeders. Therefore, these results corroborate the studies by Borowska and Lewczuk (2023) and Pimentel et al. (2018).

Borowska and Lewczuk (2023) compared traditional subjective 100-point judging systems with linear measurements in assessing dressage and jumping Warmblood mares. They found that morphometric measurements, which consider variations in shoulder length, loin length, and croup shape, are more significant for scoring. Dressage mares, with better movement and morphological balance, were better evaluated through these measurements than jumping mares. The subjective 100-point system did not reveal these differences as it lacks detailed descriptions. Therefore, the authors recommend using morphometry for breeding and judging equines.

Pimentel et al. (2018) assessed direct, indirect, and total effects of linear and angular body measurements on subjective morphological scores of Criollo horses in competitions. They reported that judge subjectivity resulted in significant score variations. The measurements most affecting total scores were neck length, upper neck circumference, rump angle, and chest width. Together, these factors explained 83% of variation in morphological scores.

The lack of phenotypic standardization (Figure 4 A, B, and C) also highlights a lack of common objective among MM horse breeders. Some of them focus on selecting for sport traits, while others seek horses with morphofunctional traits suitable for robust farm work. This raises the question: should MM horses be selected with a single phenotypic standard for the various desired functions?

Current subjective methods are insufficient to select animals with dual aptitude. However, by using weightings of traits summarized by PCA in primary dimensions, we hypothesize that selection indices could be created. These indices would reflect the importance of each morphofunctional characteristic in different desired functions for these animals. We acknowledge the limitations of this study, but it represents the initial step toward proposing objective methods that may efficiently capture phenotypic differences in MM breed horses. The breed standard serves as a guide but needs enhancement with more detail and objectivity.

This objective approach aims to eliminate subjective analysis of morphology and gait scores by employing a formula that calculates an estimated score each animal should receive based on its body measurements and their relevance to the desired function. This methodology applies not only to MM horses but also provides valuable insights for other breeds and animal species, thereby enhancing accuracy in identifying phenotypic variability. We recommend replicating the methodology used in this study across other breeds and species with a larger sample size to achieve greater method accuracy.

Therefore, these evidences suggest a re-evaluation to improve the morphofunctional evaluation process of MM animals. The inclusion of measures that proved relevant in the PCA can provide a more comprehensive and refined view, contributing to more accurate matings. This addition may benefit the breed by improving the selection criteria for marching equines, thus opening the way for further investigations.

5. Conclusions

The low association of the scores calculated in the PCA for morphometric variables with the subjective scores for morphology and gait scores, combined with the fact that only six of the 12 mandatory linear measures in the genealogical registration process were related to the variability of the animals studied, suggests the revision of the current systematic aiming to improve sensitivity in the search for phenotypic traits of interest to guarantee the current breed standard.

For future research, associating bioestatiscal and data science, are recommended to validate this approach in larger samples and explore its adaptation for other breeds, as well as consider the inclusion of new variables to increase selection accuracy.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

Conceptualization: Freire, A.; Souza, F. A. C.; Ribeiro, A. O.; Meirelles, S. L. C.; Araujo, K. V.; Souza, J. C. and Moura, R. S. **Data curation:** Ribeiro, A. O.; Meirelles, S. L. C.; Araujo, K. V.; Souza, J. C. and Moura, R. S. **Formal analysis:** Freire, A.; Ribeiro, A. O.; Souza, J. C. and Moura, R. S. **Funding acquisition:** Araujo, K. V.; Meirelles, S. L. C. and Moura, R. S. **Investigation:** Freire, A.; Souza, F. A. C.; Ribeiro, A. O.; Meirelles, S. L. C.; Souza, J. C. and Moura, R. S. **Methodology:** Freire, A.; Ribeiro, A. O.; Meirelles, S. L. C. and Moura, R. S. **Project administration:** Meirelles, S. L. C. and Moura, R. S. **Resources:** Meirelles, S. L. C.; Araujo, K. V. and Moura, R. S. **Supervision:** Moura, R. S. **Validation:** Freire, A.; Meirelles, S. L. C. and Moura, R. S. **Visualization:** Freire, A.; Souza, F. A. C.; Ribeiro, A. O.; Araujo, K. V. and Moura, R. S. **Writing – original draft:** Freire, A.; Ribeiro, A. O.; Souza, J. C. and Moura, R. S. **Writing – review & editing:** Freire, A.; Ribeiro, A. O.; Meirelles, S. L. C.; Araujo, K. V.; Souza, J. C. and Moura, R. S.

Conflict of interest

The authors declare no conflict of interest.

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