

# The Youngest Tasmanian Wolf: Scanned

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## Aims

*Thylacinus cynocephalus* (Harris, 1808) also known as Tasmanian wolf or Tasmanian tiger was the last living species of marsupial family Thylacinidae, whose last known specimen died in 1936. Since then zoologists can study only museum specimens including those interested in development of this species so similar to Canidae (wolves, jackals, foxes etc.). Recently the world youngest specimens were rediscovered and described in the collection of Department of Zoology, Faculty of Science, Charles University in Prague (Czech Republic): a jar containing four pups preserved in unknown liquid<sup>1</sup>.

As the faculty is interested in as much detail information about the samples as possible without any disruption of the sample, non-invasive methods were looked for, and microCT seemed as the best choice. SkyScan 1176 localized at the Czech Centre for Phenogenomics (Vestec, Czech Republic), which is able to scan large, fixed samples, fitted well for this goal.

## Method

Setting up the scanning procedure was the crucial part of obtaining the data. The samples bound to glass plate by a thread were supposed to be quite soft (with low level of ossification), with no contrast agent and placed in a large jar made from thick glass and with relatively huge amount of liquid. Moreover, no one could be sure, how a more then hundred years long preservation influences suitability of the samples for microCT.

SkyScan 1176 was set up for full, 360° scanning with image pixel size of 8.67 µm and no filter. The final voltage, current and exposure time were set up for 90 kV, 265 µA, and 3,000 s. Reconstruction was provided in NRecon 1.6.9.15 (Bruker) with parameters for smoothing, ring artifacts correction, and beam hardening correction set up for 3, 19, and 4 % respectively and intensities threshold from 0.000 to 0.013.

The reconstructed digital slices undergone definition of area of interest and several operations for removing noise signal and smoothing the surface of developing bones in CTAnalyzer 1.14.4.1 (Bruker). Resulting black and white bitmaps were converted in FiJi ([imagej.nih.gov/ij](http://imagej.nih.gov/ij)) to be suitable for ITK-Snap 3.6 (Yushkevich & Gerig), where final segmentation of bones were provided according to their morphology and spatial situation.

## Results

Although nothing from the soft tissue was visible, the morphology of developing bones was clear in all four samples enabling further data analysis. The samples show characteristics typical for marsupial mammal of this stage, e.g. well developed mouth apparatus with angular processes of mandible rotated medially, and well developed front limbs compared to the hind ones.

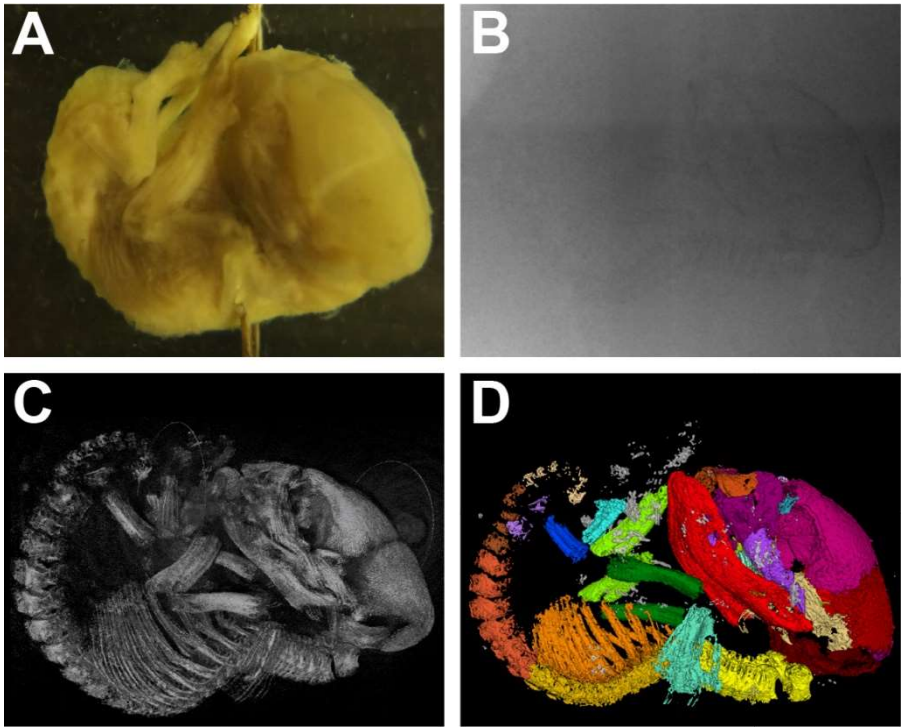


Figure 1: Pup of *Thylacinus cynocephalus*: A) original sample, B) scout-scan of the sample in A, C) bone morphology of the sample from A in CTvox 3.2 (Bruker), D) bone segmentation of the sample from A in ITK-Snap. Head of the sample on the right, abdomen to the top. See disproportion between frond and hind limbs development.

### Conclusion

MicroCT is usable also for nondestructive analysis of soft and rare biological samples of old age with long preservation time, when sustaining integrity of sample is needed.

### Acknowledgements

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### References:

1. Sleightholme SR, Robovsky J & Vohralík V, "Description of four newly discovered Thylacine pouch young and a comparison with Boardman (1945)", *Australian Zoologist* 36(2), pp 232-238, 2012