



## Collecting Landmarks

### Important Requirements for Landmarks

Remember the following requirements for landmarks:

- a. Each image must have the same number of landmarks;
- b. The landmarks on each image must be in the same order;
- c. Landmarks are ordinarily placed on homologous points, points that can be replicated from object to object based on common morphology, common function, or common geometry;
- d. You may have to flip some images so that are not reversed left to right (e.g., if most of your images show the right side, flip left side images so that they mimic right side)

<b>Collecting Landmarks</b> .....	<b>1</b>
<i>Important Requirements for Landmarks</i> .....	1
<i>ImageJ or Fiji (“Fiji is just ImageJ”)</i> .....	2
<i>tpsDig (PC only)</i> .....	4
<i>Mathematica</i> .....	5
<i>geomorph package for R</i> .....	6
<i>Phylonimbus</i> .....	6

## ImageJ or Fiji (“Fiji is just ImageJ”)

*Fiji* – updated version of ImageJ, open-source software for image processing  
<http://fiji.sc/>

### To collect landmarks

1. Place all your images in a single folder by themselves
2. Start Fiji
3. open the first image
4. Choose the *multi point* tool (located in the main tool bar using the pull-down arrow on the single point tool). You can adjust the color, size, and numbering of points under Edit > Options > Point tool.

### Repeat the following steps for each image

5. Carefully place each of your points on the image, always in the same order (you can drag the points using the arrow tool if you need to adjust them).
6. When all points are placed, click CTRL-M to record them in the measurement window (also available under Analyze > Measure menu)
7. Click CTRL-SHIFT-O to open the next image. If the points from the previous image do not automatically disappear, they can be deleted by ALT-clicking on them.

### When landmarks have been recorded for all specimens

8. In the Measurement window, right click to “Select All” then “Copy”. Paste the result into Excel.
9. The x and y coordinates of your points are in the 4th and 5th columns.
10. In Excel, insert blank rows before and after the block of rows for each specimen.
11. Above the x-coordinates, enter the text "LM=" followed by the number of points (e.g. LM=5)
12. Below the x-coordinates, enter the text "ID=" followed by the taxon name (e.g. ID=Archaeopteryx)

	A	B	C	D	E	F	G	H	I
1				LM=6					
2	1	Ateleaspis_te	0	255	51	0	0	255	
3	2	Ateleaspis_te	0	260	433	0	0	255	
4	3	Ateleaspis_te	0	300	272	0	0	255	
5	4	Ateleaspis_te	0	219	269	0	0	255	
6	5	Ateleaspis_te	0	446	43	0	0	255	
7	6	Ateleaspis_te	0	73	42	0	0	255	
8				ID=Ateleaspis					
9				LM=6					
10	7	Benneviaspis	0	190	261	0	0	255	
11	8	Benneviaspis	0	191	62	0	0	255	
12	9	Benneviaspis	0	211	154	0	0	255	
13	10	Benneviaspis	0	171	153	0	0	255	
14	11	Benneviaspis	0	373	112	0	0	255	
15	12	Benneviaspis	0	13	108	0	0	255	
16				ID=Benneviaspis					
17				LM=6					
18	13	Boreaspis_ce	0	160	336	0	0	255	
19	14	Boreaspis_ce	0	158	72	0	0	255	
20	15	Boreaspis_ce	0	172	132	0	0	255	
21	16	Boreaspis_ce	0	147	133	0	0	255	
22	17	Boreaspis_ce	0	314	135	0	0	255	
23	18	Boreaspis_ce	0	3	132	0	0	255	
24				ID=Boreaspis					
25				LM=6					
26	19	Dicranaspis_	0	182	267	0	0	255	
27	20	Dicranaspis_	0	178	41	0	0	255	
28	21	Dicranaspis_	0	198	101	0	0	255	
29	22	Dicranaspis_	0	164	102	0	0	255	
30	23	Dicranaspis_	0	350	44	0	0	255	
31	24	Dicranaspis_	0	11	40	0	0	255	
32				ID=Dicranaspis					
33									

Figure 1: screen shot from Excel showing what data look like at step 11 above.

### Save the landmarks in TPS format

1. In Excel, highlight columns 2 & 3 from the first “LM=6” to the last “ID=Dicranaspis” and copy.
2. In Word, use Paste Special to paste these rows as **unformatted text**. If you don't paste as unformatted text then you will have trouble importing the data into other programs.
3. Replace tabs, which are inserted by default when you paste from Excel, with spaces: Edit > Find > Advanced Find and Replace.
  - a. Click the arrow in the lower left to show the options.
  - b. Click the “replace” tab at the top to open both Find and Replace input bars.
  - c. Put your cursor in the “Find what” bar, then select Tab Character from the pulldown menu at the bottom labeled “Special” (or type in ^t)
  - d. Put your cursor in the “Replace with” bar, then enter a single space.
  - e. Click “Replace All”
  - f. Close the Find and Replace window.

4. Save the Word document **as plain text** using File > Save As with a name that ends in .txt or .tps (choose Plain Text from the pulldown File Format menu at the bottom of the dialog box). Click OK on the next dialog box.

```
LM=6·  
255·51  
260·433  
300·272  
219·269  
446·43  
73 42  
ID=Atelcaspis·  
LM=6·  
190·261  
191·62  
211·154  
171·153  
373·112  
13·108  
ID=Benneviaspis·  
LM=6·  
160·336  
158·72  
172·132  
147·133  
314·135  
3·132  
ID=Borcaspis·  
LM=6·  
182·267  
178·41  
198·101  
164·102  
350·44  
11 40  
ID=Dicranaspis·
```

Figure 2: screen shot from Word showing what data look like in “TPS” format at step 3 above.

### tpsDig (PC only)

1. Install *tpsUtil* and *tpsDig* programs from Rohlf’s morphometrics site (<http://life.bio.sunysb.edu/morph/> )

2. Place all images in a single folder by themselves.
3. Use tpsUtil to create a new data file from images.
  - a. Select operation.
  - b. Select folder by clicking on one of the images.
  - c. Select output file by choosing folder and giving the file a new name.
  - d. Confirm which images you want under Actions.
  - e. Click “Ok” to create empty file.
4. Open empty data file in tpsDig. Your images should appear in the program.
  - a. Use cross-hair tool to add landmarks;
  - b. Right click to delete landmarks;
  - c. Use arrow tool to move landmarks;
  - d. Use arrow buttons to move to next image;
  - e. Use + and – buttons to enlarge or reduce image on screen;
  - f. Use “Save data” to save coordinates into TPS file (overwrite existing data).
5. Import TPS file into Mathematica using the *tpsImport[]* function in the Polly Morphometrics add-in.

```
data = tpsImport["/Users/pdavidpolly/Documents/Data/Mandibles.TPS"];
```

## **Mathematica**

If you are dexterous you can collect your landmark data directly in *Mathematica* using the Get Coordinates tool (see *howto/GetCoordinatesForPointsInAPlot* in the *Mathematica* Documentation Center).

1. Place all of your images in the same folder by themselves
2. Use the SetDirectory[] function to select the folder. You can use Insert -> File Path to make this easier, but you have to (a) select an image in the folder and (b) remove the name of that image from the path as follows:

```
SetDirectory["/Users/pdavidpolly/Documents/Mandibles/image1.jpg"]
SetDirectory["/Users/pdavidpolly/Documents/Mandibles/"]
```

3. Save the image file names in a variable using the FileNames[] function. Selection of files can be controlled by giving an argument that specifies which file names to load. \* means any character, so “\*.JPG” would load all files ending in capital JPG, “DSCN\*” would load all files starting with DSCN.

```
filenames = FileNames["*.JPG"]
```

4. Import the images into a list.

```
imgs = Table[Import[filenames[[x]]], {x, Length[filenames]}];
```

5. Display all the images, or selected ones.

```
imgs
```

```
imgs[[1]]
```

```
imgs[[1;;5]]
```

6. Activate the “Get Coordinates” tool by right clicking on one of the images, or by starting Drawing Tools under the *Window* menu and choosing the cross-hair tool.
7. Select landmarks by clicking on the image with the tool. You may need to enlarge the image by resizing it or by choosing a magnification option from the right click menu. Click Ctrl-C (Cmd-C on Mac) or Copy from the Edit menu to copy the coordinates.

**Note:** remember to copy the coordinates before choosing another tool or entering text in the Notebook otherwise the points will disappear.

8. Paste the landmarks into the Notebook, and repeat for each image. You will need to format them by placing a set of curly braces “{” around the entire collection of landmarks and separating the landmarks from each image with a comma.

```
lands = {  
  [Paste landmarks here] ,  
}
```

This will result in the following:

```
lands = {  
  {{1221.5`, 653.5`}, {1167.5`, 539.5`}, {1238.5`, 438.5`}, {1049.5`, 469.5`}},  
  {{806.5`, 633.5`}, {850.5`, 633.5`}, {1083.5`, 830.5`}, {1156.5`, 631.5`}}  
}
```

## ***geomorph package for R***

See instructions in the Quick Guide to *geomorph* manual by Emma Sherratt.

## ***Phylonimbus***

*Phylonimbus* is an online, web-based landmarking system developed by Dominic White that is located at <https://www.phylonimbus.com/morphometrics/>

1. Create an account
2. Under the morphometrics link at the top left, choose “Create new project” (specify 2D or 3D at bottom of next page)
3. See the “view documentation” link for further instructions.