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1 Introduction

MorphoJ is an integrated software package to perform geometric morphometric analysis. The main purpose of MorphoJ is to provide a user-friendly environment to include the set of analysis commonly done in Geometric morphometrics. This is free software that can be run on Windows, Macintosh and the linux/unix operating system. MorphoJ can be downloaded from http://www.flywings.org.uk/MorphoJ_page.htm.

MorphoJ provides a single, integrated environment for geometric morphometrics so the user can concentrate on biological and statistical aspects of analysis (Klingenberg, 2011).

The complete sequence of preparation, analysis and presentation of result involves several steps. This is shown below:



Throughout this user guide we shall use two datasets. One of them is Mormyrid.tps. This dataset includes 25 individuals of mormyrid fishes that have a great amount of shape variation within the group. The other one is Lythrurus_Lateral.NTS, for a genus of North American minnows with comparatively less shape variation.

2 Preparation

The input to MorphoJ analysis is a data file that can be text (.txt), from Rohlf's statistical package (.tps), or NTSPC format (.nts). The final output of MorphoJ analysis is a set of statistical results and visualizations. But as we can see from the flow chart above, there are several steps involved before starting an analysis with MorphoJ.

In this user guide, we shall put the digital images in a dedicated folder and show each step with examples and explanations.

- 1. **Digital Image**: Take digital images and put all the images in a designated common folder. Download tpsutil and tpsDIG2 from The Morphometrics at SUNY Stony Brook website (http://life.bio.sunysb.edu/morph/).
- 2. Digitize: Build tps file

- a. Open tpsutil.
- b. From 'Select operation' choose 'build tpsfile from images'
- c. Click input, find your images and click any image from this folder
- d. Click output, Create a name for the tps file that will be created.
- e. Click setup actions window. All images are checked, uncheck any that you don't want in the file 'Include'.
- f. Make sure that 'Include path' is unchecked. This will include only the image name in the tps file. This is helpful if digitizing file will be saved in the same folder as images, and will result in a short name for the images.
- g. Click 'Create' and the tps file is ready to be digitized.

3. Digitize Images using tpsdig:.

- a. Choose: File>Input source>File and select the tps file you created.
- b. First image should appear (may take a few moments). Explore all tabs, find out how to zoom in and out, Delete a specimen, Set scale, Mark landmarks or outlines, change size and color of symbols and labels.

For each images you need to

- 1. Set the scale:
 - a. Options>Image tools (or hammer icon). The last tab is 'Measure'
 - b. Click set scale, measure ruler, change Reference length and units if needed, click OK.
- 2. Digitize landmarks
 - a. Change size or color of dot: Image tools, the first tab is 'Color'.
 - b. Label landmarks: In options Label landmarks (or # icon).Change size and color of number if desired in Tools>Color.
- 3. Save file. When you save the file choose: File>save data>Overwrite. It is important to choose 'Overwrite' instead of 'Append'. Use 'Append' if you want to combine two different datasets (this can also be done with tpsutil.
- 4. Examine tps file with text-reading software if desired.

A landmark file with 18 landmarks created using tpsdig will look like this:

LM=18 11.00000 173.00000 26.00000 131.00000 47.00000 122.00000 IMAGE= Lythrurus_Lateral. 211L.JPG ID=0 SCALE=0.17 In tpsDig the digitized file could be saves as .tps or .NTS

The following link also gives a brief overview of how to place landmarks in digital images.

http://www.morpho-tools.net/softwareguide/GM%20guide%20v4%20OLs.pdf

3 MorphoJ User Interface

MorphoJ does not create a data file for geometric morphometrics. MorphoJ also can not analyze outline data. MorphoJ can import raw landmark data from tab- or commadelimited text files or from other morphometric software: the TPS series (http://life.bio.sunysb.edu/morph/soft-tps.html), NTSYSpc (Rohlf 2008) and Morphologika (O'Higgins & Jones 1998). The MorphoJ web page has an excellent user guide which is available online at

http://www.flywings.org.uk/MorphoJ_guide/frameset.htm?index.htm

What follows is a step by step simple user guide for the beginners. For advanced analysis the online MorphoJ user guide is a good reference.

3.1 MorphoJ Main Window

MorphoJ has a graphical user interface from which all analyses can be done. There can be multiple analyses for one or multiple data sets. The datasets and analyses are organized into a project.

🛷 M	orphoJ 1.03d*						
File	Preliminaries	Variation	Covariation	Comparison	Genetics	Help	
Pro	ject Tree R	esults Gr	aphics Re	ports			
🕘 n	ew project						

At the top of the MorphoJ window there is a menu bar with seven menus named as follows: **File**, **Preliminaries**, **Variation**, **Covariation**, **Comparison**, **Genetics** and **Help**. These are the menus used to complete an analysis using MorphoJ.

There are four tabs under the menu bar: **Project Tree**, **Results**, **Graphics** and **Reports**. Each of these menu bars allows you to view different aspects of the analyses.

Create new project: To create a new project click on the file menu and select **Create new project** which opens the following dialog box:

Name for the new project newProject	
Dimensionality of the data: 2 dimensio 3 dimensio Name for the new dataset newDataset	ons Object symmetry? O yes ons Object symmetry? O yes
File type: text NTSYSpc TPS 	O Morphologika
Look In: 🗂 My Documents	▼ 届 台 □ 器 문
101MSDCF	🗖 bridge1
Adobe Stock Photos	🗂 Bundles
🗂 Ariidae seq	Classes
ArmbrusterTEaching	Communications
🚍 A SIH	📑 data
BabyRose	Dell WebCam Central
Bluetooth Exchange Folder	deptforms
	•
File Name:	
Files of Type: All Files	 ✓
	Create Dataset Cancel

- 1. At the very top of the dialog box is the text box for naming the new project. Select a project name and choose the **Dimensionality of data**, if it is a 2D or 3D dataset (usually 2D). 'Object symmetry' is if all of your landmarks have bilateral symmetry, for a lateral view, this would be unchecked. The 2nd text box is **Name for the new dataset** and choose the **File type**. **File type** is what kind of data file is going to be imported in MorphoJ. For our analysis we are using Mormyrid.tps as data file and it is a 2D data set.
- MorphoJ always opens in 'My Documents' in Windows and 'Documents' in MacOS. Navigate to the file. After selecting the correct data file select Create Dataset, which adds the data file in MorphoJ.

ame the new pro	oject and select the data file and appropriate options					
Name	e for the new project Mormyrid_Tutorial					
Dimen	ssionality of the data: 2 dimensions Object symmetry? yes					
	 3 dimensions Ino 					
Name	for the new dataset Mormyrid					
File type: text NTSYSpc TPS Morphologika Look In: Tutorial Image: Commyridlinks.NTS Image: Commyridlinks.NTS Mormyrids.morphoj Image: Commyrids.tps Image: Commyrids.tps						
orphometric an	nalysis.docx					
File <u>N</u> ame:	mormyrids.tps					
Files of <u>Type</u> :	All Files					
	Create Dataset Cancel					

3. Once the data set has been created, click on the report tab which gives a message "the project **'Mormyrid_Tutorial'** has been loaded successfully".



The following screen shot shows the elements of the user interface and how it looks as we create the new project and data set.

💕 MorphoJ 1.03d*	
File Preliminaries Variation Covariation Comparison Genetics Hel	p
Project Tree Results Graphics Reports	
Mormyrid_Tutorial	

- 4. To create another data set under the same project select **File** menu and click on the **Create New Data** set and do the selection as appropriate with the data set.
- 5. To save a project select **File** menu and select **Save** or **Save Project As**. When you save a project it saves as given projectname.morphoJ. For our example here the project name is Mormyrid_Tutorial.morphoJ
- If there is a project already saved it could be always opened by selecting File>Open project or Open Recent Project, which will open the most recent projects.

4 Procrustes Fit

The first step in a morphometric analysis is to extract shape information by Procrustes superimposition on the data set. MorphoJ runs a full Procrustes fit and projects the data onto tangent space. To run a Procrustes fit select the **Mormyrid** data set under the **Project Tree** window, select **Preliminaries** menu and click on **New Procrustes Fit** which opens the following dialog box:

Note: This choice of orientation	n will affect the presentation	n of data, but not the result of statistical tests (
O Align with first specimen (r	not recommended if there is	s object symmetry)
Align by principal axes		
Align using specific landma	rks	
Indicate the landmarks or lan	dmark combinations for the	e alignment of the mean shape,
e.g. "L5" or "0.3*L2 + 0.7*L7" Left point for first axis:		
Right point for first axis:		-
Upper point for second axis:		(3D data only!)
	Perform Procrustes Fit	Cancel

There are 3 choices in this dialog box and the default is aligning by principal axes. After selecting the default click Perform **Procrustes Fit** and it does give a consensus for all the landmarks. It also shows a graphical representation of the data clouds for each individual landmark.

After the Procrustes fit the graphical representation of the dataset looks like the following:



5 Data Outliers

It is always very important to check the data set to determine if there are any data outliers to determine if there are any problems in the data set. To check for outliers in a dataset, click the Mormyrid Project Tree, select the Mormyrids dataset and then select **Find Outliers** in the **Preliminaries** menu.

🐠 M	orphoJ 1.03d*						<u> </u>
File	Preliminaries	Variation	Covariation	Comparison	Genetics	Help	
Pro	New Procrust	tes Fit		S			
a M	Find Outliers .						
 ? − [Include or Exc	clude Obser	vations				
	Generate Cov	ariance Ma	trix	Inates	inates		
	Extract New O	Classifier Fr					
	Edit Classifier	'S					
	Edit Covariate	es					
	Select Landm	arks					
	Subdivide Dat	aset By					
Combine Datasets							
	Average Observations By						
	Link Datasets	i					
	Create or Edit	Wireframe					

After clicking find outliers the output window shows an interface like the following in the graphics tab:



On the upper left side the diagram shows the average shape in blue dots and deviation from the average in red line. The panel on the lower left corner "**Included**" shows each individual specimen with their Id and the number of their diviation from the average.By selecting any of them shows which landmark deviated most. The upper-right panel shows how much each individual specimen vary from the average shape of the entire sample for Mormyrid data set. This diagram is just an approximate guide to the quality of the data. If any individual specimen is not desired in the data set we can exclude that specimen and get a new dataset for further analysis. To get the excluded individual someone can always get that from "**Excluded**" panel and select the specific Identifier need to be included.

6 Exclude Observations

Some time it is important to use some of the individuals (i.e. groups, male or female) of a data set for particular analysis. The easiest way to do this is going under project tree tab, select **preliminaries** menu and then select **include or exclude observations**. After selection the following dialog box appears:

秒 Mo	rphoJ 1.03d*				
File	Preliminaries Variation	Covariation C	comparison	Genetics	Help
Proje	ect Tree Results Gra	aphics Repo	rts		
🕘 ne	w project				
P 🗐	Mormyrids				
_ '	Include/exclude obser	rvations			×
	Create a new datase	t Name: Mo	rmyrid_test		
	Included:		Excluded:		_
	0		10		
	1		15		
	12	Include	1		
	13		1		
	14	Exclude			
	10				
	Select by classifier				
		(Cancel	Accept	

In this dialog box the top right corner has **Create a new dataset** button. If the included or excluded observations need to be a in a separate new dataset, this button should be selected which enables "Name" box to have a new name for the dataset. Here is an example which shows a separate dataset has been created named as "Mormyrid_test" and where 3 observations have been excluded. At the end click **Accept** tab and it creates a new dataset under the existing project.

The user interface looks like the following as we create a new dataset:

🐠 M	orphoJ 1.03d*					
File	Preliminaries	Variation	Covariation	Comparison	Genetics	Help
Pro	ject Tree Re	sults Gr	aphics Rep	oorts		
₽ [ew project Mormyrids	test				

Note:

There might be more than one data set in an existing project. MorphoJ runs an analysis on the data set highlighted in the **Project Tree** tab, so it is improtant to select the correct data set to run an analysis.

7 Covariance Matrix

After procrustes super impositon its very important to run the covariance matrix. Covariance matrices are used for a lot of the morphometric analyses like Principal component analysis. MorphoJ can generate Covariance matrix from various types of data i.e. shape data set after the procurstes superimposition has been done or regression residuals.

First select the **Mormyrid dataset** in the main project tree and select covaraince matrix from **preliminaries** menu. Then the following dialog box appears:

Select the data set and d	ata type		
elected:		Available:	
Aormyrids	Include	Mormyrid_test	
	Exclude		
Data types (Select all types	to be used)	O Pooled within-group covarian	ces
Procrustes coordinates			

There are two datasets in this project. At the top left corner "**Selected**" has the data set selected to generate covariance matrix and Mormyrid test is excluded which is under Available box. Data types shows what kind of data are present in the current data set. After selecting data set and data types click **Execute** which opens the following window:

🌮 MorphoJ 1.03d*	
File Preliminaries Variation Covariation Comparison Genetics Help	
Project Tree Results Graphics Reports	
Mormyrid_Tutorial P I Mormyrids CovMatrix, Mormyrids, Procrustes coordinates	

In this window we can see the covariance matrix under Mormyrids data set.

8 Classifiers

For this part of the tutorial we will be using Lythrurus data set as the morymrid photos did not have names. After importing the data file and running the Covaraince matrix the project window should look like the following:



Select the Lythrurus_Lateral data set. Under **Preliminaries** menu click **Extract New Classifier from Id String** and the following dialog box appears:

🦻 Extract new classifier from ID strings					
Dataset: Lythrurus_Lateral					
Existing classifiers: Identifier strings:					
Lythrurus_alegnotus_Al					
Lythrurus_alegnotus_At					
Lythrurus_alegnotus_Al					
Lythrurus_alegnotus_Al					
Name for new classifier:					
Species					
First character: Last character:					
11 16					
(Positive - count from left; negative - count from right)					
Cancel Execute					

On the right side of the dialong box **Identifier Strings** list shows the file name for the studied individuals. On the left side the list shows if there are any existing identifiers. Here we name Species for the **Name for new classifier**. To extract a classifier choose the first letter that you will want to use. In this case, we want species. All species are in Lythrurus, which is 9 letters long, there is then an underline, so the first letter of the species is 11. Choosing 6 letters will ensure that you get enough to determine species for all photos. Select **Execute**.

It is always a good to check the results after extracting classifiers by selecting **Edit Classifier** under **Preliminaries** menu and the user interface opens the following dialog box

norphoJ 1.03d*					<u>_ U X</u>	
File Preliminaries Variation Covariation Comp	arison Genet	tics Help				
Draiget Trage Pagulte Graphics Paparts						
Project free Results Graphics Reports			1.			
Lythrurus_Lateral PCA: CovMatrix, Lythrurus_La	ateral, Procrus	stes coordinates	Edit classifiers in	'Lythrurus_Lateral'		
Identifier	Species					
Lythrurus_alegnotus_AUM27058_11_44.87L.jpg	alegno					
Lythrurus_alegnotus_AUM27058_12_36.97L.jpg	alegno					
Lythrurus_alegnotus_AUM27058_13_35.33L.jpg	alegno					
Lythrurus_alegnotus_AUM27058_14_40.49L.jpg	alegno					
Lythrurus_alegnotus_AUM27058_15_44.27L.jpg	alegno					
Lythrurus_alegnotus_AUM27058_16_39.03L.jpg	alegno					
Lythrurus_alegnotus_AUM27058_17_32.45L.jpg	alegno					
Lythrurus_alegnotus_AUM27058_18_39.59L.jpg	alegno					
Lythrurus_alegnotus_AUM27058_19_37.65L.jpg	alegno					
Lythrurus_alegnotus_AUM27058_20_32.10L.jpg	alegno					
Lythrurus_alegnotus_AUM27058_7_39.93L.jpg	alegno					
Lythrurus_alegnotus_AUM27058_8_42.33L.jpg	alegno					
Lythrurus_alegnotus_AUM27058_9_39.91L.jpg	alegno					
Lythrurus_alegnotus_48858_1_45.67L.jpg	alegno					
Lythrurus_alegnotus_48858_2_41.90L.jpg	alegno					
Lythrurus_alegnotus_48858_3_40.97L.jpg	alegno					
Lythrurus_alegnotus_48858_4_42.93L.jpg	alegno					
Lythrurus_alegnotus_48858_5_43.73L.jpg	alegno					
Lythrurus_alegnotus_48858_6_40.84L.jpg	alegno					
Lythrurus_ardens_TU71017_10_48.78L.jpg	ardens					
Lythrurus_ardens_TU71017_11_37.09L.jpg	ardens					
Lythrurus_ardens_TU71017_12_56.13L.jpg	ardens					
Atomic addres TU74047-40-0440Line addres						
Constant Inc.						
Species Add classifier		Rename	classifier			
Delete classifier	s)	Replace	values			
Combine classifie	ers			Cancel	Accept	

The list on the left of this dialog box shows the new classifier and if no editing is needed, just select **Accept** button.

9 Wireframe

A wireframe is a set of lines which connect the land marks in a graph. It makes it easier for the viewer to check the confuguration of the landmarks and to visualize the results.

At first select the Mormyrid dataset under project tree window and click on **Preliminaries menu** bar and then click **Select or edit wireframe**. The new graphics tab appears:

秒 MorphoJ 1.03	d*									
File Preliminar	ies Varia	tion Covaria	ation Con	nparison	Genetics	Help				
Project Tree	Results	Graphics	Reports							
Create wirefra	me									
¹ 2 ₃ 4	6	•7	•8	•2●	1 0		•11 •12	14 15	1 3	Name for the wireframe: Mormyrids wireframe Lmk 1 Lmk 1 Link landmarks Delete link Cancel Accept

It is very important to have an idea about the landmarks and the organism has been used in the study. The next step is to link each land mark where a solid line is desirable. And the easiest way to do is it to click on one landmark and then drag to the next and at the end click Accept button. As the wireframe is created the user interface widnows

looks like the following:



There are options that a wireframe could be hard or soft. As the wireframe is created the project window shows a tab with wireframe.

File Preliminaries Variation Covariation Comparison Genetics Help Project Tree Results Graphics Reports Image:
Project Tree Results Graphics Reports Image: Second strain s
 new project Mormyrids CovMatrix, Mormyrids, Procrustes coordinates Mormyrids wireframe
 Mormyrids Southatrix, Mormyrids, Procrustes coordinates Mormyrids wireframe

To edit an existing wireframe, click on the the wireframe in the project tree and then under **Preliminaries** menu select **Create or Edit Wireframe** option. A new graphics tab shows as **Edit wireframe**. Here in the link landmarks table it shows all the links of the existing wireframe. Any link could be selected and deleted and then press the **Accept** button.

There is an option in MorphoJ where you can choose not to include all the landmarks in a data set. This a great oppoortunity to test if some of the landmarks are necessary or you can exclude those. So to create a new dataset with some of the landmarks select **Mormyrids** data set in project tree window and, **then** click **preliminaries** menu and click on **Select Landmarks**.

The dialog box looks like the following:



On the upper right corner there is a dialog box **Included** has the list of the landmarks that would create the new data set. The next dialog box contains the landmarks which have been selected for deletion. In this example 3 landmarks have choses to be deleted and it is shown in the left diagram as hollow circle. As this is 2D data set, by default it chooses **No** object symmetry.

10 Principal Component Analysis

Principal Component Analysis (PCA) is very important for a lot of studies as it shows major shape variation. It is one of the most widely used methods for multivariate analysis. In MorphoJ, PCA is calculated from the covariance matrix. To run a PCA select **CovMatrix** from the **Project Tree** window and click on **Principal component Analysis** (PCA) from the **Variation** menu. During selection the project tree window looks like the following:

File Preliminaries Variation Covariation Comparison Genetics Help Project Tree Re Principal Component Analysis Image: Cove and the second analysis Image: Cove analysis Image: Cove and the second analysis Image: Cove analysis
Project Tree Re Principal Component Analysis Mormyrid_Tutorial Matrix Correlation Principal Coordinate Analysis for Covariance Matrices CovMatrix, Principal Coordinate Analysis for Covariance Matrices Contrast Covariance Matrix to Landmark Coordinates Procrustes ANOVA Procrustes ANOVA Procrustes ANOVA
Mormyrid_Tutoria Mormyrids Mormyrids Mormyrids Matrix Correlation Principal Coordinate Analysis for Covariance Matrices Contrast Covariance Matrices Convert Covariance Matrix to Landmark Coordinates Procrustes ANOVA
Procrustes ANOVA

As we click on the PCA it gives two types of output, graphical and printed output. Graphical output has three tabs which are **PC shape Changes**, **Eigenvalues** and **PC Scores**. Clicking on any of these tabs show the graphical output. If some one selects the the **PC shape changes** tab the following window appears:



There are options that the PC could be changed and other modifications to the graph is possible as well. If we right click, the text box shows up with the options:

Transformation Grid, Warped Outline Drawing, and Wireframe Graph. These will give you different ways of visualizing changes along the different PCA axes. For a **Warped Outline Drawing,** an outline will have to be created in a program like the tps package and entered under **Preliminaries**.



File Preliminaries Variation Covariation Comparison Genetics Help Project Tree Results Graphics Reports PCA: CovMatrix, Mormyrids, Procrustes coordinates PC PC shape changes Eigenvalues PC scores Choose PC to Display Set Scale Factor Flip diagram Resize Landmark Points Pransformation Grid Warped Outline Drawing Print Export Graph to File Close Panel Transformation Grid Wireframe Graph 10	ff MorphoJ 1.03d*		
Project Tree Results Graphics Reports PCA: CovMatrix, Mormyrids, Procrustes coordinates PC shape changes Eigenvalues PC scores PC shape changes Eigenvalues PC scores PC Set Scale Factor Flip diagram Set Scale Factor Flip diagram Resize Landmark Points Transformation Grid Warped Outline Drawing 10 Print Export Graph to File Other Transformation Grid Vireframe Graph 10 Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel Image: Close Panel<	File Preliminaries Variation Cova	variation Comparison Genetics Help	
PCA: CovMatrix, Mormyrids, Procrustes coordinates PC shape changes Eigenvalues Choose PC to Display Set Scale Factor Flip diagram Rotate diagram Rotate diagram Resize Landmark Points Change the Type of Graph Transformation Grid Warped Outline Drawing 10 Vireframe Graph 10 10 11 11 14 12 15 13 14	Project Tree Results Graphic	cs Reports	
PC shape changes Eigenvalues PC scores Choose PC to Display Set Scale Factor Flip diagram Rotate diagram Rotate diagram Resize Landmark Points Change the Type of Graph Transformation Grid Print Export Graph to File Close Panel 10 11 14 12 15 4 8	PCA: CovMatrix, Mormyrids, Procru	ustes coordinates	
Choose PC to Display Set Scale Factor Flip diagram Rotate diagram Resize Landmark Points Change the Type of Graph Print Export Graph to File Close Panel 10 10 11 14 12 15 13	PC shape changes Eigenvalues	s PC scores	
Set Scale Factor Flip diagram Rotate diagram Resize Landmark Points Change the Type of Graph) Print Export Graph to File Close Panel 10 11 14 12 15 13	Choose PC to Display		
Flip diagram Rotate diagram Resize Landmark Points Change the Type of Graph Print Export Graph to File Close Panel 10 10 11 14 12 13	Set Scale Factor		
Rotate diagram Resize Landmark Points Change the Type of Graph Print Export Graph to File Close Panel 10 10 11 14 12 15 13	Flip diagram 🕨		
Resize Landmark Points Change the Type of Graph Print Export Graph to File Close Panel 10 10 11 14 12 15 13	Rotate diagram 🕨		
Change the Type of Graph Print Export Graph to File Close Panel 10 10 10 10 10 11 14 10 11 14 10 10 10 11 12 13	Resize Landmark Points		
Print Warped Outline Drawing Export Graph to File Close Panel	Change the Type of Graph ▶	Transformation Grid	
Export Graph to File Wireframe Graph Close Panel	Print	Warped Outline Drawing	
Close Panel	Export Graph to File	Wireframe Graph	
11 14 2 ₃ 18 4 8	Close Panel		
$\frac{14}{12}$	140	- 11	
$^{2}_{3}$ 18 12 15 13	· · · · ·	7	
4		• ¹² •15	4 13
	4	L 8	
L9		9	

The Eigenvalue graphical window shows the relative percentage of principal components, where all PCs account for 100%. In shape analyses, the number of axes is the number of landmarks times the number of dimensions minus the degrees of

freedom (4 for 2D). If the **Eigenvalues** are chosen under graphical tab the following window shows up. In general, no more than the first 5 axes show enough variation to be useful.





Selecting **PC scores** under **Graphics tab** gives the following user interface window:

This is the scatter plot of PC scores. This graph is only produced if the covariance matrix on which the PCA is derived, directly comes from a dataset.

Right click on this scatter plot giver the following tab which gives lots of option to edit the scatter plot and also how to save and export the graph.



Showing Output Report as Text:

To see the text output for PCA choose Results and they are output as eigenvalues and PC coefficients.

The other way to see the report is selecting the Project tree window and right click on the PCScores and click result:

🌮 MorphoJ 1.03d*	💤 MorphoJ 1.03d*							
File Preliminaries Variation Covariation Comparison	Genetics Help							
Project Tree Results Graphics Reports								
🕘 Mormyrid_Tutorial								
🛉 🗐 Mormyrids								
🕈 🎦 CovMatrix, Mormyrids, Procrustes coordinates	👇 🌠 CovMatrix, Mormyrids, Procrustes coordinates							
👇 🎉 PCA: CovMatrix, Mormyrids, Procrustes coordinates								
PC scores, CovMatrix, Mormyrids, Procrustes coordinates								
Display Results								
Rename								
	Remove Item							
	Remove Item							

After clicking the following user interface window appears with the text output under the **Results** tab The text output in the Results window contains the following information:

🐠 M	orphoJ 1.03d*				
File	Preliminaries Varia	tion Covariatio	n Comparison Genetics	Help	
Pro	oject Tree Results	Graphics R	eports		
Prin	cipal Component An	alysis: PCA:	CovMatrix, Mormyrids,	Procrustes	coordinates
	Eigenvalues	<pre>% Variance</pre>	Cumulative %		
1.	0.00549040	43.597	43.597		
2.	0.00260612	20.694	64.291		
3.	0.00226382	17.976	82.267		
4.	0.00073164	5.810	88.076		
5.	0.00057228	4.544	92.621		
6.	0.00023287	1.849	94.470		
7.	0.00016908	1.343	95.812		
8.	0.00014762	1.172	96.985		
9.	0.00009382	0.745	97.730		
10.	0.00007936	0.630	98.360		
11.	0.00004873	0.387	98.747		
12.	0.00004422	0.351	99.098		
13.	0.0003094	0.246	99.344		
14.	0.00002316	0.184	99.527		
15.	0.00001631	0.129	99.657		
16.	0.00001215	0.097	99.753		
17.	0.0000810	0.064	99.818		
18.	0.0000699	0.056	99.873		
19.	0.0000580	0.046	99.919		
20.	0.00000432	0.034	99.954		
21.	0.00000265	0.021	99.975		
22.	0.00000132	0.010	99.985		
23.	0.0000109	0.009	99.994		
24.	0.0000078	0.006	100.000		
	Principal Comp	onent Coeffic	ients		

	PC1	PC2	PC3	PC4	PC5
x1	0.299560	-0.028888	-0.079528	0.023754	-0.330969
у1	0.166393	-0.165195	0.208535	-0.091882	-0.029748
x2	-0.306434	0.011898	0.102343	0.014502	-0.113483
у2	-0.302048	0.222358	-0.232372	-0.116990	-0.140060
x3	-0.317040	-0.031981	0.129470	0.071695	-0.016001
2	0 201442	0 102004	0 014079	0 067227	0 076640
•					

The text output contains two tables, one is Eigenvalues and the other is PC coefficientns.

Eigenvalues: In this table there are 3 columns. The first one is eigenvalues for each corresponding PC axis and the second one is as a percentage of the total variance. The third column is the cumulative percentage of total variance.

PC coefficients: The PC coefficients or eignevectors are in a tabular form which includes all the PC coefficients. In this coefficient table columns contain coefficients for one principal componenet.

11 Canonical Variate Analysis

Canonical variate analysis is a method used to find shape features which maximize the separation between groups. For this analysis group is assumed to be known apriori. For exploration taxonomy, CVA is generally inappropriate. You need to have a justifiable reason for defining groups. For CVA analysis select Canonical variates under comparison and the following dialog box opens:

Canonical Variate Analysis						
Name: CVA						
Dataset:						
Lythrurus_Lateral						
Data type:						
Lythrurus_Lateral, Procrustes coordinates 💌						
Classifier variable(s) to use for grouping: Species						
Permutation test for pairwise distances Number of iterations: 10000						
Cancel Execute						

At the very top of this dialog box is a drop down menu to select what kind of analysis to run and by default it does a CVA.

The **Dataset** drop down menu gives an option to choose a dataset and here in this example it is the Lythrurus_Lateral. As the dataset is selected the Data type and Classifier variables are available to be selected for the next two elements of the dialog box.

At last, there is a check box that can be selected for a permutation test of pairwise distances between the groups. Click the **Execute** button to run the analysis. **Graphical Output**: As the CVA analysis is done there are two graphical outputs under the Graphics tab. One of them is **CV Shape Changes**, which shows the shape change associated with the CV axes. The number of axes is the number of species minus one. The other graphical output is **CV scores**, which is the scatter plot of the CV scores.

To view the results, choose the **Results Tab.** The following screen shot shows some of the elements of the user interface:

🐠 Morph	💤 MorphoJ 1.03d*							
File Pre	eliminaries	variati	on Covariat	ion Com	parison	Genetics	Help	
Project	Tree	lesults	Graphics	Reports]			
Canonic	Canonical Variate Analysis: CVA							
Dataset	Dataset: Lythrurus_Lateral							
Classif	Classification criterion: Species							
Groups	Observa	ations						
1.	alegno	19						
2.	ardens	30						
3.	atrapi	25						
4.	bellus	29						
5.	fascio	20						
6.	fumeus	35						
7.	lirus_	21						
8.	matuti	31						
9.	roseip	30						
10.	snelso	6						
11.	umbrat	30						
Variati	on among	groups,	scaled by	the inv	erse of	the with	in-group varia	ation
	Eigenval	lues	% Variance	e (Cumulat	ive %		
1.	8.51887	7316	36.719		36.71	9		
2.	3.54695	5503	15.288		52.00	7		
3.	3.23147	7984	13.929		65.93	6		
4.	2.25753	3541	9.731		75.66	6		
5.	1.45884	4809	6.288		81.95	4		
6.	1.34017	7265	5.777		87.73	1		
7.	1.07740	0052	4.644		92.37	5		
8.	0.78608	8731	3.388		95.76	3		
9.	0.56248	8547	2.424		98.18	7		
10.	0.42053	3627	1.813		100.00	0		

At the very biginning of the test output it gives information about the name of the analysis, dataset, number of groups etc.

The first entries are how many groups, the criteria to make the groups and how many individuals are in each group.

The second table shows the amounts of variation among groups. In this table there are 3 columns. First column gives the amounts of variation among groups, 2nd column is the percentage of the total for each CV and last column is the cumulative percentage.

The next two entries are matrices for Mahalanobish distance and Procrustes distance among all possible pairs of groups. If the option for Permutation test was selected during the analysis it gives two matrices with P values for all the pairwise tests for Mahalanobis and Procrustes distances.

Finally is a table with canonical coefficients which is related to the original shape coordinates to the CV scores.

12 Incorporating Phylogeny

MorphoJ can be used to map shape data onto phylogenies using squared-change parsimony (Maddison 1991). There is also a permutation test to determine if the morphometric data contains phylogenetic signal or not. Phylogeny files are imported into MorphoJ in the NEXUS file format. For this section we will be using Lythrurus.NTS data file.

Mapping onto phylogeny:

First you must have a phylogeny. Ideally, this phylogeny should have branch lengths, as branch lengths provide a better estimate for ancestral values in squared-change parsimony. This phylogeny can be created in most phylogenetic programs by saving the output tree with branch lengths. You cannot simply build this tree, but must have the original dataset input into the phylogenetic programs and can then build the tree. The taxa in the tree must match those in the shape analysis. Tree files can be edited in text reading programs.

To map a phylogeny onto shape data, the first step is to import a phylogeny file as NEXUS tree format (.tre). Before that everyone needs to make sure the project has PCA done so the main user interface looks like the following:

秒 MorphoJ 1.03d*	- D ×
File Preliminaries Variation Covariation Comparison Genetics Help	_
Project Tree Results Graphics Reports	
 ↓ Lythrurus_Lateral ↓ Matrix, Lythrurus_Lateral, Procrustes coordinates ↓ Matrix, Lythrurus_Lateral, Procrustes coordinates ↓ PCA: CovMatrix, Lythrurus_Lateral, Procrustes coordinates ↓ PC scores, CovMatrix, Lythrurus_Lateral, Procrustes coordinates 	

To import the phylogeny file click on File menu and click on **Import Phylogeny File**, which opens the following user interface:

秒 Import phylo	geny file	x
Attach phyle	ogeny to an existing data set?	
Lythrur	us_Lateral 💌	
🖲 Use (classifier for matching to node names:	Species 🔻
Choose the NE	XUS file with the phylogeny:	
Look In:	Tutorial	
DSC_9462	.jpg	Guide to how to use morph.
DSC_9472	.jpg	🗋 Landmarks and measurme
DSC_9607	.jpg	Lythrurus tree
DSC_9620	.jpg	🗋 Lythrurus.morphoj
DSC_9975	.jpg	mormyrid_links.NTS
DSC_9981	.jpg	🗋 mormyridlinks.NTS
Geometric	Morphometrics2.pptx	Mormyrids.morphoj
•		
File <u>N</u> ame:	Lythrurus tree	
Files of <u>Type</u> :	All Files	-
		Open Cancel

There are two buttons to choose the current dataset and choose the classifier. For this example the dataset is Lythrurus_Lateral and the classifier is species have been selected. The choose the tree file and select **Open.** When the tree opens the project tree window looks like the following:



In this Project Tree the Phylogeny Tree is saved as Stored 1, to have an appropriate name for the tree, user can change it in the Nexus file. To check if the Phylogeny Tree has been imported proeprly a left mouse click on Stored1 and selecting **Display Graphs** shows the following dialog box:



To map morphometric data onto phylogeny, select PC scores in the project Tree window and select **Map onto phylogeny** under **Comparision** menu. The user interface would look like the following:

🜮 MorphoJ 1.03d*			
File Preliminaries Variation Covariation	Comparison Genetics Help		
Project Tree Results Graphics Rep	Canonical Variate Analysis		
Uthrurus ↓ □ Lythrurus_Lateral ↓ M CovMatrix, Lythrurus_Lateral, Procru ↓ M PCA: CovMatrix, Lythrurus_Lateral	Discriminant Function		
	Map Onto Phylogeny		
	Collect Statistics on Tree Set		
PC scores, CovMatrix, Lythrurus_Lateral, Procrustes coordinates			
└─ 🏆 Lythrurus_Tree			

After selecting PC Scores, the following dialog box appears

秒 Map data o	nto pl	hylogeny	×
Phylogeny:	Lyth	nrurus_Tree	
Name:	Lyth	rurus_Tree	
Data:			
Datas	et:	PC scores, CovMatrix, Lythrurus_Lateral, 💌	
Data t	ype:	PC scores	
Class	ifier fo	or taxa: Species	
Weighting	j by bi	ranch lengths (if available)	
Mapping v	with re	poted tree. (This is the usual option.)	
Permutat	ion te	st for phylogenetic signal	
Number of it	eratio	ns: 10000	
		Cancel Execute	

At the very top of the dialog box there are a few text boxes that give an option to choose the dataset and phylogeny tree.

The last button is **Permutation test for phylogenetic signal** which starts with the null hypothesis of not having any phylogenetic signal in the data set.

After all these selections click **Execute** and the following graphical user interface appears:



The graphical presentation depends on what kind of dataset has been used to overlay the phylogeny. A left click anywhere in this window gives a popup menu. This popup menu can be used to change the orientation of graph, scale factor, which PC axes to



show, and to export the graph file and save it.

In this example we have mapped phylogeny tree on the PC data. Selecting the Result tab gives the result for this analysis and you can find the results of the permutation test. For this result the P-value = 0.7355, so no phylogenetic signal. Phylogeny can be mapped also onto Canonical Variates.

13 Conclusion

This user guide shows the most common features of MorphoJ to perform Geometric Morphometrics in taxonomic and phylogenetic research. It does not include every analysis that can be done with this very powerful program. We provided only basic information on the proper use of these analyses, and you should seek out more information on statistical analyses.

Reference: MorphoJ user's guide at http://www.flywings.org.uk/MorphoJ guide/frameset.htm?index.htm

Klingenberg, C. P. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources* 11: 353-357.