

Measuring amphibian morphometrics with ImageJ

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Reference: Browne RK, 2014. Measuring amphibian morphometrics with ImageJ. Amphibian and Reptile Conservation. Protocols 2.

These methods use ImageJ™ freeware available at <http://rsb.info.nih.gov/ij/>

The accurate measurement of amphibian morphometrics is essential for taxonomy, studies of growth and development, and studies of fluctuating asymmetry.

The use of digital images and ImageJ™ enables the indefinite archiving of the source image for future reference and gives results that are often more accurate than calliper measurements of struggling animals. Some life stages of amphibians, particularly tadpoles, are difficult to measure by hand without risking damage.

Measuring anuran snout-urostyle length (SUL) and digit length from digital images

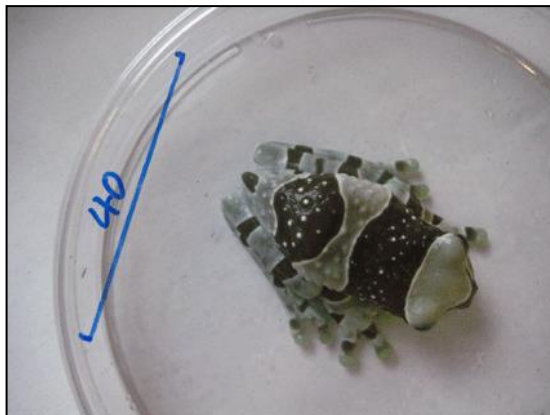
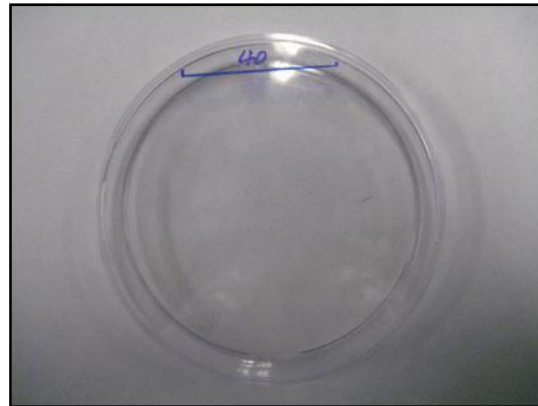
This method uses ImageJ™ freeware available at <http://rsb.info.nih.gov/ij/>

Periodic measurement of snout-urostyle length (SUL) of frogs is necessary in captive populations to monitor growth and general health. Excessive handling can be stressful for frogs, and spread disease, and it is often difficult to obtain an accurate measure using callipers. It is possible to accurately measure frogs using digital images in conjunction with image analysis packages such as ImageJ™, thus minimizing handling and stress to the individual. Note that the weight of the frog can be taken simultaneously by weighing the frog and the Petri dish together, and subtracting the weight of the Petri dish.

(See <http://zims.isis.org/aark/Research%20articles/Methods/Measuring%20caecilians.pdf>).

Measuring SUL

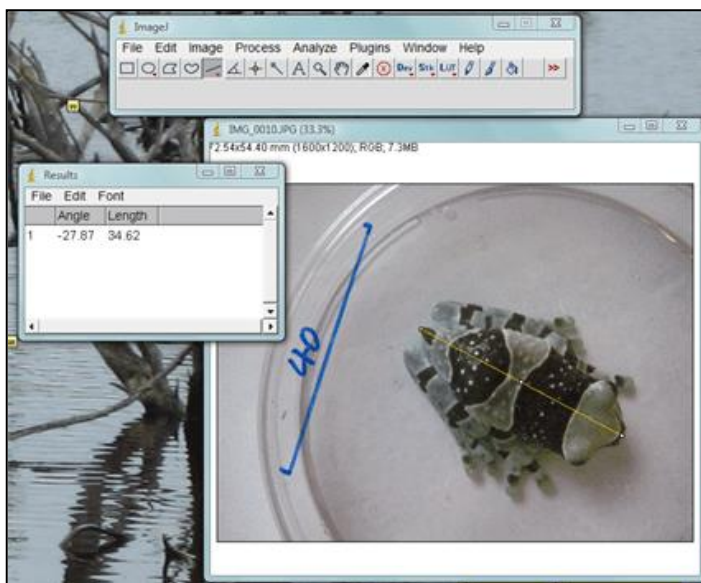
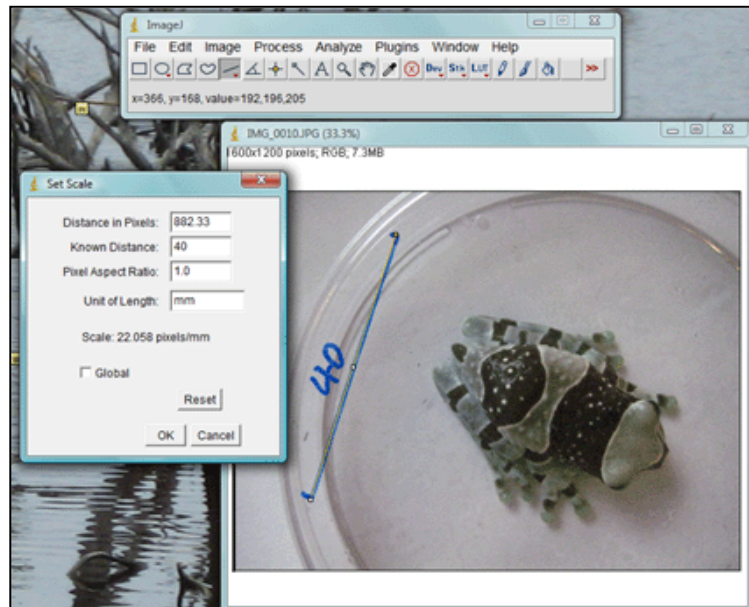
1. Mark a standard Petri dish with a bar of known length, eg. 40mm. This is used to scale the image in ImageJ™.



2. Place the frog in the Petri dish, replace the lid and hold against a white background. Take a digital image, ensuring the frog and the whole scale are visible. Make a note of the image number and which frog it correlates to (eg. tank number, individual number, code if tagged, etc.). NB. Often, dorsal patterning can be used to identify individuals as they grow.

3. Once the image is downloaded to a computer, start up ImageJ™ and open the image; **File > Open**

4. Select “straight line” from the icon bar. Draw a line along the scale bar. Choose **Analyse > Set scale** and enter the Known Distance (eg. 40) and the Unit of Length (eg. mm) and click “OK”.



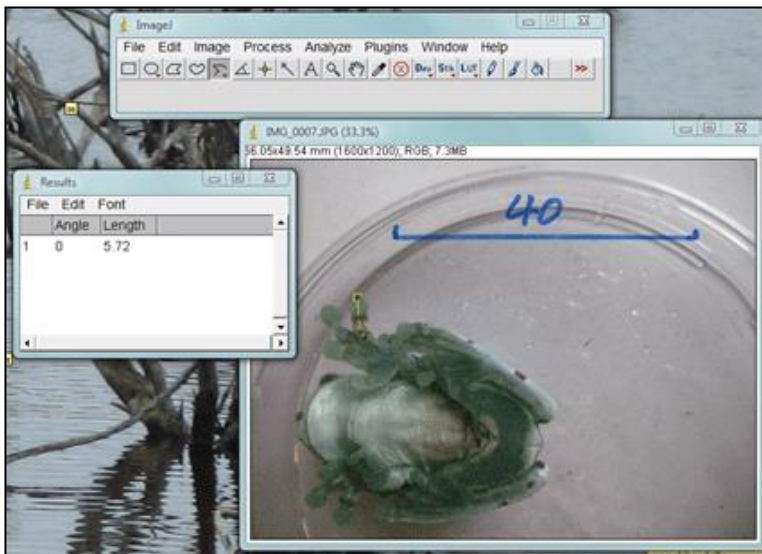
5. Again using the “straight line” tool, draw a line from the tip of the snout to the tip of the vent. Choose **Analyse > Measure**. The length of the line, and therefore the SUL, will appear in a “Results” window.

Measuring digit length

This method can also be used to measure anura digit length, which can be used to investigate the effect of hormones on development through differences in length between males and females. By ensuring the

feet are flat on the surface of the Petri dish, a digital image can be taken of the ventral side of the frog, making it possible to measure the length of the digits.

Digits are traditionally numbered with that nearest the body (the “thumb”) as number 1, moving round to the outermost digit numbered 4 (forelimb) or 5 (hindlimb).



Again, the scale can be set using the same method as before (see above). The digits can then be measured by right clicking the “Straight line” tool in the tool bar and selecting “Segmented lines”. This allows the straight line to bend round corners for more accurate measurement of digits. A line can then be drawn from the tip of the toe pad to the basal crease, and the length acquired by selecting [Analyse > Measure](#).

The digits of larger frogs can be measured using the same method by holding the frog and pressing the foot flat against the Petri dish in line with the scale.

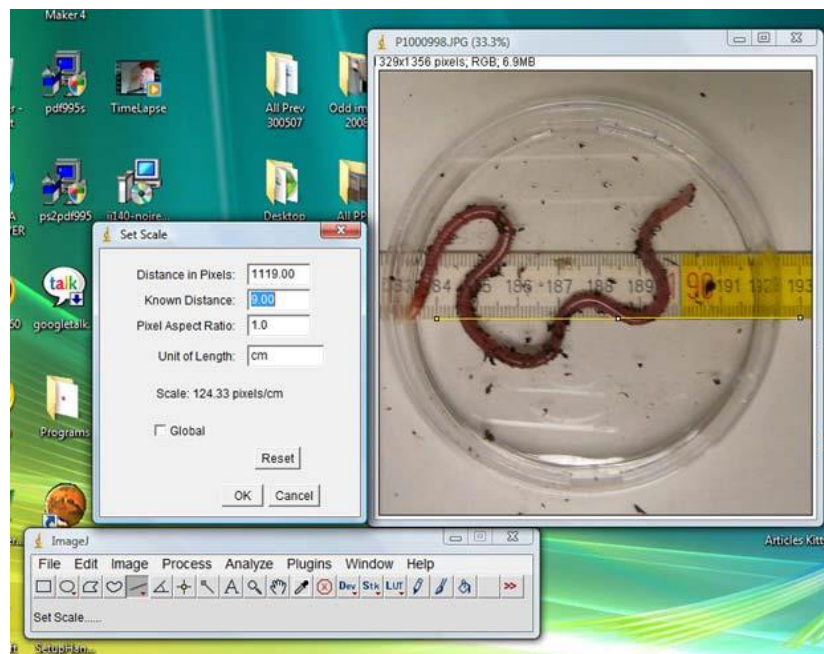


Measuring caecilians from digital images

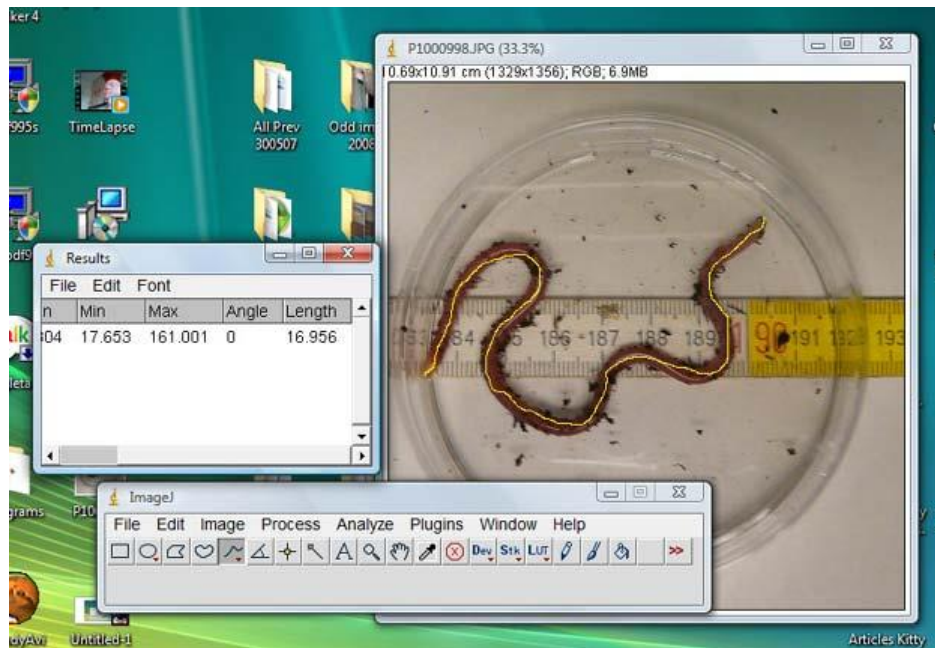
Caecilians can be hard to measure because of their constant motion and dislike at being stretched out. Excessive handling of any amphibian is stressful. The following method enables both the weighing and measuring of caecilians with very little stress. First of all weigh a moist Petri Dish with lid on. Place the caecilian in the dish and cover with lid. Weigh the combination and calculate the caecilian weight.

Then put the Petri dish over any metric ruler.

1. Take image of caecilian with ruler with centimetres in photo. The precision of the results depends on the number of pixels per cm, and the accuracy of the scale but can be easily to 0.01mm. In Figure 1 the ruler is accurate to 1mm.
2. Make sure ruler is horizontal in image or rotate image with program until image is horizontal
3. The number of resolution of the image and the length of the caecilian will decide the accuracy of the measurement
4. Open ImageJ™ program
5. File > Open
6. Select “straight line” from icon bar
7. Draw line along known number of cm on ruler. ie 9 cm.
8. Analyse > set scale
9. The set scale window will appear with distance in pixels of the drawn line
10. In the box for known distance put the length of the drawn line. ie. 9cm



11. Go back to “straight line” form icon bar and right click
12. Three choices appear “straight line”, “segmented lines” and “freehand lines”
13. Select freehand lines
14. Draw line along curved path of caecilian
15. Go to “Analyse” > “Measure”



16. The length is to the right of the results (in this case 16.96 cm)

The accuracy of the results is normally within 3% as completed above.

Discussion of the precision and accuracy of other methods of measuring caecilians are found in: Measey GC, Silva JB, Di-Bernardo M. 2002. Testing for Repeatability in Measurements of Length and Mass in *Chthonerpeton indistinctum*(Amphibia: Gymnophiona), Including a Novel Method of Calculating Total Length of Live Caecilians. <http://john.measey.com/pdf/Measey.techs.pdf>