Electronic supplementary material for

'Fossils': a new, fast, and open-source protocol to simulate muscle-driven biomechanical loading of bone

New, fast python protocol to simulate loading of bone

Narimane Chatar[1*], Romain Boman[2], Valentin Fallon Gaudichon[1,3], Jamie A. MacLaren[1,4], Valentin Fischer[1]

*Corresponding author

[1] Evolution & Diversity Dynamics lab, UR Geology, Université de Liège, Liège, Belgium

[2] Department of Aerospace and Mechanical Engineering, Non-Linear Computational Mechanics (MN2L) Research Group, Université de Liège, Liège, Belgium

[3] Institut des Sciences de l'Evolution de Montpellier (ISEM), Université de Poitiers-Montpellier, Montpellier, France

[4] FunMorph Lab, Department of Biology, Universiteit Antwerpen, Antwerpen, Belgium
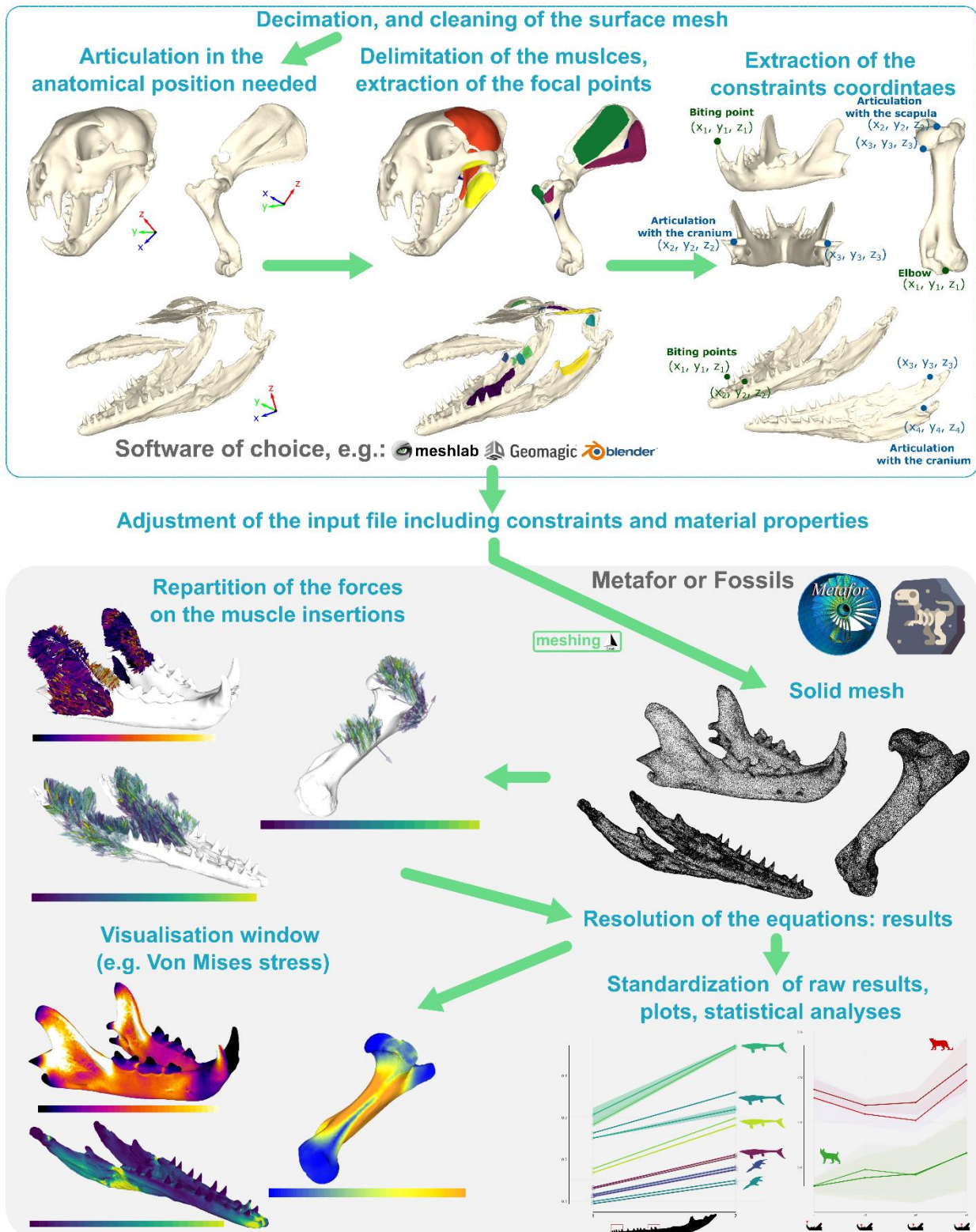
Figure S1: *Complete workflow of the protocol. Steps in the blue box can be done with a 3D processing software of choice (e.g. Meshlab, Geomagic, Blender). Our three examples are a CT-scan of the mandible of Panthera pardus (AMNH-113745) initially published by* Tseng et al. (2016)*, a surface scan of the mandible of Tylosaurus nepaeolicus (FHSM-VP2209), and a surface scan of the humerus of Tapirus indicus (NHMW 1938). Examples of results from Fallon Gaudichon et al. (2021) and* Chatar et al. (2022)

Basic mesh preparation for FEA

Data acquisition

1. CT data

It is usually recommended that the size of data remains lower than 10% of the available RAM on the computer used for segmentation. For instance, at least 20 GB of RAM are needed to segment an image stack of 2 GB.

Different software available:

a) Free and open source: Fiji (Image J) https://imagej.net/software/fiji/downloads

    3D Slicer https://download.slicer.org/

b) Academic license available: Dragonfly https://theobjects.com/dragonfly/get-non-commercial-licensing-program.html

c) Paying option: Avizo

Segment the region of interest and export as 3D mesh (stl, ply, etc.). If you use 3D slicer you can combine our protocol with 'geniso', a tool developed by d'Otreppe et al. (2012) to deal with multi region mesh.

2. CT data

Photogrammetry, laser scan or white light scan: Export directly as surface mesh (stl, ply, etc.)

Mesh processing

Different software available:

a) Free and open source: Meshlab https://imagej.net/software/fiji/downloads
b) Paying option   Geomagic

1. Close all the holes, make the mesh completely watertight.
2. If you use a software with a dedicated 'manifold' tool use it to make sure the mesh is manifold (which is not systematically the case after step 1).
3. Smooth the mesh to avoid any spikes or highly creased edges.
4. Decimate the mesh to the desired number of triangles.
5. Align anatomical plans of the structure along the three axes. Use the same alignment for all specimens, e.g. for a skull: mid sagittal axis is parallel to xy and lateral plane parallel to yz.
6. Articulate the structure of interest with other adjacent bones to draw the muscle insertions.
7. Draw all the muscle insertions (on the structure of interest and its surrounding bones) involved in the movement simulated and export the insertions drawn on the structure of interest as 3D objects (stl, ply, etc.).
8. Use any tool available in the software of choice to extract the coordinates of the center of mass of each muscle insertions on the bone surrounding the structure of interest. Store those coordinates.
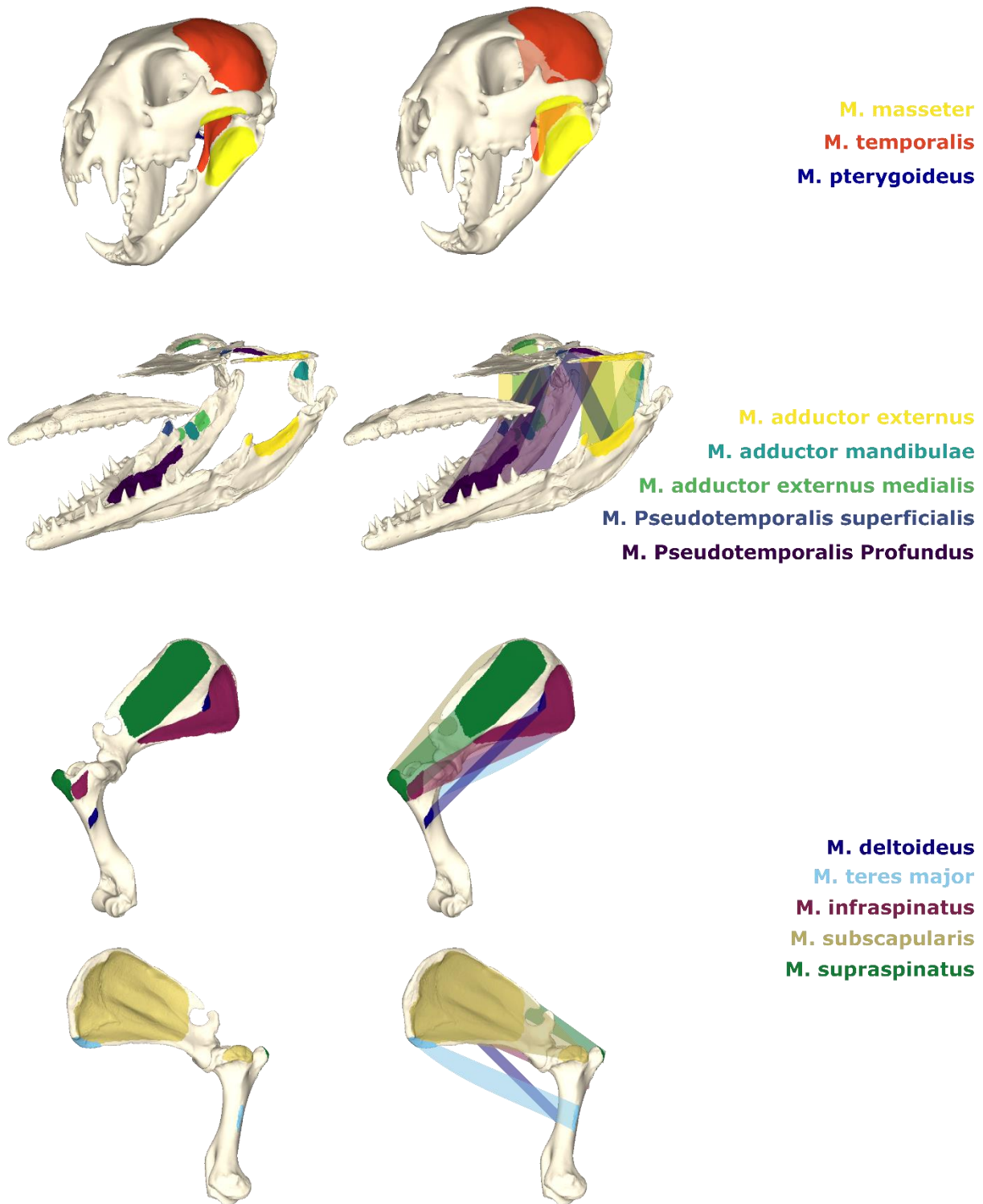
M. masseter
M. temporalis
M. pterygoideus

M. adductor externus
M. adductor mandibulae
M. adductor externus medialis
M. Pseudotemporalis superficialis
M. Pseudotemporalis Profundus

M. deltoideus
M. teres major
M. infraspinatus
M. subscapularis
M. supraspinatus

Figure S2: *Muscle insertions drawn on the* Panthera pardus *skulls (top), the* Tylosaurus nepaeolicus *skulls (middle) and the* Tapirus indicus *shoulder (bottom).*
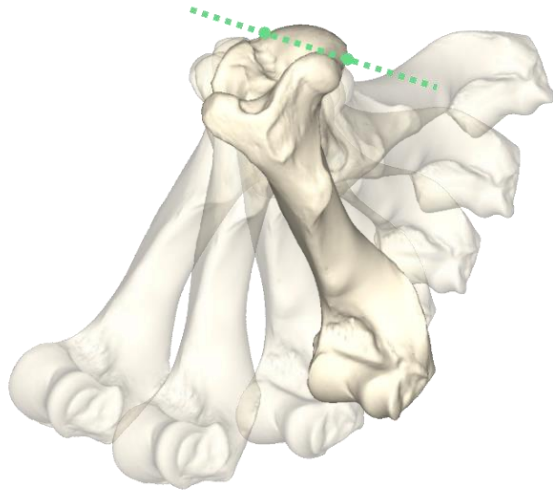
2 fixations at the articulation

➡️ **Rotation axis**
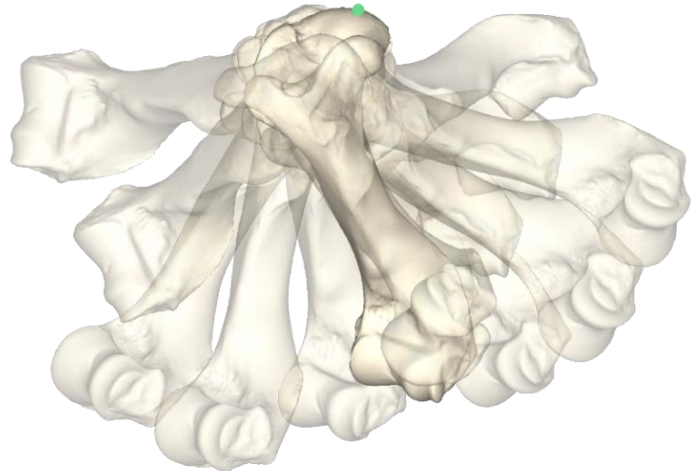
1 fixation at the articulation

➡️ **Rotation point**

Figure S3*: Rotations possible with two fixation points on the humeral head of* Tapirus indicus *(left) and only one (right).*

Comparison of the three loading scenarios

In the leopard mandible the stress is globally lower when the mandible is loaded using a tangential or a uniform traction and while the reaction force measured on the canine appears somewhat similar (max 6% of variation) in the three scenarios tested the internal energy and especially the maximum displacement are highly variable (up to 63% of variation for the maximum displacement). The global distribution of stress in the tapir shoulder appears more comparable in the three scenarios but the reaction force is much more variable here (24% of variation) along with the internal energy (28% of variation) and the maximum displacement (31% of variation). The variations observed in the mosasaur mandible are quite low for the reaction force as for the other biting simulation (4%) but higher in terms of internal energy (13%) and up to 24% in terms of max displacement.

Table S1: Comparison of the three different loading scenarios implemented in Metafor on the three different mesh using a Lenovo ThinkBook 16p G2 with 40 Gb of RAM.

|	Reaction force (N)	Internal energy (mJ)	Max displacement (mm)	Resolution time
Panthera pardus Initial number of triangles in the surface mesh: 200k				
Tangential + normal	157.62	171.93	2.08	9m 25s
Tangential	148.129	111.766	0.77	8m 28s
Uniform	156.725	143.5	1.59	8m 38s
Tapirus inducus Initial number of triangles in the surface mesh: 50k				
Tangential + normal	3,334.07	13,012.60	3.37	2m 11s
Tangential	4343.16	9348.32	2.33	1m 45s
Uniform	3972.06	9863.54	2.44	1m 48s
Tylosaurus nepaeolicus Initial number of triangles in the surface mesh: 500k				
Tangential + normal	3971.70	116,072.00	56.69	50m 26s
Tangential	3800.55	100,582.00	43.21	48m 16s
Uniform	3834.94	104,897.00	45.67	48m 2s
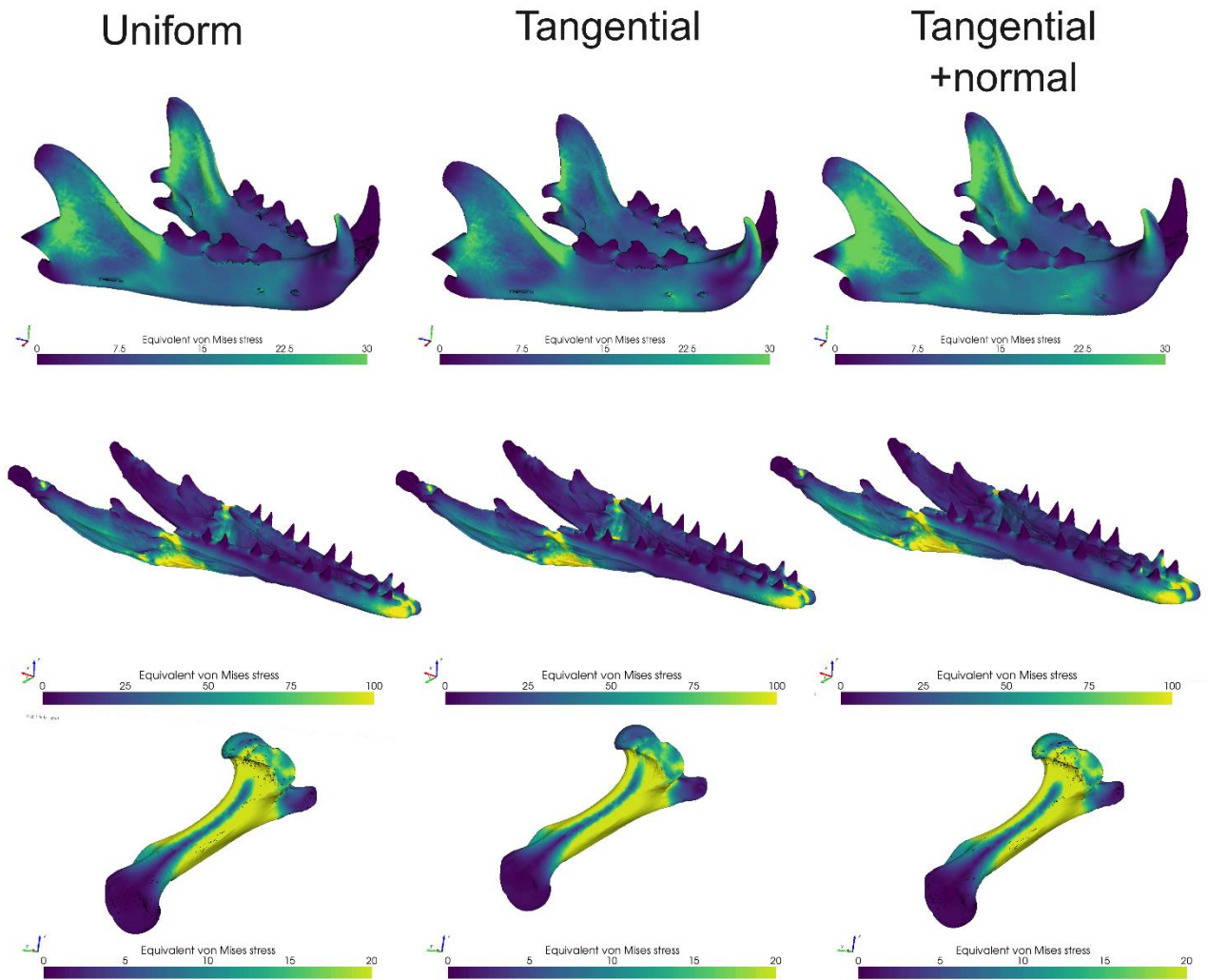
Figure S4: *Comparison of the Von mises stress contour plots in the three different loading scenarios implemented in Metafor for our three examples.*

Launching Metafor

Metafor is launched using the "Run Metafor" icon, then the python path base needs to be changed to the folder containing the script for the analysis (Figure S3a). The python scripts should appear in the Modules section (Figure S3b); the change of directory is visible in the history window "History" (Figure S3c), located above the command line (Figure S3d). This file should also contain the STL file of the model to be analysed, as well as all the 3D muscle insertion files (as STLs). To run the analysis, three python files are required: the input file specific to the specimen/analysis (in the case of the mosasaurid mandible: "TYLnep_500k_7_1.py" file), "bonemodel.py", and "boneload.py". Those three python files need to be placed in the same folder, and should appear all together in your python path. Once the three scripts appear in the python path, right-click on the specimen script (e.g. "TYLnep_500k_7_1.py") and load it, then click on the arrow icon in the toolbar (Figure S3e) to launch the analyses. Metafor will then call Gmsh to mesh your model and run the FE analysis with the parameters and constraints defined in the python script. Once all the equations are solved, the visualisation window should open automatically and a result file will be created. Once the computation starts, every time increments will be detailed in the main panel (Figure S3f) and any problem encountered during the meshing or the equation resolution will be notified in this same panel.

e) Results, normalisation and visualisation

Metafor produces a general result file as a .txt ('resFile.txt') which is saved in a new folder with the name of the corresponding .stl, in the "workspace" folder. This file contains a series of outputs, including the internal energy, reaction forces on the biting points, and mechanical efficiency. The internal energy corresponds to the work of external forces, and is a scalar value given in $mJ.mm^3$ which quantifies the energy stored in each specimen subjected to an elastic deformation, and which can be normalised by dividing it by the model volume, in mm^3. In our mosasaurid example, this internal energy was stored as 'Internal energy: 116072' in line 124. Reaction forces on the

constraint points are given in newtons (N), and can be normalised by dividing them by the total input force of the analysis to obtain the mechanical efficiency. For the mosasaurid example, these values are stored as 'teeth_Fz : -4106.15' on the last line of the result text file. Mechanical efficiency is a scale-invariant value which provides information about the efficiency with which the total muscle forces can be conveyed (in this case) into reaction force on constraint points (Dumont et al., 2011).

Metafor automatically opens a visualisation window when the resolution is complete; different parameters can then be adjusted in the configuration panel (the "Config" in the bottom left corner of the window). This window can be used to visualise various parameters, such as the Equivalent von Mises stress, in the form of a contour plot (Figure S4a), which can be assigned different colour palettes for maximal accessibility. Palettes include Gauss 100 (Figure S4b), viridis (a protanopia-friendly palette; Figure S4c), or Matlab jet (Figure S4d), among others. Colour palettes can be adjusted in the "Colors" tab of the configuration panel; the background colour can also be altered if the default white does not provide a good contrast (Figure S4e). As recently suggested by (Lautenschlager, 2021), the classic "rainbow" colour scale (Figure S4a) is not well suited to represent the underlying stress distribution of FEA models; in an effort to improve representation and clarity of stress distributions, the large panel of colour maps available in Metafor is a great advantage. To modify the scale, go to "ScalarValues" in the configuration window. In this section of the configuration panel, the "field" value also enables the plotting of other parameters rather than just the von Mises stresses, such as the displacement. To display a more continuous colour scale, go to "Colors" and increase "Number of colors". Contour plots for the leopard show an example with 50 colours in the scale, whereas those for the mosasaurid were performed with 20 colours (Figure S4). To visualise traction vectors (direction and traction force on a triangle edge), go to "VectorValues" in the configuration window; in this section, deselect "Hide" in "field" subsection, and for a better visualisation select in "representation" > "Arrows",

allowing changes in the external forces scale by modifying "max" in "colormap" and dimensions of the arrows with "amplitude" in "scaling" subsection. This will show the force repartition on the muscle insertions (Figure 4). To export an image capture of the visualisation window, go to "Capture" in the configuration panel.
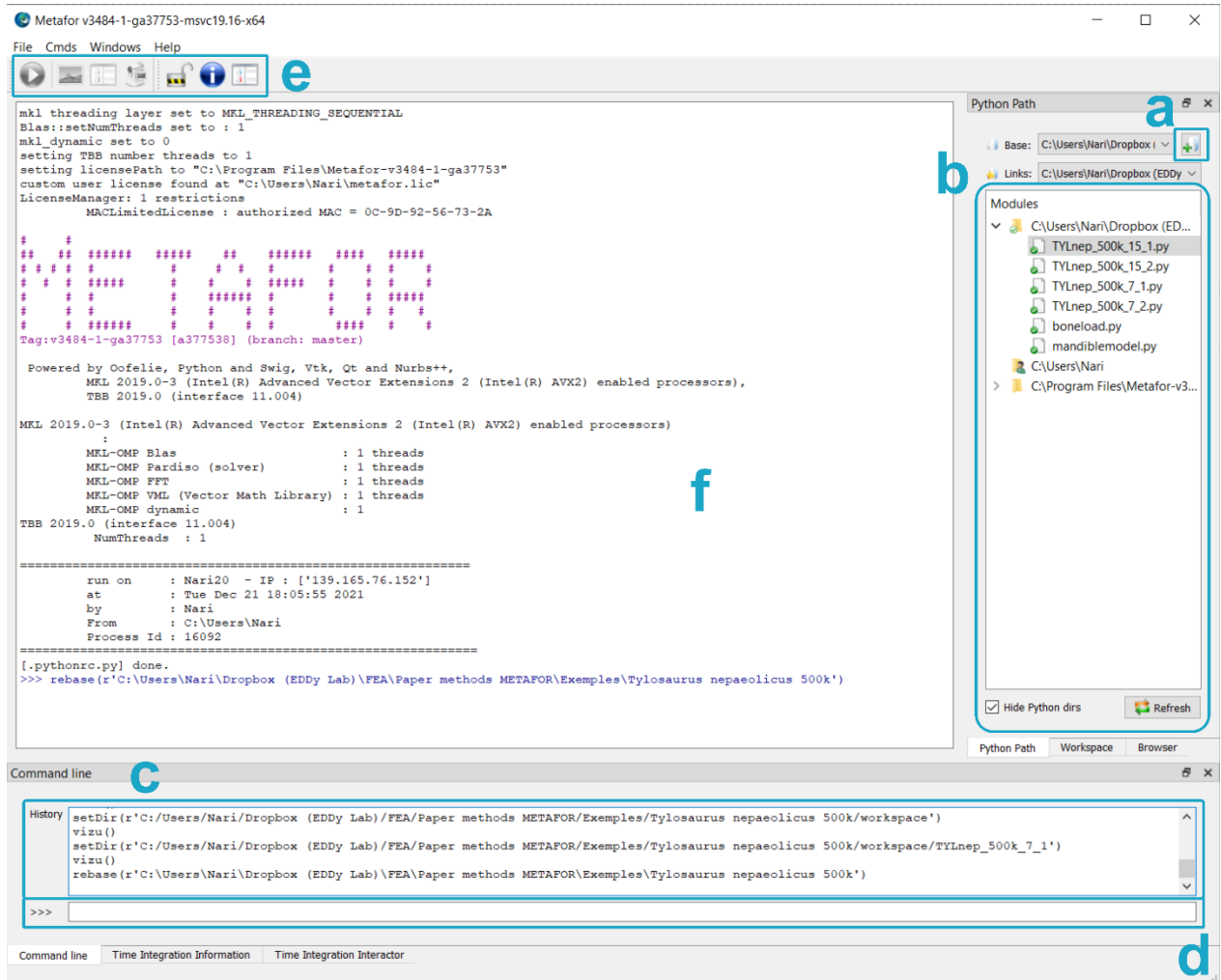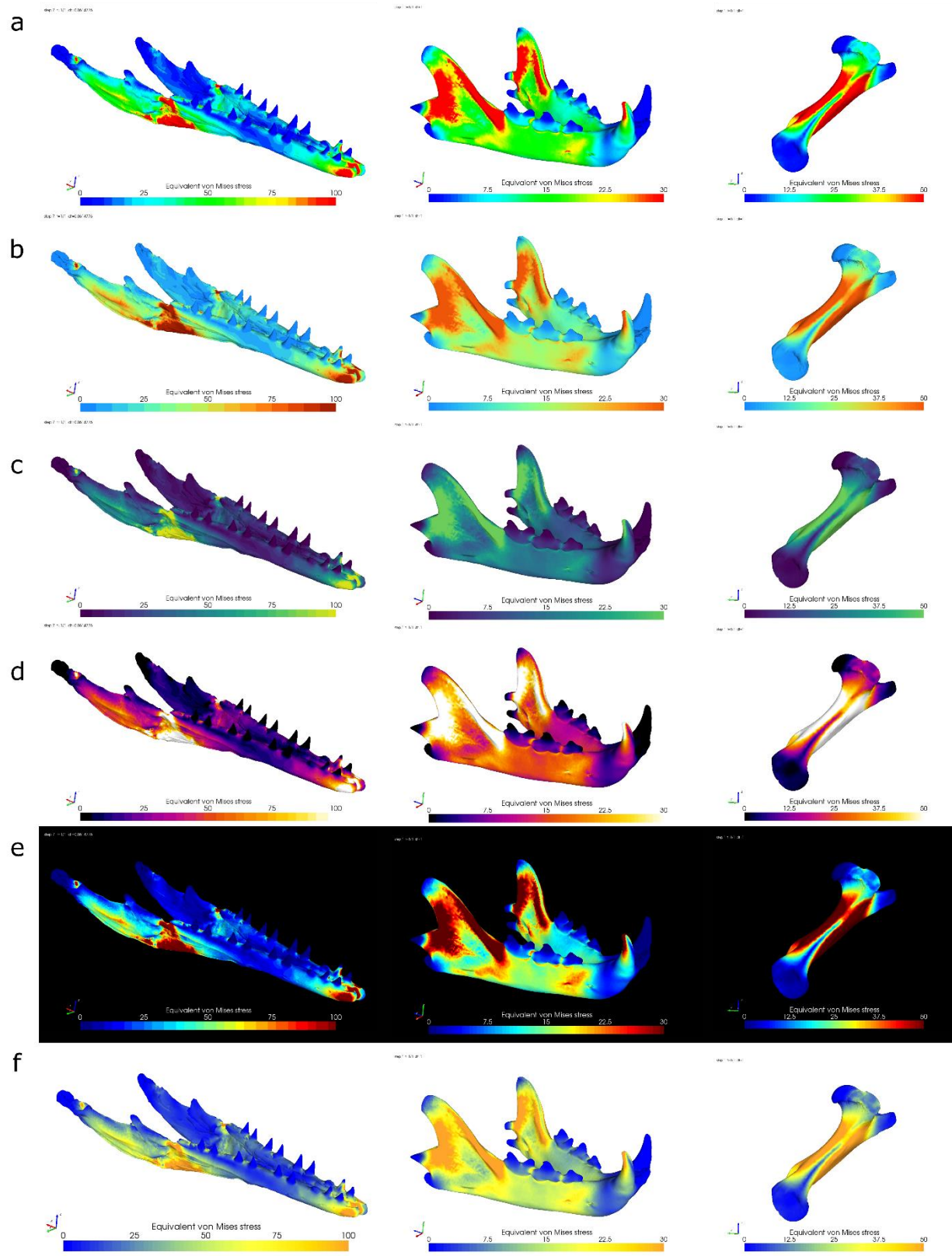

Figure S5: *Metafor window*

Figure S6: Some of the different colour palettes implemented in the Metafor visualization window.
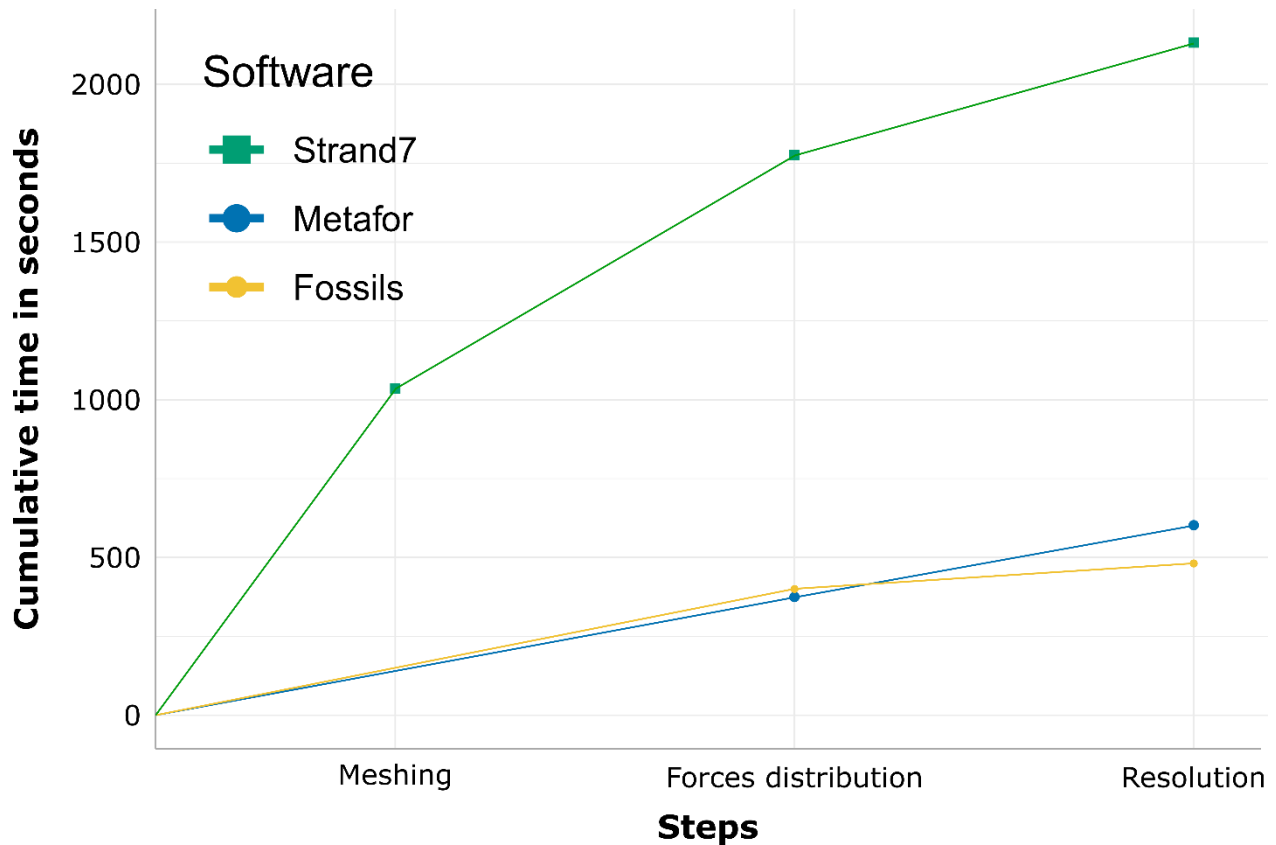
Figure S7*: Cumulative resolution time (in seconds) for the same analysis in Strand7, Metafor and Ansys for the leopard mandible.*

Supplementary references

Chatar, N., Fischer, V., & Tseng, Z. J. (2022). Many-to-one function of cat-like mandibles highlights a continuum of sabre-tooth adaptations. *Proceedings of the Royal Society B: Biological Sciences*, *289*(1988). https://doi.org/10.1098/rspb.2022.1627

D'Otreppe, V., Boman, R., & Ponthot, J.-P. (2012). Generating smooth surface meshes from multi-region medical images. INTERNATIONAL JOURNAL FOR NUMERICAL METHODS IN BIOMEDICAL ENGINEERING Int. J. Numer. Meth. Biomed. Engng, 28, 642–660. https://doi.org/10.1002/cnm.1471

Fallon Gaudichon, V., Boman, R., Chatar, N., Maclaren, J., & Fischer, V. (2021, September). Uncovering the biomechanical disparity of Cretaceous marine reptile feeding via finite element analysis. 69th Symposium on Vertebrate Palaeontology and Comparative Anatomy.

Lautenschlager, S. (2021). *True colours or red herrings?: colour maps for finite-element analysis in palaeontological studies to enhance interpretation and accessibility*. https://doi.org/10.1098/rsos.211357

Tseng, Z. J., Grohé, C., & Flynn, J. J. (2016). A unique feeding strategy of the extinct marine mammal kolponomos: Convergence on sabretooths and sea otters. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1826). https://doi.org/10.1098/rspb.2016.0044