

Dear Editor

I have read carefully the Author's letter of reply for the manuscript "*On the morphological variability of Ichniotherium tracks and evolution of locomotion in the sistergroup of amniotes*" by Michael Buchwitz and Sebastian Voig. Many of the answers seem essentially tautological. It seems the Authors repeat what they have done (which is already clear from the manuscript) rather than explaining why they did not follow for some of the analyses the minimum standard protocols, which I suggested in the review according to the existing literature about PCA's methodology. At the end, I have the distinct feeling that they are eluding the core of the question.

One thing I find curious is how the Authors have put so much effort into trying to defend the results in order to not to change anything, rather than re-run just a simple analysis to show that their results remain solid even by following the standard protocol. Especially because it took less than a half hour to me to do re-run some of the analysis, which I provide briefly below.

The scatter plots that follows shows the PCA results using a standard protocol, were linear measurements are transformed into logarithms and are not mixed with ratios and angular values. All the analyses were performed using the original dataset provided by the Authors in the supplementary materials. I have conducted a first analysis only on the pes measurements. As expected, the loadings show that the first component essentially indicates the absolute size of the specimens. So, it results more informative the scatterplot of component 2 against component 3 (shown below). The morphospace of the three sites (Birkheide, Gottlob and Bromacker) are mostly overlapping, not justifying a morphometric separation. Also the scatter plot of component 1 versus component 2 is substantially different from that shown in Figure 10 of the original manuscript, with a substantial overlap between Birkheide and Bromacker specimens.

A second interesting result, already predicted only on the theoretical level in the first revision, derived from analyses of loadings for the second principal component (which explains changes in morphology and not in absolute dimensions). The load for digit IV is very high (see loading below); this indicates that the four digit accounts substantially to the total variance, i.e. digit IV is very variable. It follows that normalizing all the measures for one of the most variable parameters is a major methodological problem, and can lead to misleading results and interpretations.

A second important analysis, that has not been conducted in the original work, is to consider both manus and pes measurements in the same PCA analysis. Even in this case, the principal component 1 essentially indicate the absolute size. Again, the scatter plot of the principal components 1 and 2 shows a consistent overlap between Birkheide and Bromacker specimens, while a greater overlap among the convex hulls of the three localities is observed in the scatter plot of PC2 vs PC3.

A final analysis was conducted on track parameters identified by linear measures; as already specified in the review (even if it was not taken into account in the submitted review), the simple PCA cannot be applied to values expressed in angles. Even worse is to mix linear measurements, ratios, and angular measurements in the same analysis. The new analysis following a standard protocol just on linear measurements led to the scatter plots reported below. In the scatter plot of the first two principal components a great overlap is observed again between the Birkheide and Bromacker specimens, and in the scatter plot of PC2 vs. PC3 the specimens from Gottlob and from Birkheide are essentially totally included within the morphospace individuated by the Bromacker specimens.

In conclusion, the simple application of a standard protocol to the dataset shows that the three morphotypes are not at all distinct on a morphometric basis (even considering the trackway parameters), and all the discussion and conclusion using the original PCA are not supported. The authors point out more than once that PCA is not the focus of their work, but only an exploratory method. Even though I disagree on this point, I believe that no Journal would accept an analysis not conducted on the light of the standard protocol (especially if the authors themselves admit that such a protocol has not been followed).

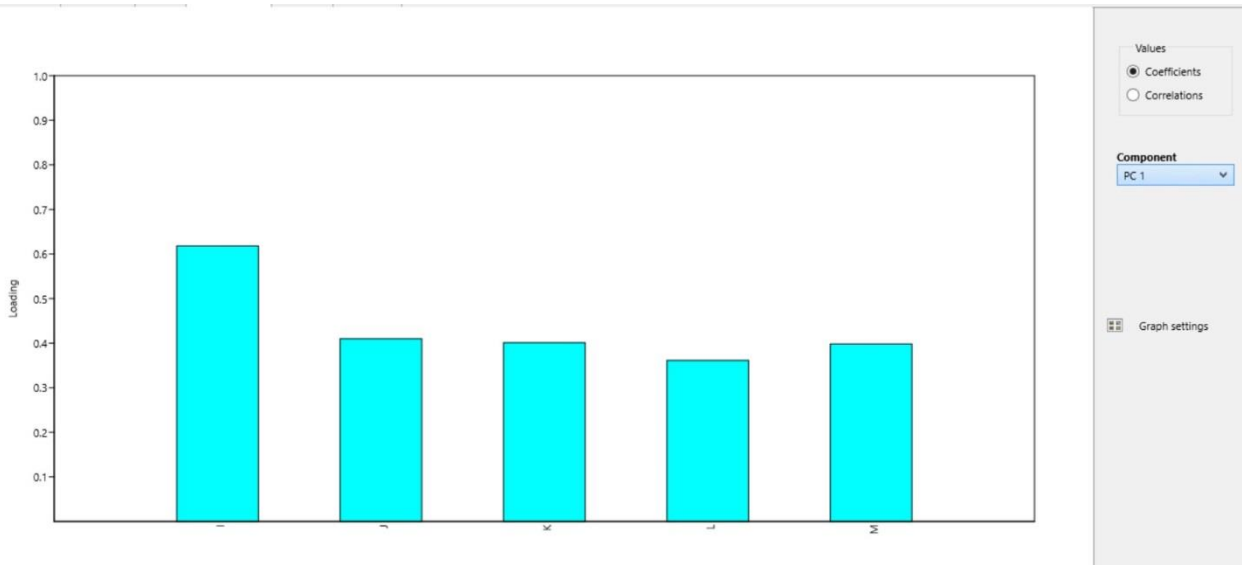
So, being able to decide only on the analyzes I re-run personally, and being unable to accept principal component analyses that mixed linear values with ratios and angles, I consider that the manuscript, in its current form, does not respect the methodological standards to be accepted for publication.

Best regards

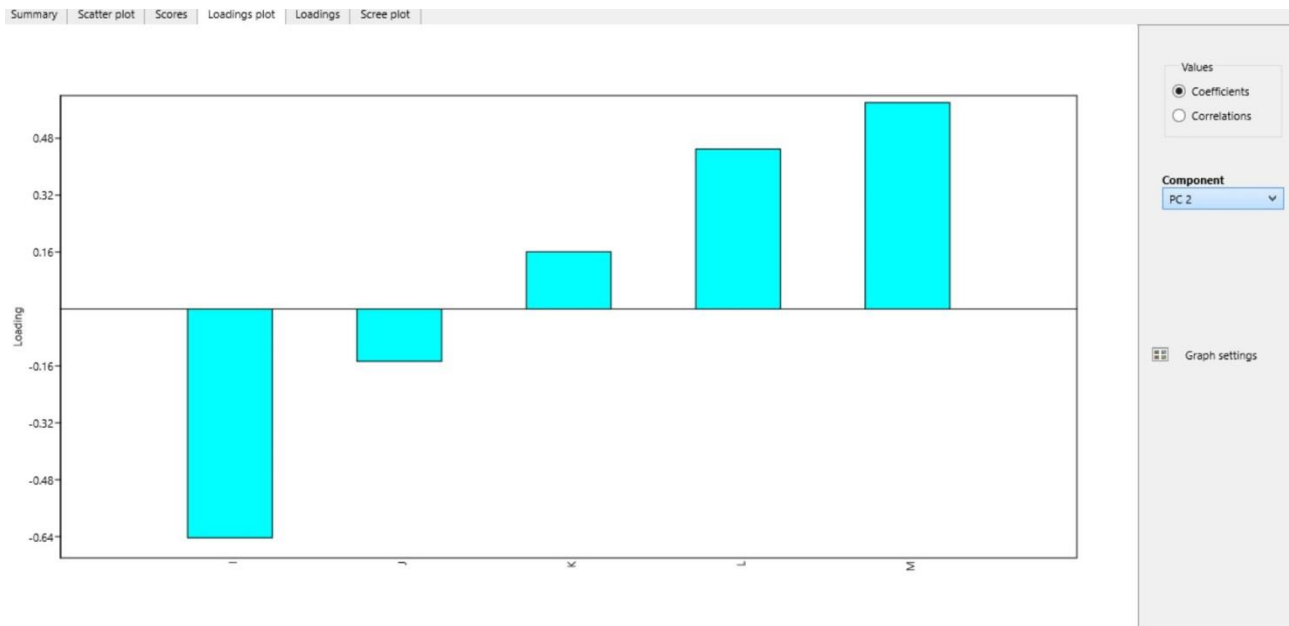
Marco Romano

Berlin

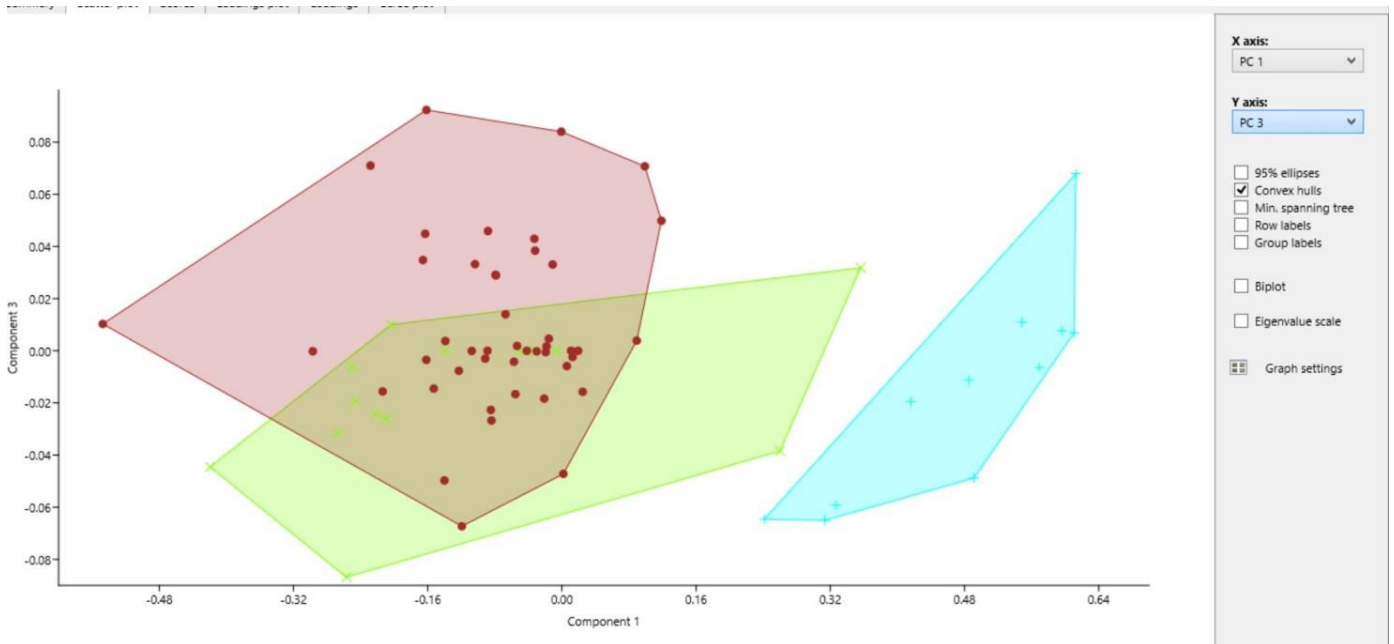
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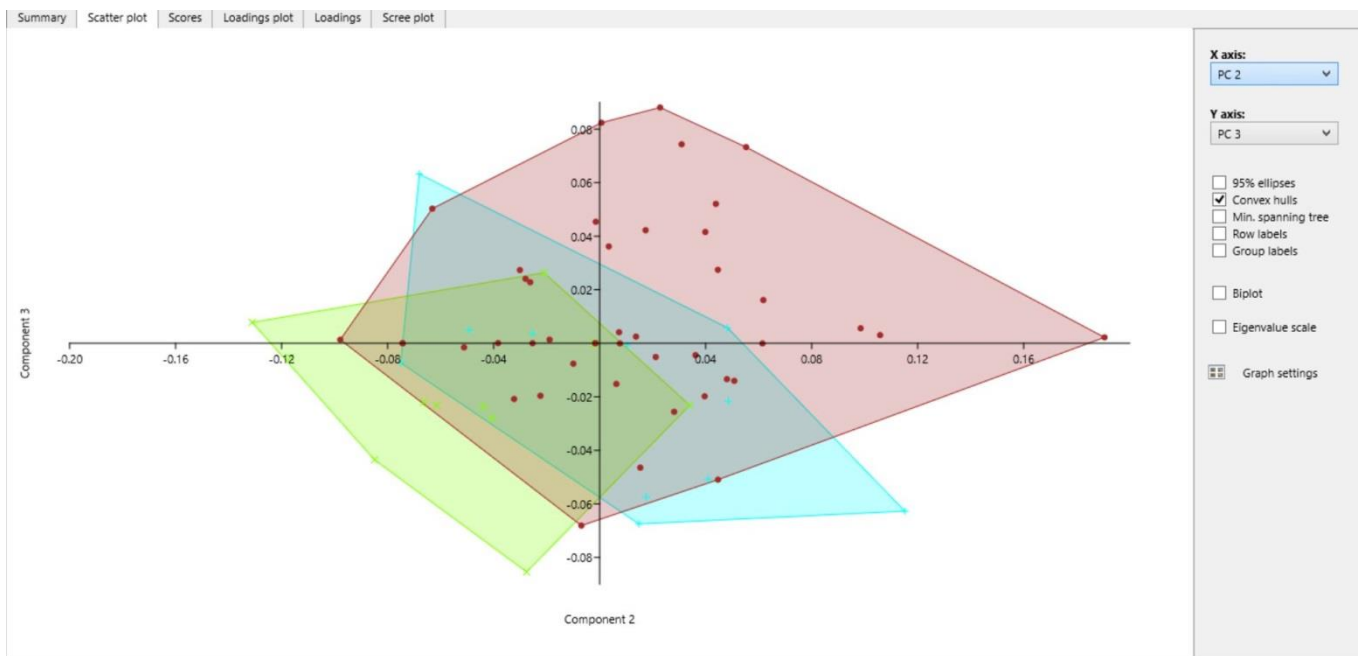
Loadings of Principal Component 1. PCA performed just on pes measurements.



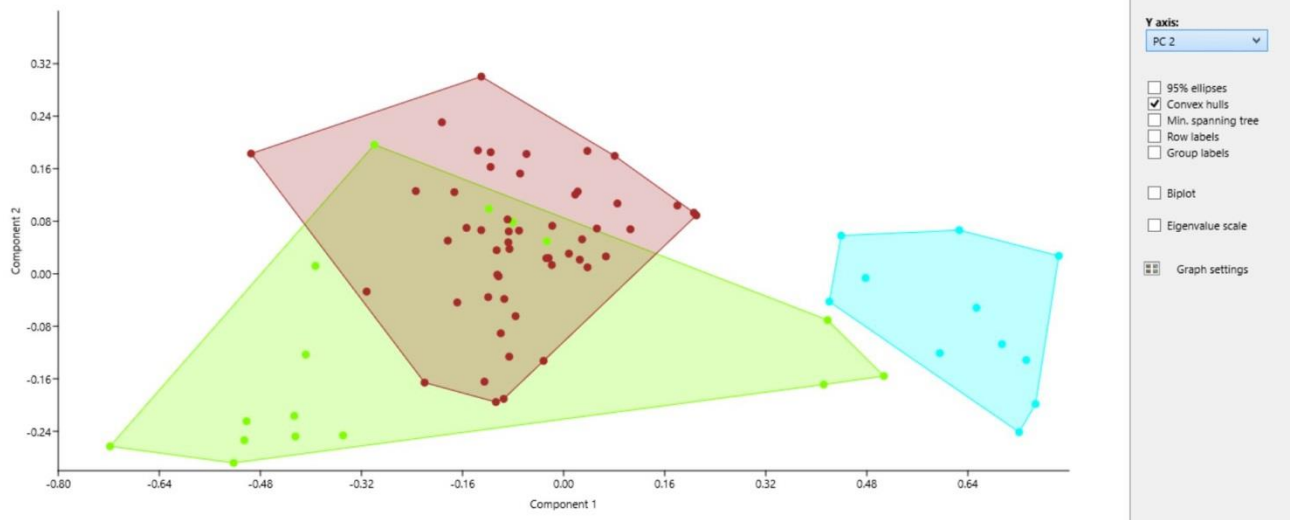
Loadings of Principal Component 2. PCA performed just on foot measurements. The fourth column indicate the loading for digit IV. By convention, are considered as significant loading greater than 0.3 and lower than -0.3.



Scatter plot of first two principal component performed just on pes measurements. Green: Birkheide specimens; Blue: Gottlob specimens; Red: Bromacker specimens

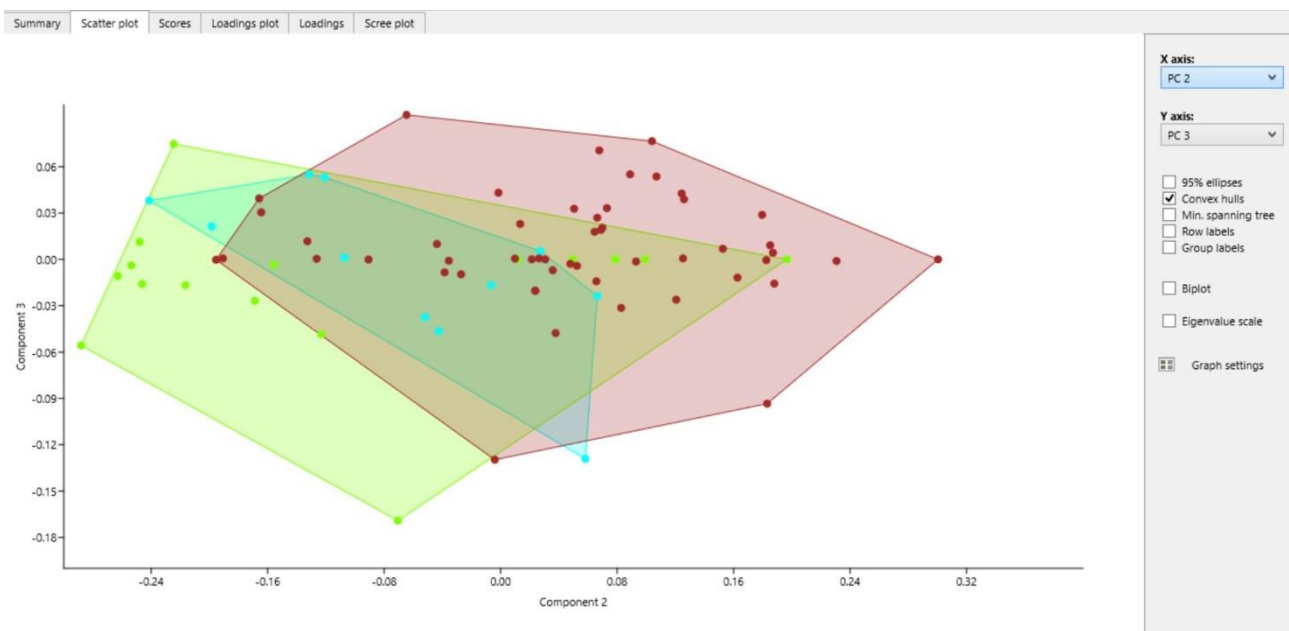


Scatter plot of PC2 vs. PC3 performed just on foot measurements. Green: Birkheide specimen; Blue: Gottlob specimens; Red: Bromacker specimens

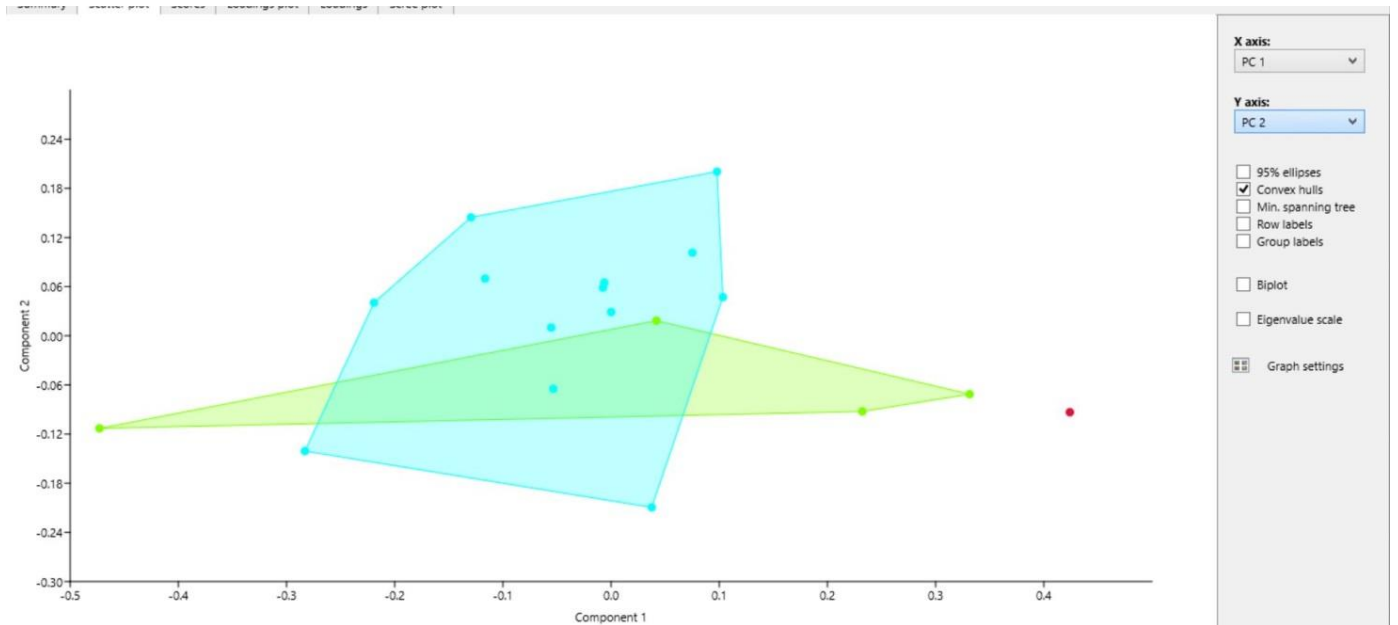


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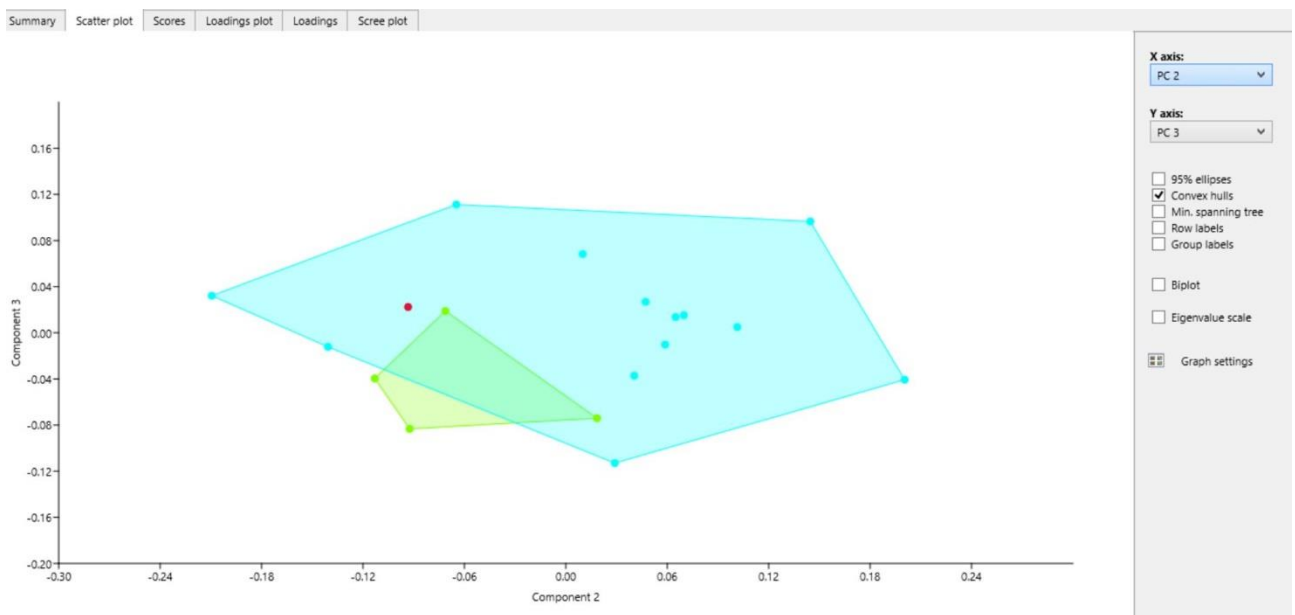
Scatter plot of PC1 vs. PC2 performed on both manus and pes measurements. Green: Birkheide specimen; Blue: Gottlob specimens; Red: Bromacker specimens



Scatter plot of PC2 vs. PC3 performed on both manus and pes measurements. Green: Birkheide specimen; Blue: Gottlob specimens; Red: Bromacker specimens



Scatter plot of first two principal component performed on linear measurements of trackway parameters. Red: Gottlob; Green: Birkheide; Blue: Bromacker



Scatter plot of PC2 vs. PC3 performed on linear measurements of trackway parameters. Red: Gottlob; Green: Birkheide; Blue: Bromacker