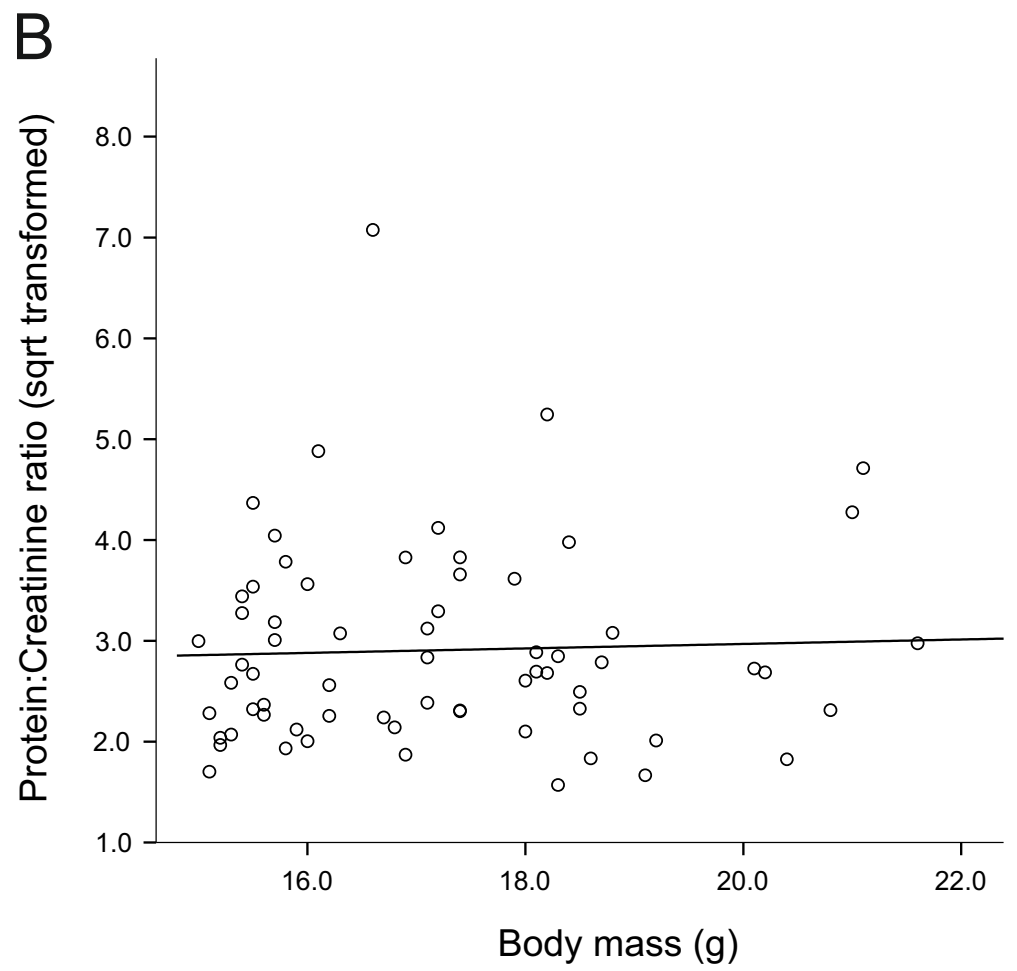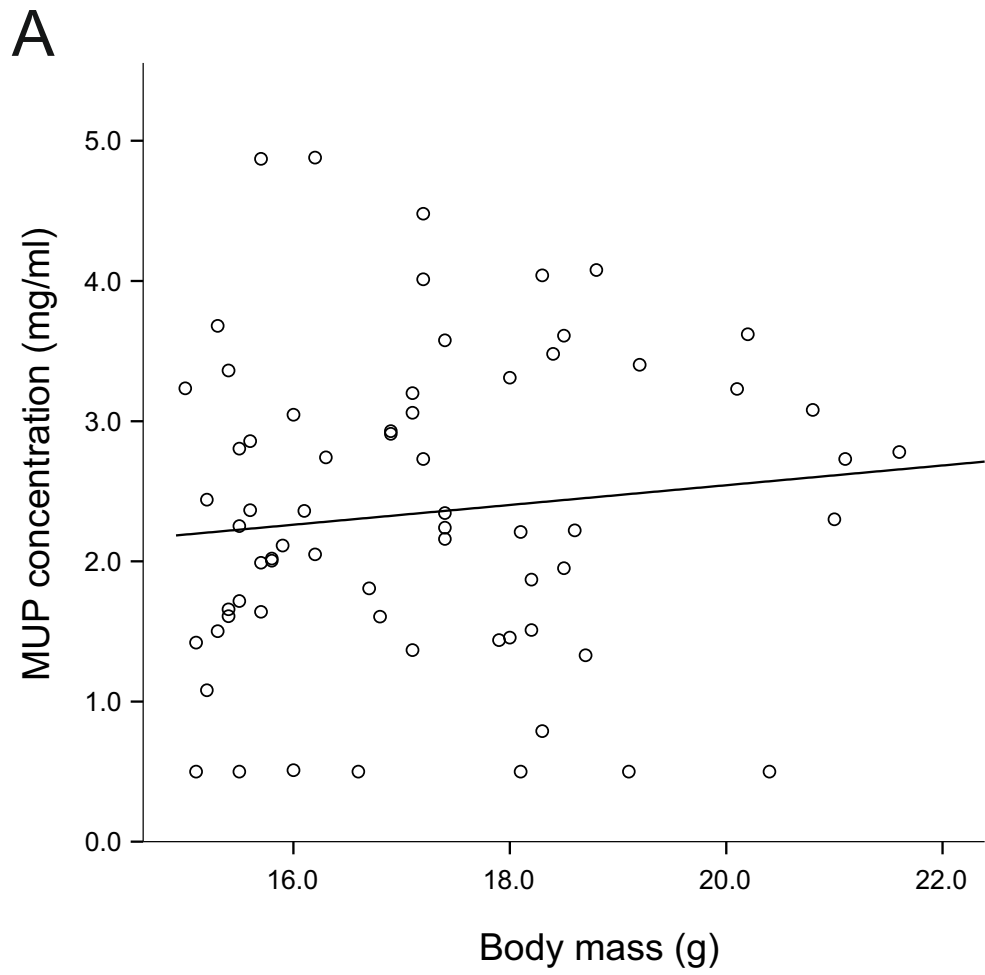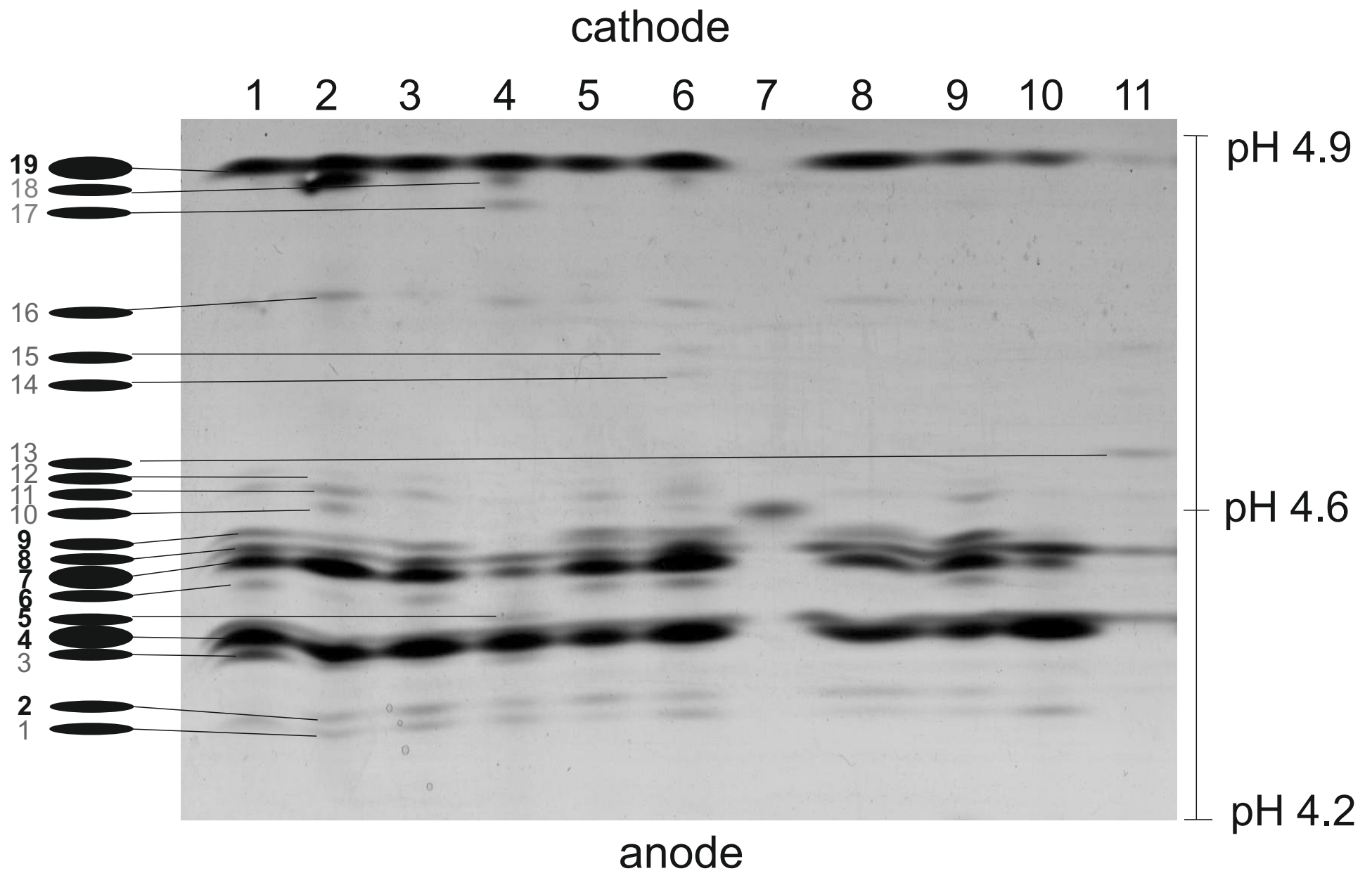Supplemental figure 1. Map of Austria indicating name of locality and number of mice sampled. Scale indicates 50 km.

Supplemental figure 2. Linear correlation of individual body mass and (A) MUP concentration and (B) protein:creatinine ratio.

Supplemental figure 3. In-house immobilized pH gradient (IPG) gel used to separate urinary MUPs by isoelectric point in narrow pH range (pH 4.2 to 4.9). Lanes 1-6 and 8-11 show individual urine samples. Standard pH 4.6 marker (lane 7, trypsin inhibitor (Sigma, St. Louis, USA)) was run on every IPG gel to identify individual bands based on their migration distance relative to the marker. Scheme on the left indicates position and band ID (number) for all 19 bands (major bands in black, minor bands in gray, see Methods). Example bands are indicated by connecting lines. 3µg of protein (1µg/µl) were applied to each lane.

cathode
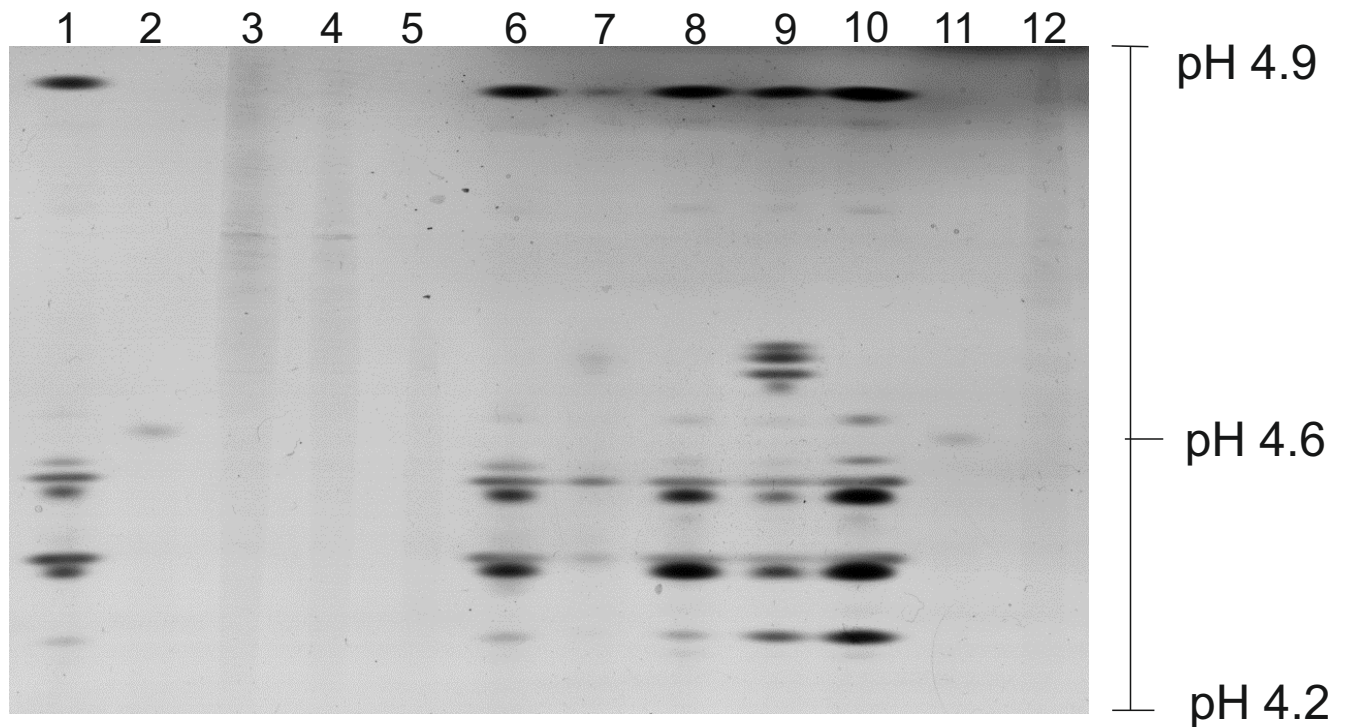
anode

Supplemental figure 4. In-house immobilized pH gradient (IPG) gel used to separate urinary MUPs by isoelectric point in narrow pH range (pH 4.2 to 4.9). Lane 2 and 11: pH 4.6 marker. Lanes 1, 6 to 10: individual male urine samples. Lane 3: mouse serum albumin (commercial preparation, > 95% pure according to manufacturer, antibodies online) at 3.33µg/µl, lane 4 and 12: mouse serum albumin at 1 µg/µl, lane 5: mouse serum albumin at 0.33µg/µl. 3µg of protein (1µg/µl) were applied to each lane. Mouse serum albumin is seen as a large number of faint bands from about pI 4.8 towards the cathode. Albumin bands with pI 4.9 and higher migrate into the cathodic electrode strip and are not detected.
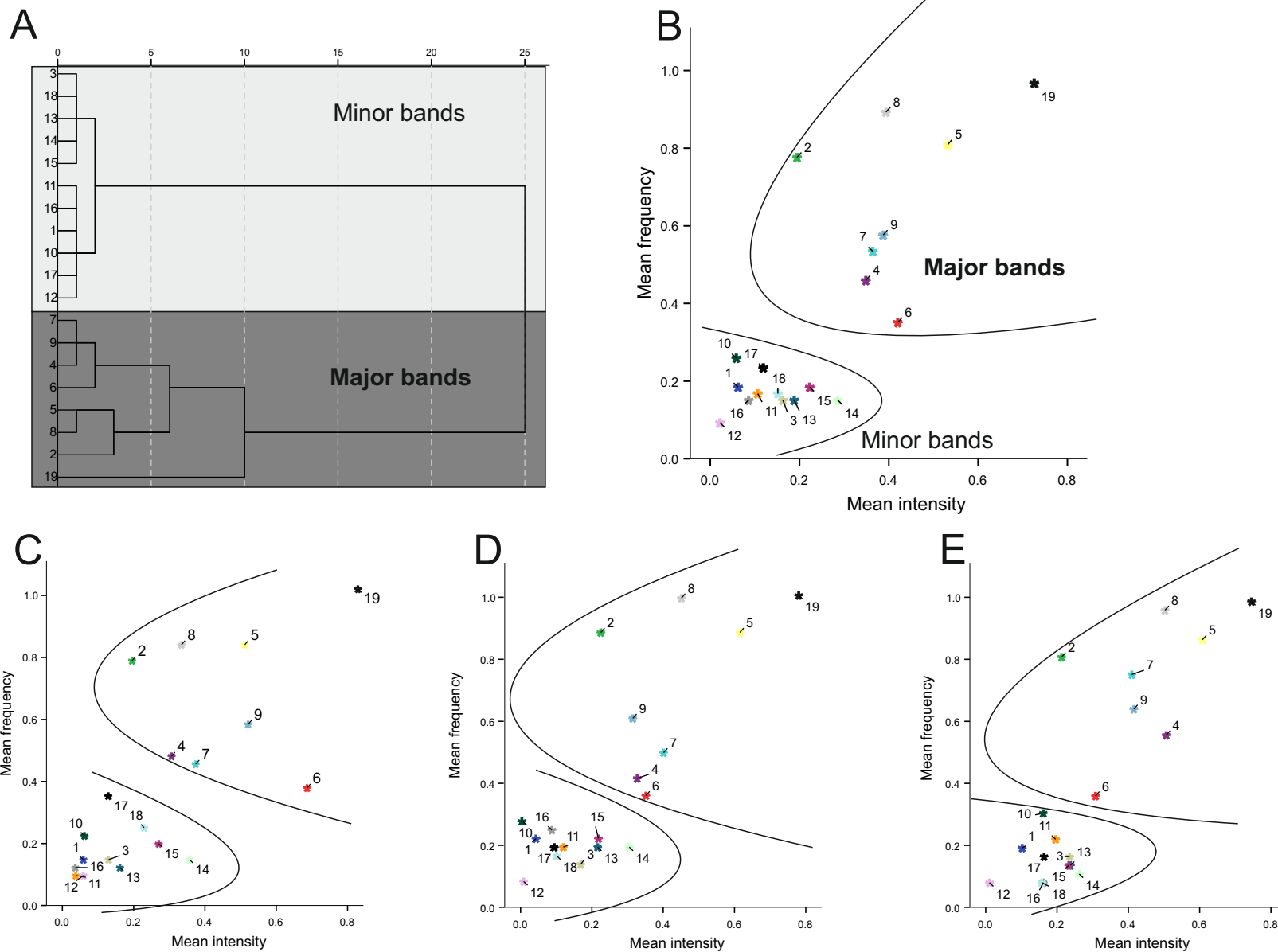
Supplemental figure 5. Hierarchical clustering of MUP bands based on frequency and intensity of each band using nearest neighbor clustering method. (A) Dendrogram showing separation of MUP bands into two clusters, major versus minor bands. (B) Major and minor bands differ in frequency and intensity when averaged over the samplings and when age classes were analyzed separately, (C) 6 weeks, (D) 10 weeks and (E) 24 weeks. Numbers in the graphs refer to band IDs as in Supplemental figure 3.
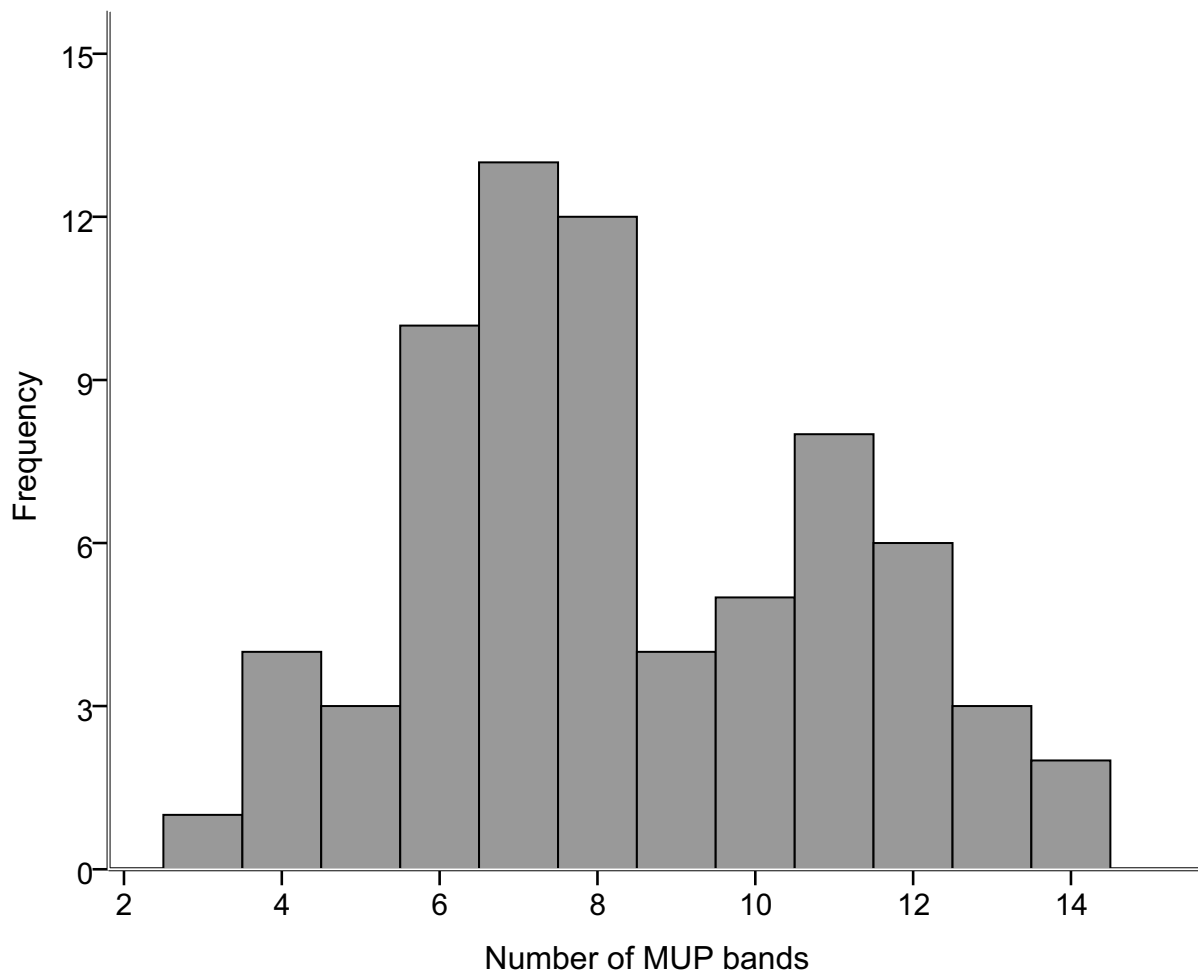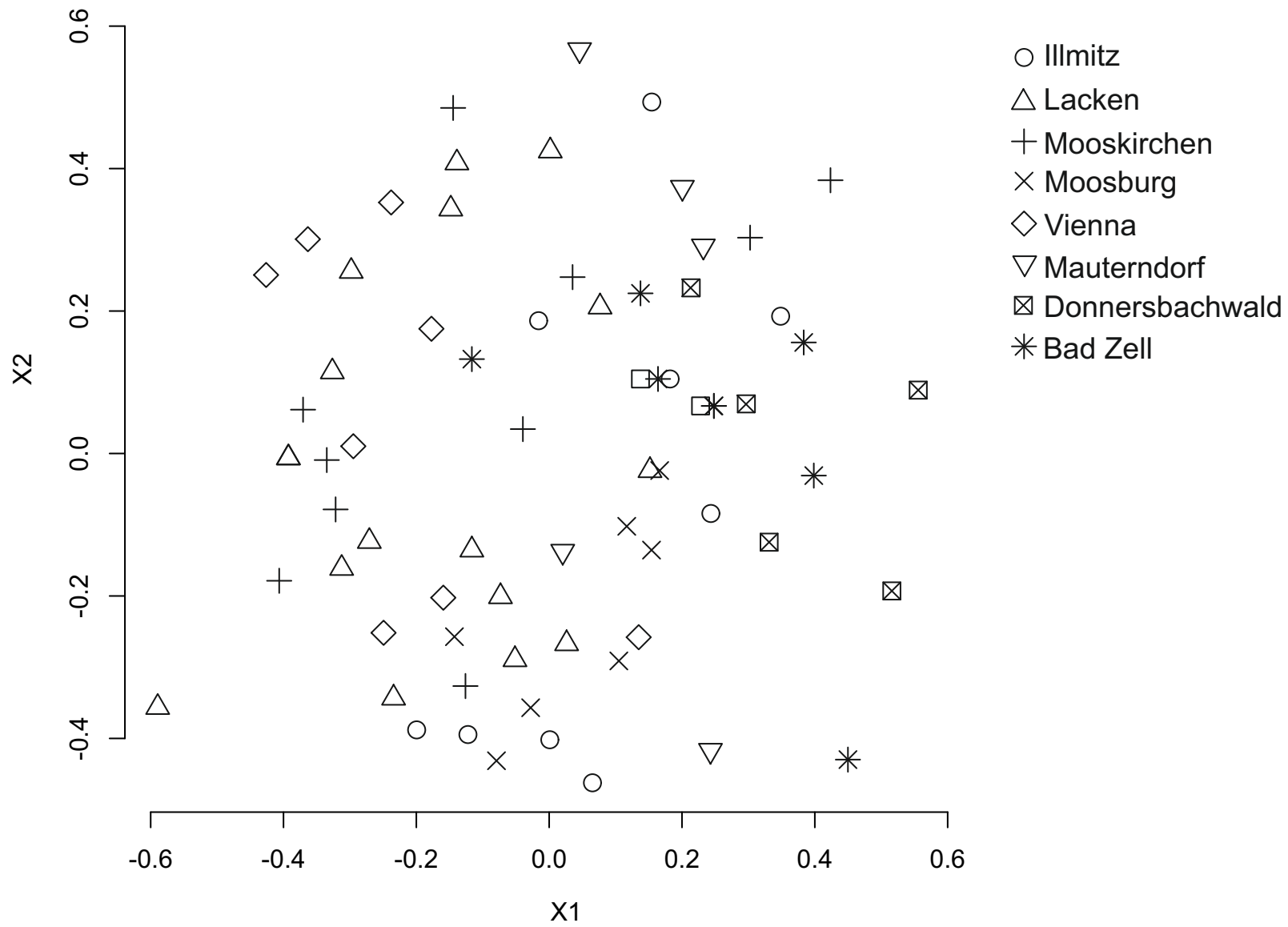
Supplemental figure 6. Histogram of total number of MUP bands per male.
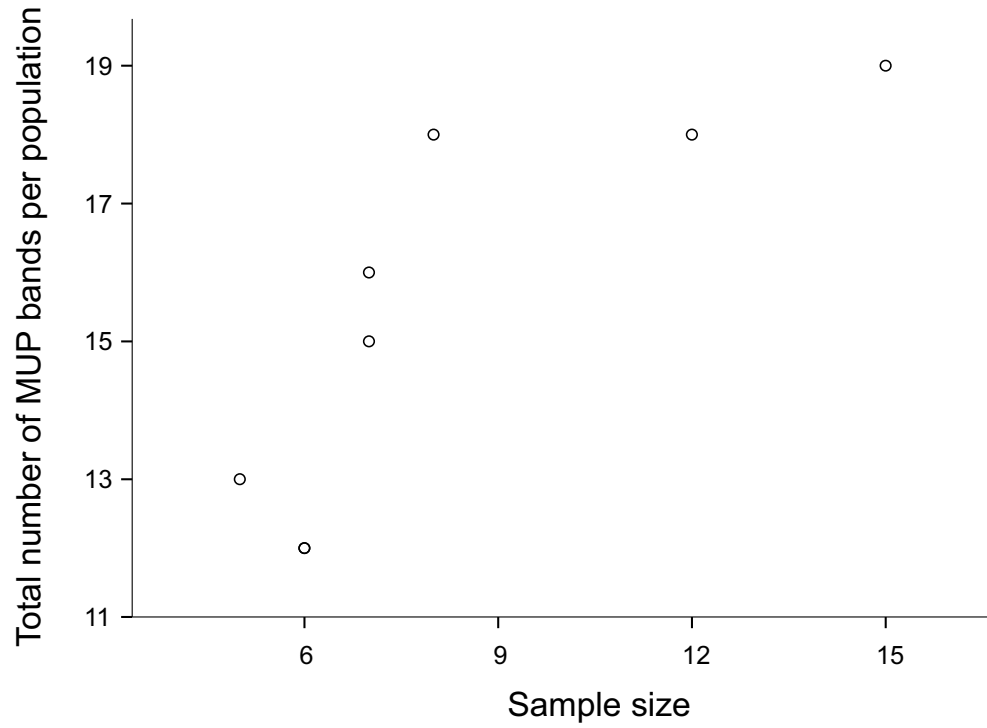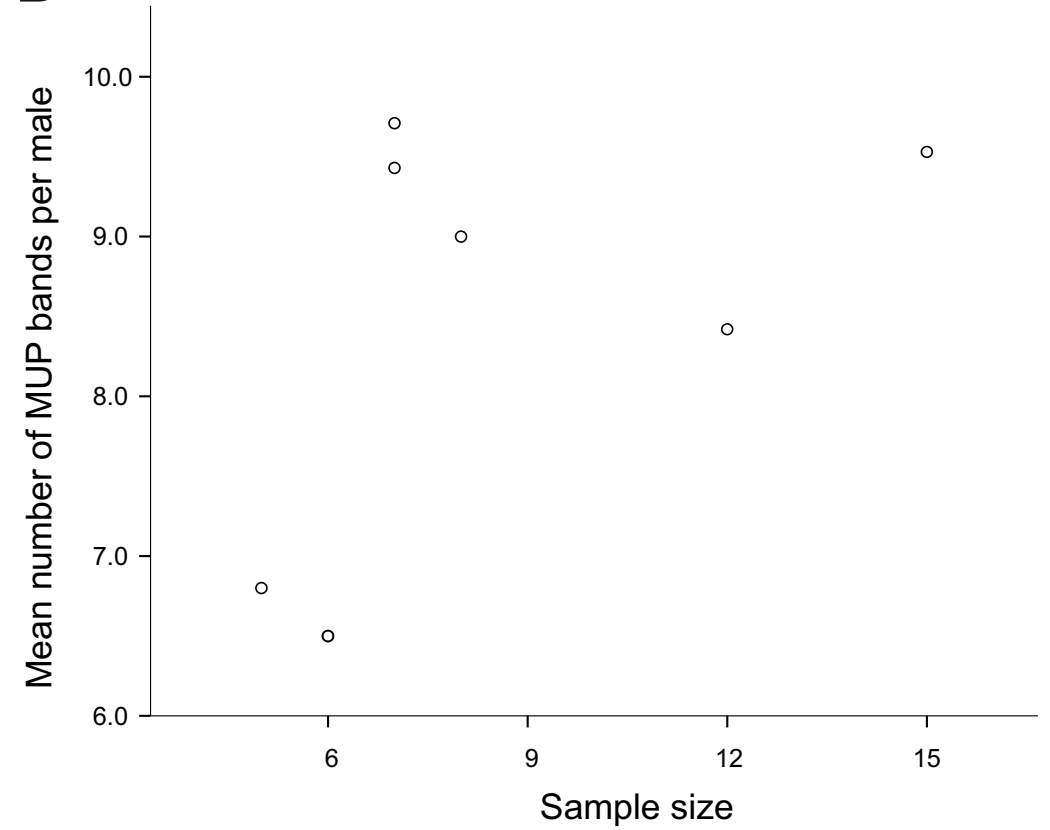
Supplemental figure 7. Correlation of number of sampled individuals with (A) total number of MUP bands found in a population and (B) mean number of MUP bands per male.
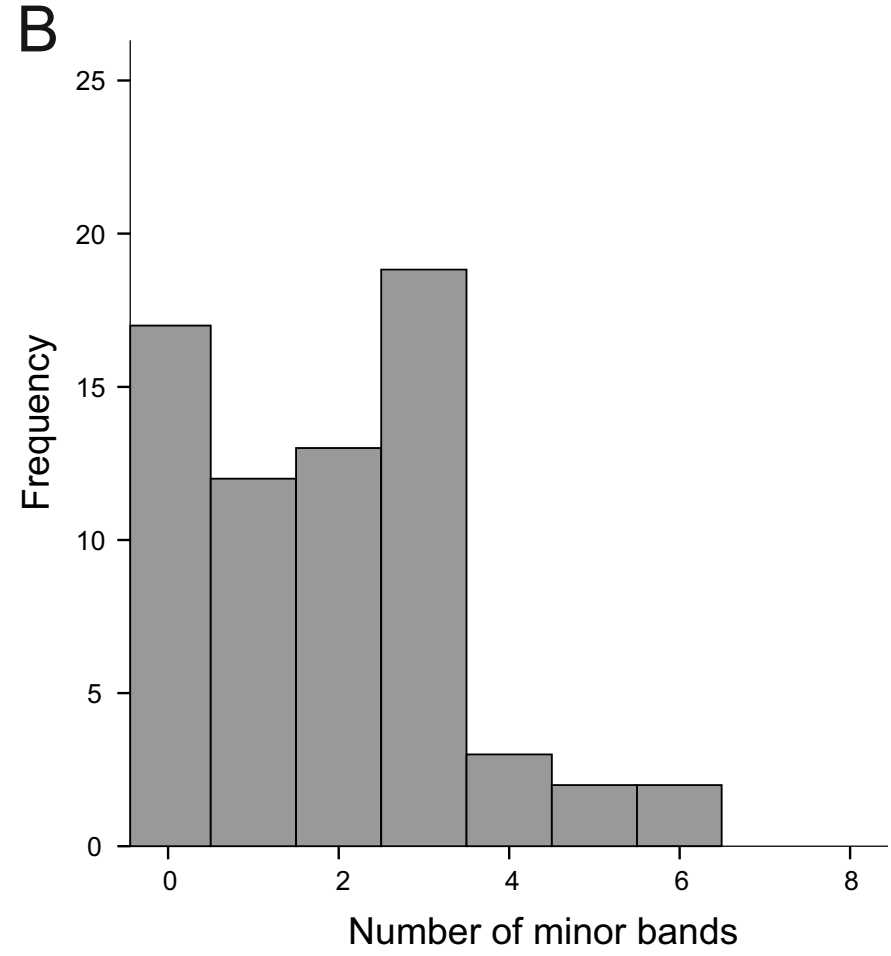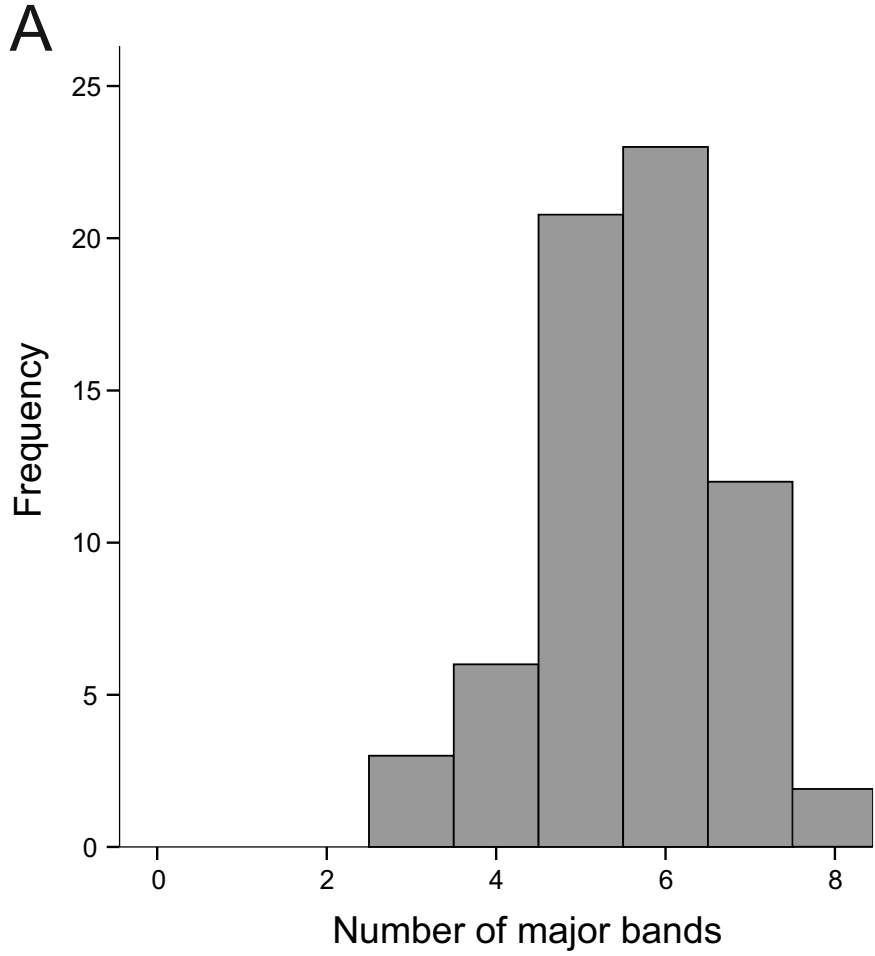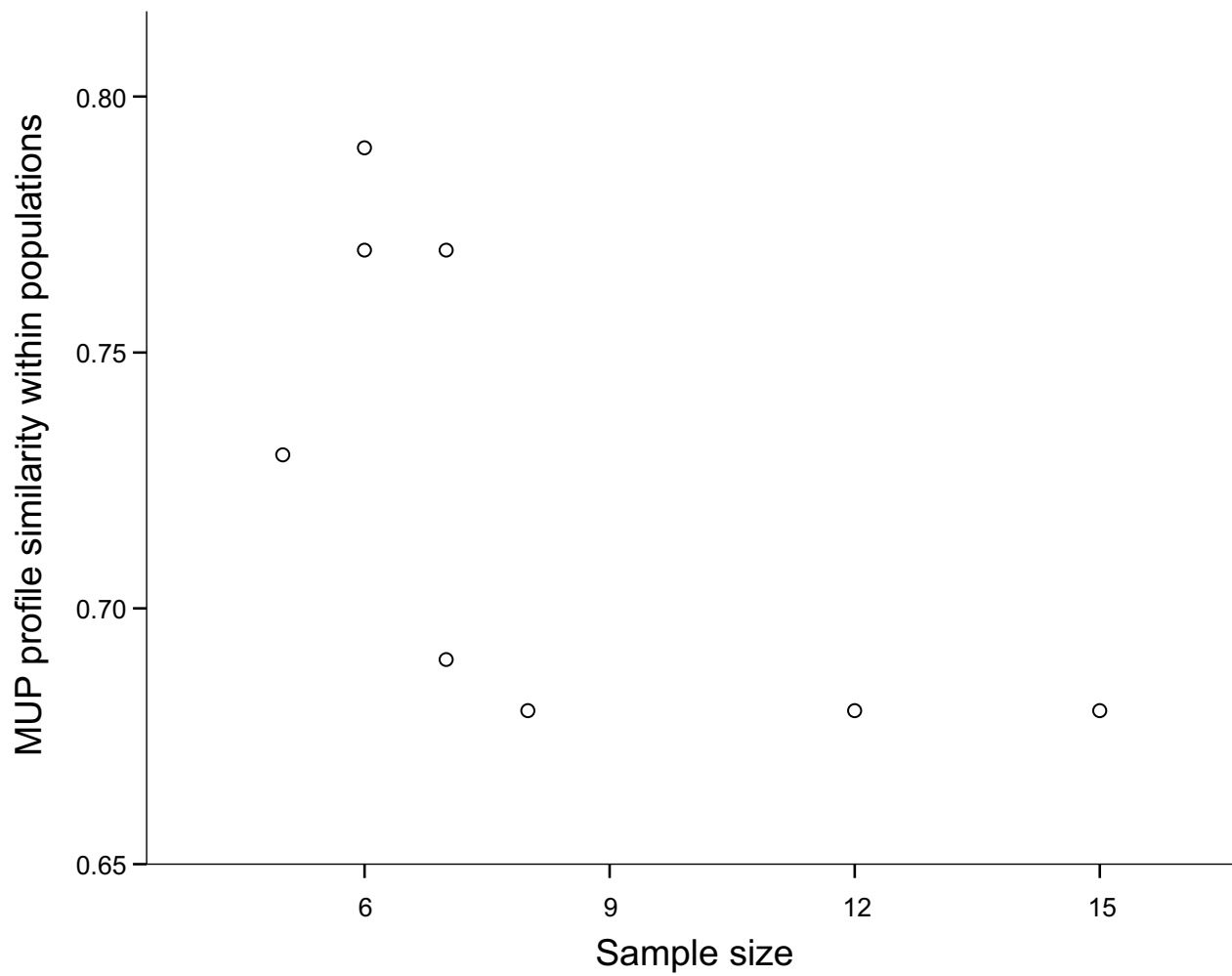
Supplemental figure 8. Histogram of number of (A) major and (B) minor MUP bands per male.
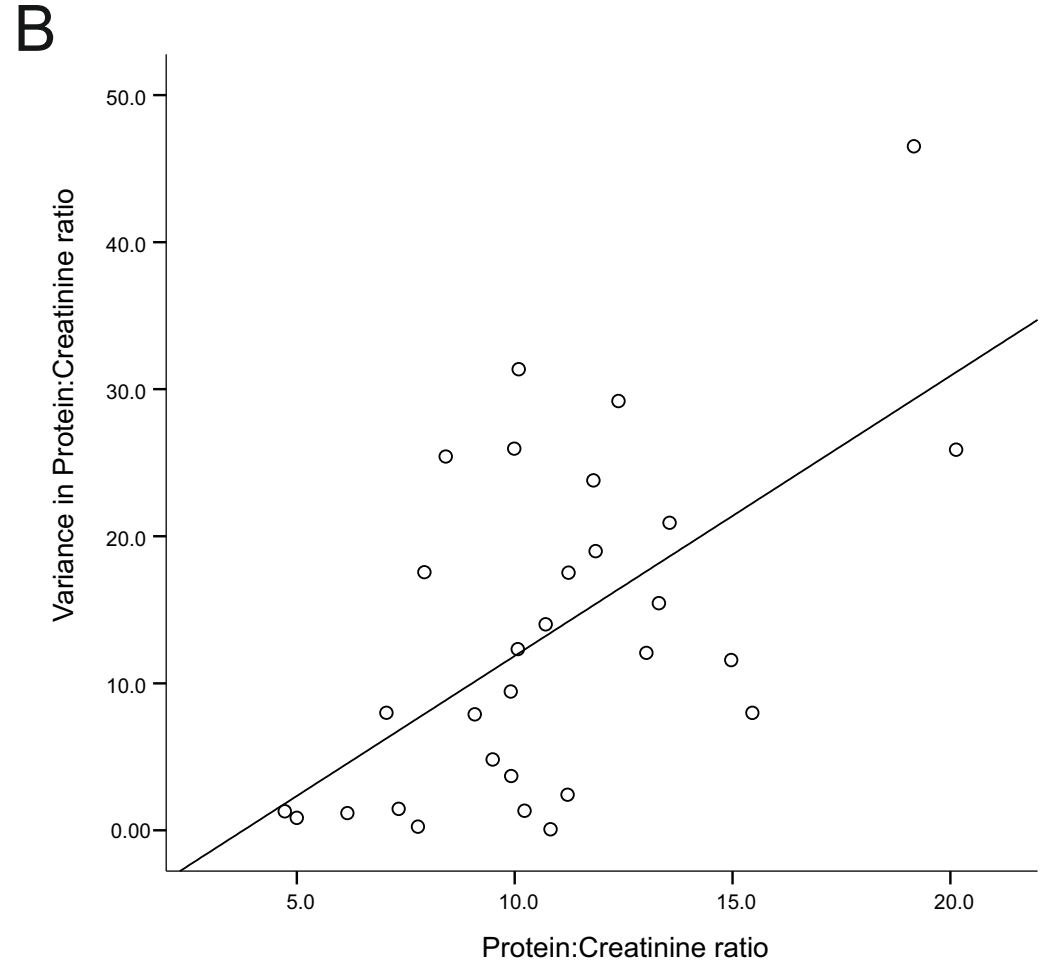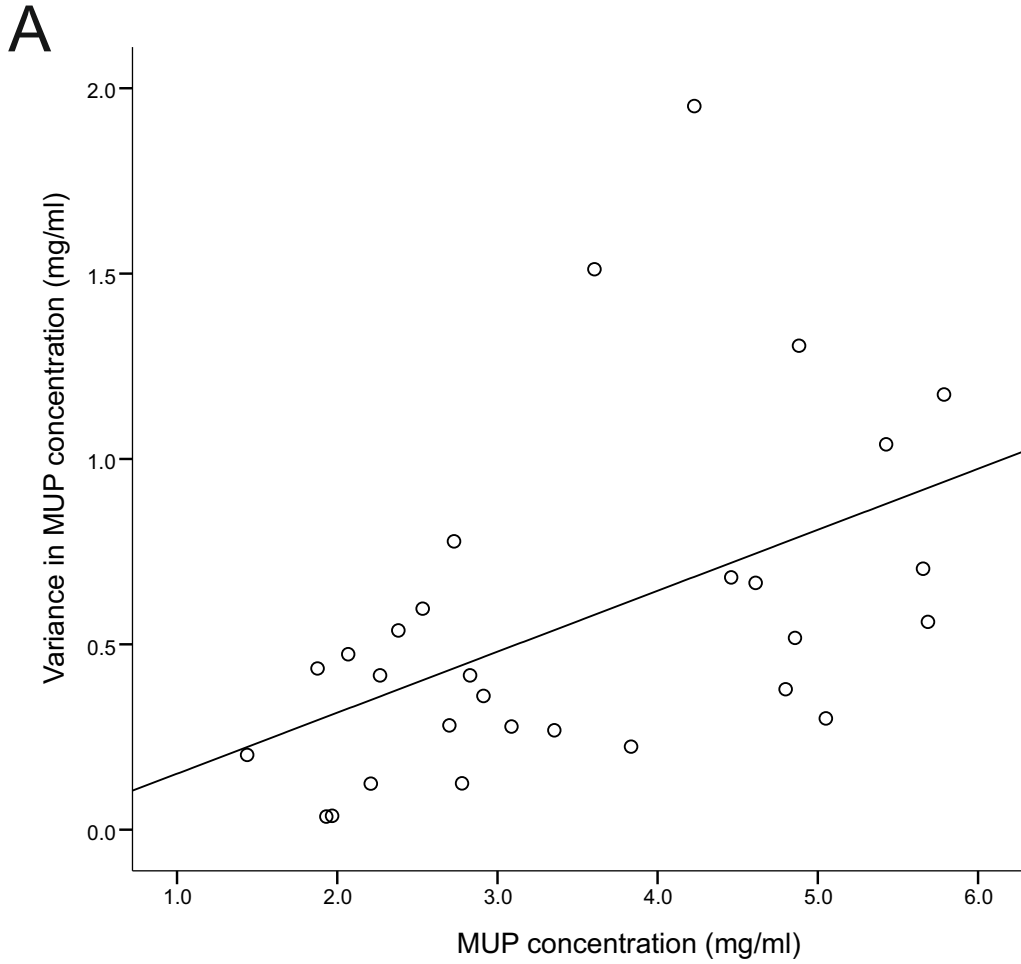
Supplemental figure 9. Nonmetric multidimensional scaling (NMDS) analysis of MUP profiles of eight populations (see legend for localities).
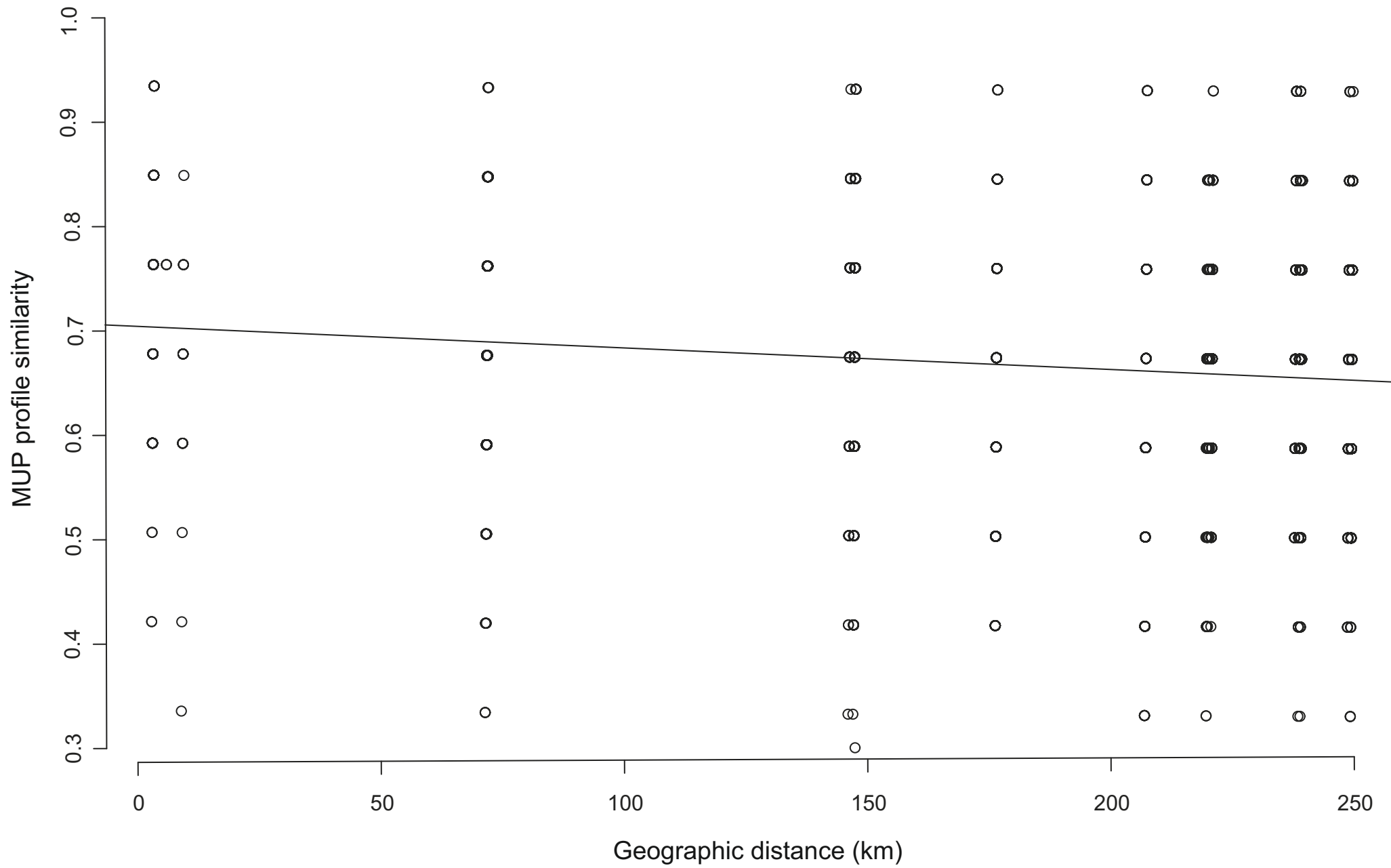
Supplemental figure 10. Linear correlation of individual MUP profile similarity and geographic distance using Mantel test.

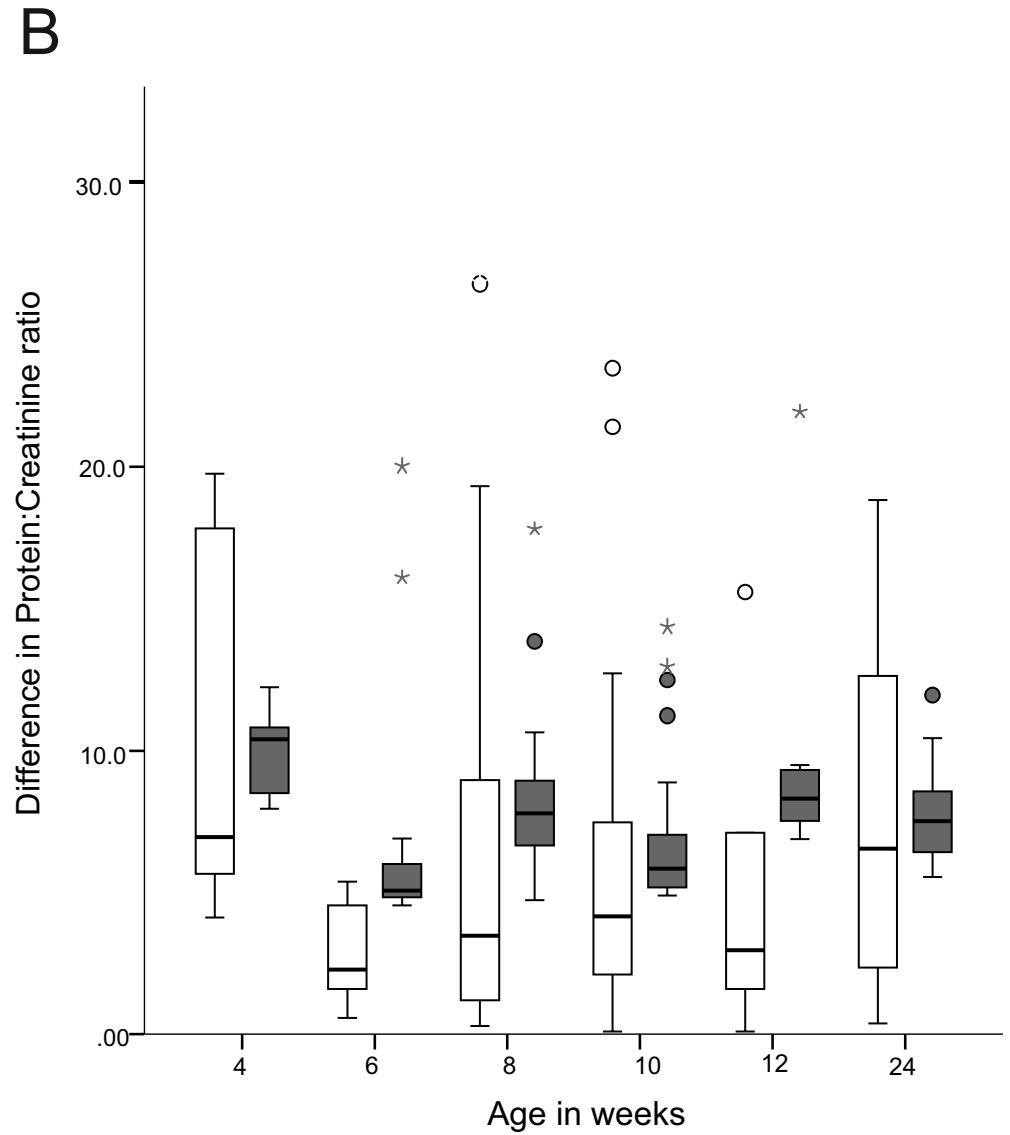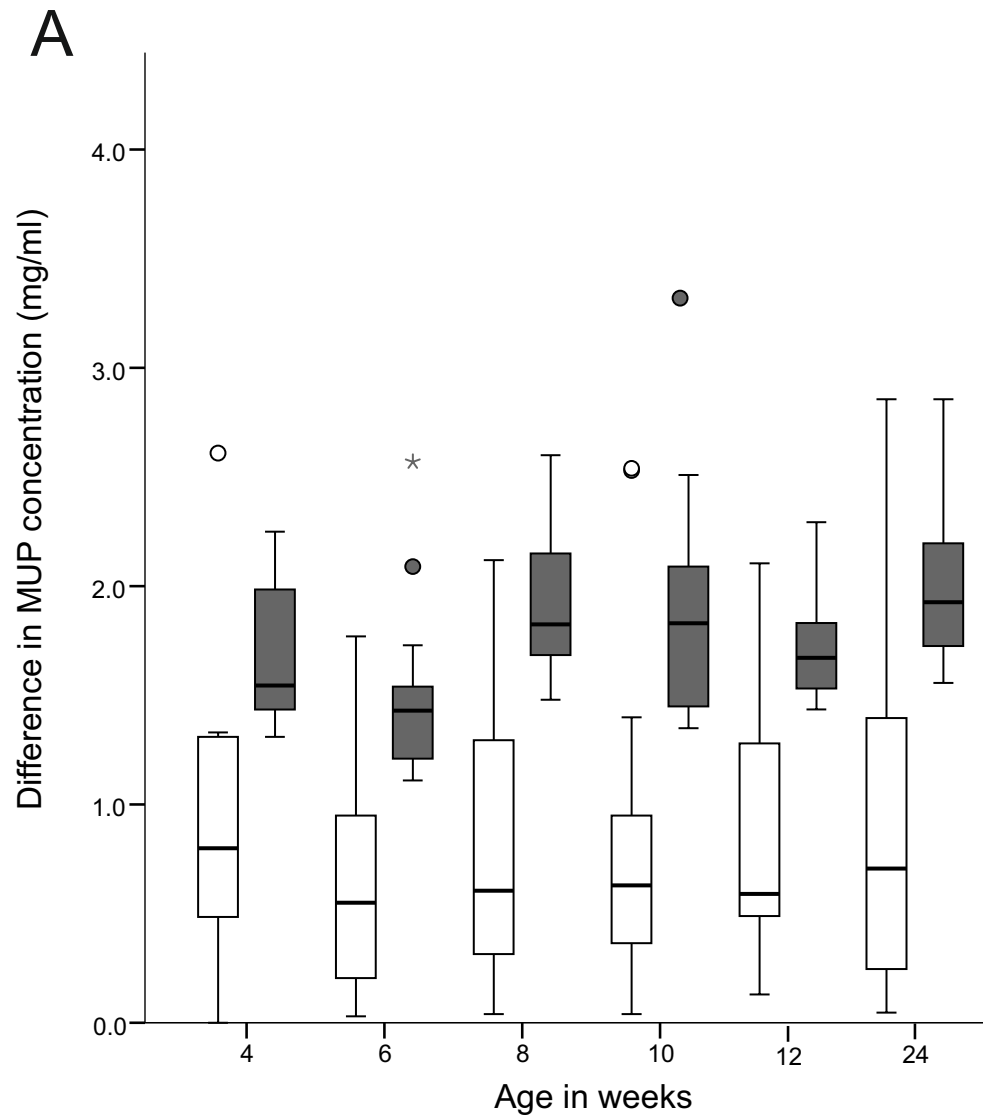Supplemental figure 11. Correlation of sample size with within population MUP profile similarity.

Supplemental figure 12. Linear correlation of individual (A) MUP concentration with variance in MUP concentration and (B) protein:creatinine ratio with variance in protein:creatinine ratio.
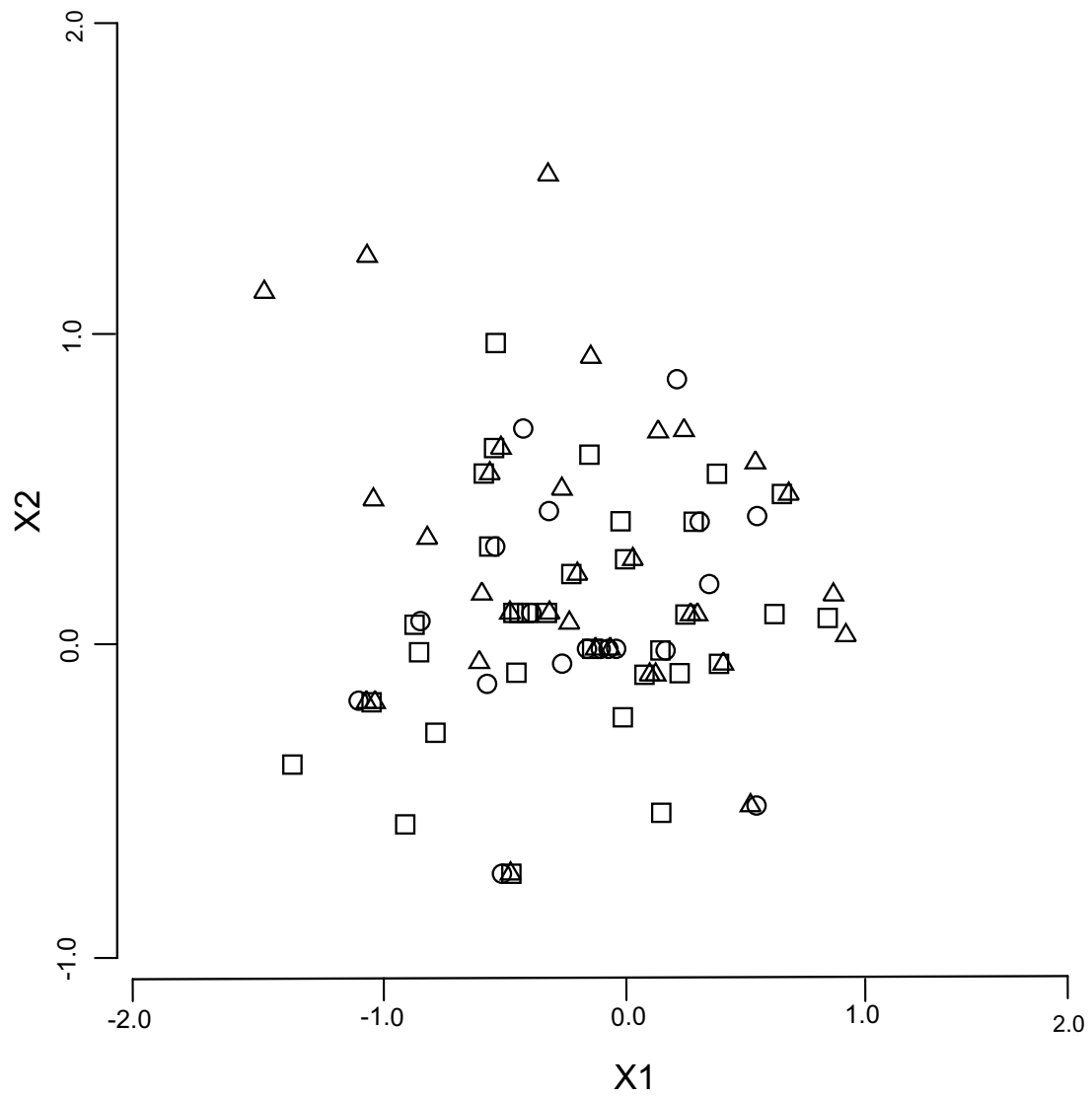
Supplemental figure 13. Nonmetric multidimensional scaling (NMDS) analysis of MUP profiles at 6 wks (circles), 10 wks (squares) and 24 wks (triangles) of age.
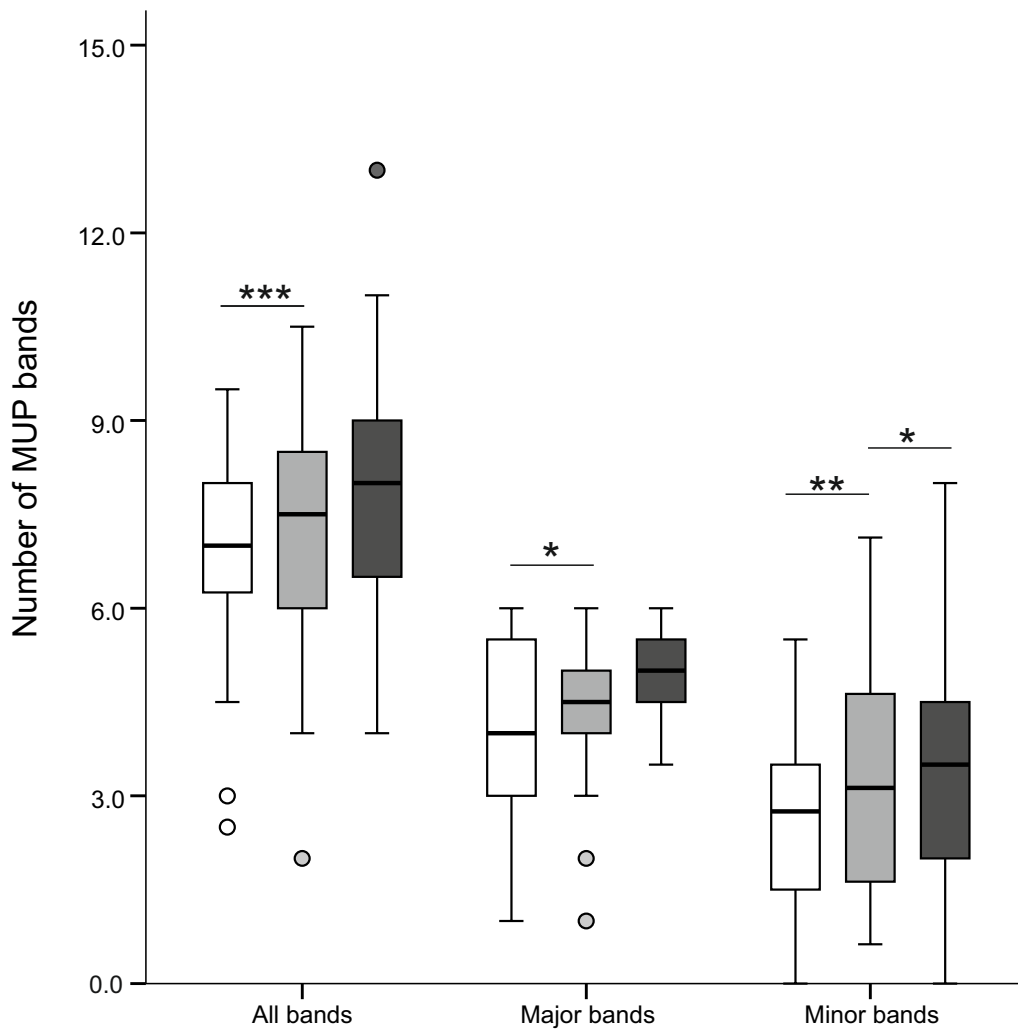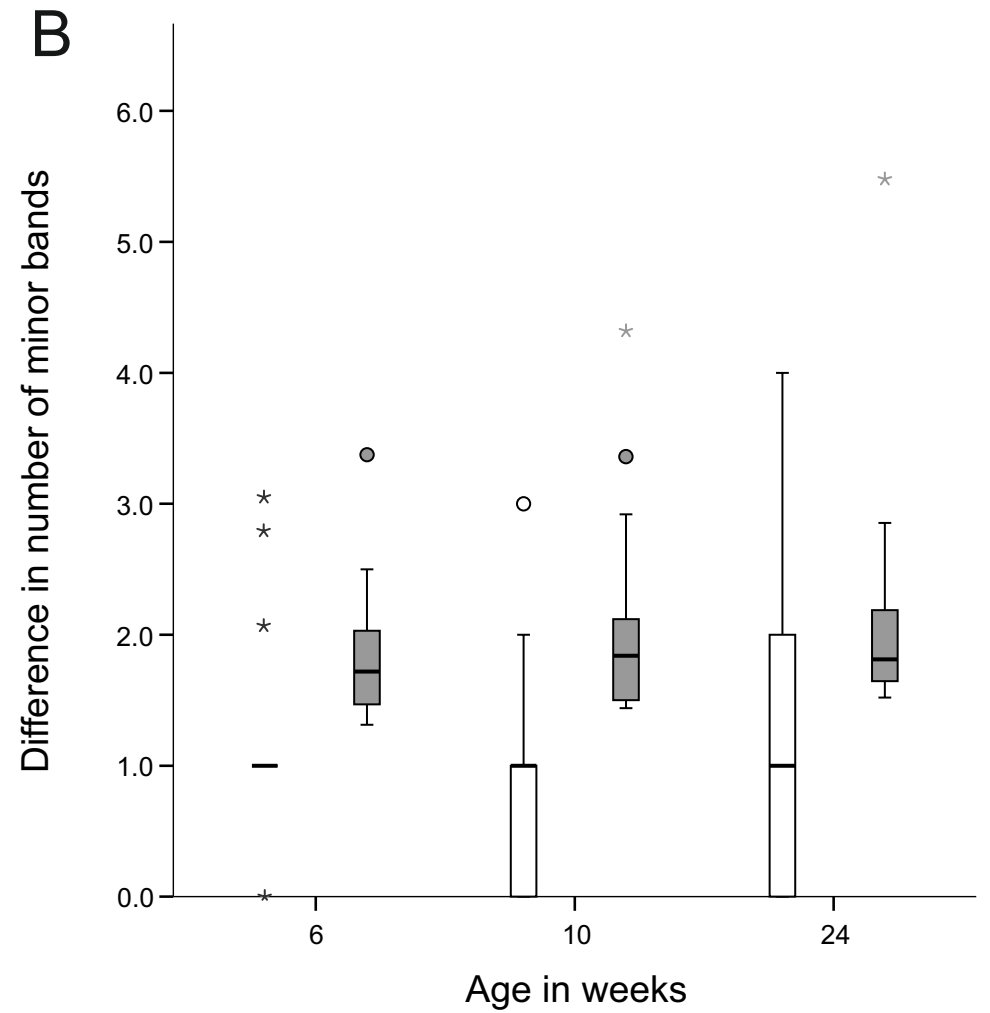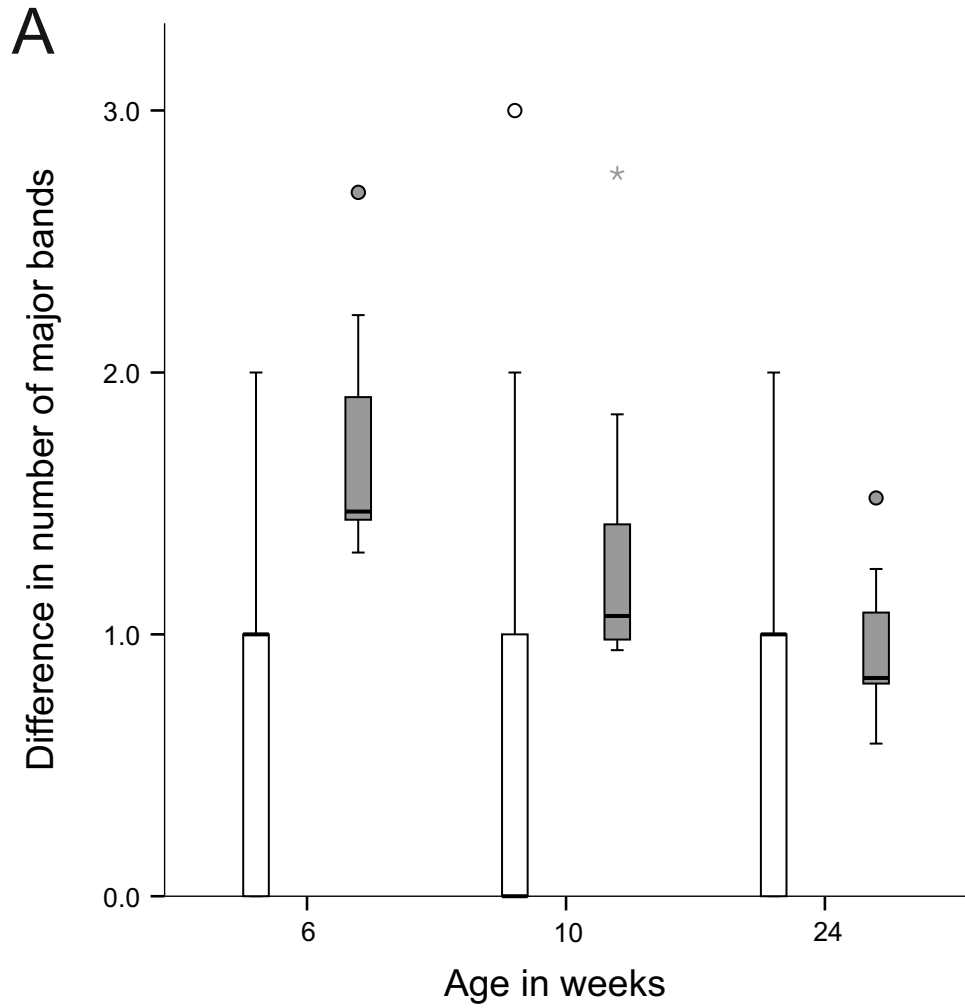
Supplemental figure 14. Boxplot of number of MUP bands at 6 wks
(white bars), 10 wks (light gray bars) and 24 wks of age (dark gray bars).
 * = p < 0.05, ** = p < 0.02, *** = p < 0.01

Supplemental figure 15. Partial correlation of individual number of MUP bands and MUP concentration (black circles, solid line) and protein:creatinine ratio (white circles, dashed line).

Supplemental figure 16. Boxplot comparing difference in (A) MUP concentration and (B) protein:creatinine ratio between brothers (white bars) and unrelated males (gray bars) over the sampling period. Circles and asterisks indicate outlier data points.
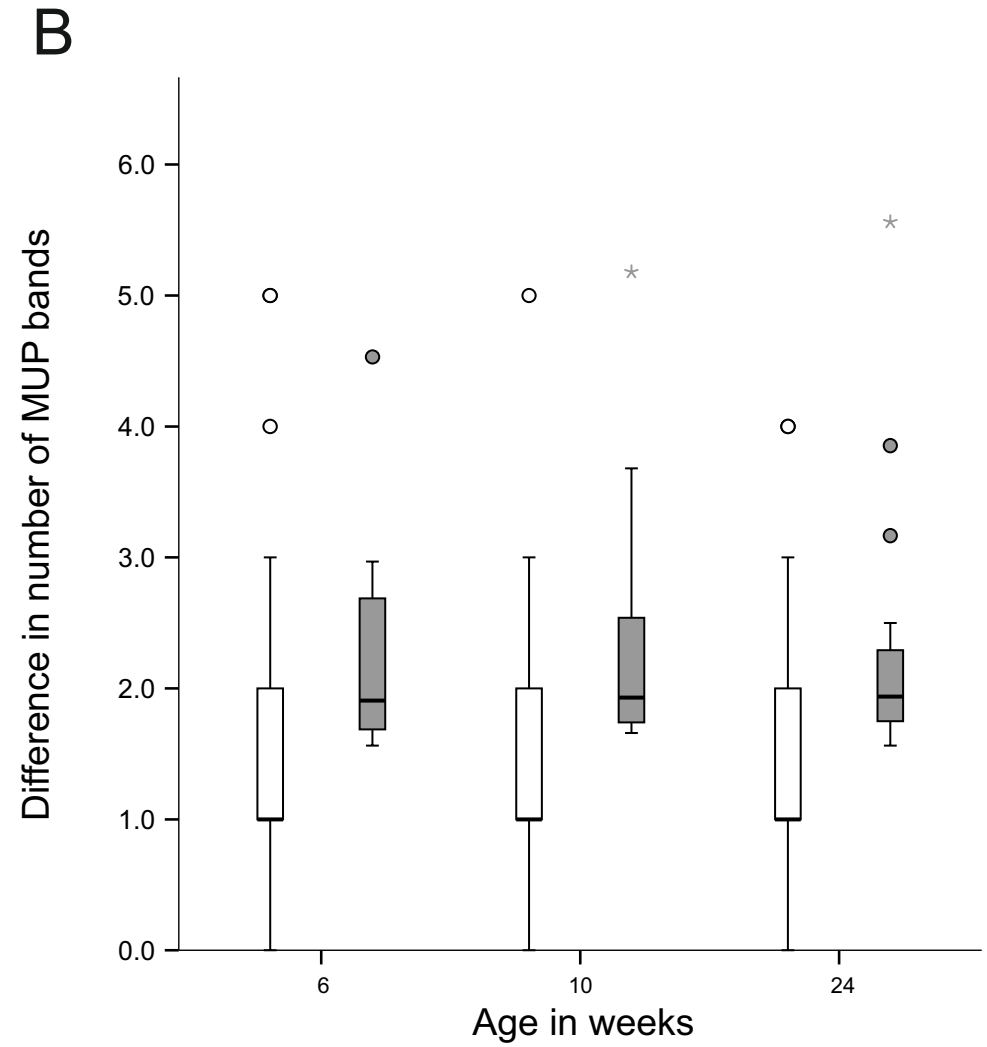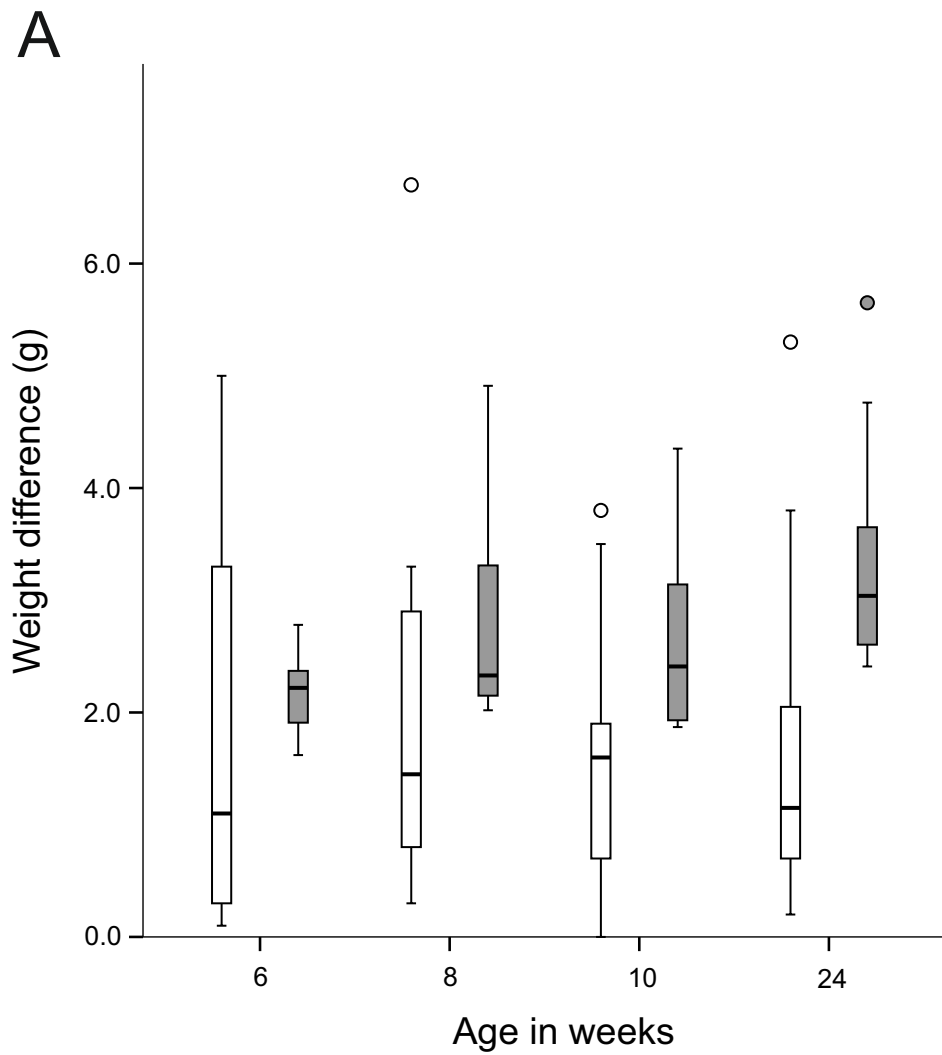
Supplemental figure 17. Boxplot comparing (A) weight differences and (B) difference in total number of bands for brothers (white bars) and unrelated males (gray bars) at different age classes. Circles and asterisks indicate outlier data points.

Supplemental figure 18. Boxplot comparing differences in number of (A) major bands and (B) minor bands for brothers (white bars) and unrelated males (gray bars) at different age classes. Circles and asterisks indicate outlier data points.