

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:

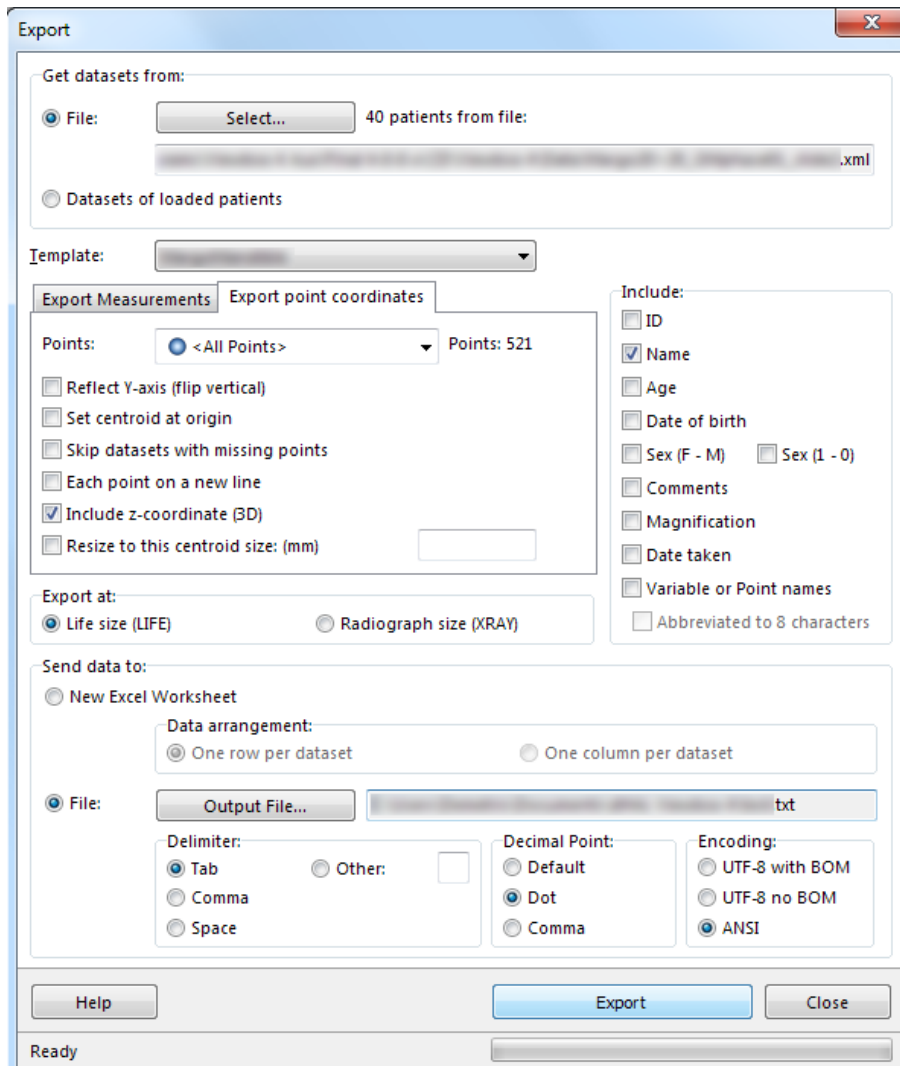
- SP001a
- SP001b
- SP002a
- SP002b
- ...

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:

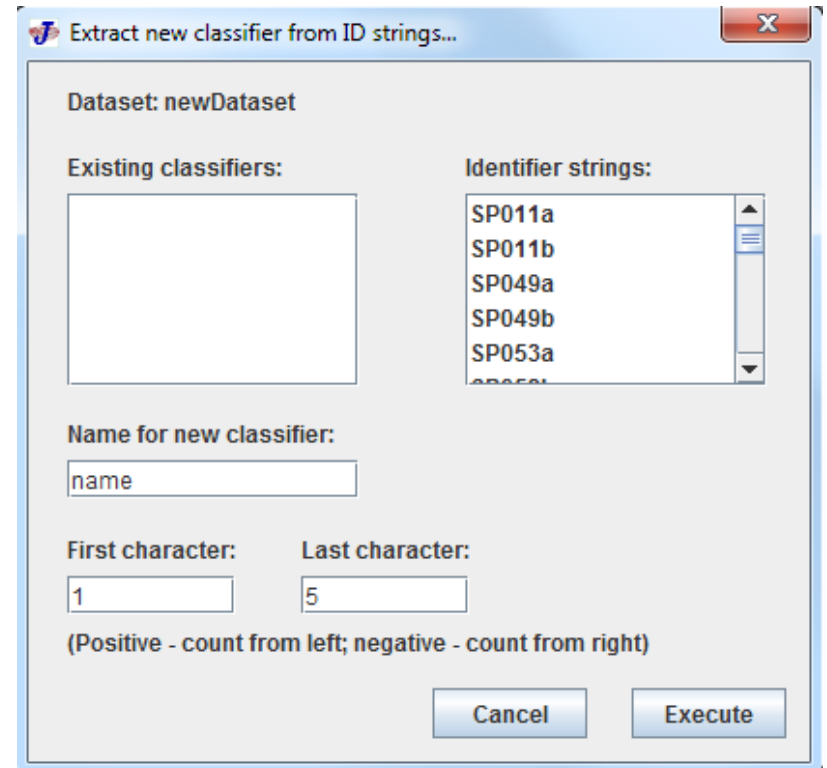
Preliminaries – New Procrustes Fit...

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':



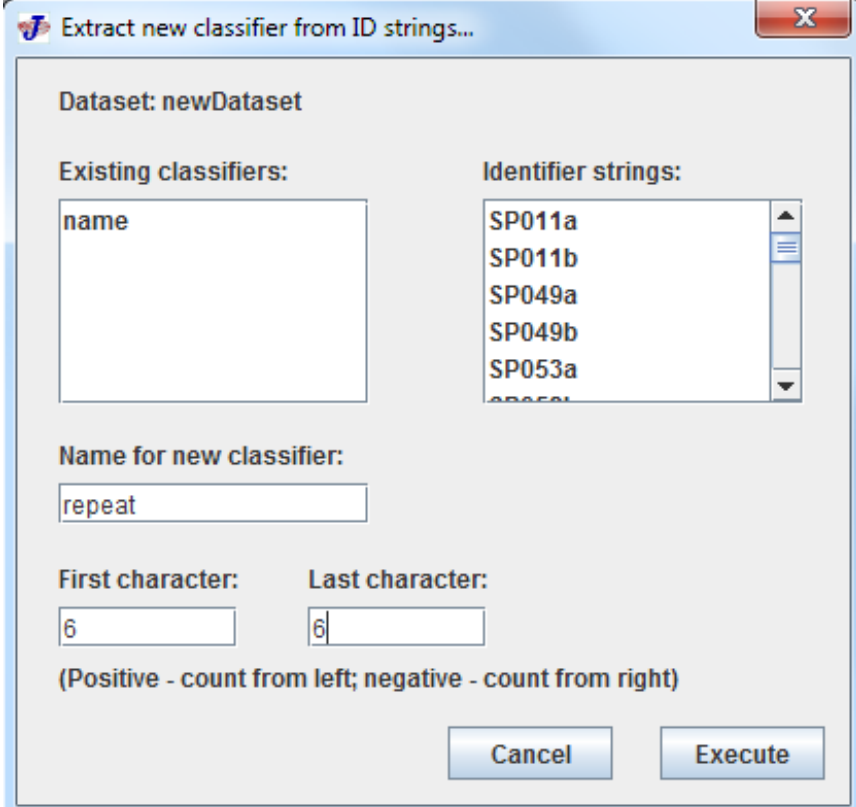
The screenshot shows a dialog box titled "Extract new classifier from ID strings...". The dialog is set to "Dataset: newDataset". It features two columns: "Existing classifiers:" (an empty list) and "Identifier strings:" (a list containing "SP011a", "SP011b", "SP049a", "SP049b", "SP053a", and "SP053b"). Below these is a text field for "Name for new classifier:" containing the text "name". At the bottom, there are two input fields: "First character:" with the value "1" and "Last character:" with the value "5". A note below these fields reads "(Positive - count from left; negative - count from right)". At the bottom right, there are "Cancel" and "Execute" buttons.

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':



Dataset: newDataset

Existing classifiers:

- name

Identifier strings:

- SP011a
- SP011b
- SP049a
- SP049b
- SP053a

Name for new classifier:

repeat

First character: 6 Last character: 6

(Positive - count from left; negative - count from right)

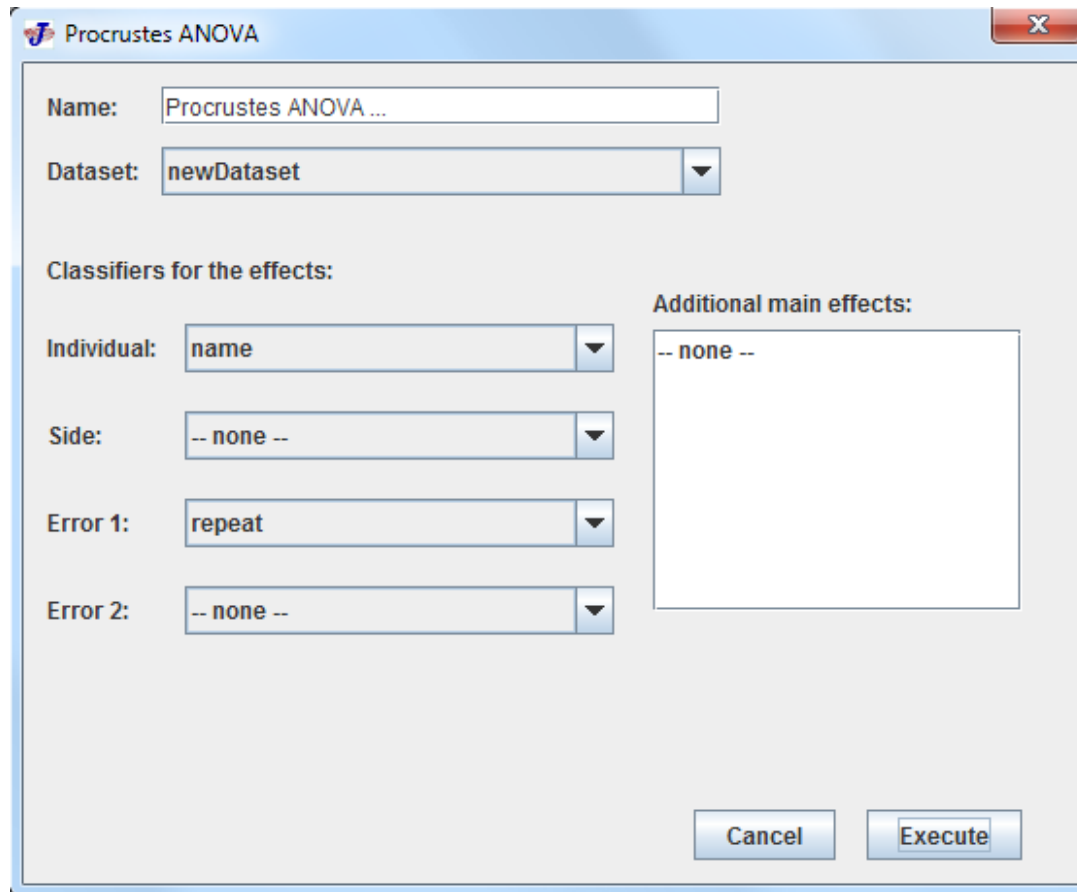
Cancel Execute

Step 4 (MorphoJ)

Perform a Procrustes ANOVA:

Variation – Procrustes ANOVA...

Set the 'repeat' classifier as 'Error 1':



The screenshot shows the 'Procrustes ANOVA' dialog box. The 'Name' field is 'Procrustes ANOVA ...'. The 'Dataset' dropdown is 'newDataset'. Under 'Classifiers for the effects:', 'Individual' is 'name', 'Side' is '-- none --', 'Error 1' is 'repeat', and 'Error 2' is '-- none --'. The 'Additional main effects' list is empty, showing '-- none --'. 'Cancel' and 'Execute' buttons are at the bottom.

Field	Value
Name	Procrustes ANOVA ...
Dataset	newDataset
Individual	name
Side	-- none --
Error 1	repeat
Error 2	-- none --
Additional main effects	-- none --

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
```

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:

$$\text{Ind_var} = (\text{MS}_{\text{individual}} - \text{MS}_{\text{error}}) / 2 = (44700 - 185) / 2 = 22257.5$$

$$\text{Total} = 22257.5 + 185 = 22442.5$$

$$\text{Ratio} = 22257.5 / 22442.5 = 0.992 = \text{repeatability}$$