

# Following the Footsteps of the South American *Equus*: Are Autopodia Taxonomically Informative?

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**Abstract** The genus *Equus* originated in the Pliocene Epoch of North America, and its arrival in South America is likely related to the Great American Biotic Interchange that took place in the transition of Pliocene to Pleistocene. Currently, there are five recognized species for the South American continent: *Equus neogeus*, *E. santaelenae*, *E. insulatus*, *E. andium*, and *E. lasallei*. The taxonomy of the genus is traditionally based in part upon the proportions of the autopodia. The aim of this study is to evaluate the diagnostic importance of the autopodia of South American *Equus* through comparative and multiple statistical analyses. Therefore, we analyzed metacarpals, metatarsals, and phalanges from all available South American *Equus*, with the exception of *E. lasallei*, which is only known

by a skull. We also examined the North American species *E. occidentalis*, as it has been interpreted to be closely related to South American *Equus*. Results showed no significant differences between the various South American species according to the dimensions and proportions of the autopodia. A continuum of gradual linear variation among the species was revealed, with superimposition between autopodial characters. The succession and overlap of species indicated that the South American *Equus* might represent a type of cline.

**Keywords** *Equus* · Cline · Taxonomy · Autopodia

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## Introduction

The genus *Equus* originated in North America during the Pliocene Epoch, with subsequent dispersal to Eurasia and Africa (MacFadden 1994; Alberdi and Prado 2004). During the Pleistocene, the genus reached its peak geographical distribution in the wild, dispersing to every continent but Australia and Antarctica (MacFadden 1994; Eisenberg and Redford 1999; Alberdi and Prado 2004). Currently, wild *Equus* is restricted to Eurasia and Africa. The end-Pleistocene extinction of the genus in the Americas may be related to the negative selection of the megafauna at the beginning of the Holocene (MacFadden 1994).

The arrival of *Equus* in South America is likely related to the Great American Biotic Interchange (GABI) that occurred at the end of the Pliocene and the beginning of the Pleistocene (Webb 1978; MacFadden 1994; Bacon et al. 2016). This biogeographic event was made possible by the uplift of the Isthmus of Panama in the Pliocene, which provided a terrestrial connection between the American continents (Webb 1978). The GABI is an event composed of four incidents of dispersal (Woodburne 2010; MacFadden 2013): GABI 1 (2.6 to 2.4 million years ago [mya]); GABI 2 (approximately 1.8 mya); GABI 3 (1.0 to 0.8

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mya); and GABI 4 (approximately 0.125 mya). According to Woodburne (2010), the dispersal of *Equus* to South America took place during GABI 4, because the earliest South American records date to the upper Pleistocene. More recently, MacFadden (2013) recognized the arrival of *Equus* earlier during GABI 3, based upon records of the genus from middle Pleistocene deposits in south Bolivia. Moreover, Webb (1978) correlated the arrival of *Equus* in South America with the beginning of the Pleistocene, when the intercontinental connection functioned as a savanna corridor.

Regardless of the biogeographic proposal for the origin of *Equus* in South America, species in the genus were established in distinct regions and localities of the continent (Fig. 1), presenting fossiliferous records dated from middle to upper Pleistocene (Prado and Alberdi 1994). The most commonly cited recent taxonomic proposals (Alberdi and Prado 1992, 2004) recognized five species of *Equus* in South America: *Equus neogeus* Lund, 1840, *E. santaeelenae* Spillmann, 1938, *E. insulatus* C. Ameghino in F. Ameghino, 1904, *E. andium* Branco, 1883 ex Wagner, 1860, and *E. lasallei* Daniel, 1948.

South American *Equus* has previously been interpreted to belong in the subgenus *Amerhippus*, which was characterized

by the morphology of the vomer, a massive jaw, and relatively short distal limb elements (Hoffstetter 1950; MacFadden and Azzaroli 1987). The subgenus *Amerhippus* was originally proposed based on one single character, the lack of lower incisor infundibula, and was also interpreted to include the North American species *E. occidentalis* Owen, 1863 (Hoffstetter 1950; MacFadden and Azzaroli 1987). However, according to Eisenmann (1979) and Alberdi and Prado (2004), the use of the lack of lower incisor infundibula as a diagnostic character is dubious because it is highly variable. Furthermore, Orlando et al. (2008) conducted DNA-based phylogenetic analyses revealing that all Pleistocene South American species of *Equus* were members of the caballine horse lineage, not a distinct subgenus as first suggested by Hoffstetter (1950). Therefore, the validity of the subgenus *Amerhippus* is questionable, and is not employed herein.

Prado and Alberdi (1994, 2004) stated that the most evident differences between the South American species can be observed in the postcranial elements, mainly in the autopodia, and that these are more related to variations in the dimensions rather than of shape. Consequently, Alberdi and Prado (1992, 2004) recognized: 1) *E. neogeus* as the species with the longest and most gracile autopodia among the South American species, being extremely similar to *E. santaeelenae*, differing mainly by the occlusal breadth of the lower cheek teeth; 2) *E. santaeelenae* as slightly more robust than *E. neogeus*, with measurements of the first phalanx (1PHIII) similar to *E. neogeus* while data from metatarsals (MTIII) more closely aligned with *E. insulatus*; 3) *E. andium* and *E. insulatus* as the species with the shortest autopodia compared to the others; and 4) *E. andium* as presenting a slightly more pronounced shortening of the autopodia than *E. insulatus*.

In the current literature, the presence of monodactylism, relatively straight and high crowned cheek teeth, and dental characters including complex enamel plications and protocones are used in the diagnosis of the genus *Equus* (MacFadden 1994). Therefore, these features are not necessarily sufficient to distinguish among different species of South American *Equus*. For this reason, the taxonomy of South American equids has traditionally been based upon the proportions of the bones of the distal appendicular apparatus, especially the autopodia (Alberdi and Prado 2004). However, a clear distinction between these species is lacking in the literature. This contribution intends to comparatively analyze the autopodia of South American *Equus* in order to evaluate their diagnostic importance.

## Materials and Methods

In this study we analyzed MCIII, MTIII, and 1PHIII from all of the recognized South American species of the genus *Equus*, with the exception of *E. lasallei*, a species known



**Fig. 1** Map of fossil records and geographical distribution of the five species of *Equus* in South America: *E. neogeus* (circle), *E. santaeelenae* (dash), *E. insulatus* (triangle), *E. andium* (square), and *E. lasallei* (star)

only from a skull (Alberdi and Prado 2004). We also included the North American species *E. occidentalis* in the analysis, as this form has been assigned by some authors to the subgenus *Amerhippus* (Hoffstetter 1950); this species was interpreted here as the outgroup. All specimens analyzed represent mature individuals. The sample represents the diversity of the South American localities and the specimens are deposited in the paleontological collections of the following institutions: Museu Nacional (MN) and Museu de Ciências Naturais da Pontifícia Universidade Católica de Minas Gerais (MCL), Brazil; Museo Argentino de Ciencias Naturales ‘Bernardino Rivadavia’ (MACN-Pv), and Museo de La Plata (MLP), Argentina; Museo de Historia Natural ‘Gustavo Orcés V’ (V and MECN), Ecuador; Museo Nacional Paleontología y Arqueología de Tarija (TAR), Bolivia; American Museum of Natural History (AMNH) and La Brea Tar Pits and Museum (formerly the George C. Page Museum) (GCPM), USA; and Muséum National d’Histoire Naturelle (MNHN), France.

Postcranial elements were analyzed both morphologically and metrically, using data acquired with the help of digital calipers with a 0.01 mm precision, following the recommendations of Eisenmann et al. (1988; Table 1).

Three different statistical analyses were also conducted. In order to determine whether the set of samples originate from the same distribution or not – that is, whether the observed differences are disparate between populations or not (McDonald 2014) – a non-parametric Kruskal-Wallis analysis of variance was executed. This analysis was executed in the program Bioestat 5 (Ayres et al. 2007) using Dunn’s method as a post hoc test. In the Kruskal-Wallis analysis, we evaluated measurements related both to the Gracility Index (Alberdi and Prado 2004) and to biomechanical aspects of the limb (such as anteroposterior and mediolateral diameter of the diaphysis that usually relates to bone resistance), those being measurements 1, 3, 4, 5, and 10 of MCIII and MTIII and 1, 3, 5, 7, and 8 of 1PHIII.

Data were also analyzed via Principal Component Analysis (PCA) for the comparison of quantitative characters, in order to evaluate the morphometric variations within the population and examine any evident patterns of phenetic variation (Neff and Marcus 1980; Reis 1998; Missagia 2014). This analysis was run in the program Past (Hammer 2012). The missing values of the matrix were replaced through the Iterative Imputation method, which replaces the missing value by the average value of the corresponding column for an initial PCA, which serves as the basis for computing values of a regression, replacing it until it reaches convergent value (Missagia 2014). Only specimens sufficiently complete to enable at least three measurements were considered; this procedure eliminated noise from the replacement of missing values by the statistical program used.

Furthermore, the Gracility Index was calculated according to the following formula:  $\frac{\text{minimal breadth (measure 3)} \times 100}{\text{maximal length (measure 1)}}$ ; in accordance with Hussain (1971; Alberdi and Prado 2004) and Alberdi (1974; Alberdi and Prado 2004). A linear regression between the index and the maximal length was obtained using the Reduced Major Axis regression (RMA) method, which allows to describe the relation between two variables as well as to estimate a variable in regards of another (Lapponi 2000). Together with this analysis, the coefficient of determination ( $R^2$ ) was calculated; it is a percentage that evaluates how much a variable can be explained by another one according to the presented values (Lapponi 2000).

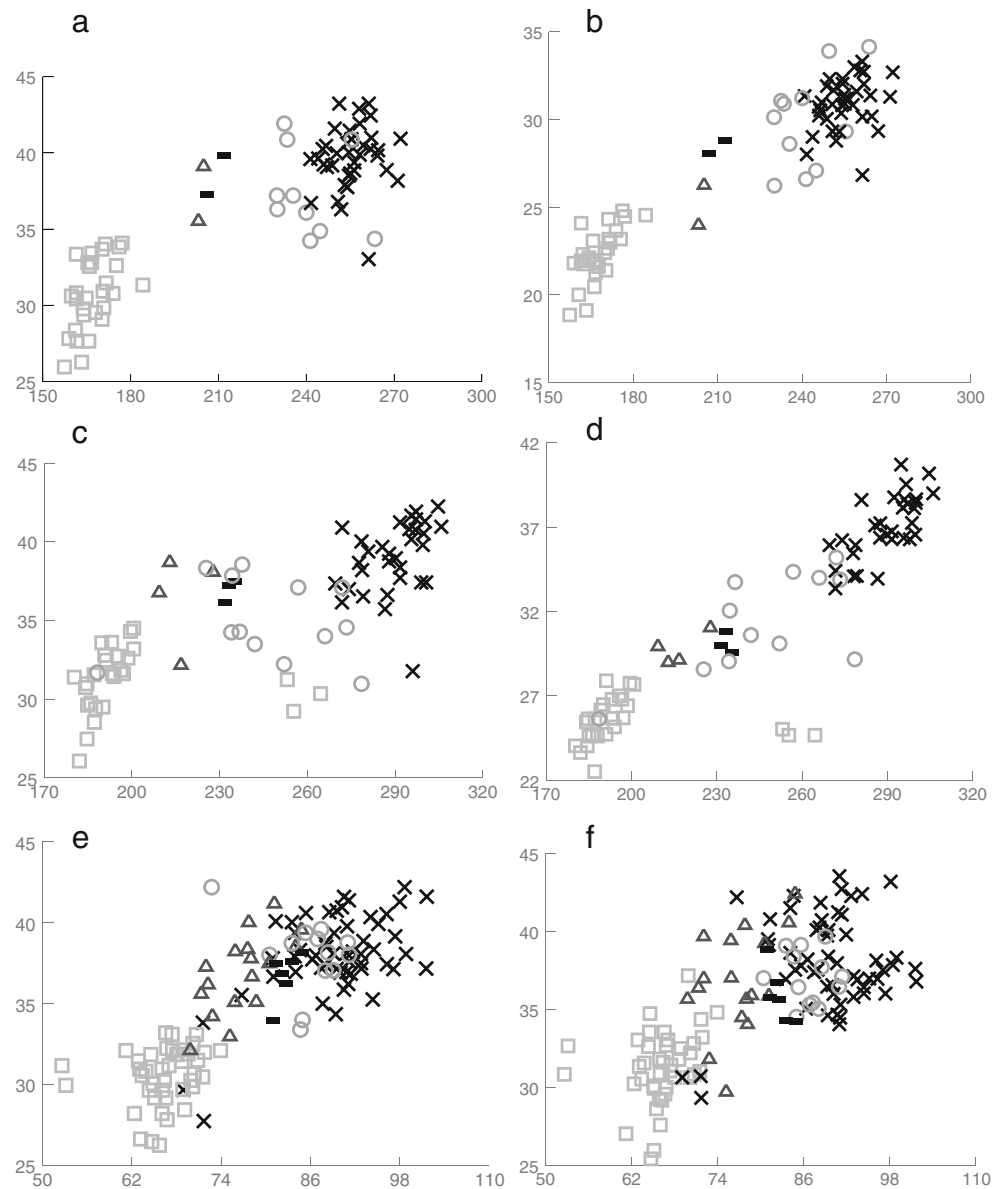
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Results

The comparative morphological analyses (Fig. 2) carried through the comparison of measures 1 and 3, 1 and 4 of MCIII and MTIII, and measures 1 and 3, 1 and 5 of 1PHIII of the species *E. occidentalis*, *E. neogeus*, *E. santaeelenae*, *E. insulatus*, and *E. andium* do not distinguish species based solely on size. Rather, the specimens are scattered in a continuum of linear gradual variation in which different species succeed and overlap one another. Regarding the MCIII (Fig. 2a,b), there is an overlap between *E. occidentalis* and *E. neogeus* and between *E. santaeelenae* and *E. insulatus*, although the small sample size of the latter two species for those bony elements probably is insufficient to observe if there is a superimposition with other species. With respect to the MTIII (Fig. 2c,d), there is a partial superimposition between *E. occidentalis*, *E. neogeus*, *E. santaeelenae*, and *E. insulatus*. In contrast, the 1PHIII (Fig. 2e,f) clearly shows a continuum, with major superimposition between all the species.

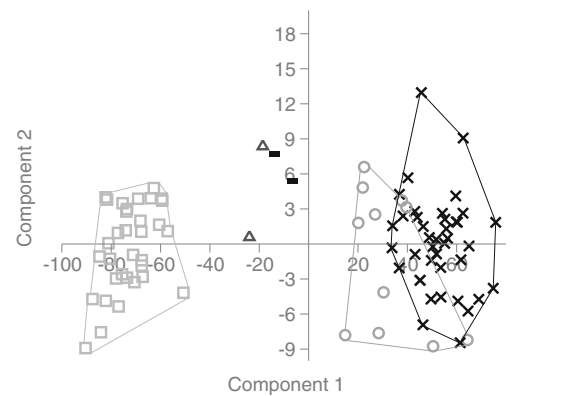
The PCA analysis on data from MCIII (Fig. 3) yielded positive values for the coefficients of PC1, indicating that this component describes variation in size among the groups, while PC2 presented positive and negative values, indicating a variation of shape or proportion between the groups. PC1 is responsible for 98% of the variation, and indicated that measures 1 and 2 were the most significant variables. PC2 is responsible for 0.5% of the variation, with measures 2 and 5 as the most significant variables. Considering PC2, there is a clear superimposition between *E. occidentalis* and *E. neogeus*, but in this case PC1 allows us to once again observe the continuum between the species, with partial overlap between *E. occidentalis* and *E. neogeus* as well as between *E. santaeelenae* and *E. insulatus*; the only species that is distinguished from the others in this context is *E. andium*. It is important to highlight that the small sample size of

**Fig. 2** Comparative morphological analysis: correlation between measures  $1 \times 3$  and  $1 \times 4$  of MCIII (graphs A and B, respectively) and MTIII (graphs C and D, respectively) and measures  $1 \times 3$  and  $1 \times 5$  of IPIII (graphs E and F, respectively) of *E. occidentalis* (cross), *E. neogeus* (circle), *E. santaeeleanae* (dash), *E. insulatus* (triangle), and *E. andium* (square)

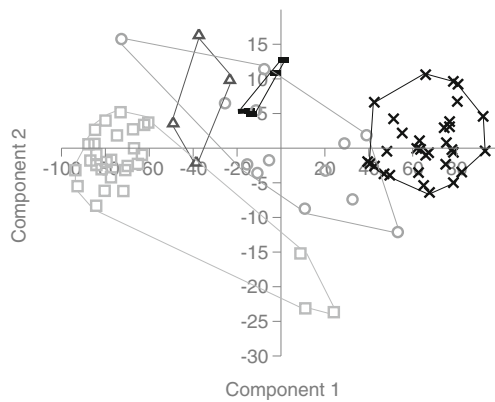


*E. santaeeleanae* and *E. insulatus* might be limiting the superimposition with *E. neogeus* and *E. andium*, mainly because the superimposition with those latter species is observed in other appendicular elements.

The PCA analysis conducted for data from MTIII (Fig. 4) produced positive values for the coefficients of PC1, while PC2 again presented coefficients with positive and negative values. PC1 is responsible for 97% of the variation of the species, presenting measures 1 and 2 as the most significant variables, while PC2 is responsible for 1% of the variation of the species, yielding measures 5 and 10 as the most significant variables. Regarding PC1, once again a continuum between the species is observed, with superimposition between *E. neogeus*, *E. santaeeleanae*, and *E. insulatus*. Although PC2 is only responsible for 1% of the variation, it is noted that, combined with the PC1 variation, it is possible to



**Fig. 3** Scores projection of Principal Components 1 (98%) and 2 (0.5%) based on 15 measures of MCIII for the species *E. occidentalis* (cross), *E. neogeus* (circle), *E. santaeeleanae* (dash), *E. insulatus* (triangle), and *E. andium* (square)

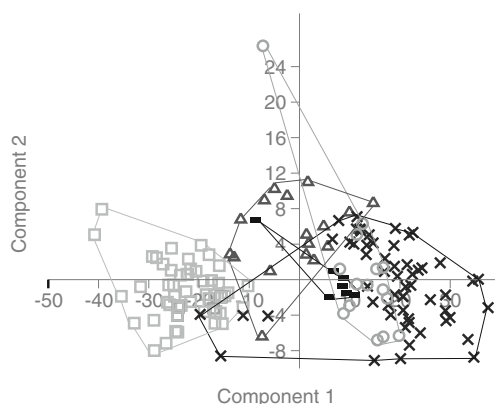


**Fig. 4** Scores projection of Principal Components 1 (97%) and 2 (1%) based on 14 measures of MTIII for the species *E. occidentalis* (cross), *E. neogeus* (circle), *E. santaeelenae* (dash), *E. insulatus* (triangle), and *E. andium* (square)

distinguish *E. andium*, as well as *E. occidentalis*, from the others species.

The PCA analysis conducted for measurements of 1PHIII (Fig. 5) yielded positive values for the PC1 and positive and negative values for PC2. PC1 accounts for 88% of the variation, with measures 1 and 2 being the most significant variables; while PC2 is responsible for 5% of the variation, giving measures 4 and 5 as the most significant variables. In regard to PC2, a complete superimposition between the species is observed and, regarding PC1, once again we observe a continuum with partial overlap between *E. neogeus*, *E. santaeelenae*, and *E. insulatus*. The North American *E. occidentalis* overlaps here with all the South American species, although the majority of the sample overlaps only with *E. neogeus*.

The Kruskal-Wallis analysis conducted for the measures of MCIII (Table 2) yielded significant differences in all measurements analyzed in comparisons of *E. andium* versus *E. neogeus* and *E. andium* versus *E. occidentalis*, as well as for measurement 10 in the comparison of *E. insulatus* versus *E. occidentalis*. The other analyses presented non-significant values.



**Fig. 5** Scores projection of Principal Components 1 (88%) and 2 (5%) based on 9 measures of 1PHIII for the species *E. occidentalis* (cross), *E. neogeus* (circle), *E. santaeelenae* (dash), *E. insulatus* (triangle), and *E. andium* (square)

The Kruskal-Wallis metric performed for measurements of MTIII (Table 3) gave results with significant values for all data analyzed in the comparison of *E. andium* versus *E. occidentalis*; for measurements 1, 4, and 5 in the comparison of *E. andium* versus *E. neogeus*; for measurements 1, 3, 4, and 10 in *E. neogeus* versus *E. occidentalis*; for measure 3 in the comparison of *E. andium* versus *E. santaeelenae*; and for measurement 1 in the comparison of *E. insulatus* versus *E. occidentalis*. Non-significant values were obtained in all other analyses.

The Kruskal-Wallis test conducted for measurements of 1PHIII (Table 4) yielded results with significant values for all the measurements analyzed in the comparison of *E. andium* with all the other species; for measurements 1, 7, and 8 in the comparison of *E. insulatus* versus *E. occidentalis*; and for measurement 8 in *E. santaeelenae* versus *E. occidentalis*. Non-significant values were obtained in all other analyses.

The coefficients of determination obtained in the analysis of the Gracility Index relative to the maximum length of the bone (Measure 1) were quite low: for MCIII,  $R^2 = 0.46$  for South American species and  $R^2 = 0.11$  for the North American one; for MTIII,  $R^2 = 0.58$  for South American species and  $R^2 = 0.07$  for that from North America; and for 1PHIII,  $R^2 = 0.11$  for South American species and  $R^2 = 0.16$  for the North American species. The generally low  $R^2$  values indicate that the variation of the Gracility Index is too high to confirm whether there is a correlation with the length of the bone. Despite these low coefficients, all analyses indicated negative allometry of the Gracility Index relative to Measure 1: the longer the bone is, the lower this index tends to be (Fig. 6). For MCIII, the regression for South American species is  $y = -0.06x + 27.23$  and, for the North American species,  $y = -0.11x + 43.82$ . For MTIII, the regression for South American species is  $y = -0.06x + 28.38$  and, for the North American species,  $y = -0.07x + 34.38$ . For 1PHIII, the regression for South American species is  $y = -0.43x + 76.79$  and, for the North American species,  $y = -0.68x + 104.10$ . The allometric patterns of the South and North American species were similar, especially for MTIII and 1PHIII. However, the low values of  $R^2$  are insufficient to infer similarities or discrepancies between them.

## Discussion

The results of this study revealed that it is not possible to distinguish the South American species based solely upon dimensions and proportions of their autopodia. All morphological analyses indicate there is superimposition of autopodial metric characters between different species, characterizing a continuum of gradual linear variation in which different species succeed and overlap one another. This conclusion was corroborated by the statistical analyses, which also showed the presence of marked overlap between the species.

**Table 1** Measurements used in this analysis, according to the Hipparion Conference (Eisenmann et al. 1988)

Measure	MTIII	MCIII	IPHIII
1	Maximal length	Maximal length	Maximal length
2	Medial length	Medial length	Anterior length
3	Minimal breadth	Minimal breadth	Minimal breadth
4	Depth of the diaphysis	Depth of the diaphysis	Proximal breadth
5	Proximal articular breadth	Proximal articular breadth	Proximal depth
6	Proximal articular depth	Proximal articular depth	Distal breadth at the tuberosities
7	Maximal diameter of the articular facet for the third tarsal	Maximal diameter of the articular facet for the third carpal	Distal articular breadth
8	Diameter of the articular facet for the fourth tarsal	Diameter of the articular facet for the fourth carpal	Distal articular depth
9	Diameter of articular facet for the second tarsal	Diameter of articular facet for the second carpal	Minimal length of the trigonum phalangis
10	Distal maximal supra-articular breadth	Distal maximal supra-articular breadth	
11	Distal maximal articular breadth	Distal maximal articular breadth	
12	Distal maximal depth of the keel	Distal maximal depth of the keel	
13	Distal minimal depth of the lateral condyle	Distal minimal depth of the lateral condyle	
14	Distal minimal depth of the medial condyle	Distal minimal depth of the medial condyle	
16		Diameter of the posterior facet for the fourth carpal	

PCA analyses reinforced that there is a superimposition among the species, especially for PC2. But the continuum became evident only by the partial overlaps between the scores of PC1. The loadings obtained in the PCA revealed that the most significant variables of PC1 of MCIII, MTIII, and IPHIII were the measures 1 and 2 of its respective post-cranial elements. They also revealed that the variation is more related to size rather than form or proportion. Thus, it is concluded that length is the most significant character to define the variation of the group, but it is not enough to distinguish the species, as clearly shown by the overlaps.

The Kruskal-Wallis analysis revealed that, in general, there is a greater distinction in measures between the extremities of the continuum, but there is not significant distinction among species that are closer in the continuum. Therefore, it is observed that *E. andium* and *E. occidentalis* always appeared in opposite extremes of the continuum and, consequently, can be easily distinguished by these measurements and proportions. These two

species also presented a more significant difference from intermediate species that appear closer to the opposite end of the continuum. For example, *E. andium* is more distinct from *E. neogeus* than from *E. insulatus* or *E. santaeelenae*.

The Gracility Index analysis suggests a certain relation of negative allometry with the length of the bone, which means that there is a certain tendency for the bone to be more slender the longer the bone is. However, the low coefficient of determination indicates that this trend is not significant. Nonetheless, this result does not corroborate the previously-proposed idea relating the Gracility Index to the type of ground in which slenderness would be more related to harder grounds and robustness would be more related to softer grounds (Prado and Alberdi 1994; Alberdi and Prado 2004). According to these authors, the South American species of *Equus* present great differences in the Gracility Index, those being related to the environment each one inhabited. Our results do not corroborate this interpretation.

**Table 2** Result of the Kruskal-Wallis statistical analysis of MCIII by comparison of the species. Significant values ( $p < 0.05$ ) were obtained for measures 1, 3, 4, 5, and 10 in *E. andium* versus *E. neogeus* and *E. andium* versus *E. occidentalis* as well as for measure 10 in *E. insulatus* versus *E. occidentalis*; non-significant values were obtained in the other analyses

	Measure 1	Measure 3	Measure 4	Measure 5	Measure 10
<i>E. andium</i> versus <i>E. insulatus</i>	ns	ns	ns	ns	ns
<i>E. andium</i> versus <i>E. santaeelenae</i>	ns	ns	ns	ns	ns
<i>E. andium</i> versus <i>E. neogeus</i>	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$
<i>E. andium</i> versus <i>E. occidentalis</i>	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$
<i>E. insulatus</i> versus <i>E. santaeelenae</i>	ns	ns	ns	ns	ns
<i>E. insulatus</i> versus <i>E. neogeus</i>	ns	ns	ns	ns	ns
<i>E. insulatus</i> versus <i>E. occidentalis</i>	ns	ns	ns	ns	$p < 0,05$
<i>E. occidentalis</i> versus <i>E. santaeelenae</i>	ns	ns	ns	ns	ns
<i>E. neogeus</i> versus <i>E. occidentalis</i>	ns	ns	ns	ns	ns
<i>E. santaeelenae</i> versus <i>E. occidentalis</i>	ns	ns	ns	ns	ns
<i>E. neogeus</i> versus <i>E. occidentalis</i>	ns	ns	ns	ns	ns

**Table 3** Result of the Kuskal-Wallis statistical analysis of MTIII by comparison of the species. Significant values ( $p < 0.05$ ) were obtained for measures 1, 3, 4, 5, and 10 in *E. andium* versus *E. occidentalis*; for measures 1, 4, and 5 in *E. andium* versus *E. neogeus*; for the measures

1, 3, 4, and 10 in *E. neogeus* versus *E. occidentalis*; for the measure 1 in *E. insulatus* versus *E. occidentalis*; and for measure 3 in *E. andium* versus *E. santaeelenae*; non-significant values were obtained in the other analyses

	Measure 1	Measure 3	Measure 4	Measure 5	Measure 10
<i>E. andium</i> versus <i>E. insulatus</i>	ns	ns	ns	ns	ns
<i>E. andium</i> versus <i>E. santaeelenae</i>	ns	$p < 0,05$	ns	ns	ns
<i>E. andium</i> versus <i>E. neogeus</i>	$p < 0,05$	ns	$p < 0,05$	$p < 0,05$	ns
<i>E. andium</i> versus <i>E. occidentalis</i>	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$
<i>E. insulatus</i> versus <i>E. santaeelenae</i>	ns	ns	ns	ns	ns
<i>E. insulatus</i> versus <i>E. neogeus</i>	ns	ns	ns	ns	ns
<i>E. insulatus</i> versus <i>E. occidentalis</i>	$p < 0,05$	ns	ns	ns	ns
<i>E. santaeelenae</i> versus <i>E. neogeus</i>	ns	ns	ns	ns	ns
<i>E. santaeelenae</i> versus <i>E. occidentalis</i>	ns	ns	ns	ns	ns
<i>E. neogeus</i> versus <i>E. occidentalis</i>	$p < 0,05$	$p < 0,05$	$p < 0,05$	ns	$p < 0,05$

As far back as Darwin (1859), naturalists have often preferred not to classify as distinct species any taxa that are closely similar and connected by intermediated gradations. Huxley (1939) proposed the term “cline” for the geographic gradients in phenotypic characters, in order to assist the taxonomy of organisms that present gradual variation according to several factors, such as geography and ecology, in a way of indicating gradual spatial variation within a population. Aleixo (2007) defined clines as geographic gradients in phenotypic characters forming intergradation zones, describing clines as diagnostic metapopulations in geographical extremities, connected by a zone with individuals with intermediate characteristics between them. Clines might be related to several phenotypic

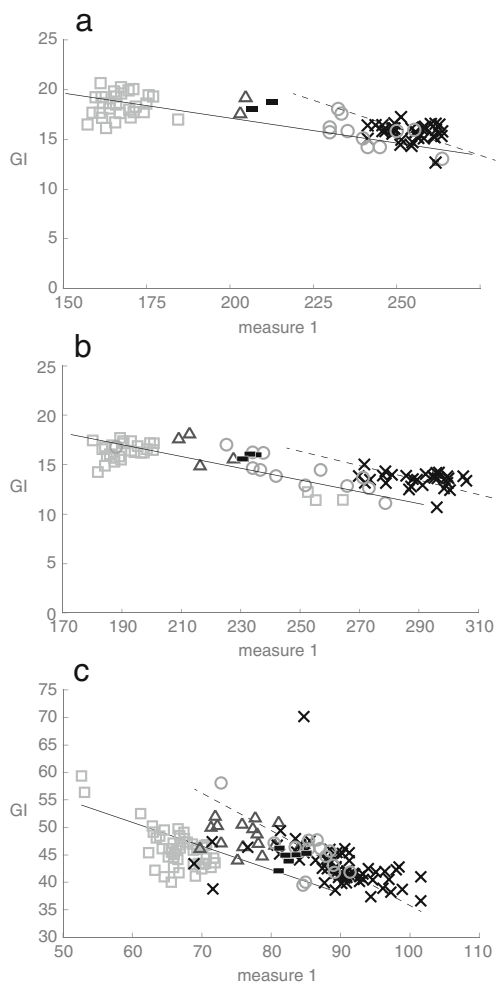
characteristics, such as size, color, pattern of physiology, resistance or even patterns between two or more distinct variables, and may or may not be correlated, directly or indirectly, to environmental gradients or other environmental factors (Huxley 1939).

Based on the results of our analyses, South American *Equus* can be recognized as a species cline. The continuum revealed by the graphs, corroborated by the statistical analyses, illustrates clinal variation in which *E. andium* and *E. neogeus* represent the extreme phenotypes, being the diagnostic metapopulations, while *E. insulatus* and *E. santaeelenae* would represent the interconnected intermediate forms. It is important to note that the North American

**Table 4** Result of the Kuskal-Wallis statistical analysis of 1PHII by comparison of the species. Significant values ( $p < 0.05$ ) were obtained for measures 1, 3, 5, 7, and 8 in *E. andium* versus *E. insulatus*, *E. andium* versus *E. santaeelenae*, *E. andium* versus *E. neogeus*, *E. andium* versus

*E. occidentalis*; for measures 1, 7, and 8 in *E. insulatus* versus *E. occidentalis* and for measure 8 in *E. santaeelenae* versus *E. occidentalis*; non-significant (ns) values were obtained in the other analyses

	Measure 1	Measure 3	Measure 5	Measure 7	Measure 8
<i>E. andium</i> versus <i>E. insulatus</i>	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$
<i>E. andium</i> versus <i>E. santaeelenae</i>	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$
<i>E. andium</i> versus <i>E. neogeus</i>	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$
<i>E. andium</i> versus <i>E. occidentalis</i>	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$	$p < 0,05$
<i>E. insulatus</i> versus <i>E. santaeelenae</i>	ns	ns	ns	ns	ns
<i>E. insulatus</i> versus <i>E. neogeus</i>	ns	ns	ns	ns	ns
<i>E. insulatus</i> versus <i>E. occidentalis</i>	$p < 0,05$	ns	ns	$p < 0,05$	$p < 0,05$
<i>E. santaeelenae</i> versus <i>E. neogeus</i>	ns	ns	ns	ns	ns
<i>E. santaeelenae</i> versus <i>E. occidentalis</i>	ns	ns	ns	ns	$p < 0,05$
<i>E. neogeus</i> versus <i>E. occidentalis</i>	ns	ns	ns	ns	ns



**Fig. 6** Linear regression analysis: relation between the Gracility Index with measure 1 (maximal length) of MCIH (a), MTIH (b), and IPIH (c) of *E. occidentalis* (cross), *E. neogeus* (circle), *E. santaeelenae* (dash), *E. insulatus* (triangle), and *E. andium* (square); trend line of South American *Equus* (straight line) and trend line of *E. occidentalis* (dotted line)

*E. occidentalis* was not considered part of this metapopulation because it presents cranial diagnostic characters that distinguish it from the South American species. It was also not included because it did not participate in the GABI event and because it is not inserted in the same geographic context as the South American *Equus*.

## Conclusions

The currently taxonomy of South American *Equus* is most often based upon the proportions of the bones of the distal appendicular apparatus, especially the autopodia. However, our study revealed a continuum of gradual variation of autopodial dimensions in which the species succeed and overlap. Results indicate that such characters are inadequate to distinguish and diagnose the South American species.

It is proposed that South American species of *Equus* most likely represent a species cline in which *E. andium* and *E. neogeus* represent the diagnostic metapopulations - that is, they represent either phenotypic extreme in this gradual variation, and the other species would be configured as the spectrum of intermediated forms of the intergradation zone.

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