

Guide to geometric morphometrics

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Relative warp analysis

Programs

The key set of programs you'll need is Jim Rohlf's tps suite (<http://life.bio.sunysb.edu/morph/>).

1. *tpsUtil*. This program will allow you to build tps files with very little hassle.
2. *tpsDig*. This program will allow you to place landmarks on your images and record scale factors, saving this data in the tps file.
3. *tpsRelw*. This aligns the specimens and computes partial and relative warps.

You will also want a text editor (e.g., *Notepad* or *Word*), a spreadsheet editor (e.g., *Excel*), and a stats package (e.g., *SPSS*, *SAS*, many others, even *Excel*). An image processing program like *Photoshop* is very helpful.

Imaging

1. Leave space around your image during photography. If your image fills the frame, it will likely be distorted near the edges. You can still crop your image afterwards (e.g., in *Photoshop*).
2. Take photos at max available resolution. Storage is cheap, so don't needlessly shrink your images just to save disk space. You may want that information later. Images should be jpegs or uncompressed tiff files. Other formats are okay, but you risk having problems with the analysis programs. I recommend using lossless compression where possible (e.g., tiffs), then converting to jpegs for the analysis if you want to work with smaller files.
3. Always use a scale bar. Take your photos with a ruler visible in the picture, preferably not on the edge of the image (to avoid distortion).

Building a tps file

Before collecting data, you need a tps file, which is basically a list of your specimens.

1. Place all of the images that you want to use (or copies of them) in the same folder. You don't have to do this, but I find it helpful.

Possible issues: tps programs have had issues with really long file paths (the file name plus all of the names of the folders it's in). To avoid this issue, place your images in My Documents or in the C:\ directory in a folder with a short name (e.g., "analysis"). You can move them out after you're done.

2. Open *tpsUtil* (Start > All Programs > tps > tpsUtil)
3. Click on "Select an operation" and choose "Build tps file" from the drop-down list.

4. Select your input directory:

- a. Click "Input" (which should now be visible).
- b. Find your directory of images
- c. Double-click one image in that directory
-instead of "Data file = ?", it should show the path to your images

5. Name your output file

- a. Click "Output"
- b. Enter a name that ends in ".tps". I recommend you save this file in the folder with your images.
-instead of "Output file = ?", it should show the path to your images

6. Build the tps file

- a. Click "Setup" (which should now be visible).
- b. Checked images will be used to build your tps file. Check/uncheck as needed. If the folder contains only the relevant images, your list should be fine.

Note: if you click "include path", you can move the tps file anywhere and it will still "find" the images, but then you can't move the images. If you leave it unselected, you have to keep the tps file in the folder with the images, but you can move that folder anywhere you want.

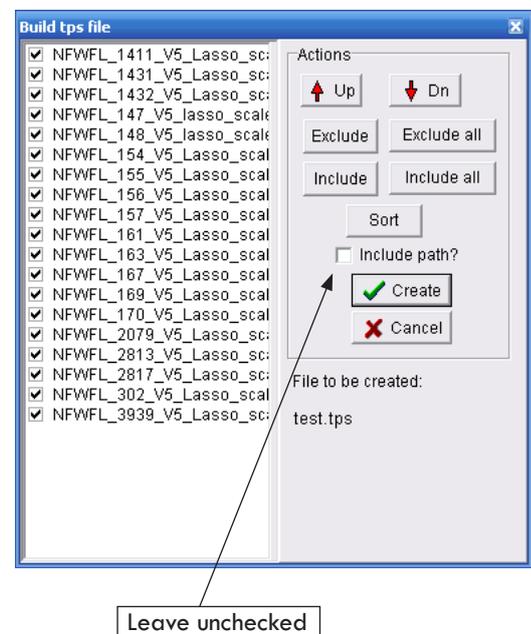
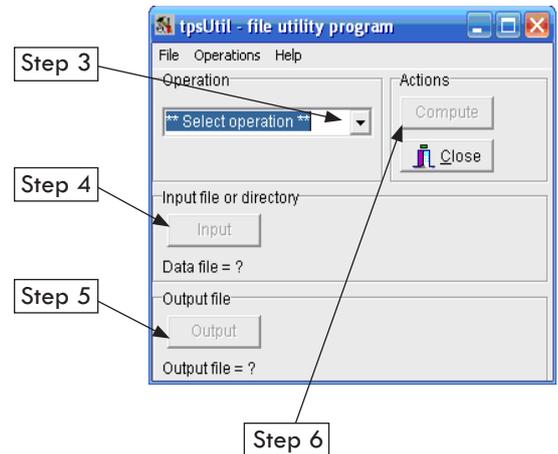
- c. Confirm that you have a file named "[something].tps" under "File to be created". If not, go back to step 5.
- d. Click "Create"
- e. Click "Close" to exit *tpsUtil*.

You should now have a file that you can open in tpsDig (<http://life.bio.sunysb.edu/morph/>).

What a raw tps file looks like (open in a text editor like Notepad):

```
LM=0
IMAGE=MSB_125_oscoxae_lateral.JPG
LM=0
IMAGE=MSB_54742_oscoxae_lateral.JPG
```

Your specimens (given by the IMAGE= names) currently have zero landmarks (LM=0).



Landmarking your specimens

In this step you place landmarks on your images and save them into your tps file.

1. Open tpsDig (Start > All Programs > tps > tpsDig)
2. Open your raw tps file (File > Input Source > File...)



You should now be able to scroll through all of your images using either the right and left arrow keys (on your keyboard) or the red arrow buttons on the top left hand corner of the navigation screen. The file name is listed at the bottom of the screen, and information on the number of landmarks will appear there as you begin collecting data. The help files are worth a look.

3. Place landmarks. Select the crosshairs icon. Left-click to place a landmark at the appropriate location.

Remember: all landmarks must be placed in sequence. If you click a landmark (LM) with the arrow tool, the LM number will be shown at the bottom of the screen. If you've screwed up the sequence, you can move the LMs with the arrow tool. Right-clicking on a landmark with the arrow tool will allow you to delete that LM or insert a new LM before the selected LM.

Make sure you know what your landmarks are before you start and be ready to make adjustments them after you start. This happens sometimes with ambiguous (e.g., non-Type I) landmarks. Consistency in landmark placement is critical, so it is important to spend a good amount of time preparing and thinking about where your landmarks will go.

4. Set scale. Go to Options > Set Scale. In the pop-up window select the length of your scale (e.g., if you have a 10cm ruler, enter 10.0). In your image, place the cursor (now an arrow with a cross) on one end of your ruler. Left-click, then set the arrow at the other end and left-click again. The scale window should now show your scale factor. Click OK.

Do you need to set scales? The ability to set scales in *tpsDig* is extremely useful and I believe it should always be used. It allows you to photograph specimens without worrying about the distance between the lens and the specimen by using the scale bar in your images and inserting a scale adjustment into the tps file. Even if you know that your lens was always at an equal distance, it does not hurt to set a scale. You can set the scale for each specimen as you landmark, or come back to it later.

5. Save your landmark data. File > Save data > Save > Overwrite. Because these programs are made by individuals and not corporations, they are prone to minor glitches (that can make you lose valuable information). Therefore, save often! Or don't, and learn the hard way.

Repeat this process for each specimen. Your tps file should now look like this:

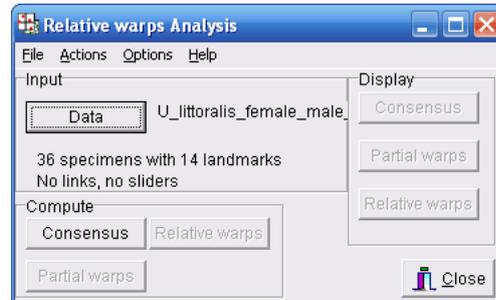
```
LM=4
563.00000 732.00000
432.00000 749.00000
435.00000 678.00000
572.00000 685.00000
IMAGE=MSB_125_cranium_lateral.JPG
ID=1
SCALE=0.006664
```

Note that each specimen starts with "LM=" and ends with the scale factor. Occasionally, you may find yourself manually editing files in Notepad. It can be confusing having the specimen name at the end of the specimen data. Be careful about this. The ID number doesn't matter; you can concatenate files so that there are multiple specimens with the same ID. I haven't yet seen a problem with doing so.

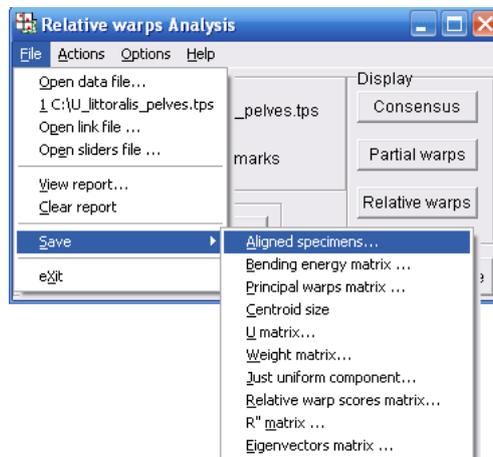
Aligning data and computing the partial and relative warps

First and foremost, we must perform Procrustes Superimposition on the raw landmark data. The following are instructions for using tpsRelW.

1. Open *tpsRelW*
2. Open your tps file with all of the landmark data (File > Open)
3. Compute Consensus, Partial Warps, and Relative Warps by clicking on the relevant buttons in order. As you click a button, the next one becomes clickable.



4. Save the following for later statistical analysis: Weight Matrix, Centroid Size, Relative Warp Scores.



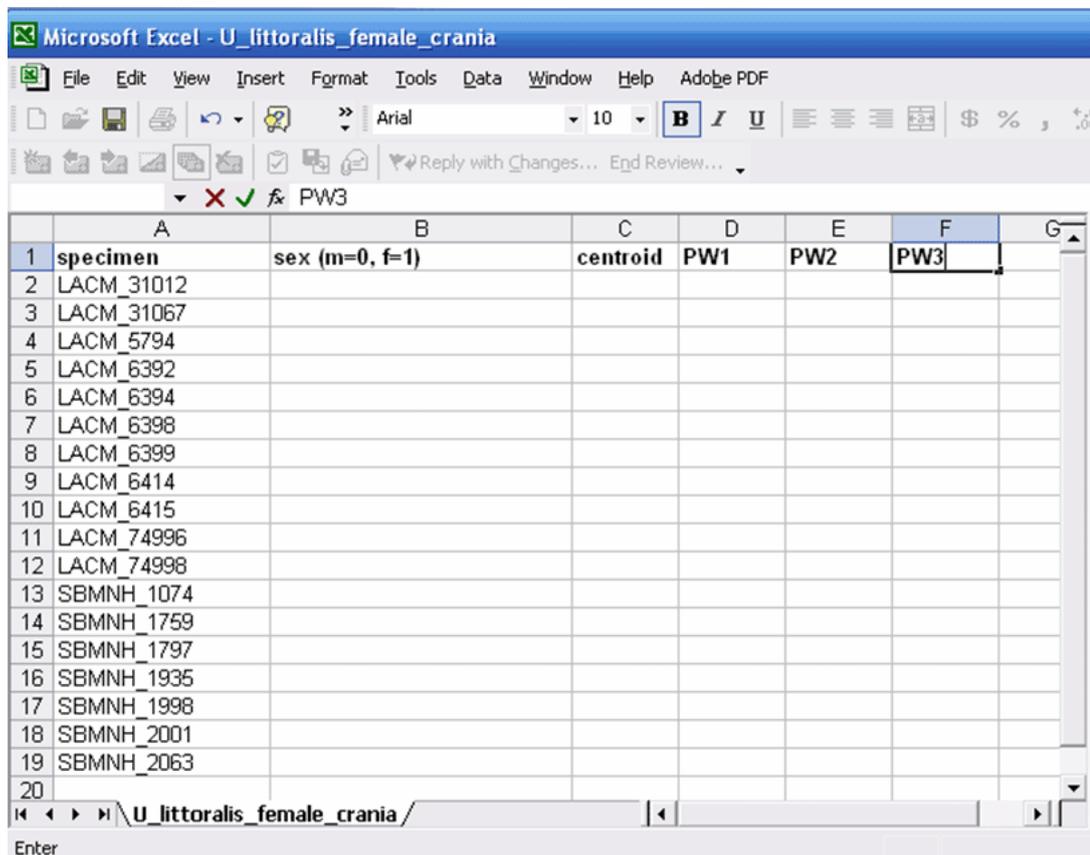
5. You may also want to save the report of the RW analysis, by selecting “View Report” in the same drop-down menu. The report will pop-up in a separate window and you can save it as a text file.

As you can see, there are many other bits of data that you can save here. I am just alerting you to the absolute basics.

Data export

You may find that you want to run analyses using statistical programs like SPSS or even in EXCEL. This will require that you transfer/transform the data. The following steps will allow you to transfer data from tps output formats into EXCEL, which should then be easy to import into any standard stats package.

1. Create an Excel spreadsheet with your specimens as rows and your variables as columns. It will look something like this:

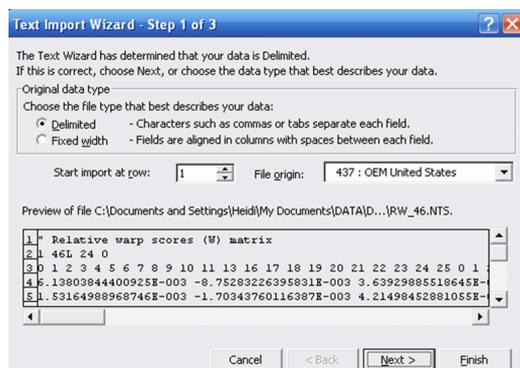


The screenshot shows a Microsoft Excel spreadsheet titled "U_littoralis_female_crania". The spreadsheet has columns labeled A through G and rows numbered 1 through 20. The data is as follows:

	A	B	C	D	E	F	G
1	specimen	sex (m=0, f=1)	centroid	PW1	PW2	PW3	
2	LACM_31012						
3	LACM_31067						
4	LACM_5794						
5	LACM_6392						
6	LACM_6394						
7	LACM_6398						
8	LACM_6399						
9	LACM_6414						
10	LACM_6415						
11	LACM_74996						
12	LACM_74998						
13	SBMNH_1074						
14	SBMNH_1759						
15	SBMNH_1797						
16	SBMNH_1935						
17	SBMNH_1998						
18	SBMNH_2001						
19	SBMNH_2063						
20							

The order of specimens in this file should be the same as that of the tps file. Using a unique ID for each specimen is highly useful.

2. Import Centroid Size.



- a. Go to *File > Open File* (make sure you choose to see all files) and select your centroid size nts file.
- b. Select *Delimited*, click *Next*.
- c. Under *Delimiters*, select *Tab*; click *Next*, then *Finish*. This should produce an Excel spreadsheet with centroid size.
- d. Copy the column with centroid sizes onto your spreadsheet. Remember that there should be as many centroid sizes as there are specimens, no more no less.

3. Import shape data.

There are two ways to do this: using macros in Excel or formatting the data in Word or another word processing program. Macros can be evil, but indispensable when you have large data sets. Below is a step by step procedure on how to format the data in Excel using macros.

- a. Open Excel. Go to File > Open File (make sure you choose to see all files). Select your weight matrix file (this file contains the pw and uniform component shape data).
- b. As before, we must convert these data: Choose *Delimited*. Under *Delimiters* choose *Tab* and *Space*. Click *Next*, then *Finish*.

You should now have a file that has columns labeled 1x 1y 2x 2y and so on plus a UniX and a UniY column.

3. Time for the macro:

- a. Highlight the first row of actual data you need to move on the spreadsheet. The first 3 rows just describe the dataset. They are NOT included in the macro (see yellow highlight and red arrows in image below). I usually delete these.
- b. Go to Tools – Macro – Record New Macro-Pick a key-stroke shortcut for your macro.

Further analysis

You can run basic analyses in some of the programs found in <http://life.bio.sunysb.edu/morph/> or in <http://www2.canisius.edu/~sheets/morphsoft.html>. However, you may find that you want to run analyses using statistical programs like SPSS or even in EXCEL.

Suggestions???

Other tips

1. Test your data collection. Take five images of each specimen (not all at once; take them on different days if you can, to make sure your photographic methods are consistent), then landmark them (also at different times). You can then look at the within-specimen variance relative to other sources, to assess the degree to which it might affect your analyses.

Dave Polly has found that small differences in the orientation of 3D objects (where you're trying to line up a plane with the optical plane) are a minimal source of variance.

2. Read the help files in the tps programs.

3. Subscribe to the morphmet listserve, and don't be shy about posting questions there if you're stuck. Also post any problems you have with the programs. Chances are very good someone's experienced and solved your problem. If not, Jim Rohlf will incorporate a fix into the next version of his programs, which everyone will appreciate.

Suggestions???