**CLIC workflow**

1. COO: Digitize LMs on individuals to compare (same number, same location each time) using a known scale.
2. TET: concatenate .txt file (if needed) in a specific order: generate .nts and .db ouput files (this data transformation allows to get the info of each specimen on one row). The .nts file is useful in case that you want to run some analysis on TPS morphometric software by J. Rohlf.
3. Excel: copy .db file without first file (just the values) and with scale info do the transformations pixels to millimeters. Copy the mm coordinates to .txt file. In the first row of the .txt file, you can copy the number of specimens per group and other info. Next software will not use the info on the first row (i.e. 12 18 20 Brazil Colombia Venezuela 18 LMs). Here, you will have the *raw data* scaled.
4. MOG: open the .txt file (raw data). Once opened MOG asks about data in the file- copy the specimen´s numbers by group on the first row using just one space between numbers (i.e. 12 18 20). Do the **Procrustes analysis** (Translation, Rotation, Rescaling)- You will just need to click on the order given by the green button. At this point, you will see a graph showing the process and indicating each group using a specific color per group. After Procrustes analysis, you will get several output files that will be needed for following analysis. Some files have PW.txt (shape variables), other PW\_CS.txt for allometry and other just CS.txt for CS analysis.
5. **CS analysis**: you will need to test for normality and homocestadicity to know if you need to do parametric (ANOVA) or non-parametric (Kruskal-Wallis) test for CS comparison among populations.
6. First question to be tested: *Are there an allometric effect*?–it means, an effect of size on shape –bigger individuals have different shapes? (other variables can be tested: sex, locations, regions, developmental stage, etc.). Open COV as follow: MANCOVA design > Different versus common slope allometric model – 1000 permutations. Choose CS\_PW.txt output file and indicate groups (i.e. 12 18 20). COV software used PW (shape variables) as dependent variables and CS as independent variable. Output: if you check the results on COV, you will find a red letters reporting multivariate regression. A p-value <0.05 indicates a significant allometric effect. Under multivariate regression result you will see Euclidian distances with Bonferroni correction and a text that indicates that after Bonferroni correction, just those values <XXX should be considered as significant. In case that you find a significant allometric effect, the next question is if this effect is the same in each group (pop/species) –you should check in the output file on COV the Wilks lambda result to know if you can correct the effect (Wilks lambda compares the slopes among groups-if they are similar, you can correct the effect, if not it could not). A Wilks lambda value <0.05 means that effect cannot be correct. In contrast, a value >0.05 indicated that it could be correct and COV offers a button to do the correction.

*Note*: when you are working with population comparison (same species) some authors suggest that allometric effect is negligible and is a biological property of the species and so, it should not be corrected. However, when you are comparing different species, it is a requirement to test this. Usually, different species have different growth patterns and this effect should be measured. See (Outomuro and Johansson, 2017)

1. **PCA**. A basic question for shape variables (Procrustes coordinates) is if shape variation data can suggest some grouping pattern. Then, PCA is an analysis that allow us to check that without a priori info about groups. However, we can realize how is the distributions of individual per group in the morphospace. You can use COV for running the analysis using the PW matrix: COV>One single matrix>PCA on convariance matrix: select \_PW.txt file .

Other way is take the Procrustes coordinates and open it on PAST, group by colour and then run PCA (results should be almost identical). PAST is nice because you can modify the color of each group if you want. In the same way, because you have more than two populations or biogeographical regions for comparison you can run a **CVA** (discriminant analysis) to test if there are significant differences among average wing shape per population/region, etc. Discriminant analysis on CLIC is done using PAD as follows: PAD > Geometric Morphometrics > Total \_PW.txt – indicate number of specimen per group, in order > Enter. Calculate Mahalanobis distances. To continue “clicks” on “C” and “CCC” to get a unsupervised classification and supervised classification, respectively (CCC is the strongest result for discriminant analysis). PAD will calculate the % of individuals that are correctly classified to each population based on wing shape.

1. COV can also be used for running **metric disparity**. After successive entries of a single matrix, COV compares MD among samples by using non parametric tests (bootstrapping, see Zelditch et al., 2004. Geometric Morphometrics for Biologists. A primer). Here you will get MD values per population/species and pairwise comparisons.
2. **ANOVA and MANOVA multifactorial** can be used for testing relations between CS or shape (Procrustes coordinates) respectively, with environmental variables. As I told you before, using SAS, R or whatever software that allow to do it and ideally, testing interactions. I know that some software allow running just test interaction with two or three variables –if it is still the case, you can do individual ANOVAs/MANOVAs with each factor and select just those significant for testing interactions.
3. **Tree analysis**. If you want to get a tree using the phonetic distances (Procrustes, Euclidian or Mahalanobis distances) to see which are the most similar and different populations based on wing shape do as follow: COV > One subd matrix > submatrix statistics > open \_PW.txt file and ENTER. Push to get Euclidian and Procrustes distances. Save reports. After that in COV you can click con “External softwares” menu and open NJ Phylip. The software will require one file: open Procrustes file recently created. Set the question as\_ R > Enter > Choose UPGMA or NJ – L --->Yes, Yes, Enter. For next question, please write “r” to write names of populations. If is not possible you can check in “external software” an option for “giving a name to each OTU”. To open the tree: COV folder > W folder > njplot.exe CLICK --- open outtree file.