Viewbox Tutorial
Digitization error: Procrustes ANOVA

Procrustes ANOVA

Calculation of the digitization error for 3D specimens, as described here, is based on:

- Klingenberg, C. P. MorphoJ: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* **11,** 353-357 (2011).
- Fruciano, C. Measurement error in geometric morphometrics. *Dev. Genes Evol.* **226,** 139-58 (2016).

MorphoJ is required.

Step 1 (Viewbox)

Digitize the specimens twice (intra-observer: one investigator, each specimen twice; inter-observer: two investigators, each specimen once) and save them using ‘name’ as the following:

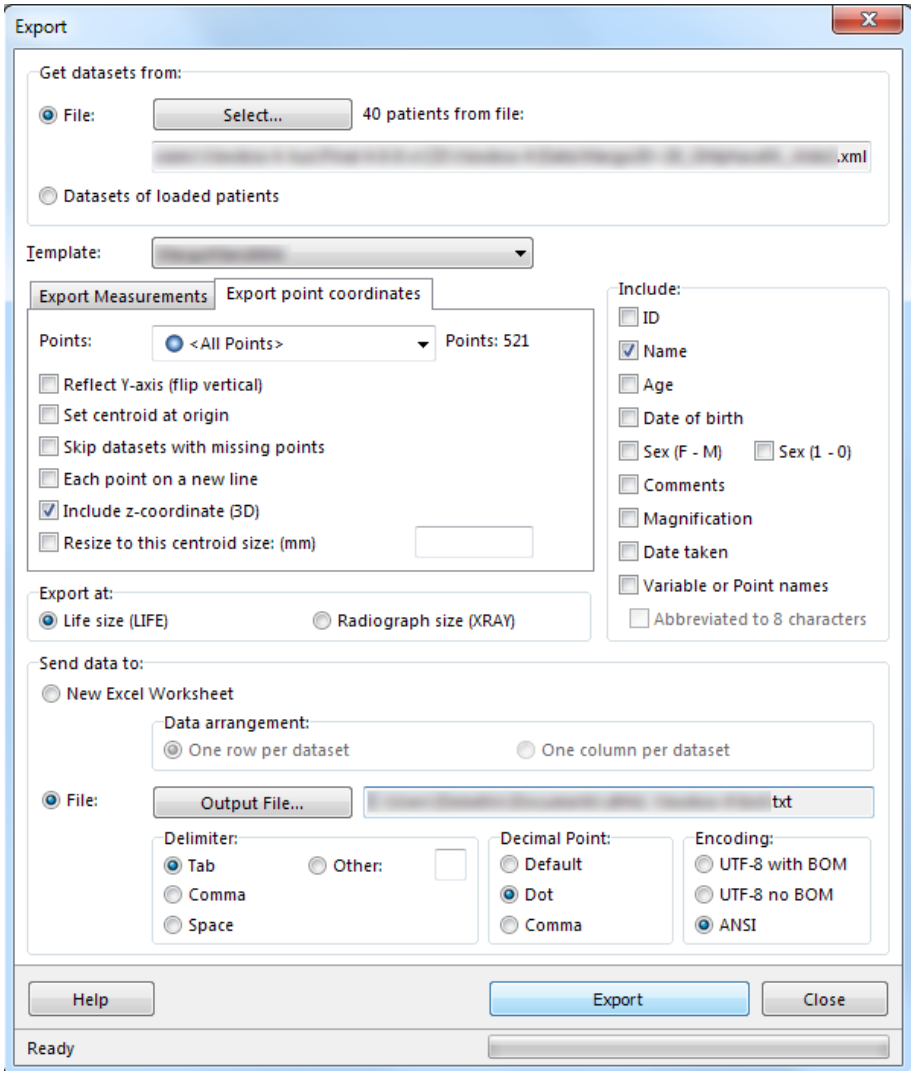
- SP001a
- SP001b
- SP002a
- SP002b
- …

where ‘a’ and ‘b’ signify the two repeated digitizations.

Note that we pad numbers with ‘0’ (i.e. SP001 and not SP1), so that all names have the same length. This is important later.

Step 2 (Viewbox)

Export the specimens to a text file using Tools – Export:



Make sure 'Include z-coordinate' is selected, if your data are 3D.
Make sure 'Name' is selected.
Note the settings for the output file:

- Delimiter: Tab
- Decimal Point: Dot (try Comma if Dot does not work when importing into MorphoJ)
- Encoding: ANSI or UTF-8 no BOM

Step 3 (MorphoJ)

Open MorphoJ and create a Dataset using the file from the previous step:

File – Create New Dataset

Make sure you set 2 or 3 dimensions, depending on your data. Then select the txt file and click ‘Create Dataset’.

Perform a Procrustes Fit:

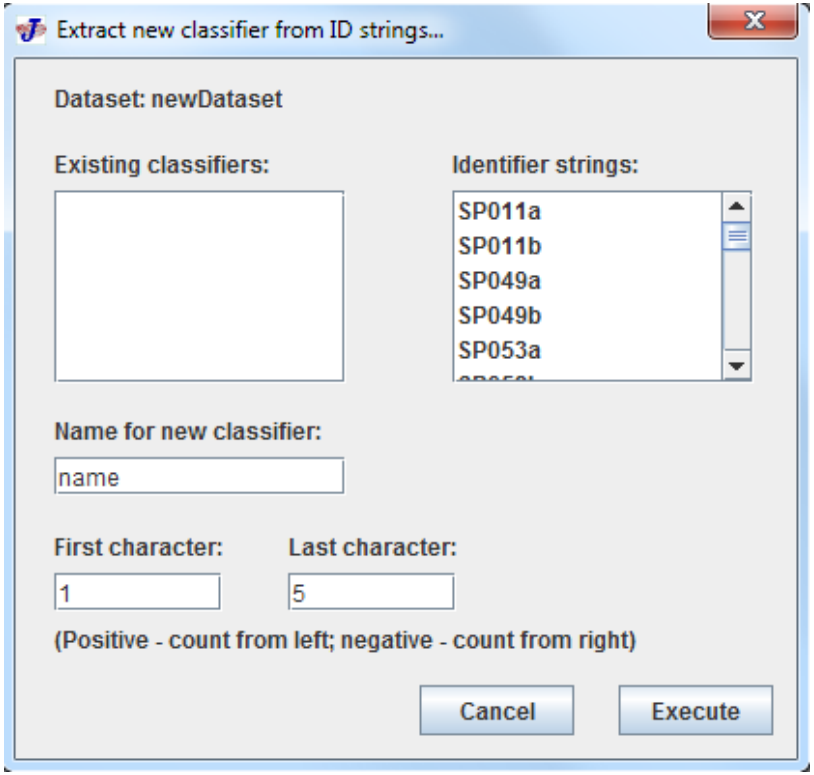
Preliminaries – New Procrustes Fit…

Step 3 - continued

Extract two classifiers. The first contains the name (e.g. SP001) and the second the repeat (a or b):

Preliminaries – Extract new classifier from ID strings…

Set the name and the first and last characters (1 and 5, because ‘SP001’ has 5 characters), click ‘Execute’:

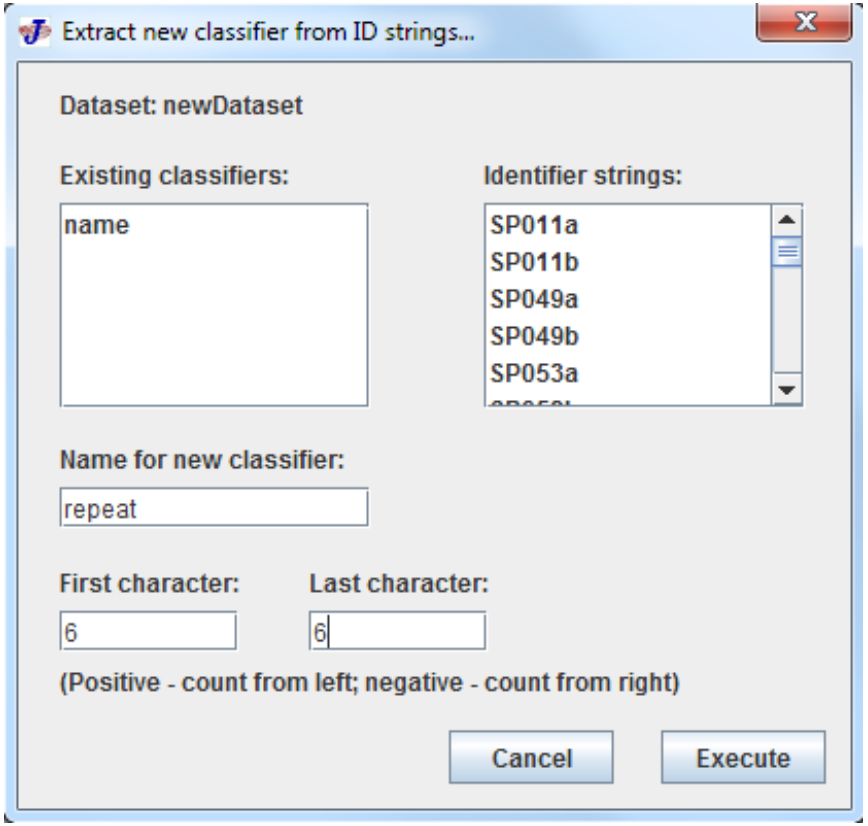


Step 3 - continued

The second classifier is based on the a or b character:

Preliminaries – Extract new classifier from ID strings…

Set the name and the first and last characters (6 and 6, because ‘a’ and ‘b’ are always at the 6th position), click ‘Execute’:

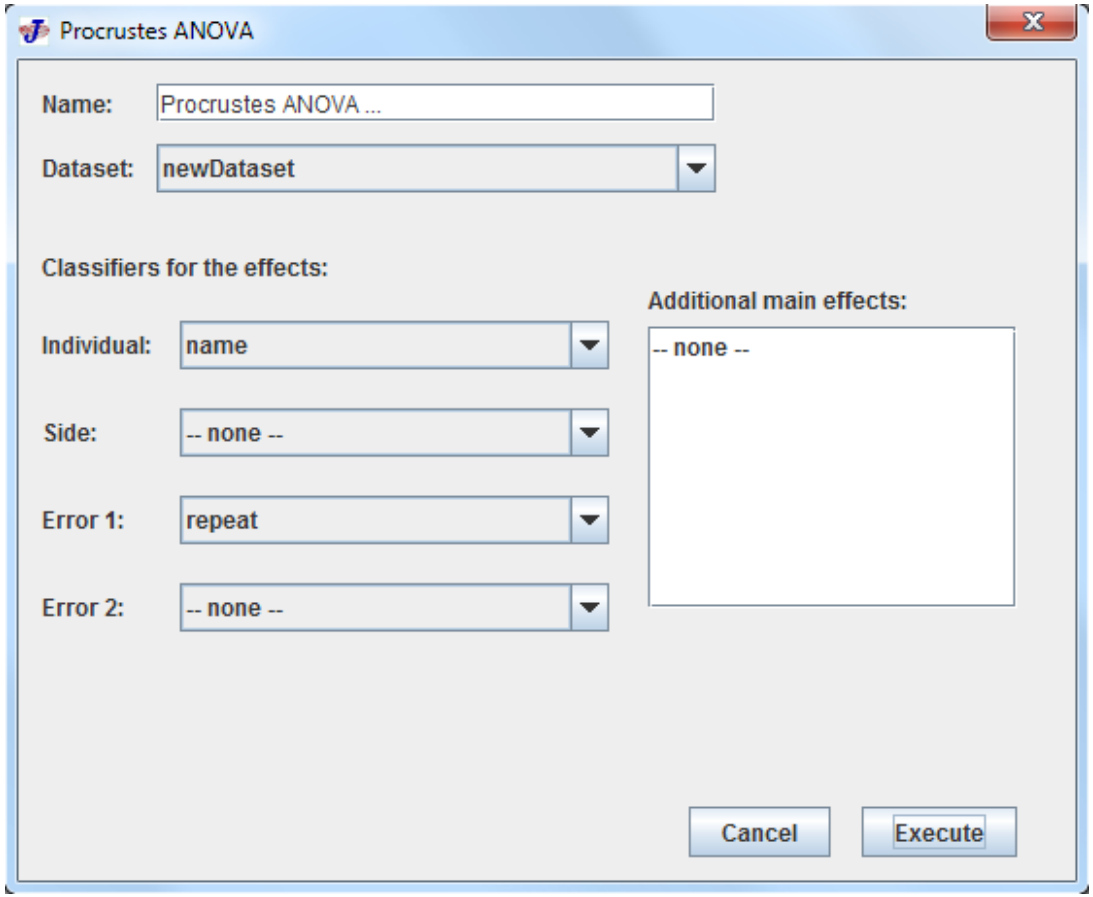


Step 4 (MorphoJ)

Perform a Procrustes ANOVA:

Variation – Procrustes ANOVA…

Set the ‘repeat’ classifier as ‘Error 1’:



Step 5 (MorphoJ): Results

These appear at the end of the 'Results' tab:

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Procrustes ANOVA: Procrustes ANOVA ...
Dataset: newDataset

Classifiers used for the Procrustes ANOVA:
Individuals: name
Error 1: repeat

Centroid size:
Effect        SS              MS            df          F         P (param.)
Individual    154155,651890   8113,455363   19          21240,51  <.0001
Error 1       7,639606        0,381980      20

Shape, Procrustes ANOVA:
Effect        SS              MS            df          F         P (param.)
Individual    0,13215122      0,0000044700  29564       242,04    <.0001
Error 1       0,00057473      0,0000000185  31120
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Based on the book "Geometric Morphometrics for Biologists. A Primer, 2nd Edition, Authors: Miriam Zelditch, Donald Swiderski, H. David Sheets", page 258, we calculate reliability of shape as:

Ind_var = (MSindividual - MSerror)/2 = (44700 - 185)/2 = 22257.5

Total = 22257.5 + 185 = 22442.5

Ratio = 22257.5 / 22442.5 = 0.992 = repeatability