

# Zebrafish morphometric analysis protocol

## Supplementary protocol for

### **Zebrafish carrying pycr1 gene deficiency display aging and multiple behavioral abnormalities**

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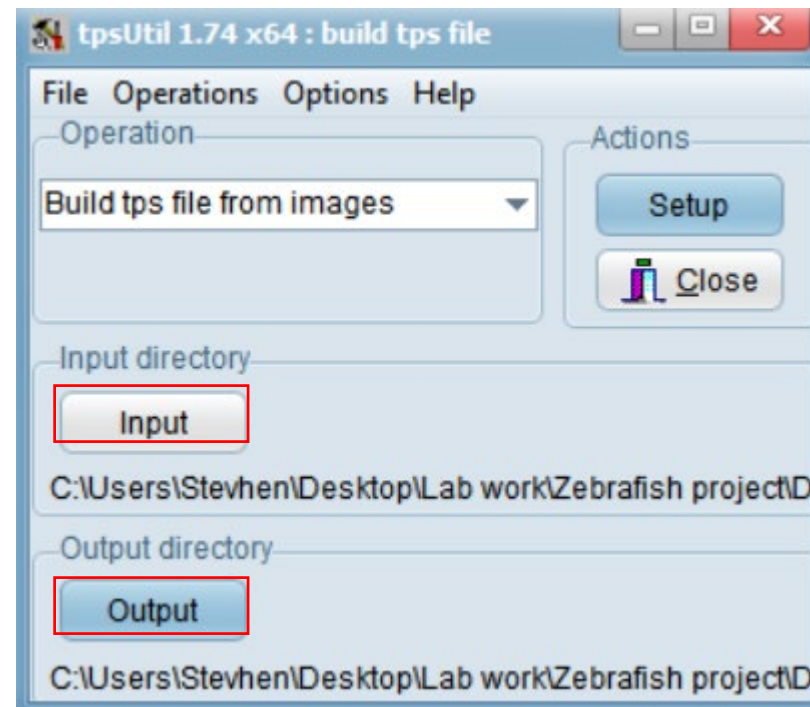
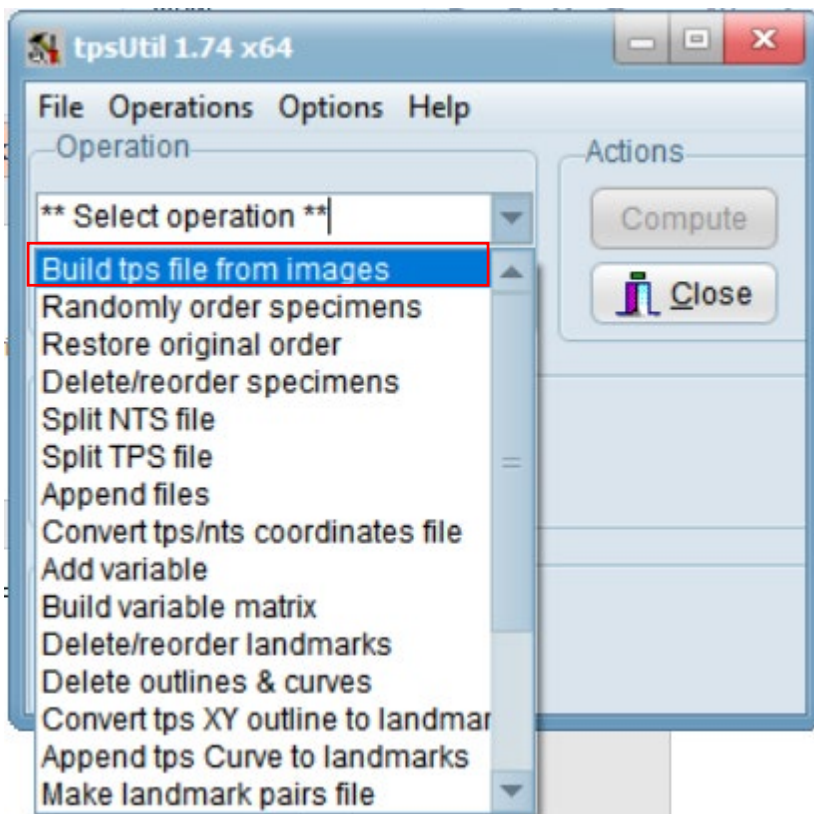
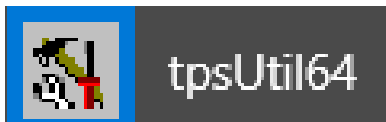
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# Analysis pipeline outline

1. Convert image file to .tps format using **TpsUtil**
2. Digitize the image landmark using **TpsDig2**
3. Procrustes analysis using **MorphoJ**
4. Generate covariance matrix using **MorphoJ**
5. Principal Component Analysis (PCA) using **MorphoJ**

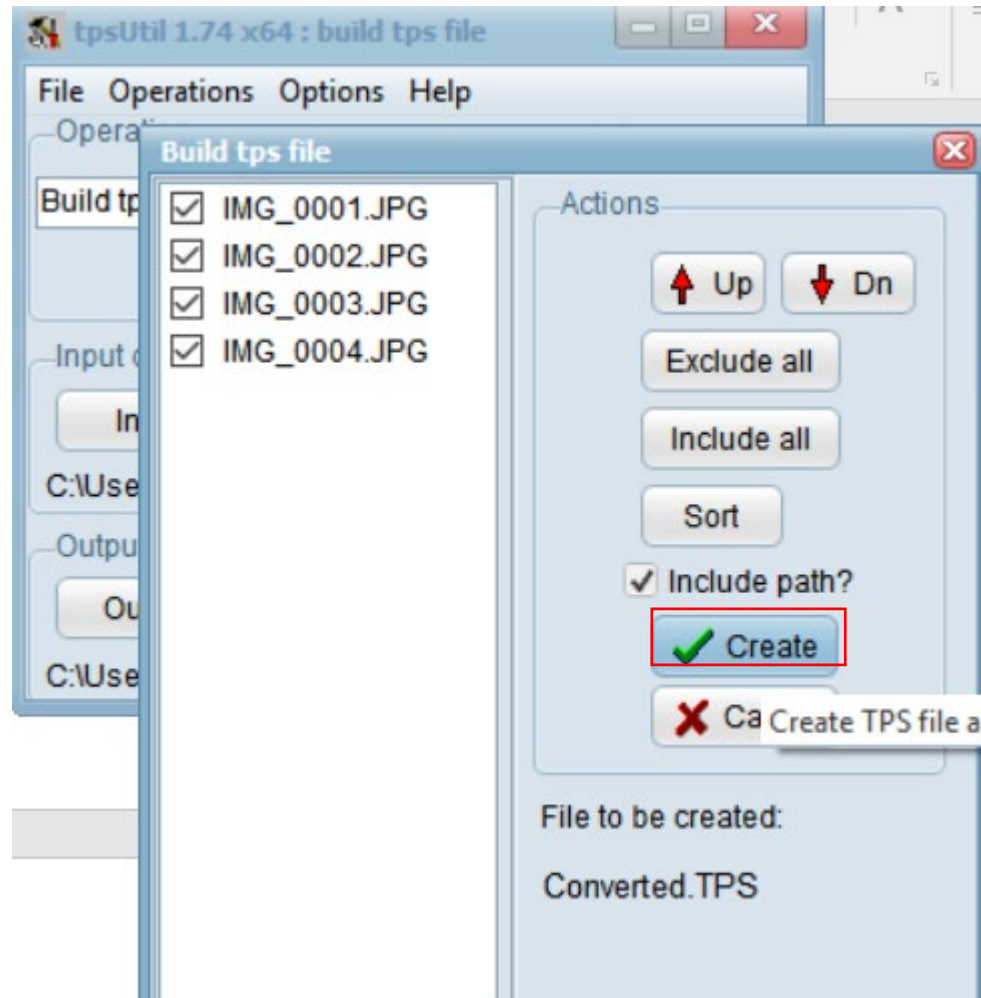
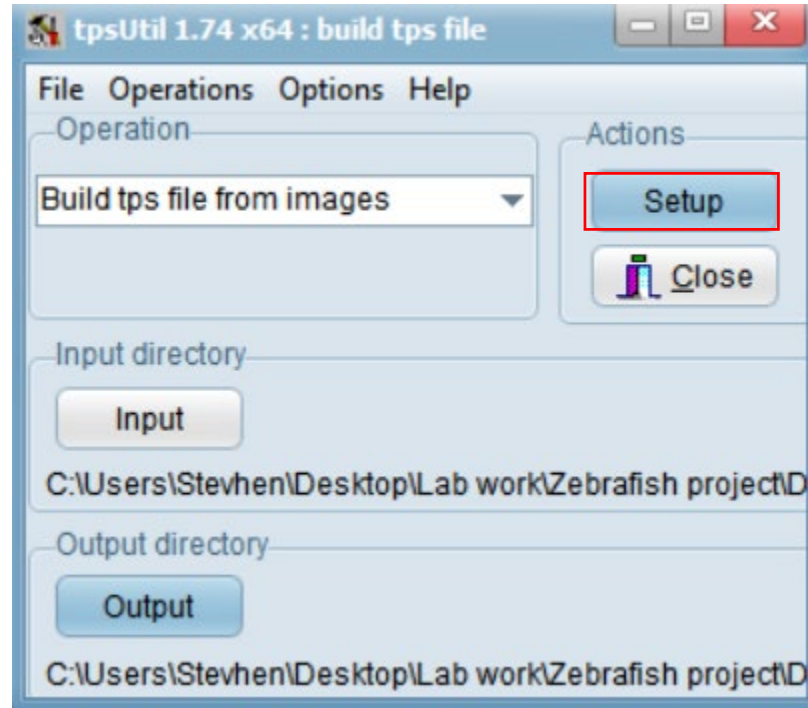
# 1. Convert image file to .tps format using TpsUtil

- Download **tpsUtil64** to convert image file to TPS file  
<http://life.bio.sunysb.edu/morph/soft-utility.html>



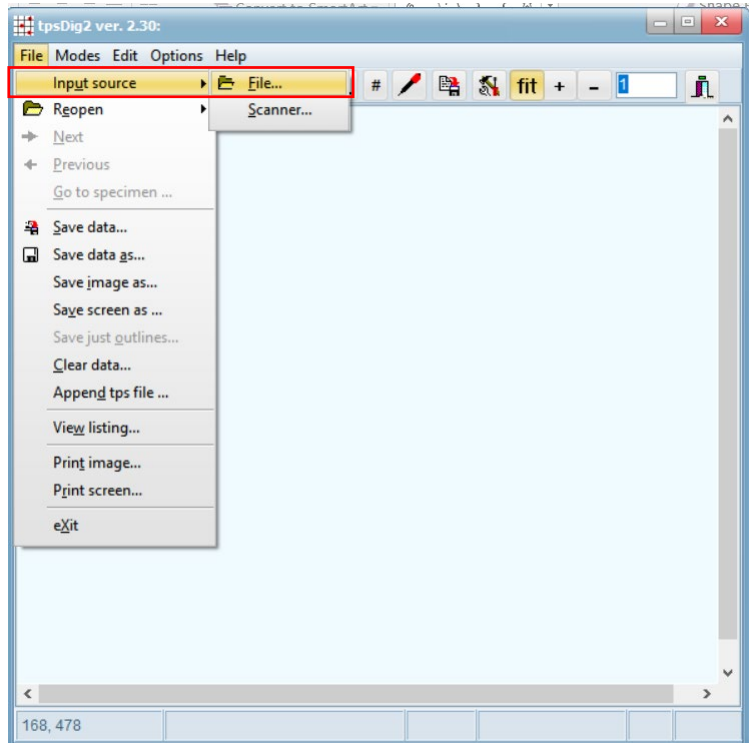
Select the Input and Output Directory

# 1. Convert image file to .tps format using tpsUtil

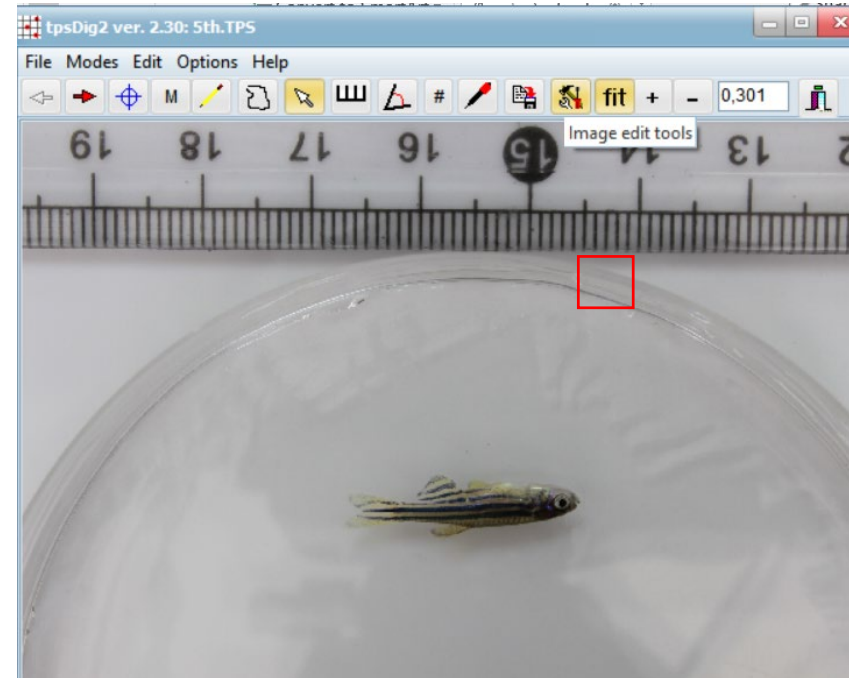


Select all the image to be measured in the TPS file and create the file

## 2. Digitize the image landmark using TpsDig2

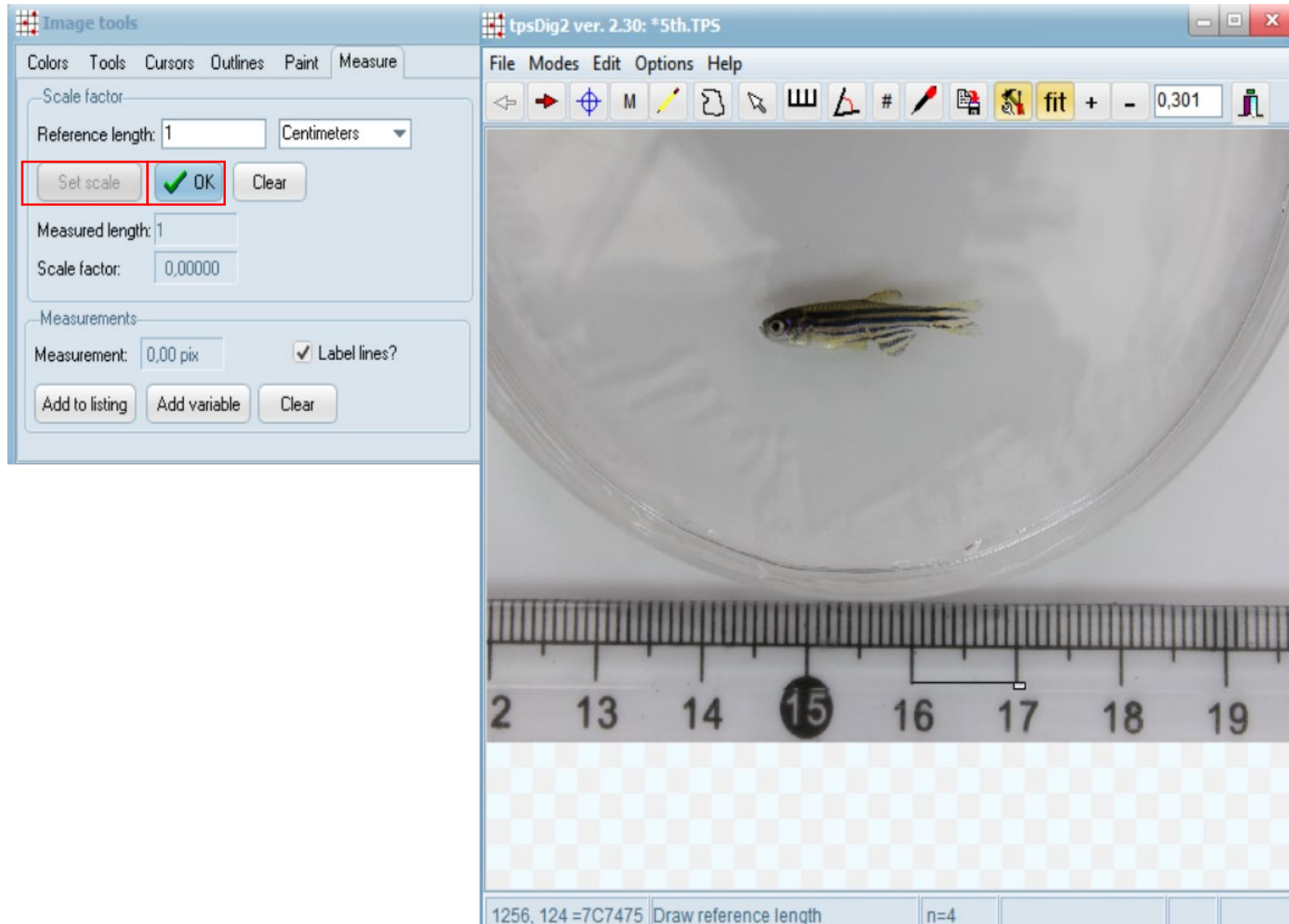


Open the .tps file by selecting the input source



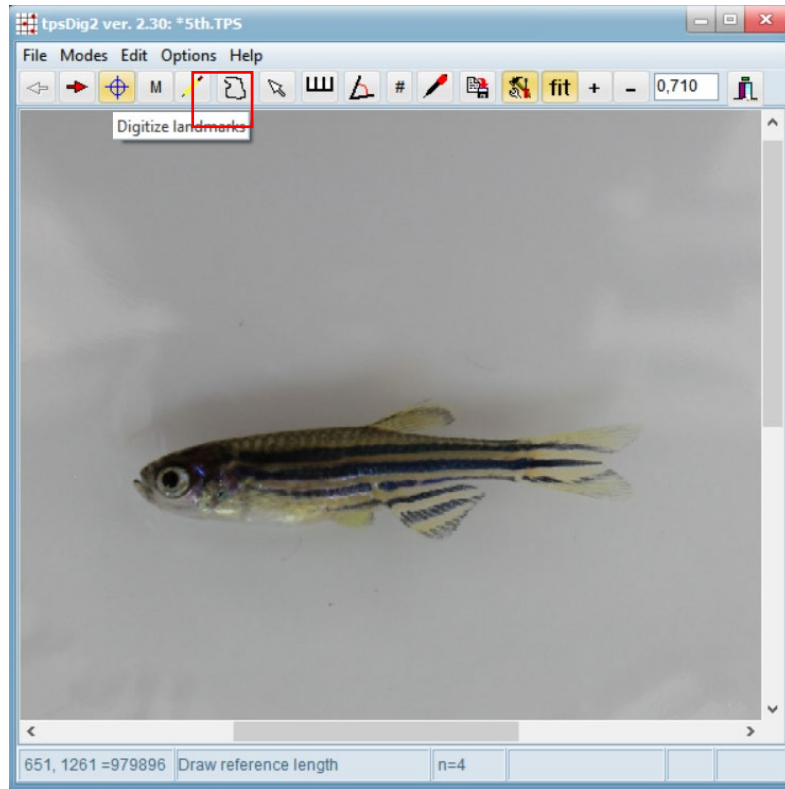
Edit the image using the edit tools image and set the scale

## 2. Digitize the image landmark using TpsDig2

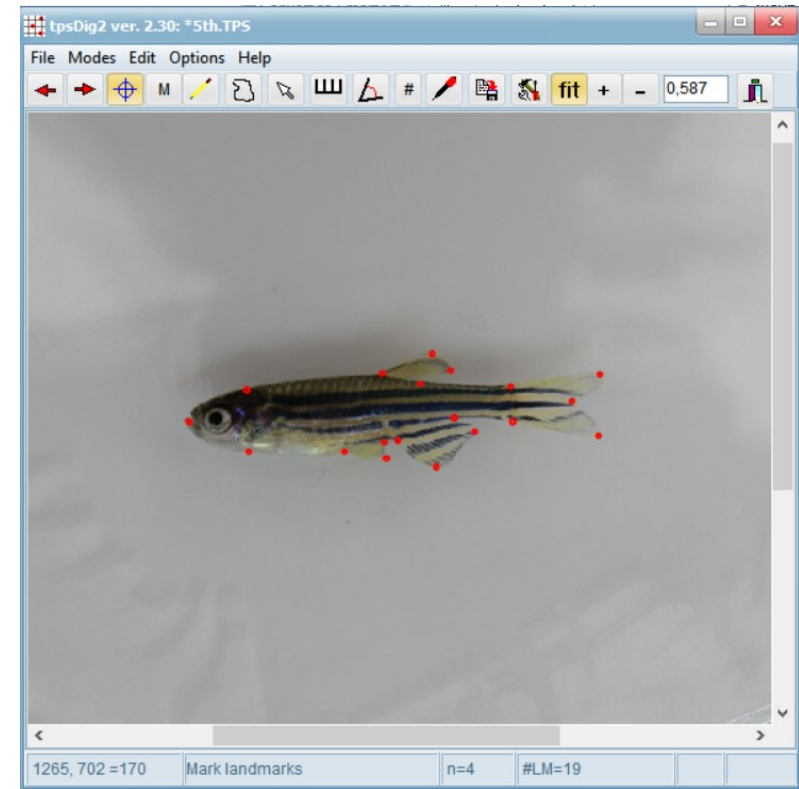


Set the scale by selecting the measure tab and press the OK button

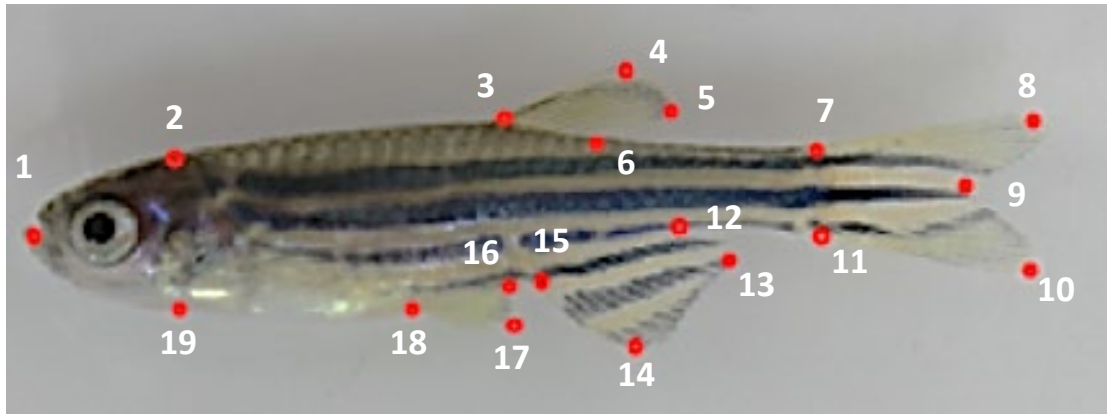
## 2. Digitize the image landmark using TpsDig2



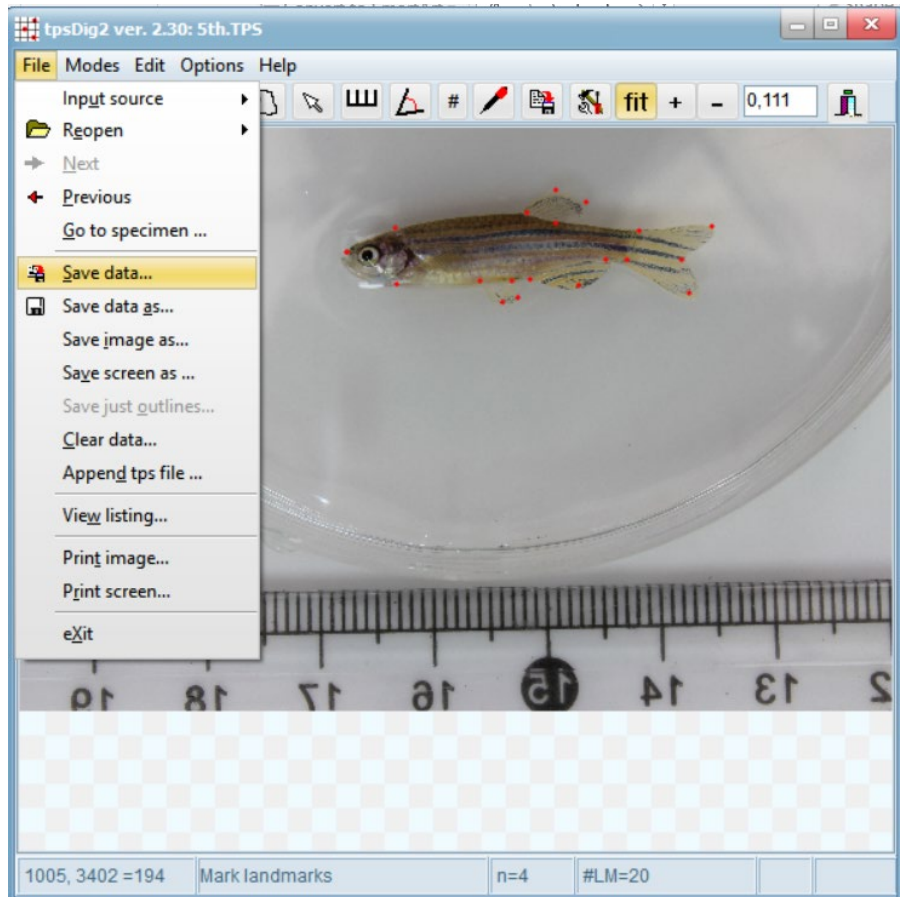
Select the  
digitize  
landmarks icon  
then click the  
landmark



In our case,  
19 landmarks were selected



## 2. Digitize the image landmark using TpsDig2



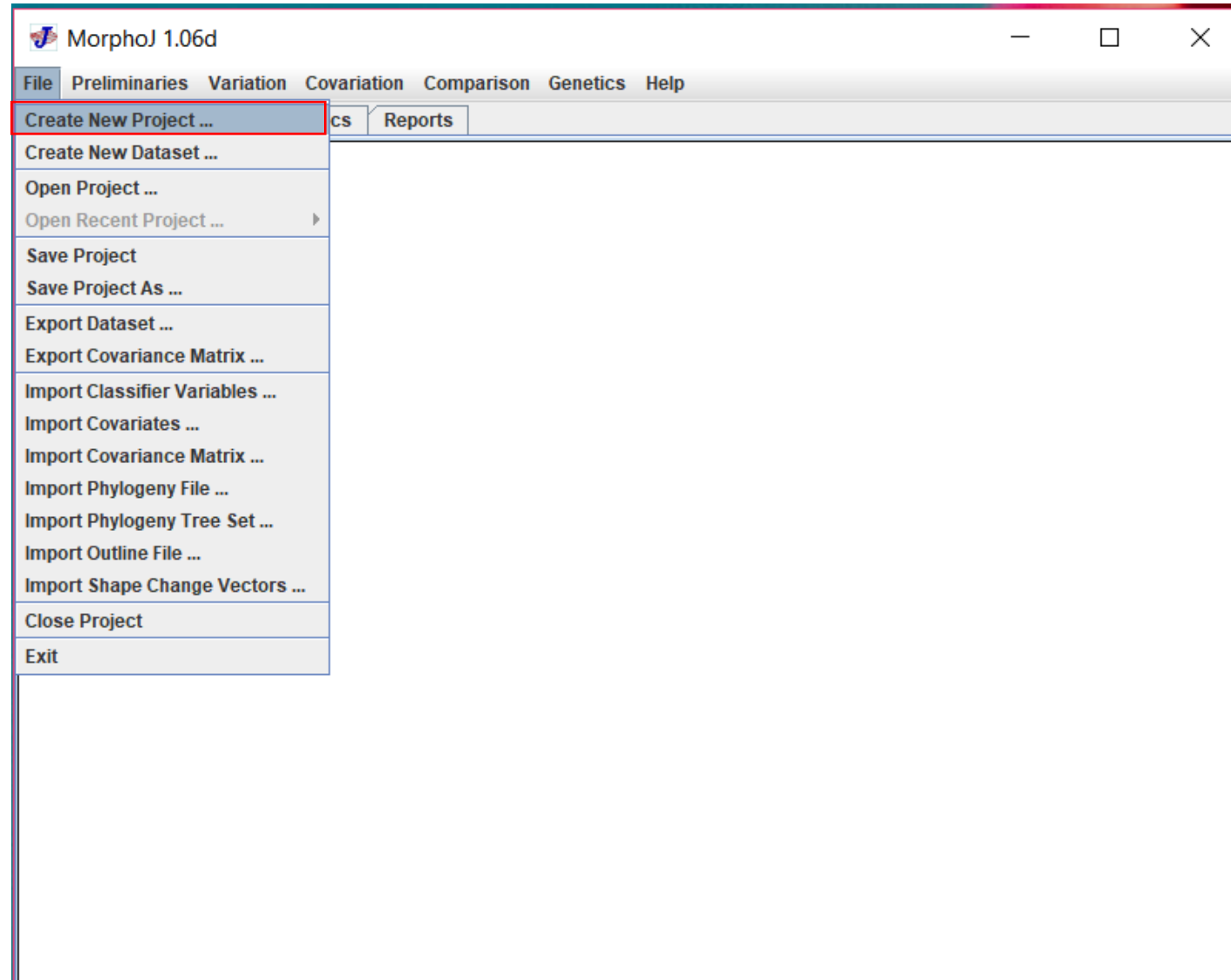
Save the file in  
.TPS after  
digitizing the  
landmark

To do the Principal component  
analysis (PCA), we need to install  
**MorphoJ** program




**MorphoJ** is an open-source program and can be downloaded freely  
[http://www.flywings.org.uk/morphoj\\_page.htm](http://www.flywings.org.uk/morphoj_page.htm)

### 3. Procrustes analysis using MorphoJ



Create New Project by  
choosing the File Tab

 Morpho

File Prelimin

Project Tree

new projec

Name the new project and select the data file and appropriate options

Name for the new project: Zebrafish Project

Dimensionality of the data: ☒ 2 dimensions ☐ 3 dimensions  
Object symmetry? ☐ yes ☒ no

Name for the new dataset: Zebrafish

File type: ☐ text ☐ NTSYSpc ☒ TPS ☐ Morphologika

Look In: Desktop

PS4 Remote Play.Ink

Steam.Ink

The Witcher 3 - Wild Hunt.Ink

tpsPLS32.Ink

VLC media player.Ink

XLSTAT 2014.Ink

6.Ink

µTorrent.Ink

caligata\_caligata\_Becheroff.txt

Digitize image Matrix.txt

Digitize1 landmarked.TPS

Discord.Ink

Dota 2.url

FISH mark.xlsx

Grand Theft Auto V.url

Ima

IMG

IMG

Left

LINE

New

Sha

File Name: Digitize1 landmarked.TPS

Files of Type: All Files

Create Dataset

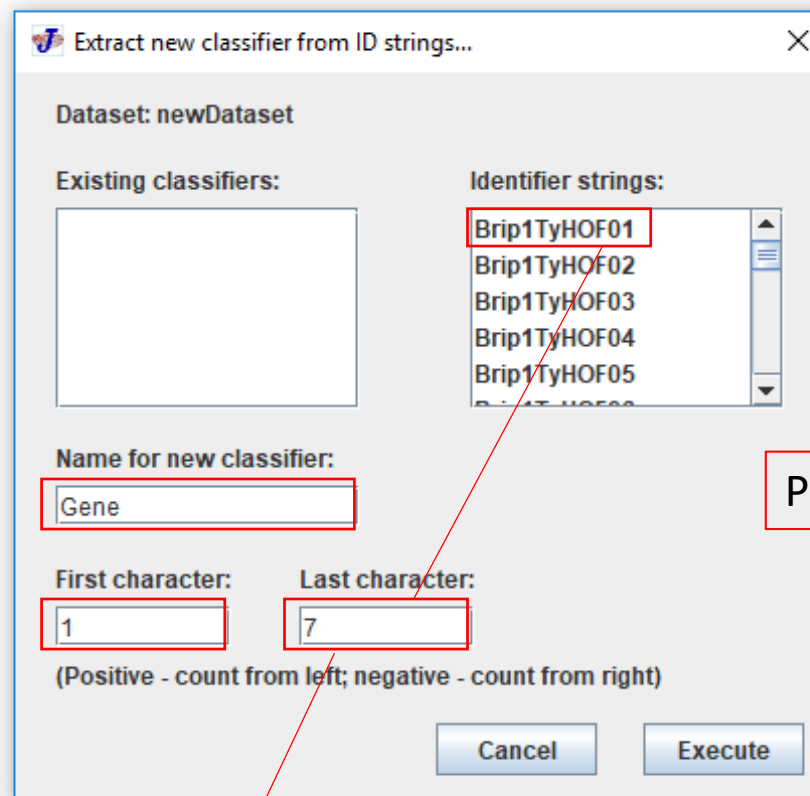
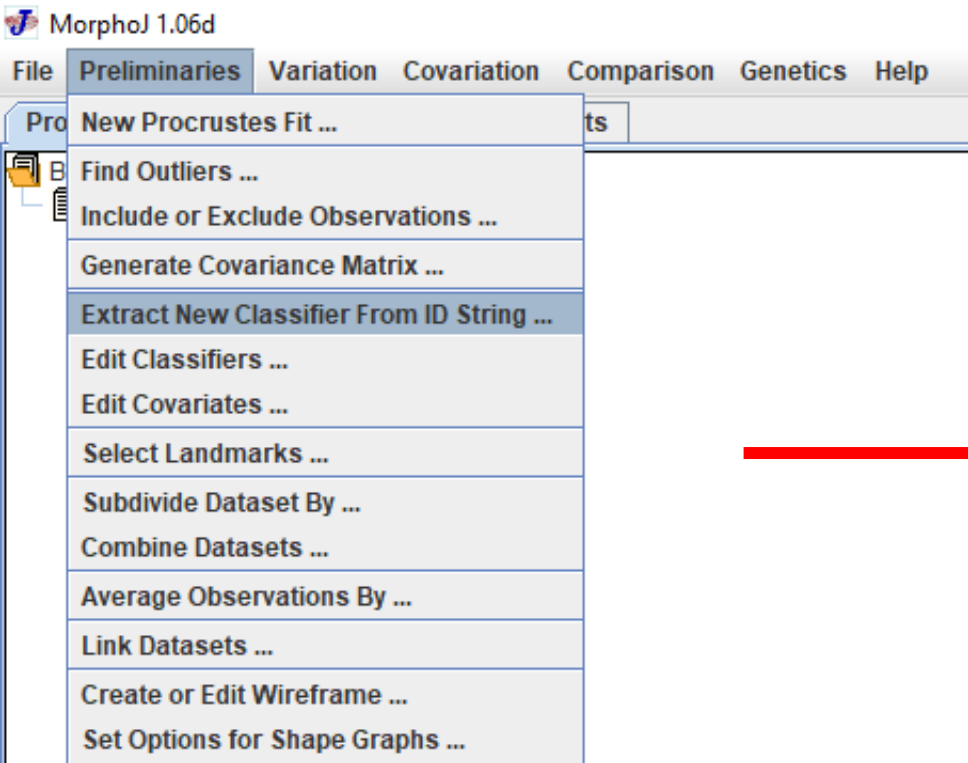
Cancel

Put a name for the project

Put a name for the dataset

Select the data file type


Select the .tps from tpsDig2 output



Put the name for a new classifier

For gene name start from 1-7 in this example

Select the newdatasetTo make a new classifier/can divide the sample by gender/gene

 Extract new classifier from ID strings... ✕

Dataset: newDataset

Existing classifiers:

gender

Identifier strings:

Brip1TyHOF01  
Brip1TyHOF02  
Brip1TyHOF03  
Brip1TyHOF04  
Brip1TyHOF05  
Brip1TyHOF06

Name for new classifier:

Gender

First character: 10 Last character: 10

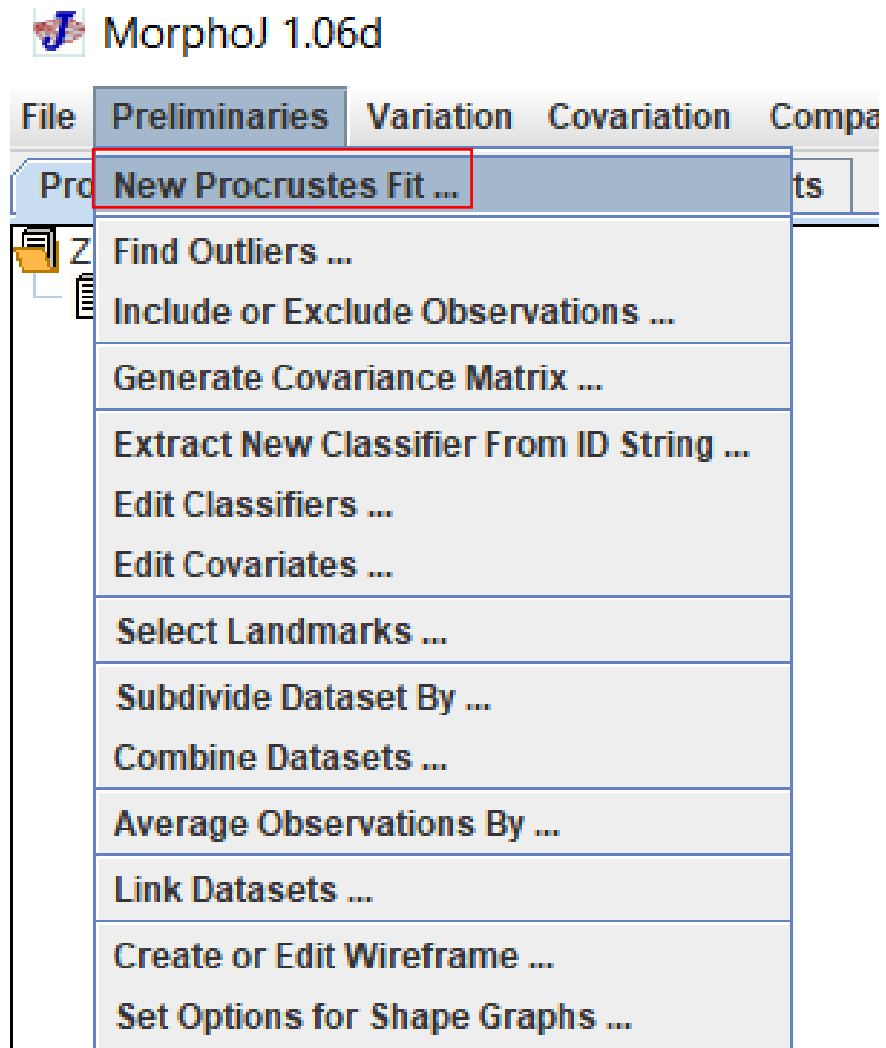
(Positive - count from left; negative - count from right)

Cancel Execute

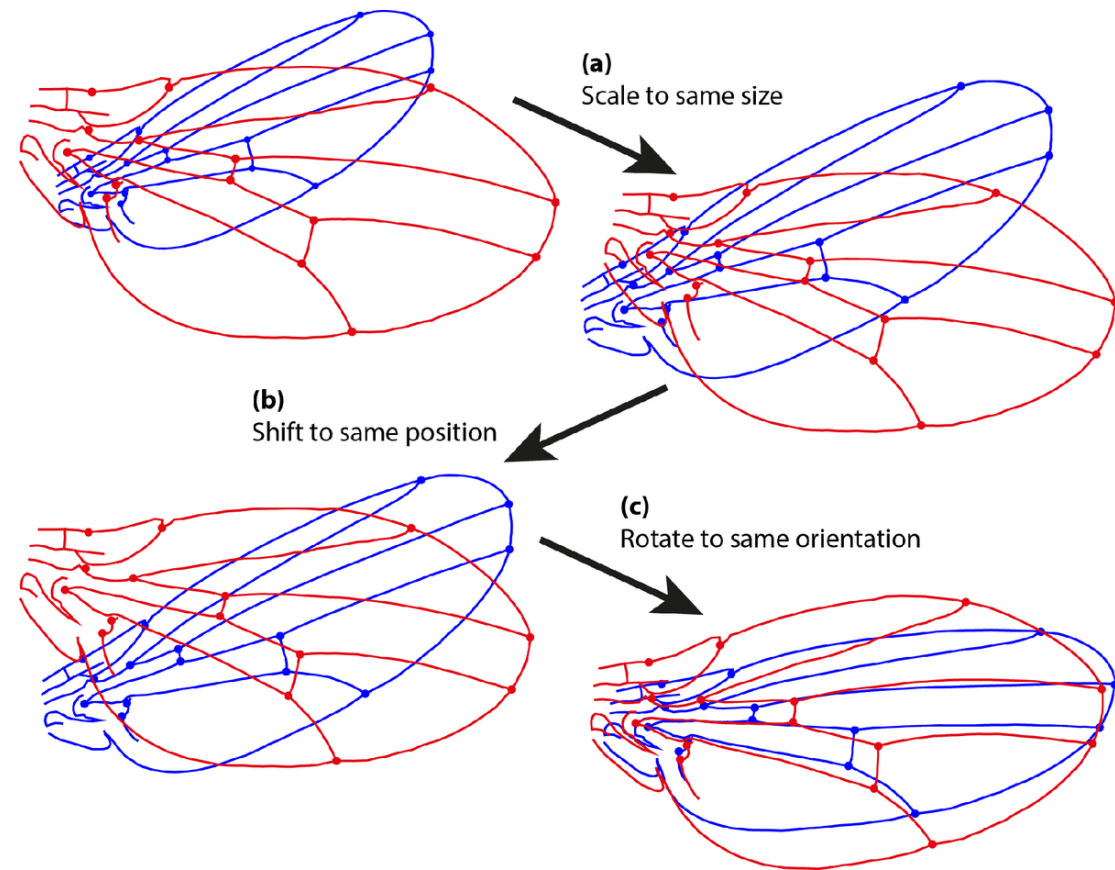
Put the name for a new classifier

For Gender name start from 10  
to 10 in this example

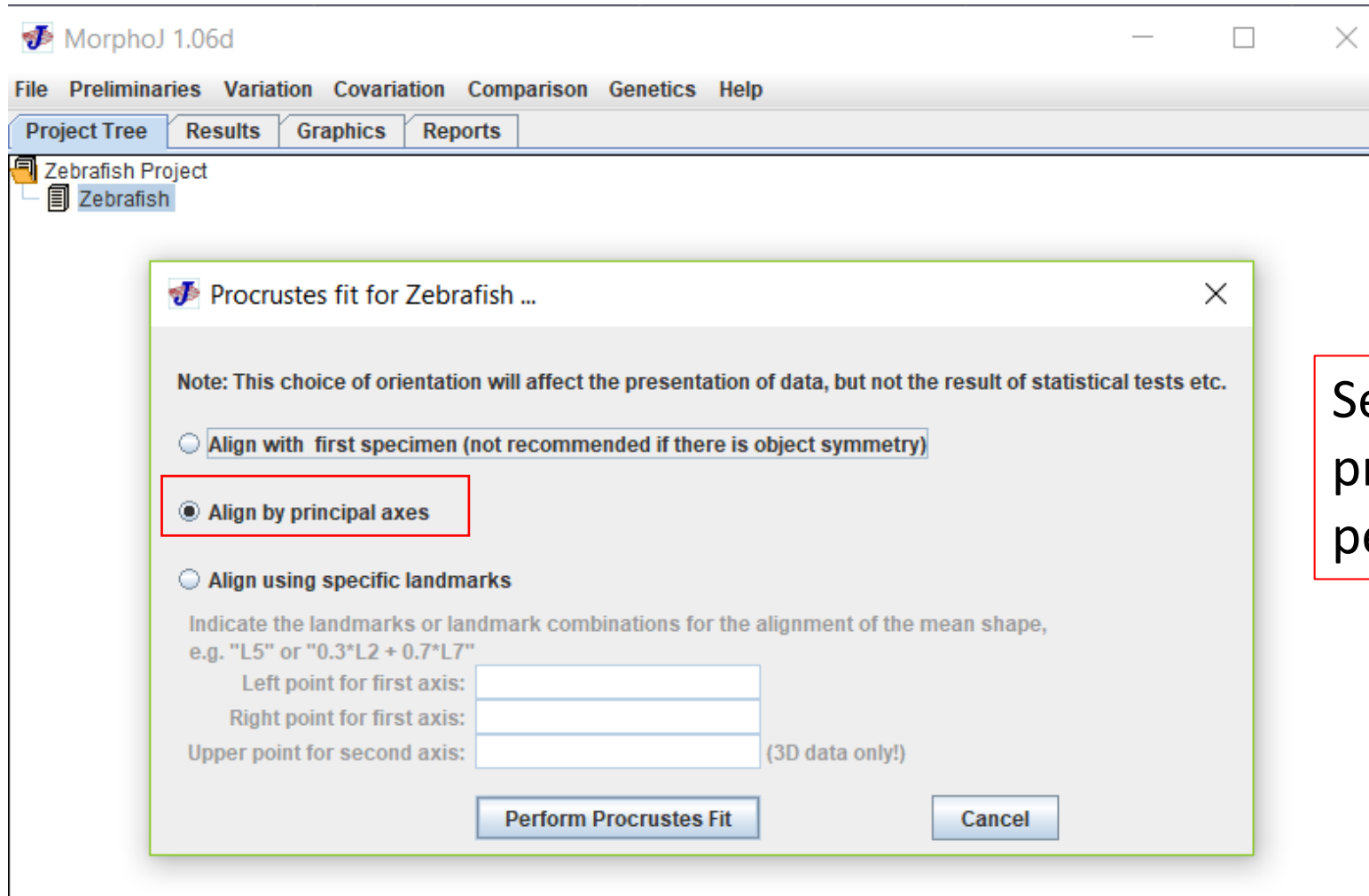
### 3. Procrustes analysis using MorphoJ



Do **Procrustes fit analysis** to analyse the distribution set of shape

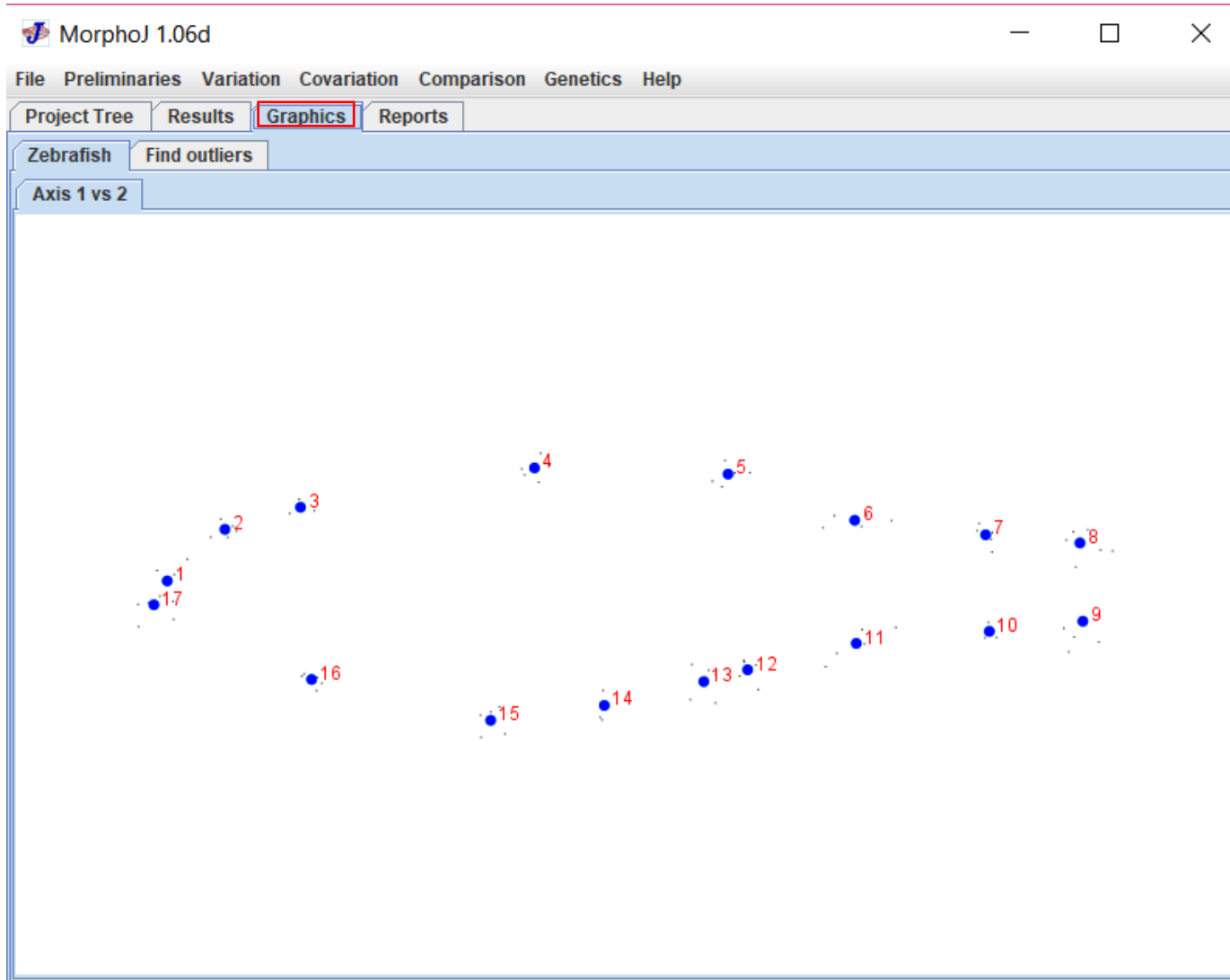


# 3. Procrustes analysis using MorphoJ



Select the Align by principal axes then perform **Procrustes Fit**

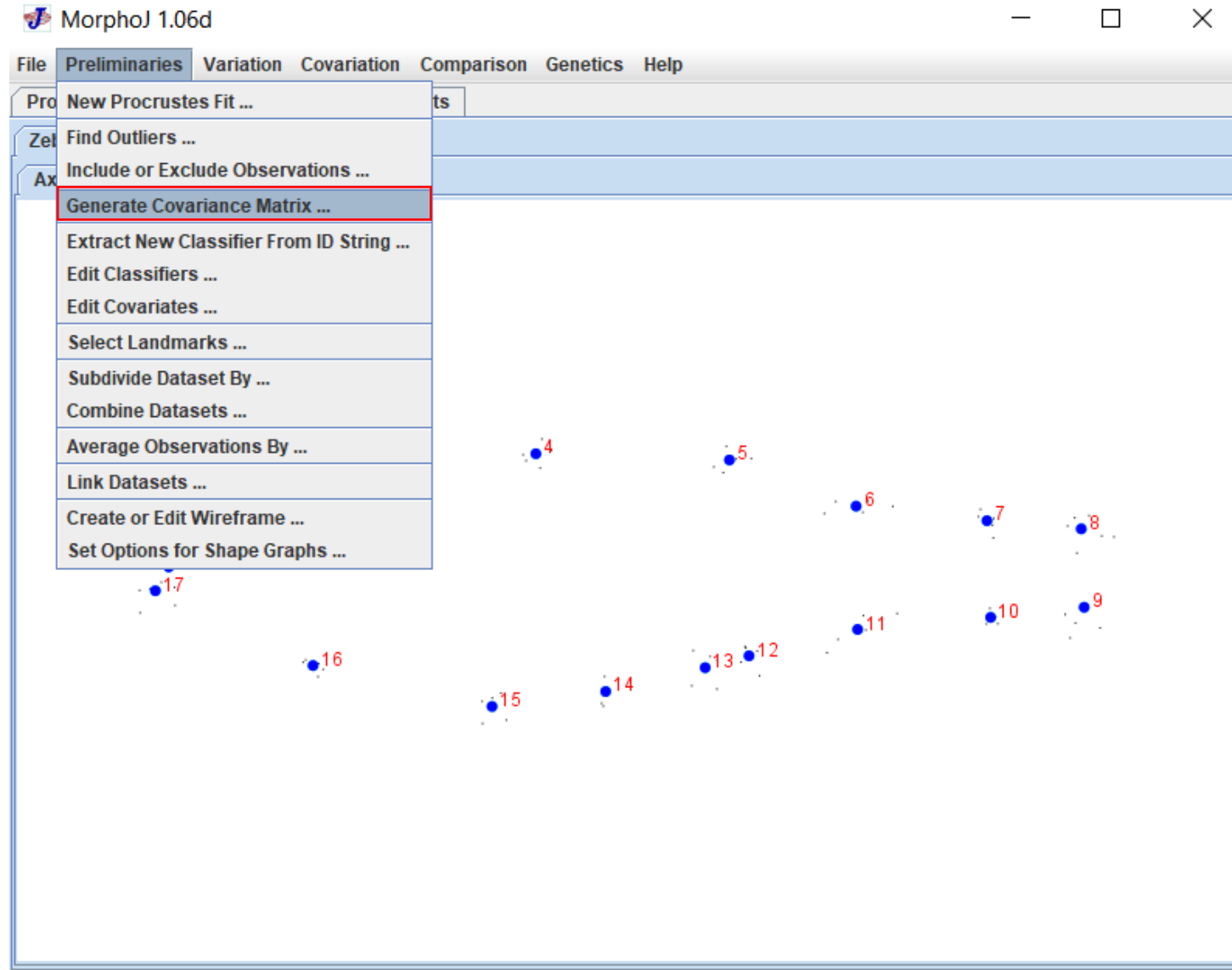
### 3. Procrustes analysis using MorphoJ



**Procrustes Fit analyses**  
result can be viewed in  
graphics tab

In this case,  
17 landmarks were selected

## 4. Generate covariance matrix using MorphoJ



Generate **covariance matrix** by selecting the preliminaries tab

## 4. Generate covariance matrix using MorphoJ

Select Data Sets to Generate Covariance Matrices

Select the data set and data type ...

Data sets

Selected:

newDataset

Include

Exclude

Available:

Data types (Select all types to be used)

Procrustes coordinates

☒ Pooled within-group covariances

gender

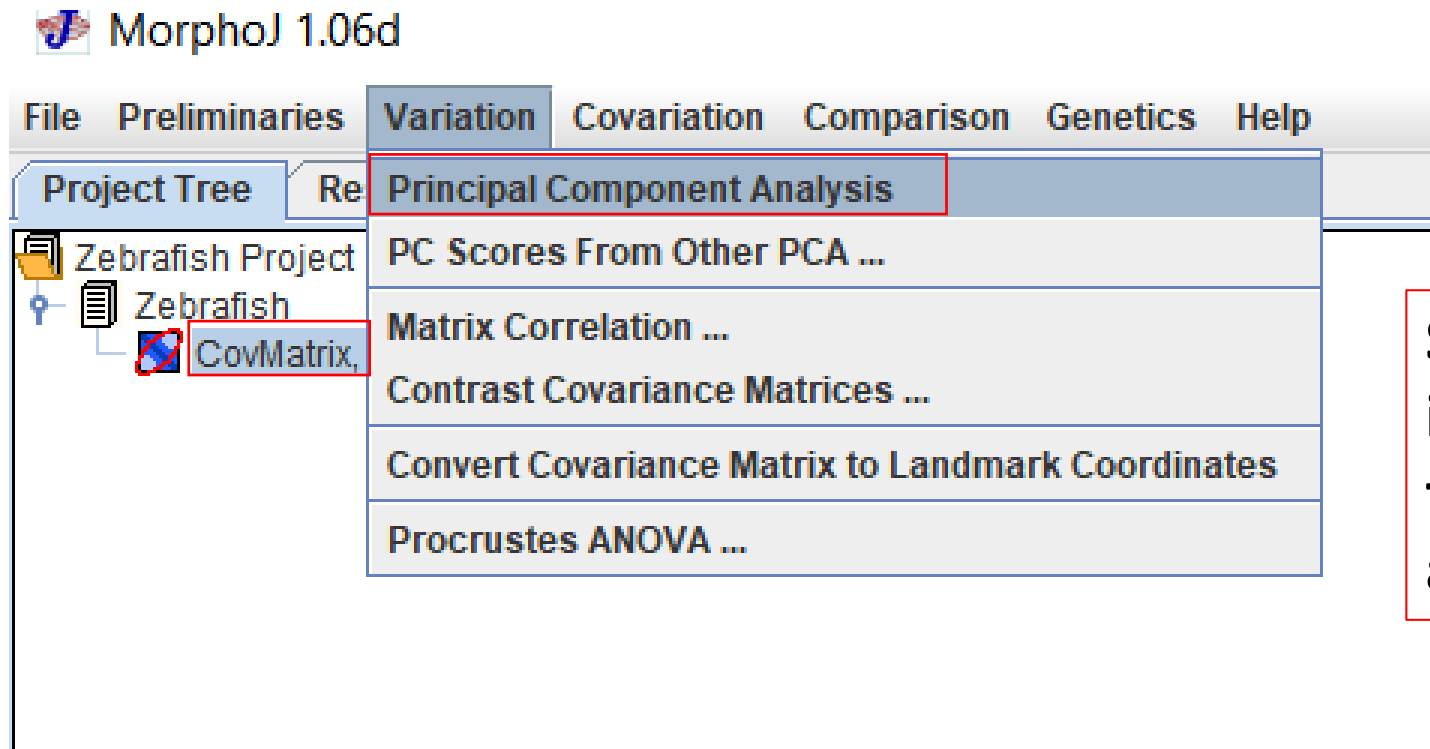
gene

Cancel

Execute

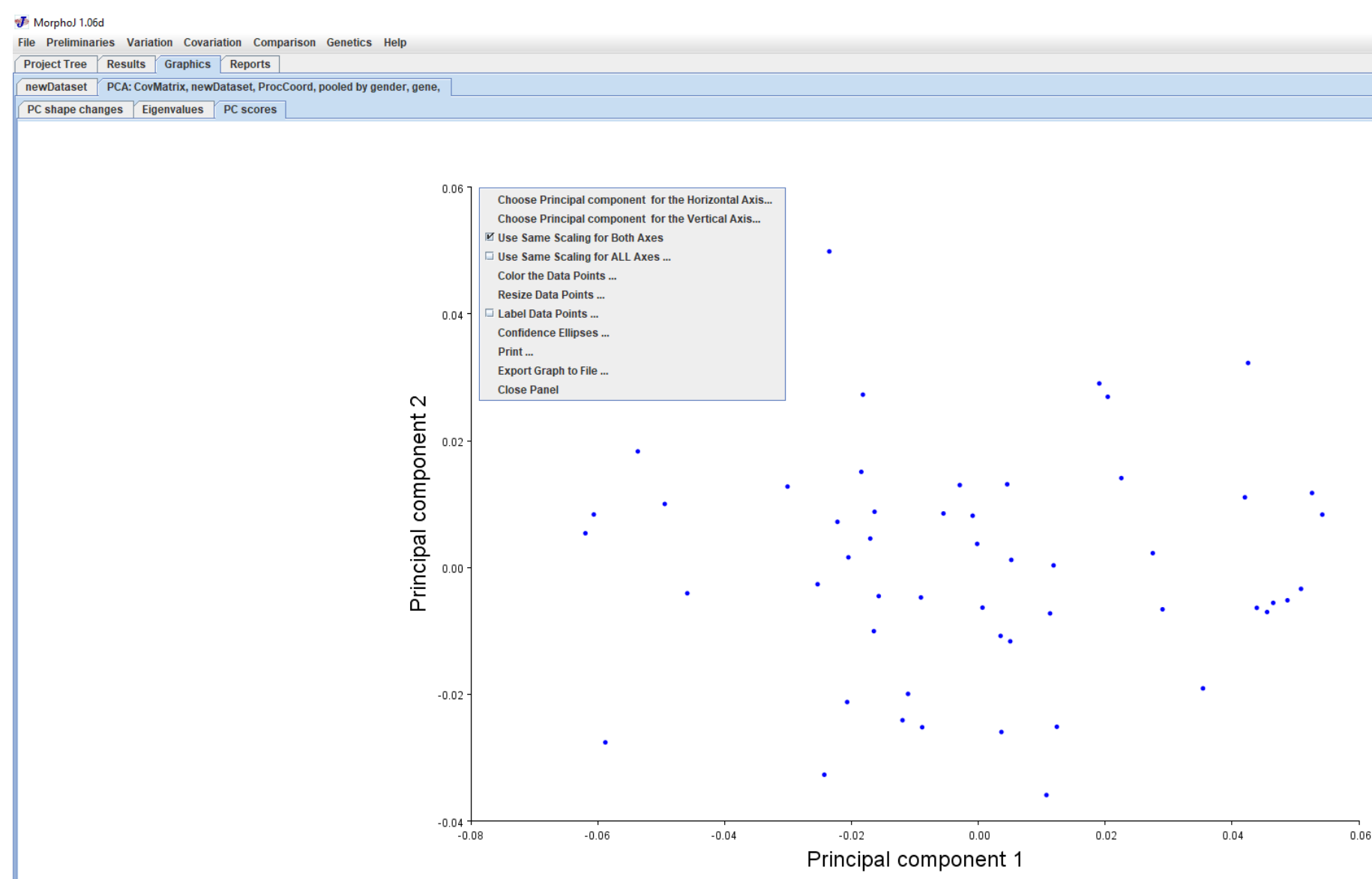
Select the dataset and  
“pooled within group  
covariance” then execute  
the program to generate  
**covariance matrices**

## 4. Generate covariance matrix using MorphoJ



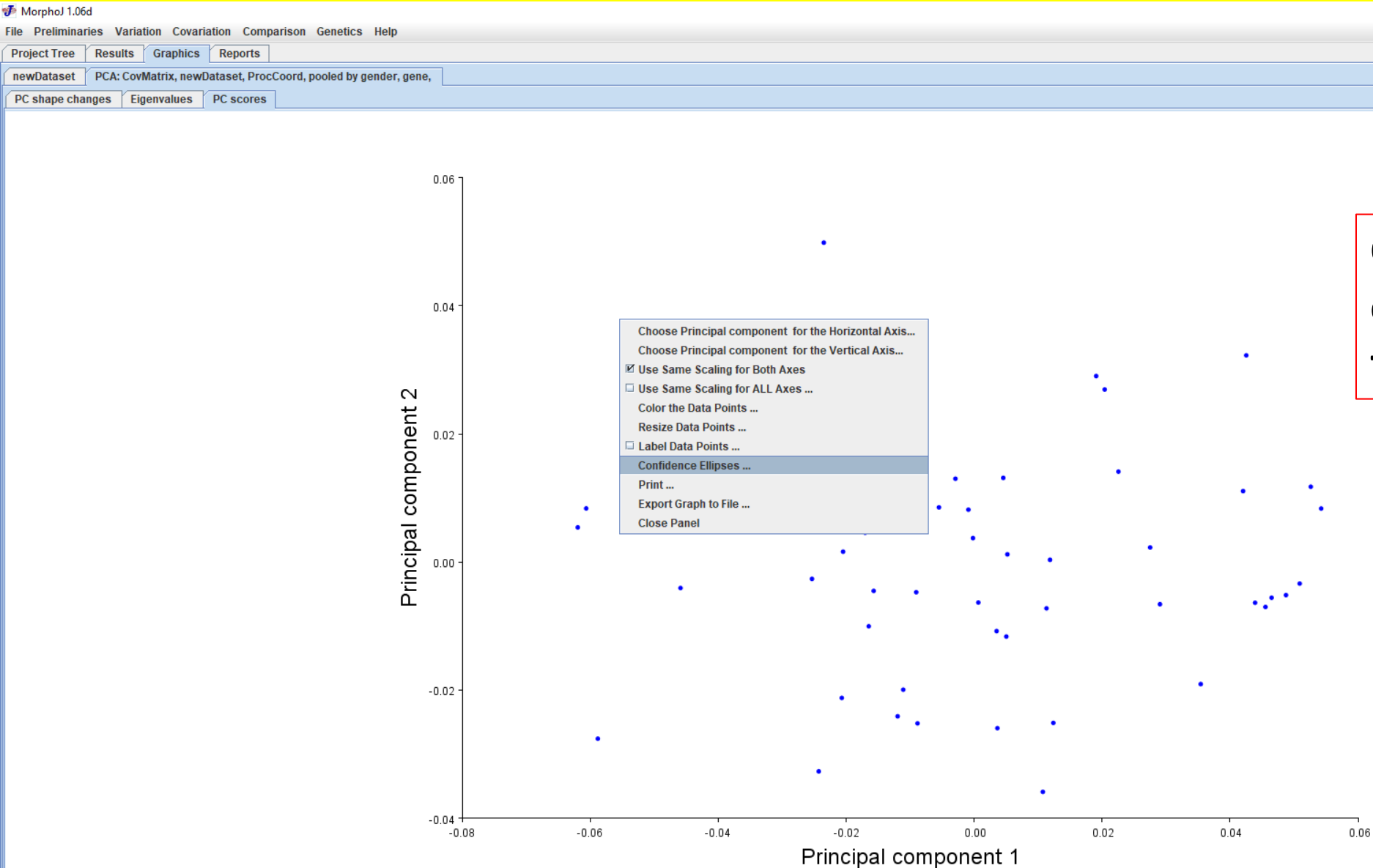
**Select the covariance matrices in the project tree tab and do the principal component analysis in the variation tab**

# 5. Principal Component Analysis (PCA) using MorphoJ



**View the results in the graphics tab and click right in the image to edit several parameter**

# 5. Principal Component Analysis (PCA) using MorphoJ



**Choose Confidence ellipses to make ellipses from the data**

# 5. Principal Component Analysis (PCA) using MorphoJ

Choose settings for the confidence ellipses ...

☒ Draw ellipse(s)

Type: ☒ Equal frequency ellipse(s) ☐ Confidence ellipse(s) for mean(s)

Probability:  (Must be between 0.0 and 1.0.)

☒ Use a classifier as a criterion for grouping observations

☒ gene

(Caution: this may override earlier choices of colors.)

☒ Clip the ellipse(s) at the margins of the graph

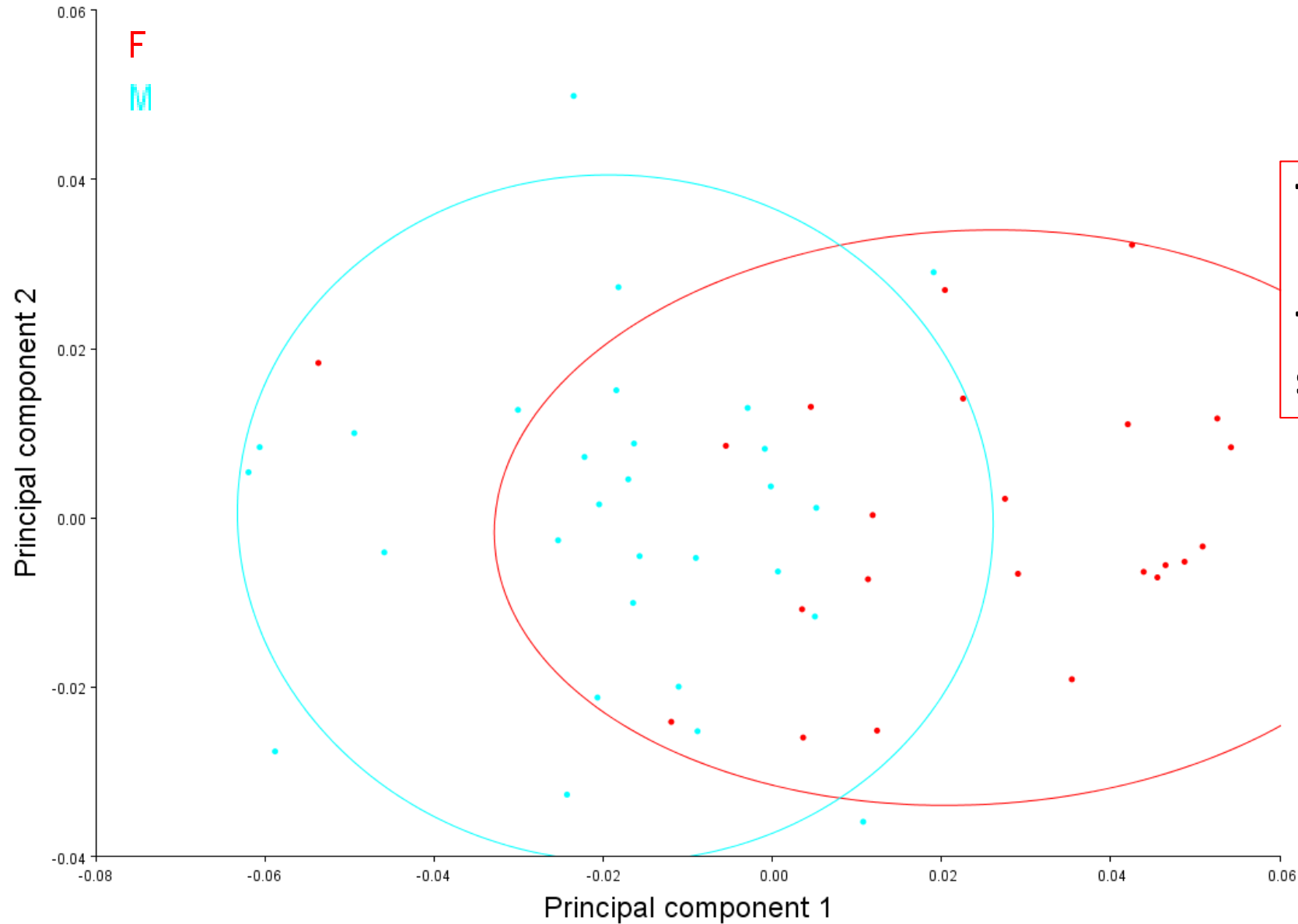
☒ Show the data points

Cancel OK

Choose Draw ellipses

Choose the category "Gene or Gender"

## 5. Principal Component Analysis (PCA) using MorphoJ



The ellipses graphic will be made like this from the sample and can be seen in the PC score tab.