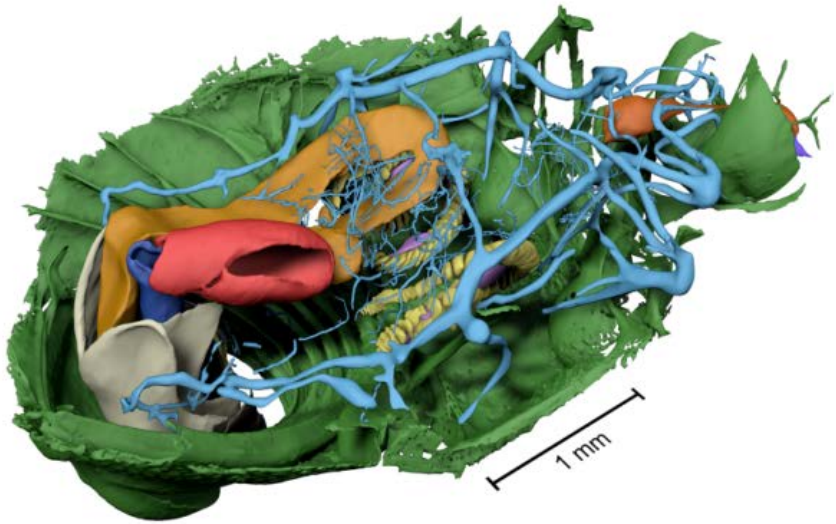
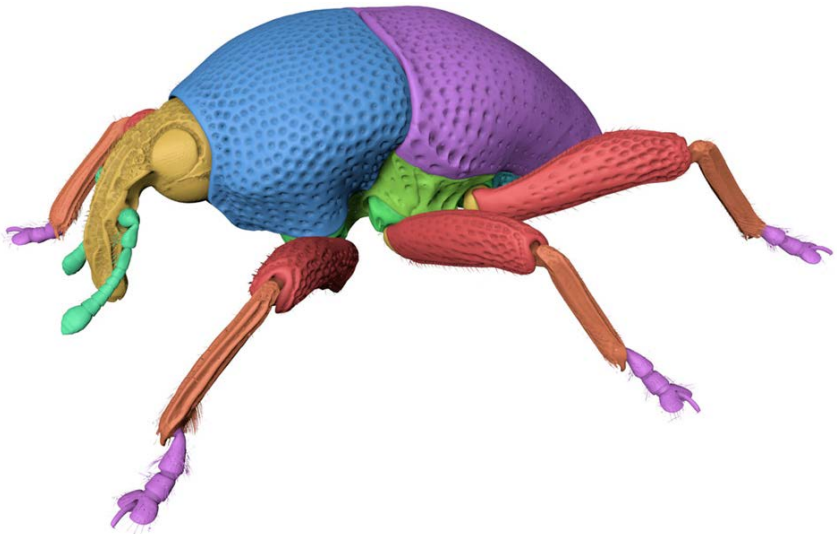
Karlsruhe Institute of Technology

Synchrotron X-ray imaging for life science and paleontology

Thomas van de Kamp

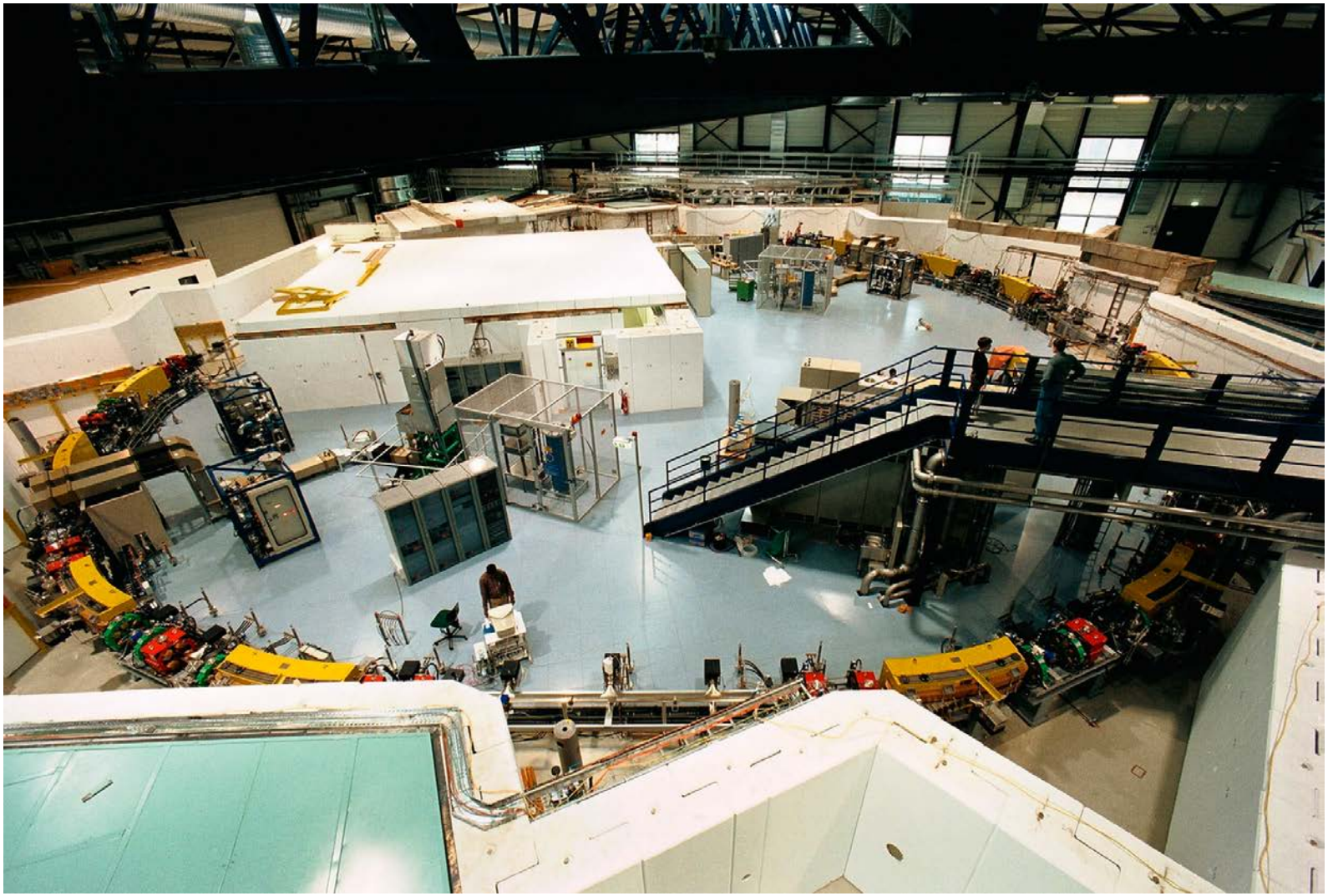
LABORATORY FOR APPLICATIONS OF SYNCHROTRON RADIATION





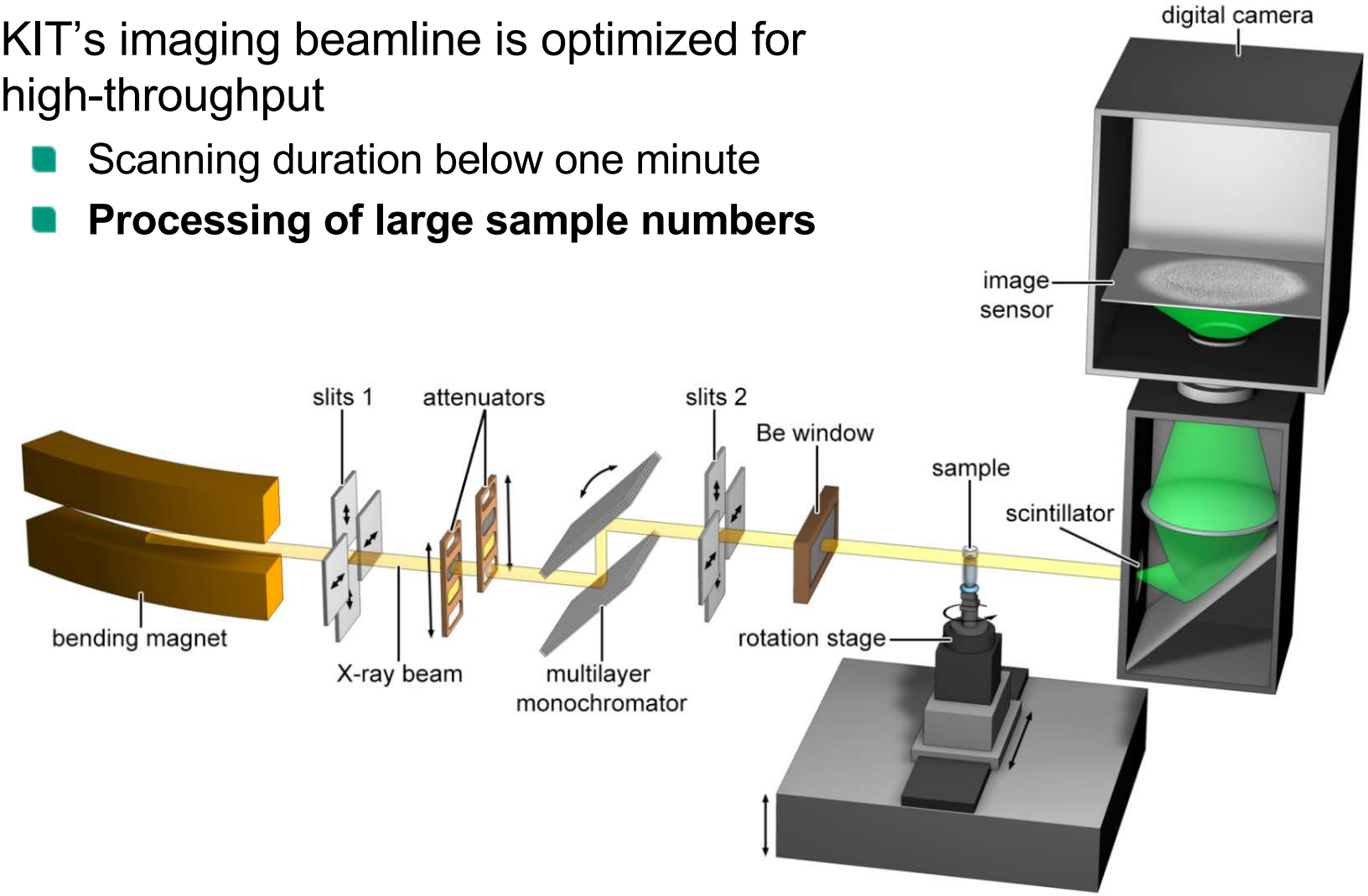
KIT light source

KIT light source



Synchrotron X-ray microtomography

- KIT's imaging beamline is optimized for high-throughput
  - Scanning duration below one minute
  - **Processing of large sample numbers**

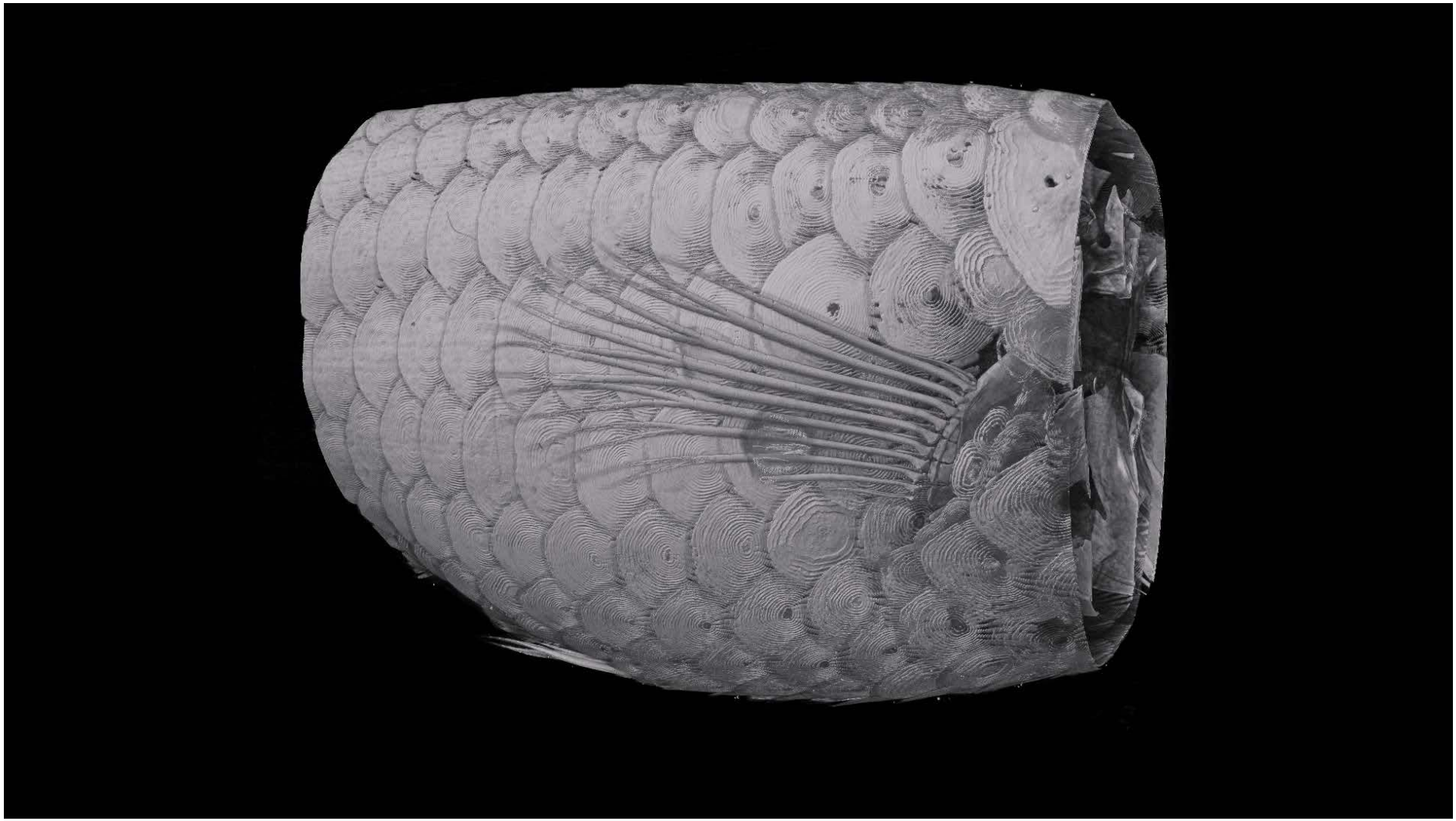


3D visualization

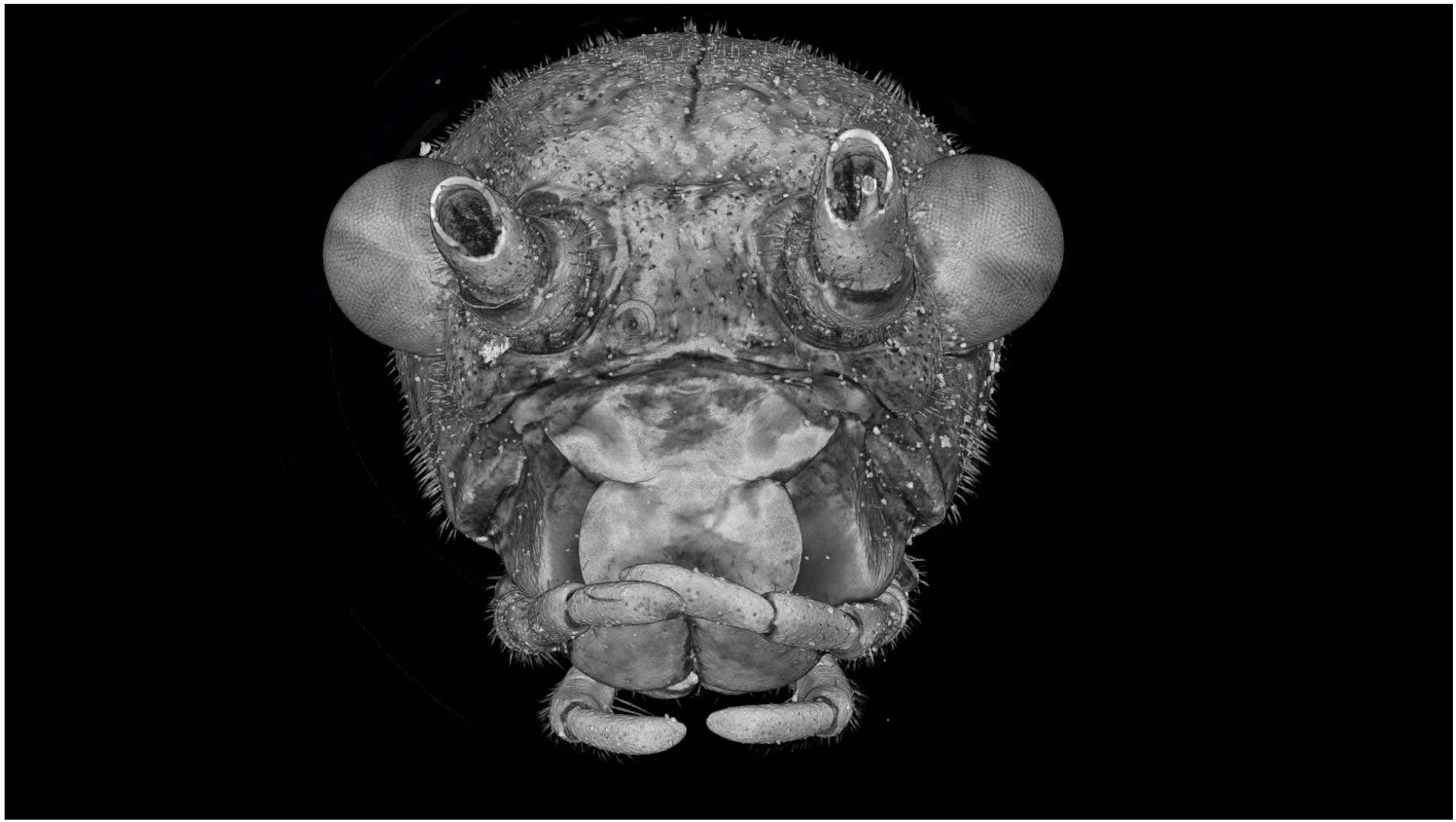
- Fast visualization based on grey levels
  - Volume rendering
  - Surface rendering
- Manual segmentation of tomograms and subsequent reconstruction of surface models

Volume rendering of a newt larva

Volume rendering of a pregnant fish

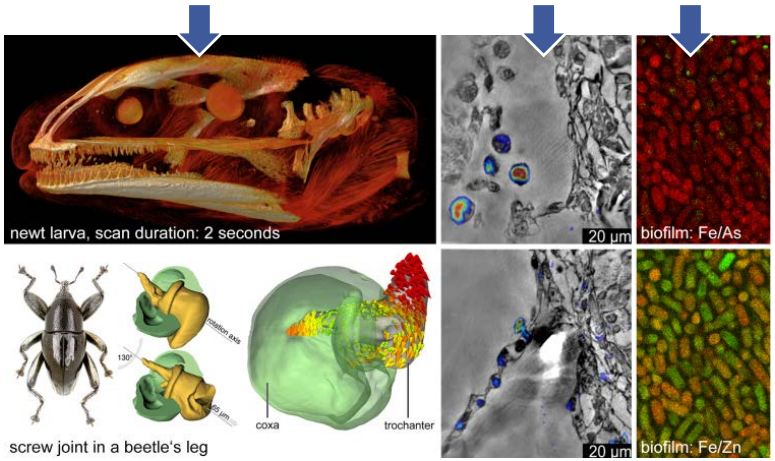


Volume rendering of a stick insect



Imaging for Life Sciences

- **Interdisciplinary team** of physicists, computer scientists and biologists
- **Methods** for hierarchical, correlated & *in vivo* imaging of organisms, organs, tissues, cells, scaffolds
  - Phenotype imaging of vertebrate model organisms
  - **Morphology and morphodynamics of arthropods**
  - Tissue engineering and biomimetics
- Morphology and function correlated to genetics and environment



- **Strategic collaborations** with universities and research institutes
- **Helmholtz** cross-program collaborations
- **International partners** from universities, research facilities and public institutions

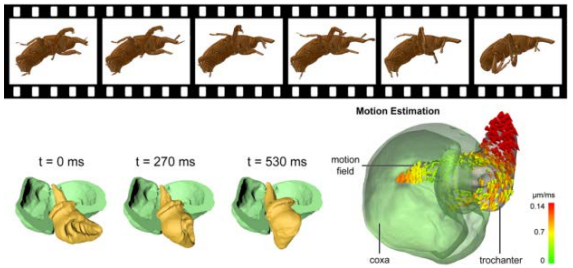
Morphology and Morphodynamics of Arthropods

- **Challenges**
  - Comparative 3D morphological analysis
  - Morphodynamics of exoskeletons
  - Combined genetics and morphology
- **Application Fields**
  - Zoology, Paleontology, Biomimetics, Environment
- **Method Development**
  - High-throughput image acquisition & reconstruction
  - Ultrafast cine-tomography
  - Remote data analysis infrastructure ASTOR/NOVA for global community

Interactive 3D reconstruction of a *Trigonopterus* weevil

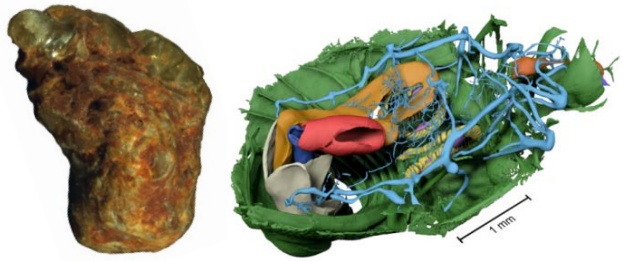
van de Kamp et al. *PLoS ONE* **9**, e102355 (2014)

***In vivo* 4D cine-tomography of a living weevil**



dos Santos Rolo et al. *PNAS* **111**, 3921 (2014)

Reconstruction of the anatomy of a 30 million-year-old beetle



Schwermann et al. *eLife* **5**, e12129 (2016)

- **Numerous collaborations with the global arthropod community**

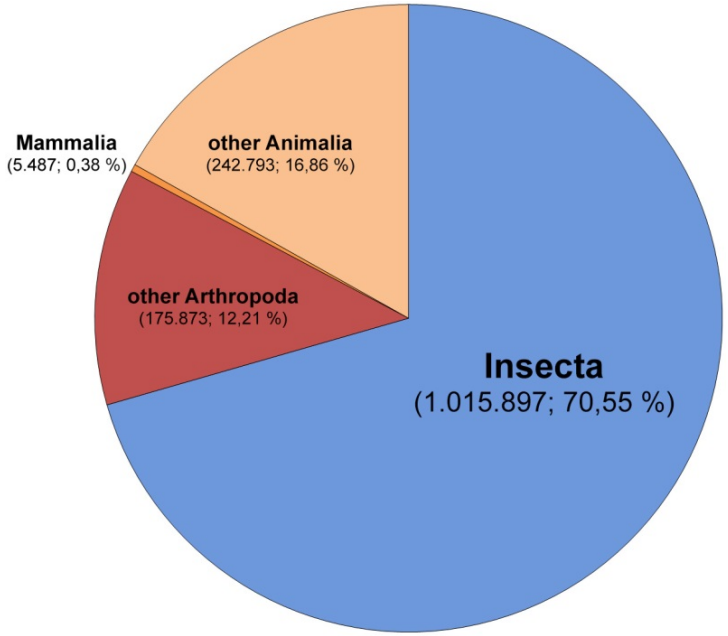
Insects

- About **70%** of all described animals are insects

- **Key functions in our ecosystems**
  - pollinators
  - decomposers of organic material
  - regulatory functions as predators and parasites
  - basis of complex food webs

    →Huge impact on economy

- **Rapid insect decline**
  - Consequences for ecosystems and economy still unclear

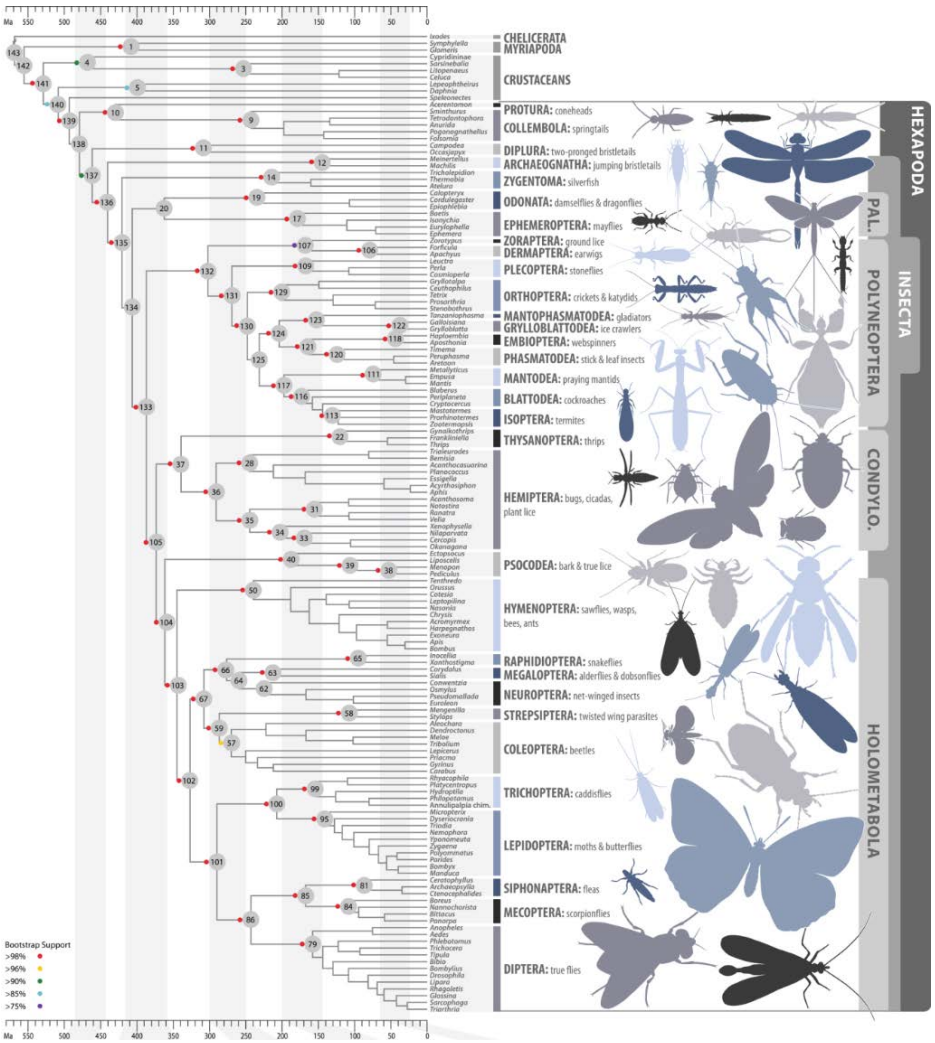


Insects

- The relative wealth of **genetic data** collected in recent years is hardly matched by comparative **3D morphological information**

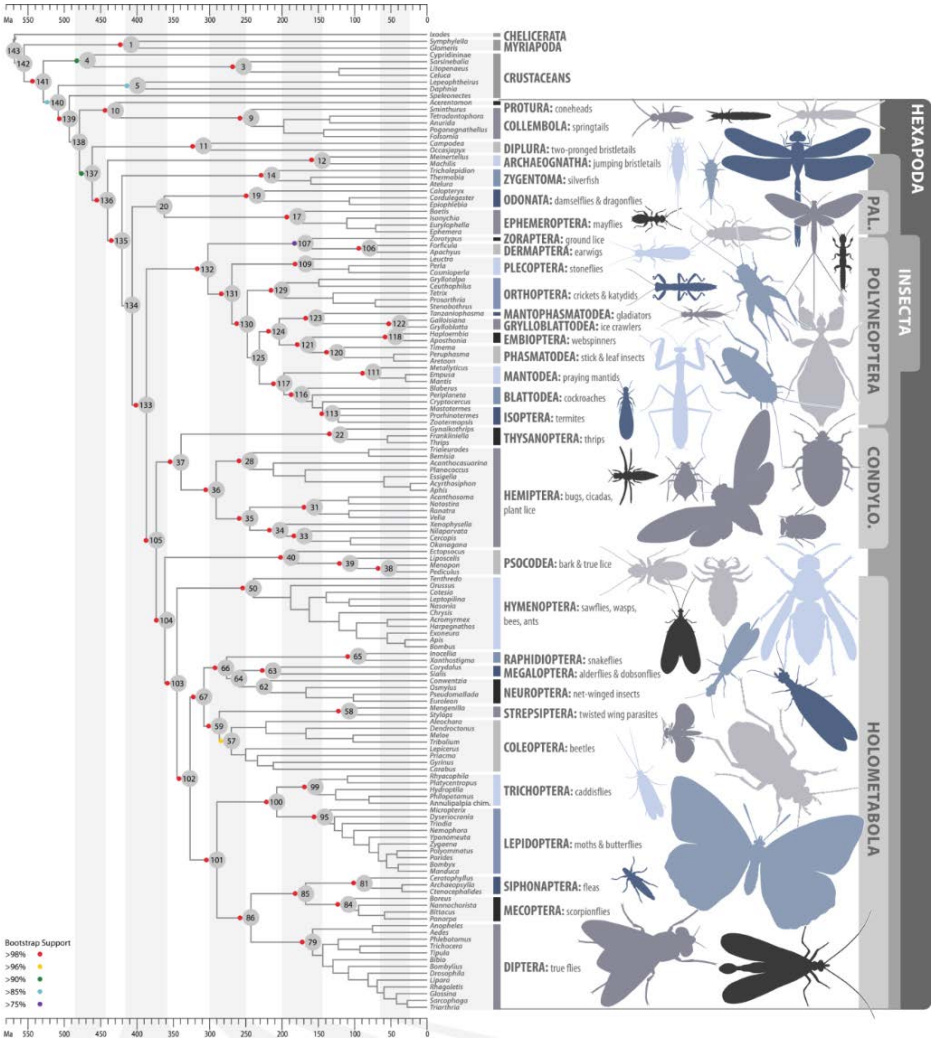
- **Quantitative collection** of the morphological information and its **combination with environmental and genetic data** would pave the way for a deeper understanding of insect biology

Misof et al. 2014: Phylogenomics resolves the timing and pattern of insect evolution. *Science* 346: 763-767.



Outline

- Insect imaging
- *In vivo* imaging
- Biomimetics
- High-throughput imaging
- Imaging of fossil insects
- Perspectives



Insect imaging

Weevils

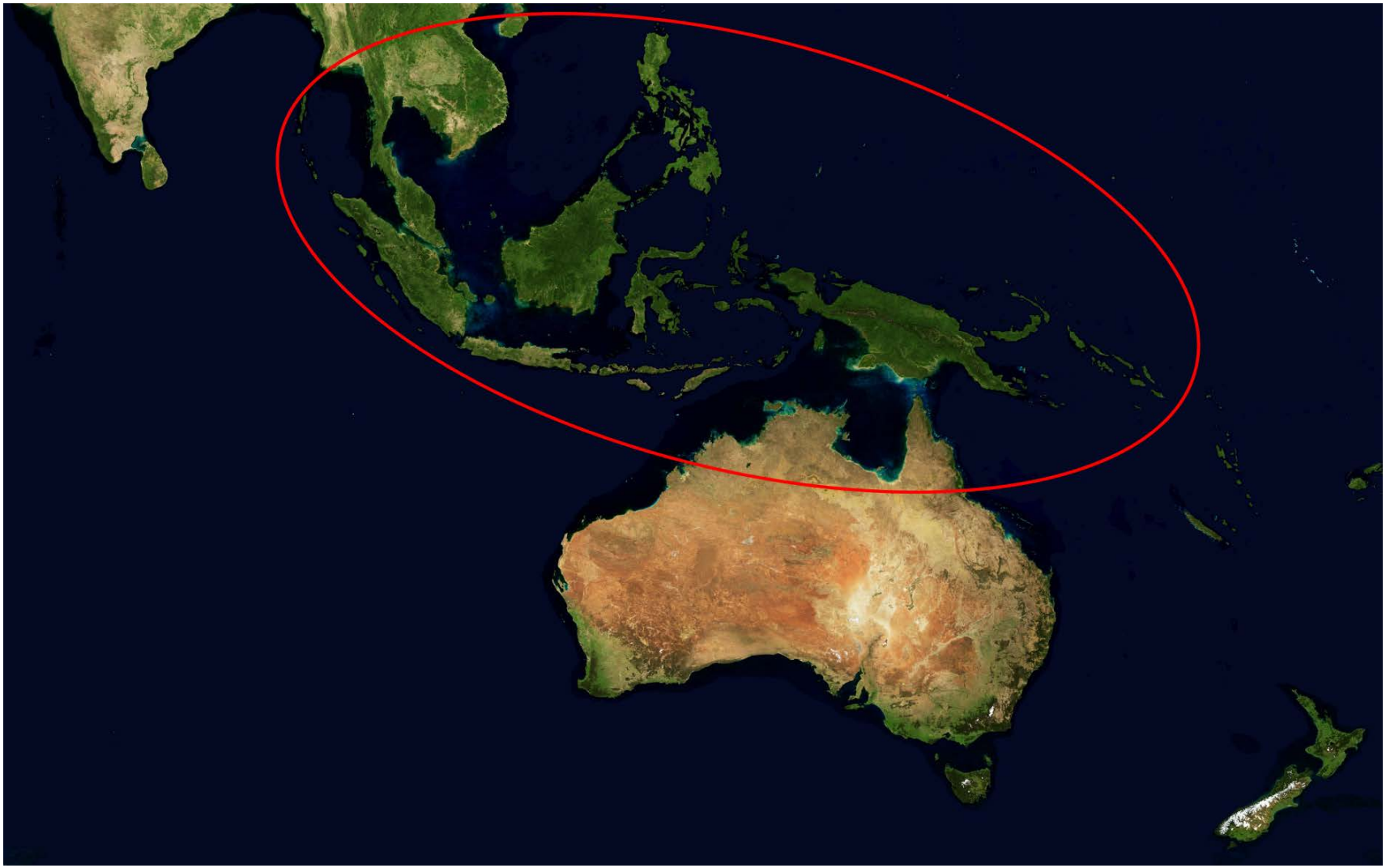
- „Snout beetles“
- ca. 62.000 species described
- Most species small and cryptic

Trigonopterus

- Flightless weevils from the Indo-Pacific
- Exoskeleton extremely robust
- Mechanical blocking of the legs during death-feigning

© Alexander Riedel

Distribution

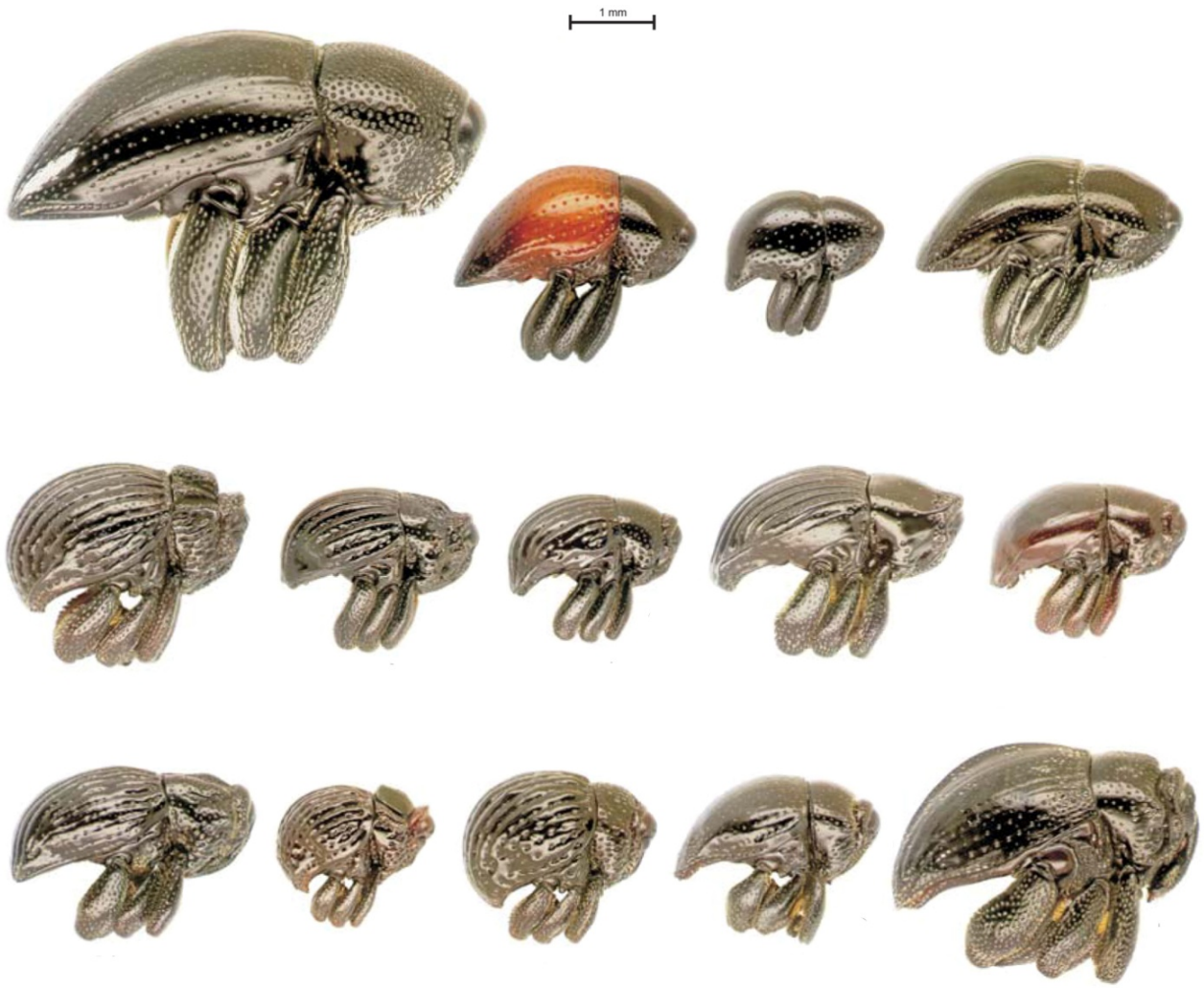


Collecting

Thanatosis in *Trigonopterus*

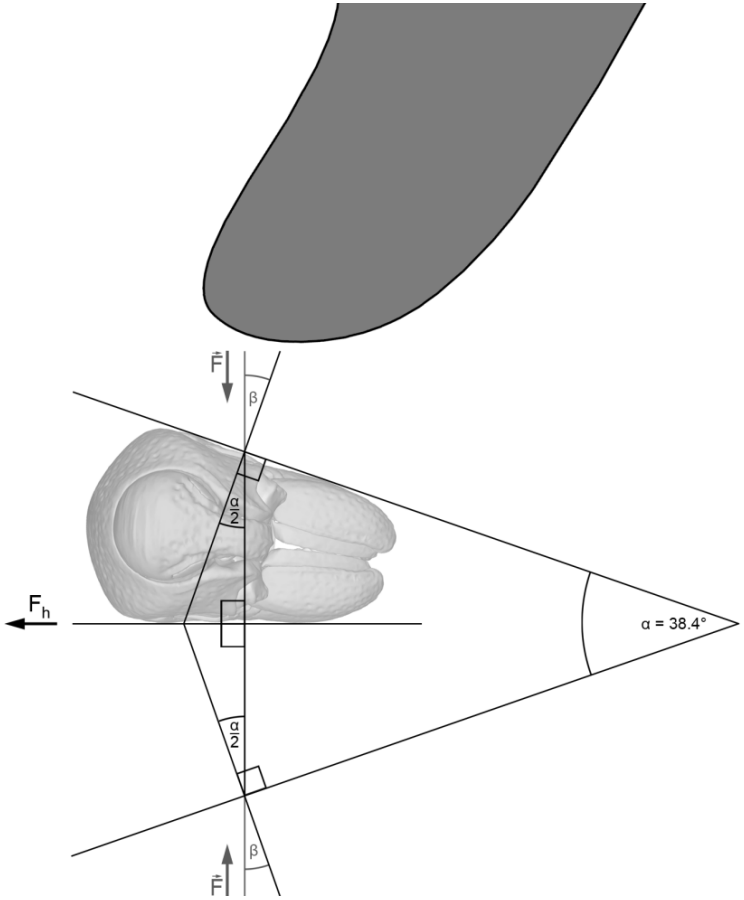
- Thanatois: „death-feigning“ triggered by an external stimulus
- Defensive behavior
- „Drop-off“ reflex
- Legs are folded under the ventral body
- Motionless for several minutes
- **In many species: mechanical blocking of the legs!**

Thanatosis in *Trigonopterus*

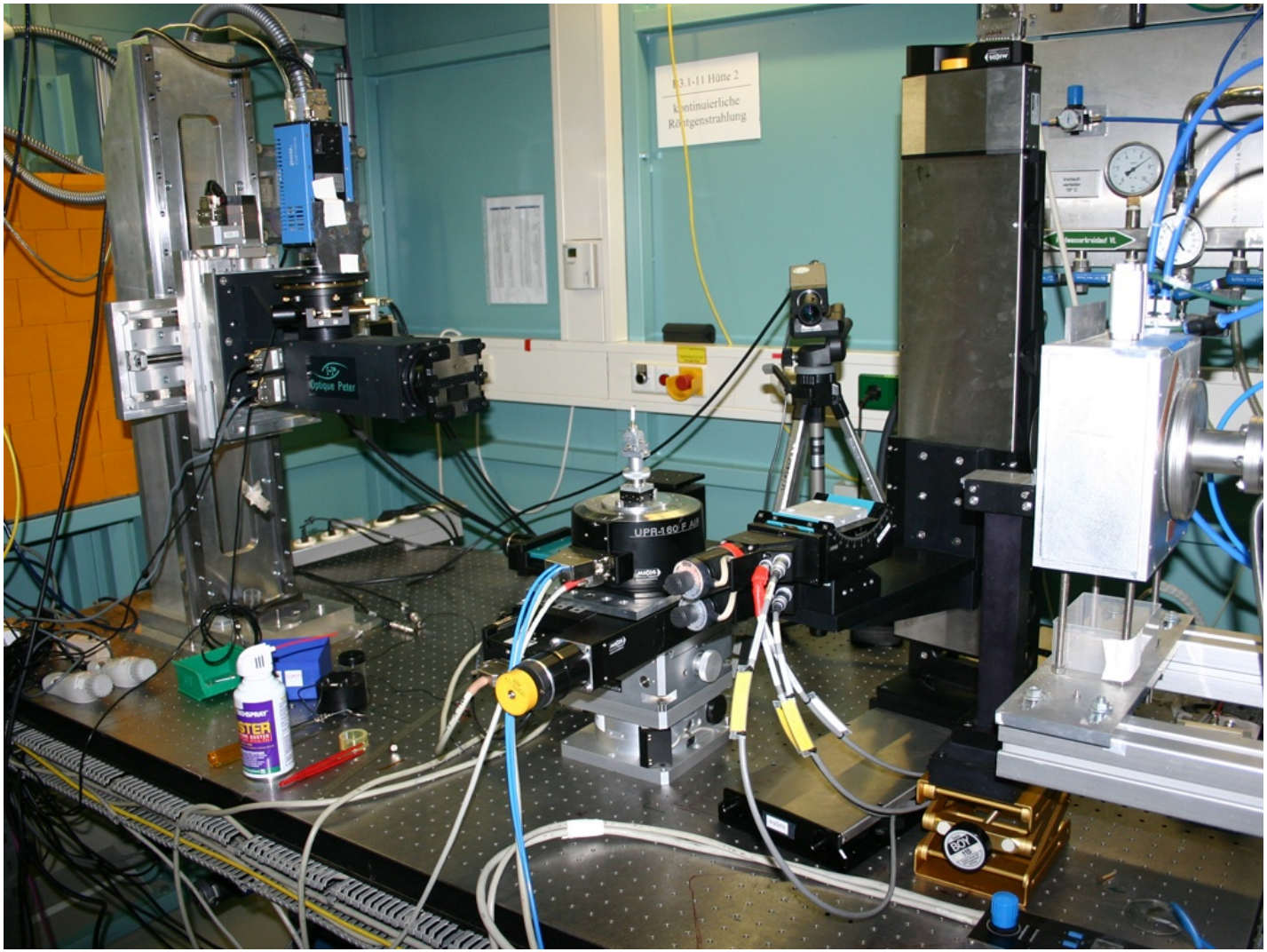


Problems

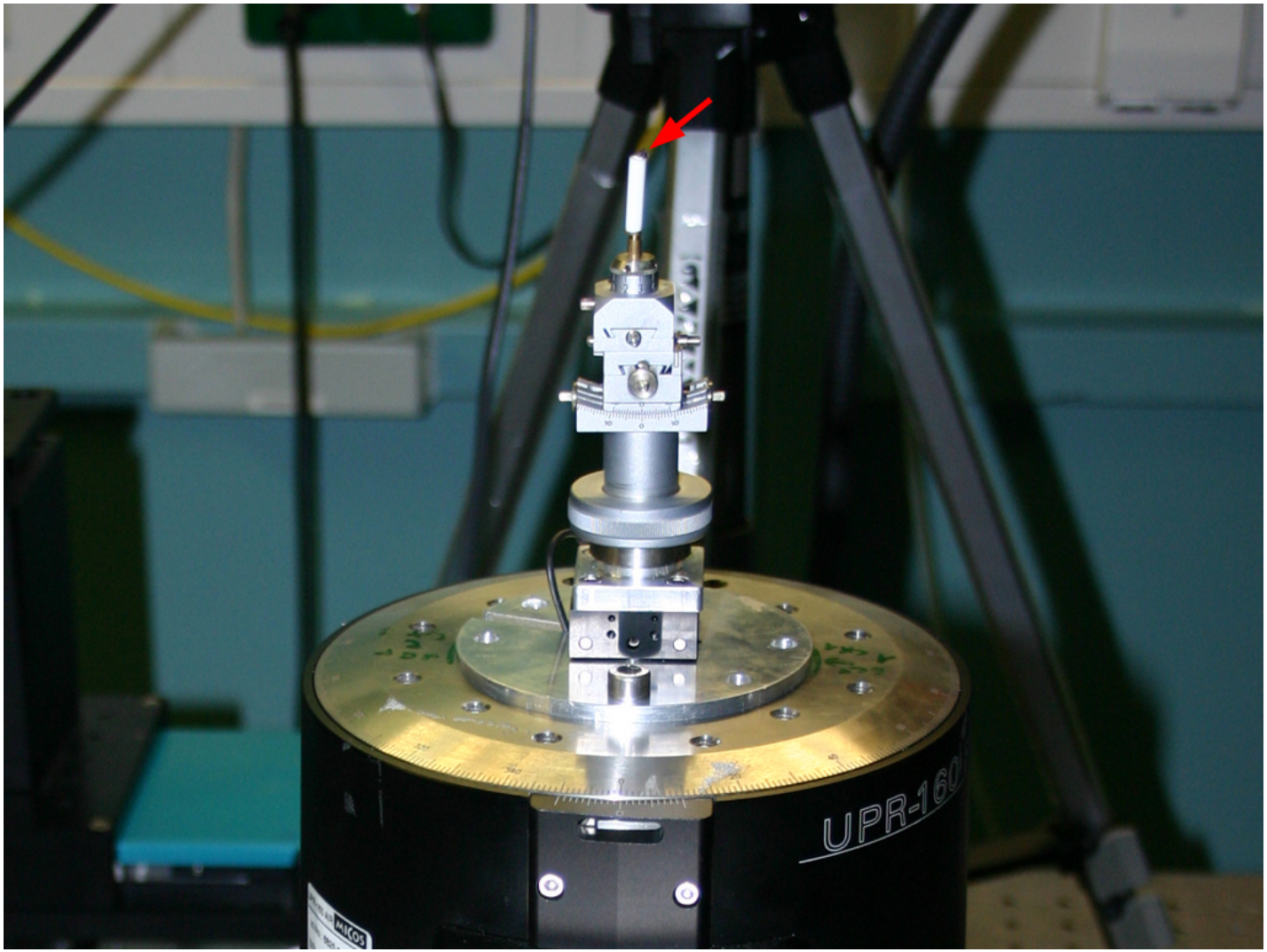
- Legs blocked also in dead specimens
- Beetles easily slip away due to their smooth body surface
- Examination by traditional morphological methods (manual dissection, microscopy) extremely difficult



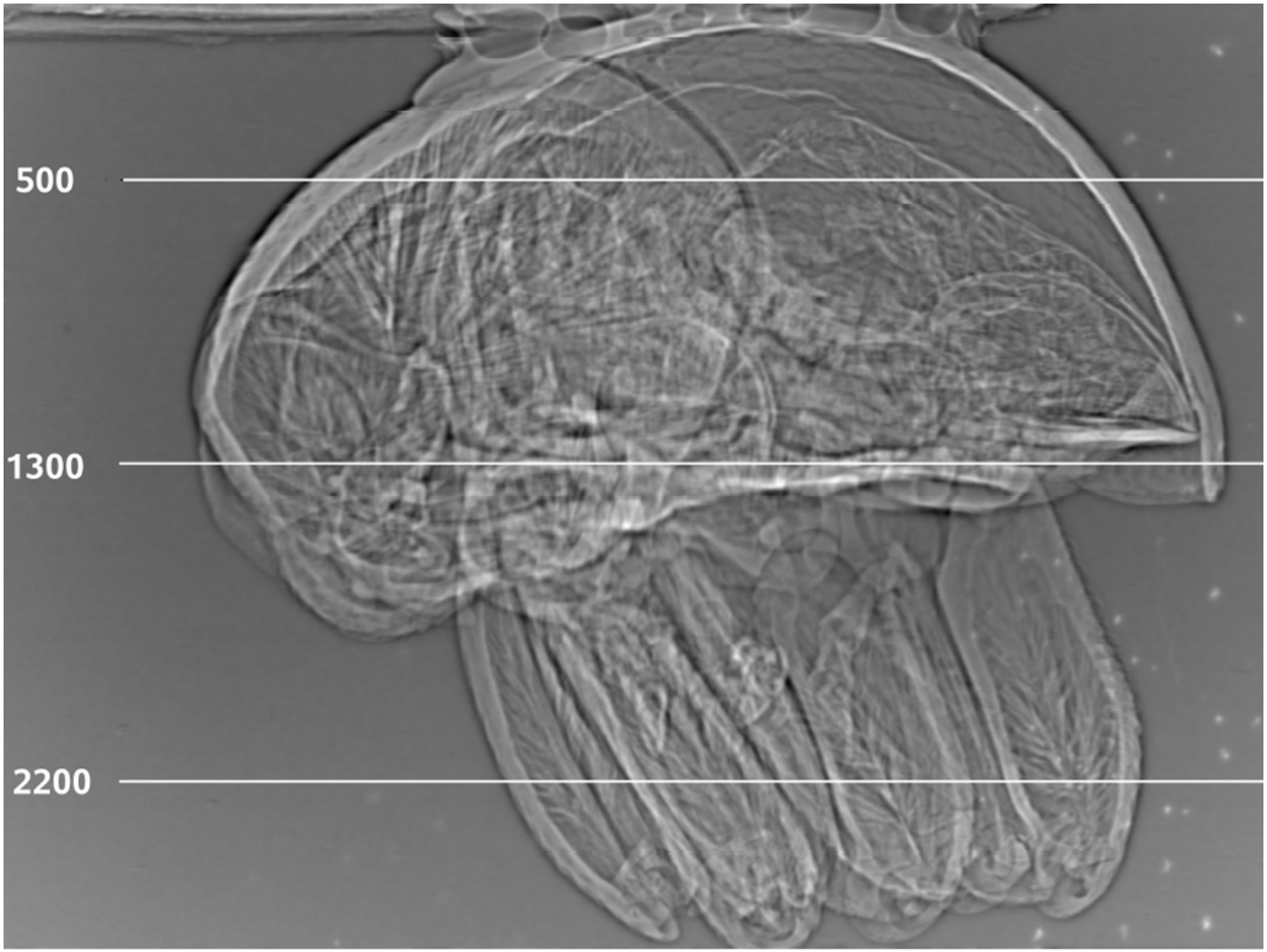
Tomography



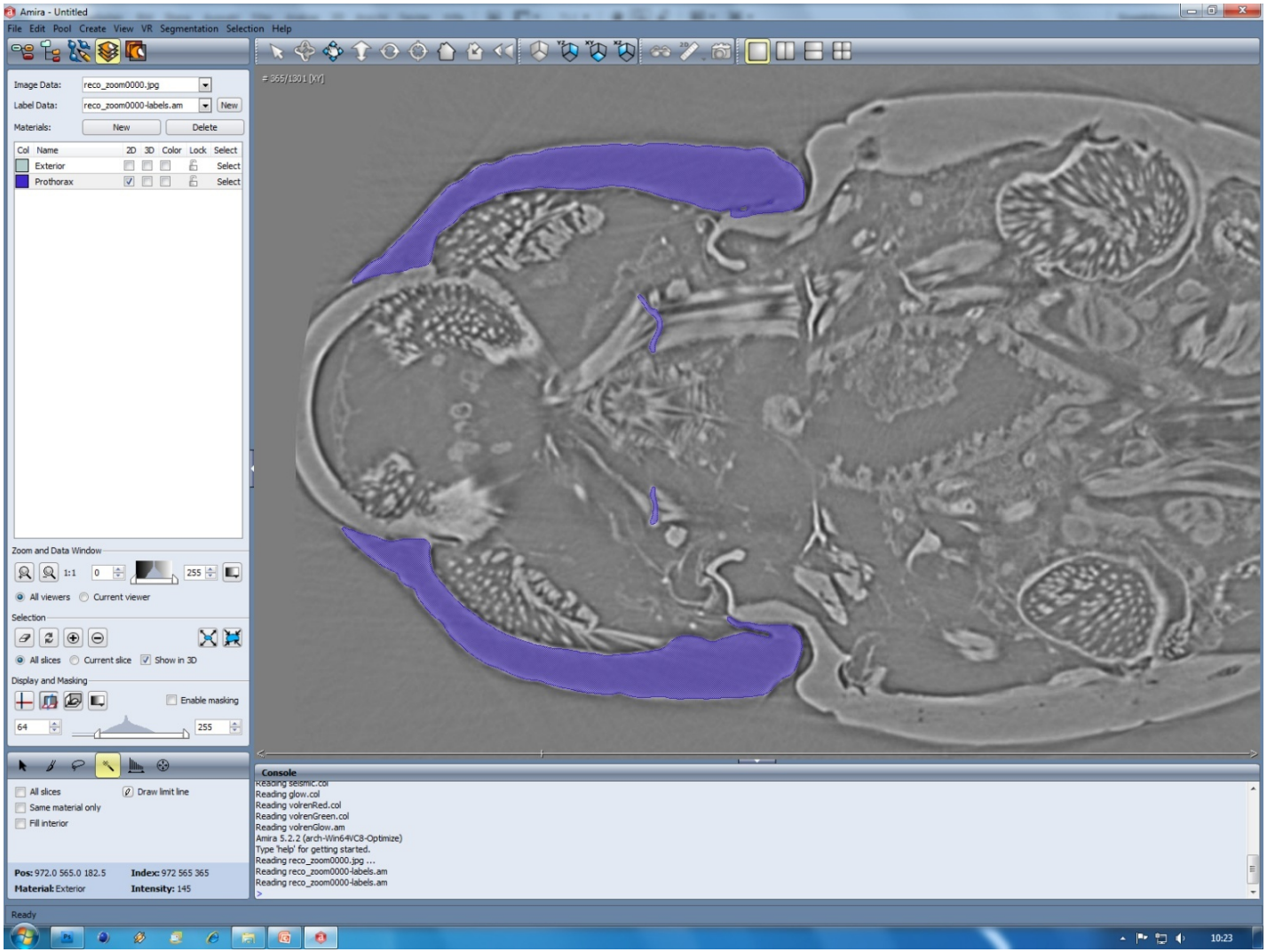
Tomography



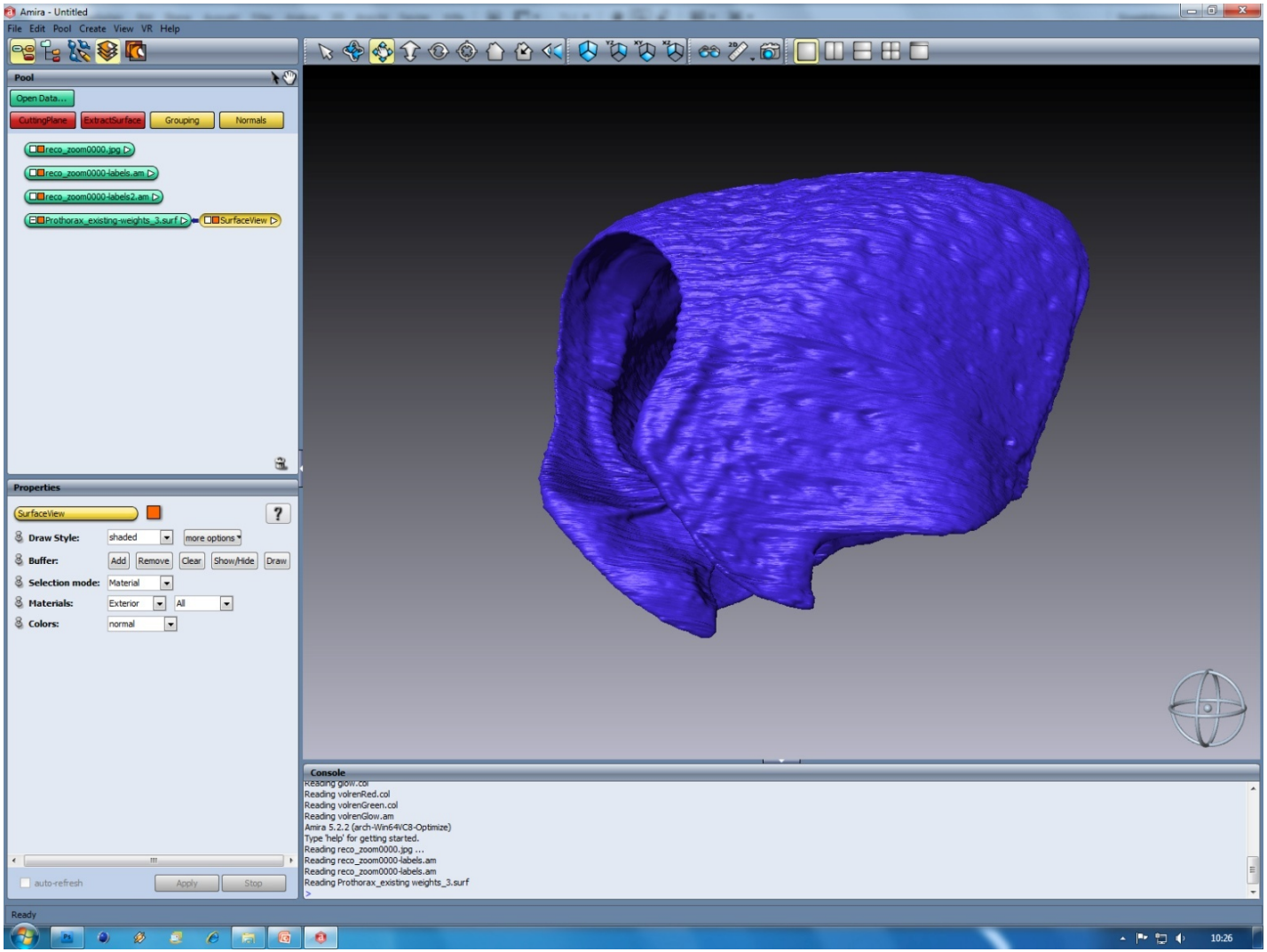
Tomography



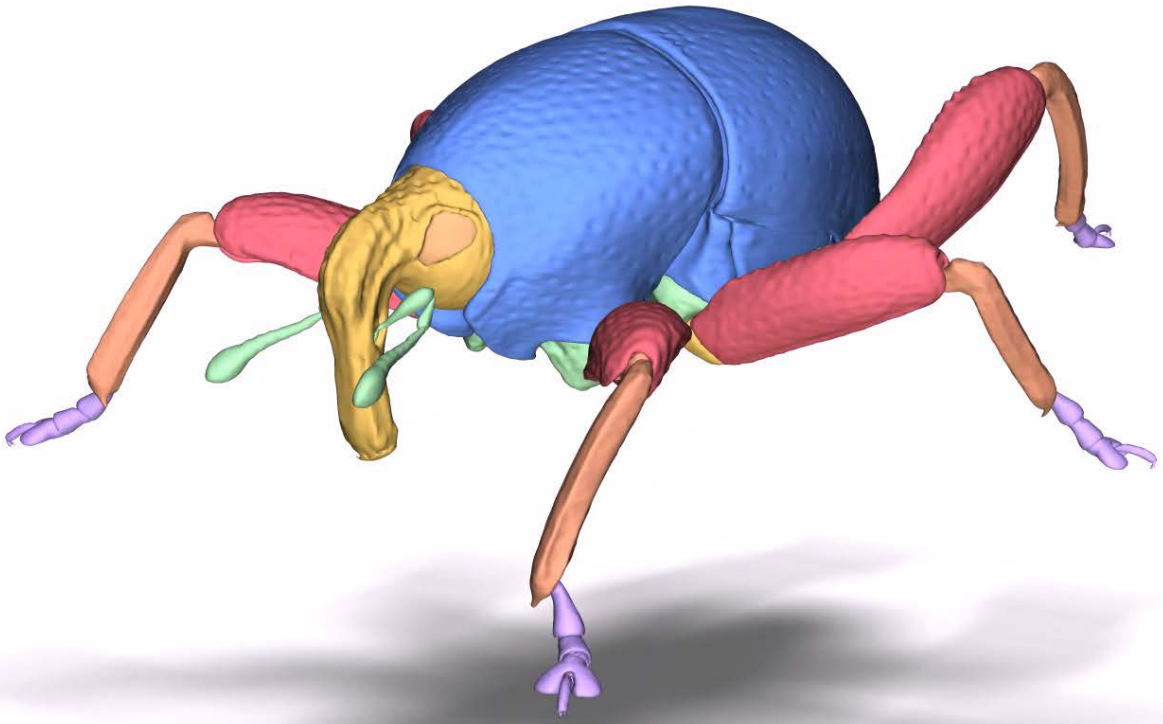
Segmentation



Surface meshes

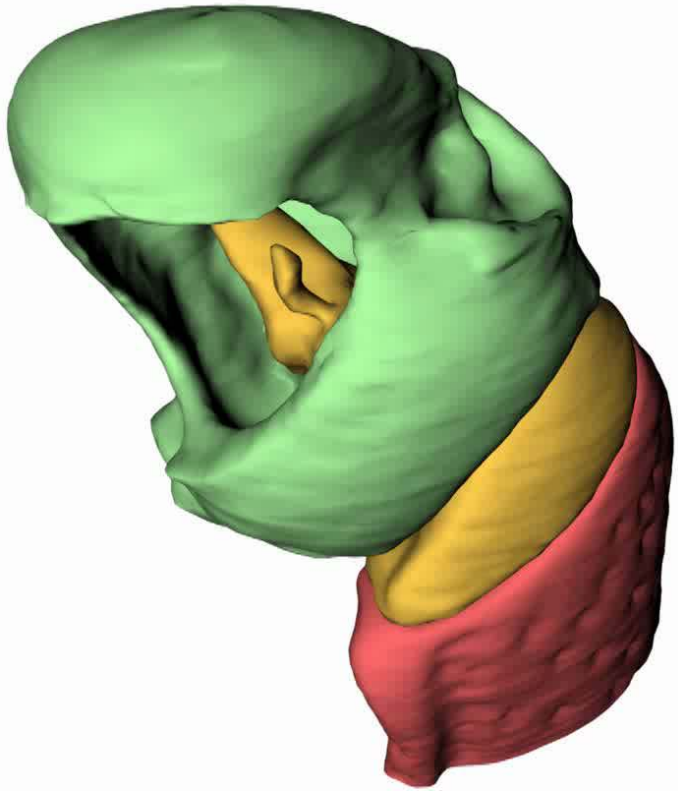


Interactive 3D models



van de Kamp et al. 2014: *PLoS ONE* 9: e102355

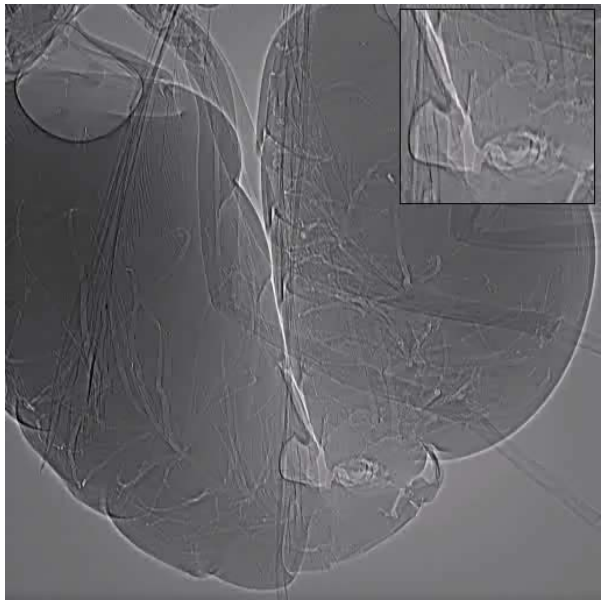
Biological screw joint

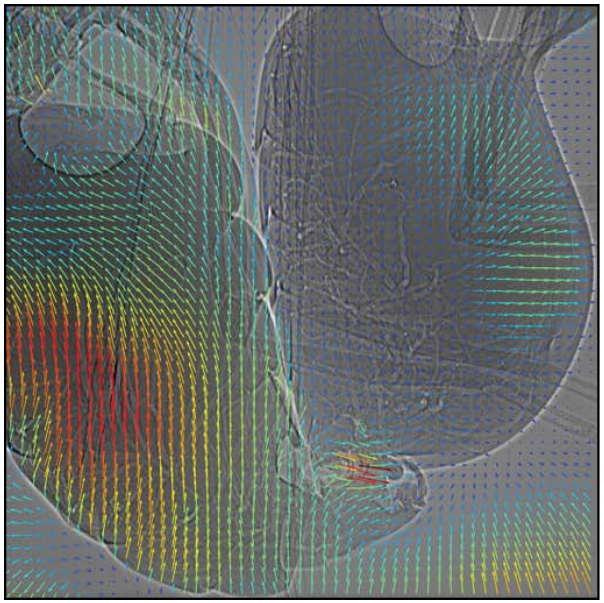


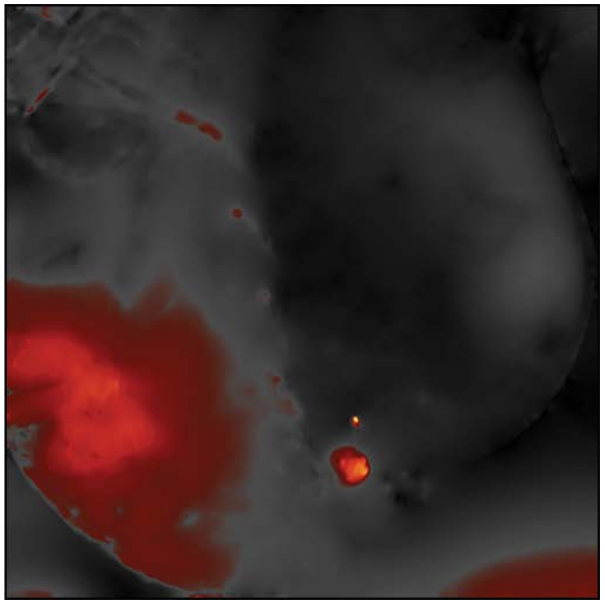
van de Kamp et al. 2011: *Science* 333: 52

In vivo imaging

Radiographic sequences







- Time information about functional dynamics
- No information about the third spatial dimension!

In vivo X-ray 4D cine-tomography

- Ultra-fast tomography
- Advanced image analysis
- Optimized setup
  - high spatial resolution
  - high temporal resolution
- **4D tomography of living samples**

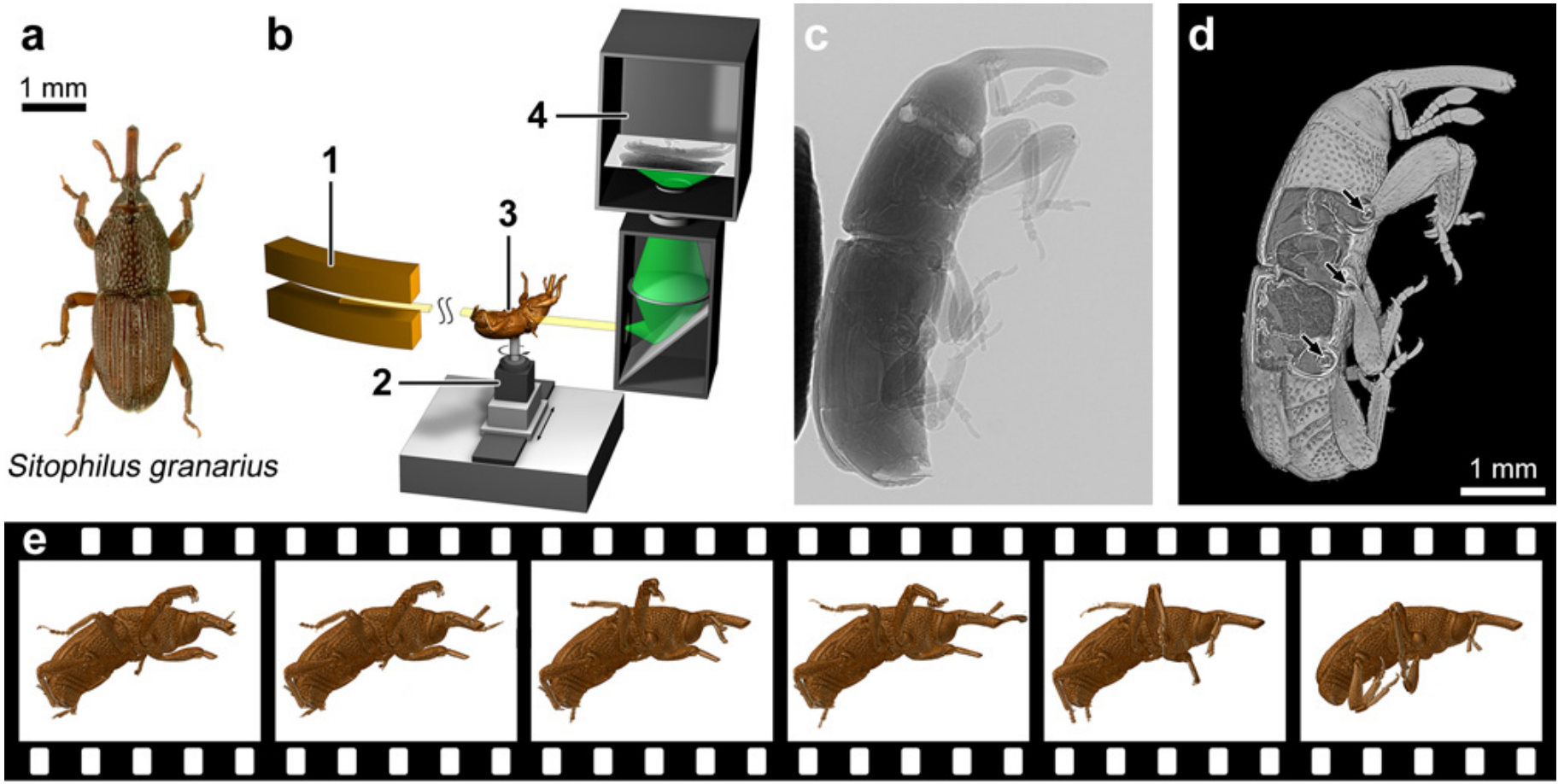
In vivo X-ray 4D cine-tomography

- TOPO-TOMO (1.5 T bm beamline; 2.5 GeV storage ring)
- White beam with maximum flux density at 14.5 keV
- Filter: 0.2 mm Al

- Overview scan
  - 20 tomograms/s
  - 250 proj./tomogram
  - 6.6 µm pixel size

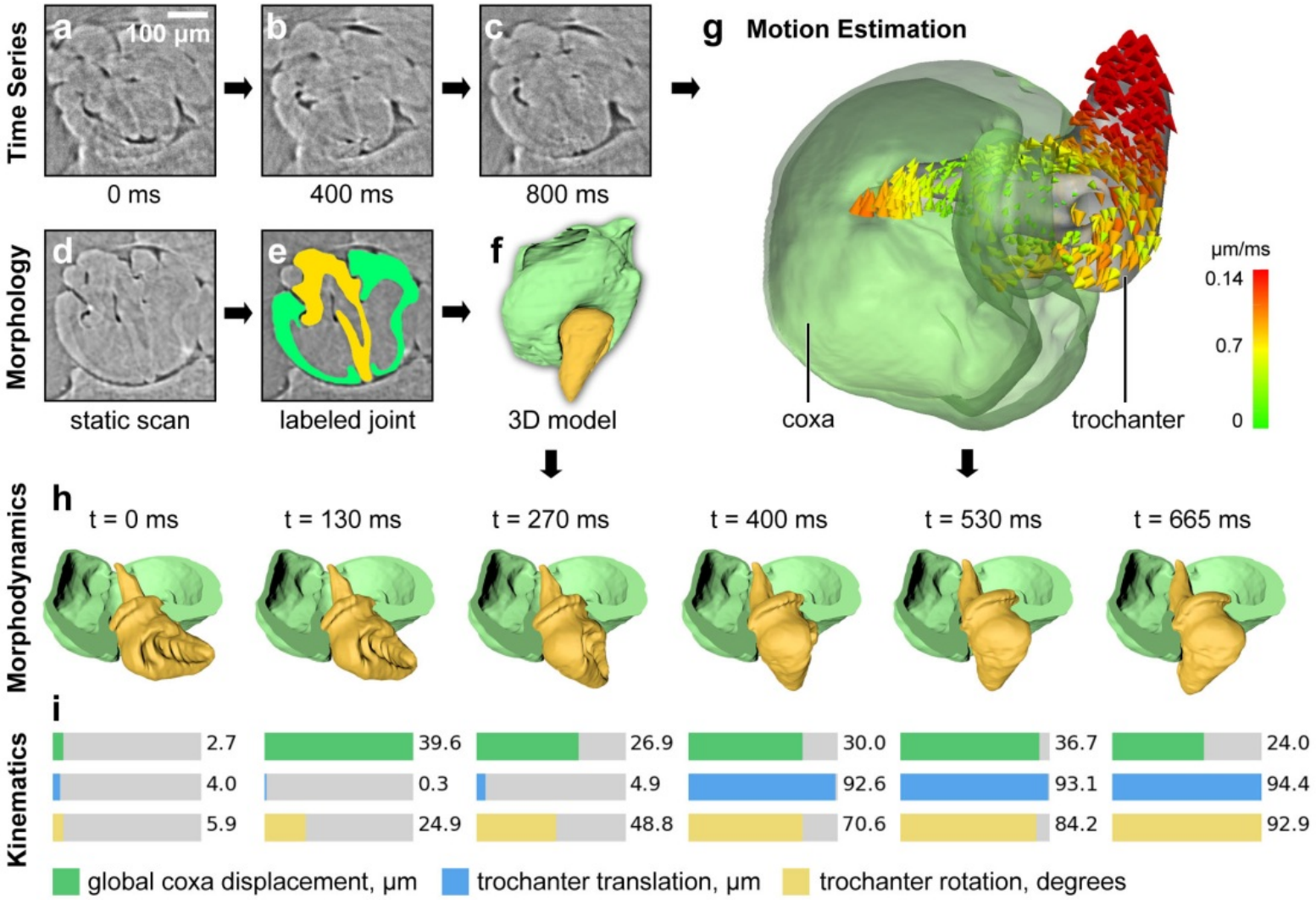
- Detail scan
  - 7.5 tomograms/s
  - 200 proj./tomogram
  - 1.22 µm pixel size

In vivo X-ray 4D cine-tomography



dos Santos Rolo, T., Ershov, A., van de Kamp, T. & Baumbach, T. 2014: *PNAS* 111(11): 3921-3926

In vivo X-ray 4D cine-tomography



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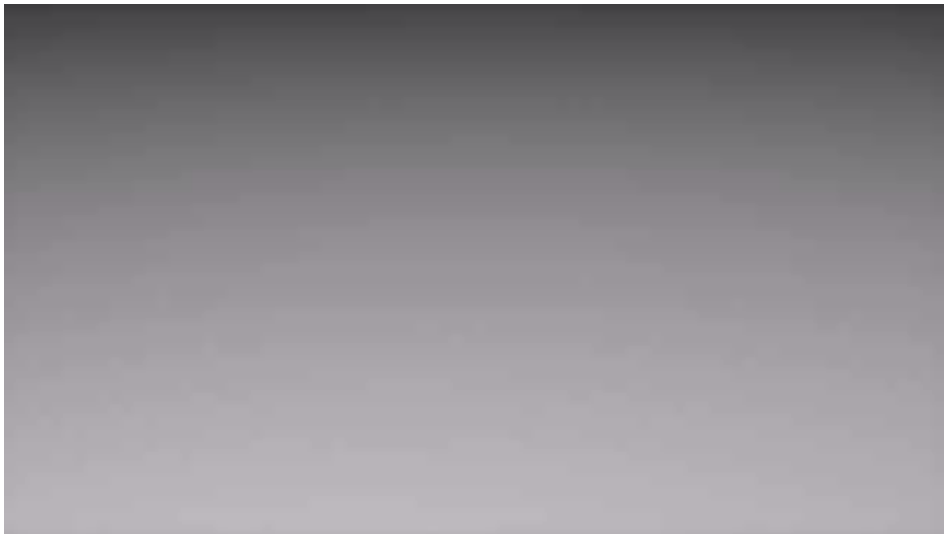
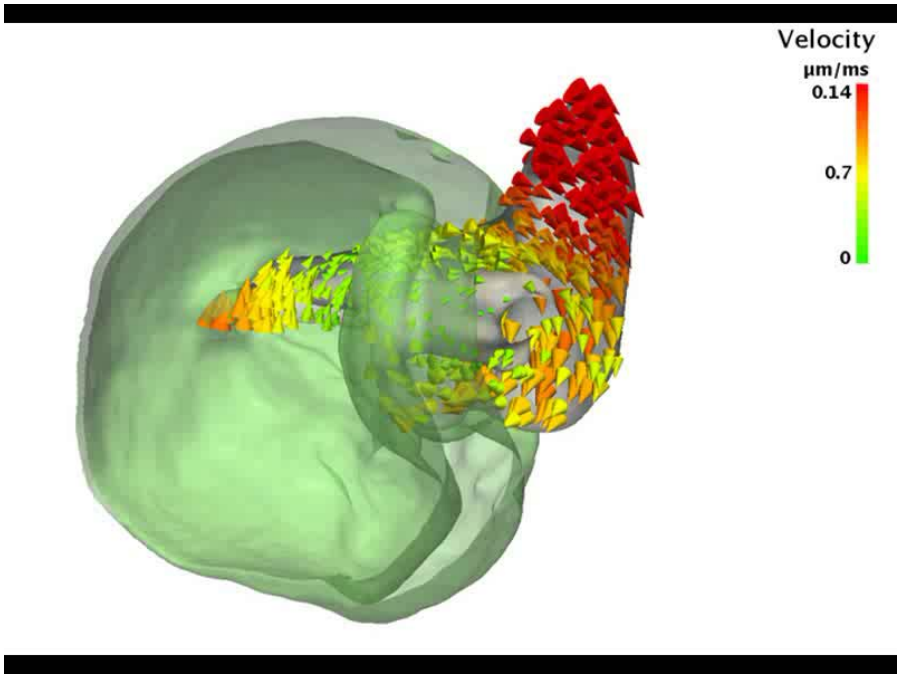
In vivo X-ray 4D cine-tomography





dos Santos Rolo, T., Ershov, A., van de Kamp, T. & Baumbach, T. 2014: *PNAS* 111(11): 3921-3926

Biomimetics

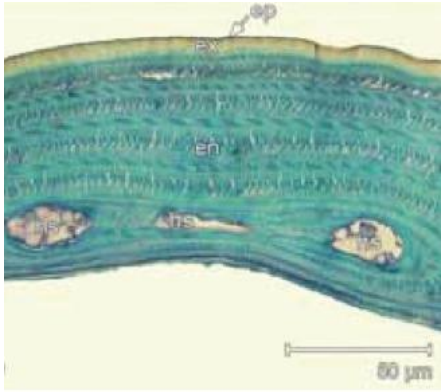
Research Pavilions

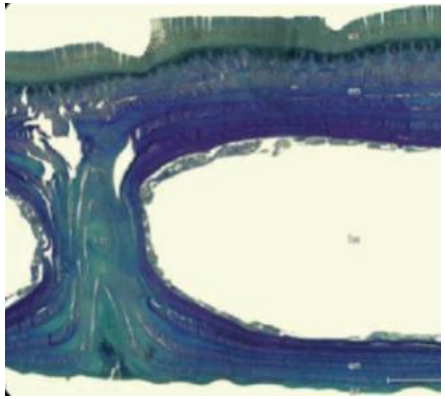
- Structures based on construction principles from nature
- Interdisciplinary collaboration
- University of Stuttgart
  - Institute for Computational Design (ICD)
  - Institute of Building Structures and Structural Design (ITKE)
- University of Tübingen
- Karlsruhe Institute of Technology
- etc.

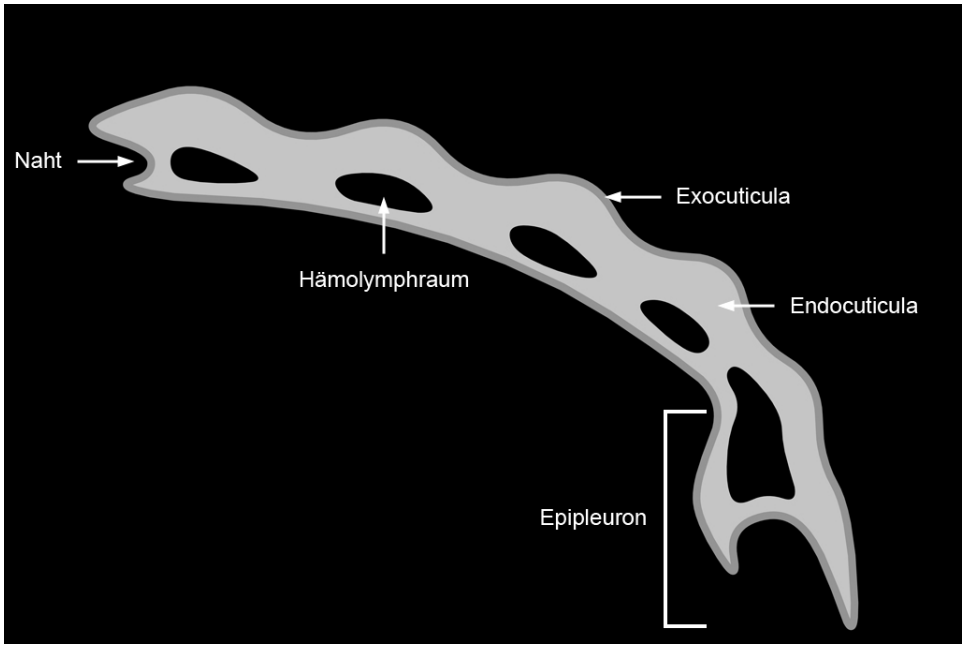
2013/2014: Example beetle elytra

- Lightweight construction
- Arthropod cuticle: Fiber composite of chitin microfibrils in a protein matrix
- Top and bottom sides are connected via columns (trabeculae)

2013/2014: Example beetle elytra



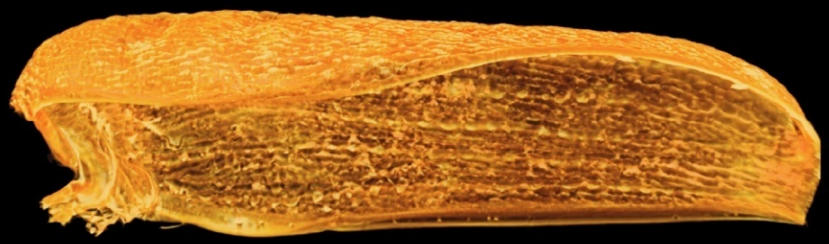




Approach

- Analysis
  - 3D data sets → fundamental construction principles
  - Light and electron microscopic investigations
- Computerized transfer
- Robotic construction

Tomographic volume



Valgus hemipterus

Cassida viridis

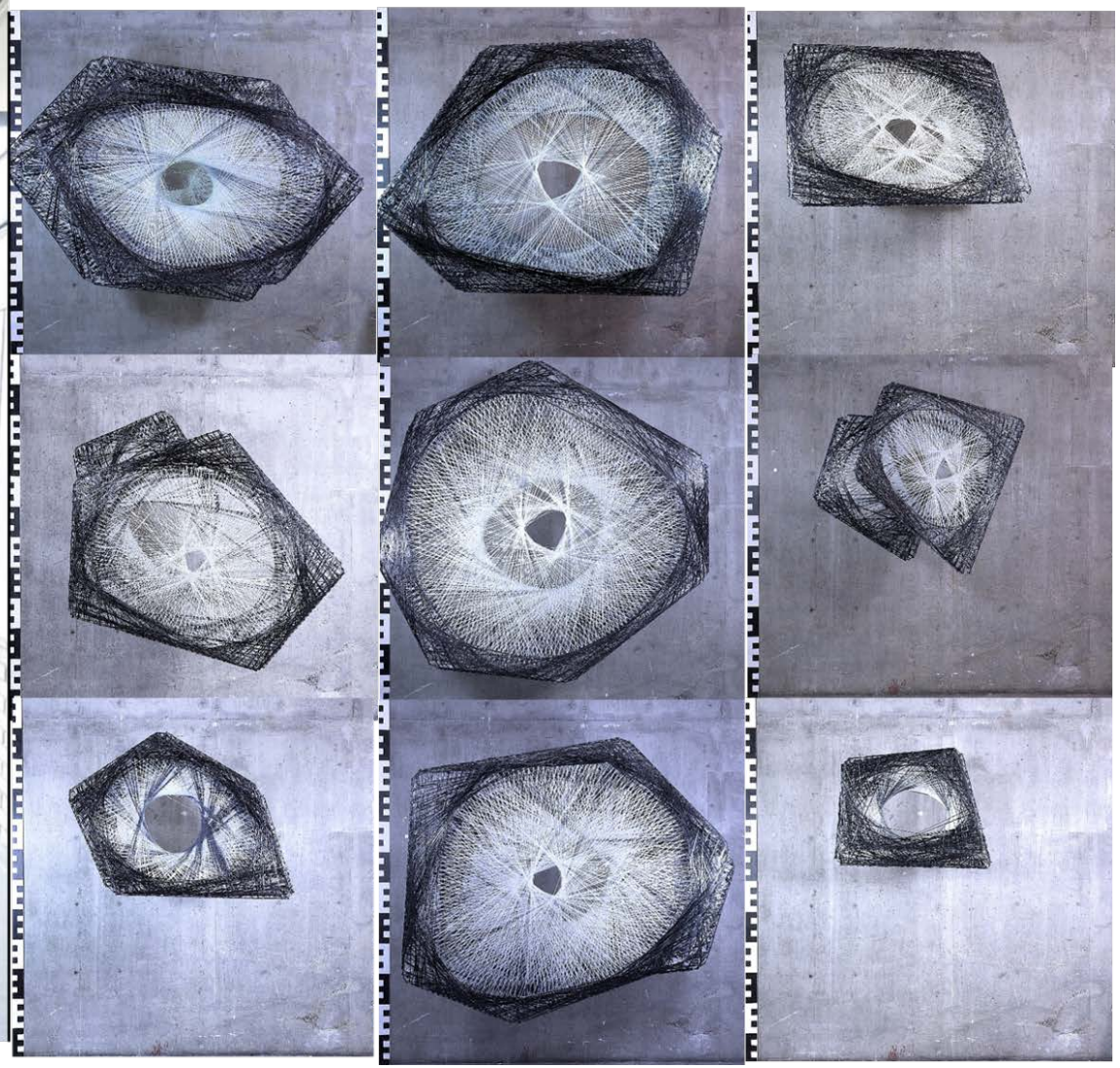
Hoplia argentea

Leptinotarsa decemlineata

Lilioceris lilii

Chrysomela vigintipunctata

Elements



High throughput imaging

High-throughput 3D Digitization of Insects

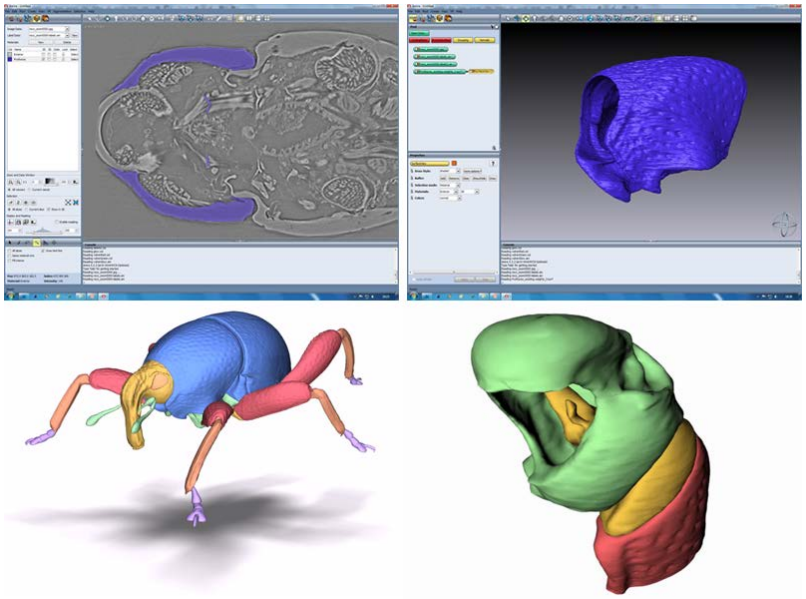
- Over **one billion specimens** in scientific collections
- State-of-the-art digital 3D imaging techniques facilitate **entirely new insights**
- **Unique possibilities** due to large beam at KIT
- **Huge potential for scientific discoveries**
  - morphology, biomimetics, paleontology, **biodiversity**
- **Challenges**
  - Huge amount of data
  - Data analysis (e.g. segmentation) is still very time-consuming
  - → **Automatize as much as possible!**

ASTOR & NOVA

- Virtual analysis infrastructure for tomographic data @ KIT & DESY
- Remote access for external users
- Development of segmentation tools
- Joint analysis of selected datasets by different research groups

Semi-automated segmentation

- Traditional approach: manual segmention of several slices and interpolation
- Interpolation prone to errors
- Correction is time consuming; results show conspicuous artifacts



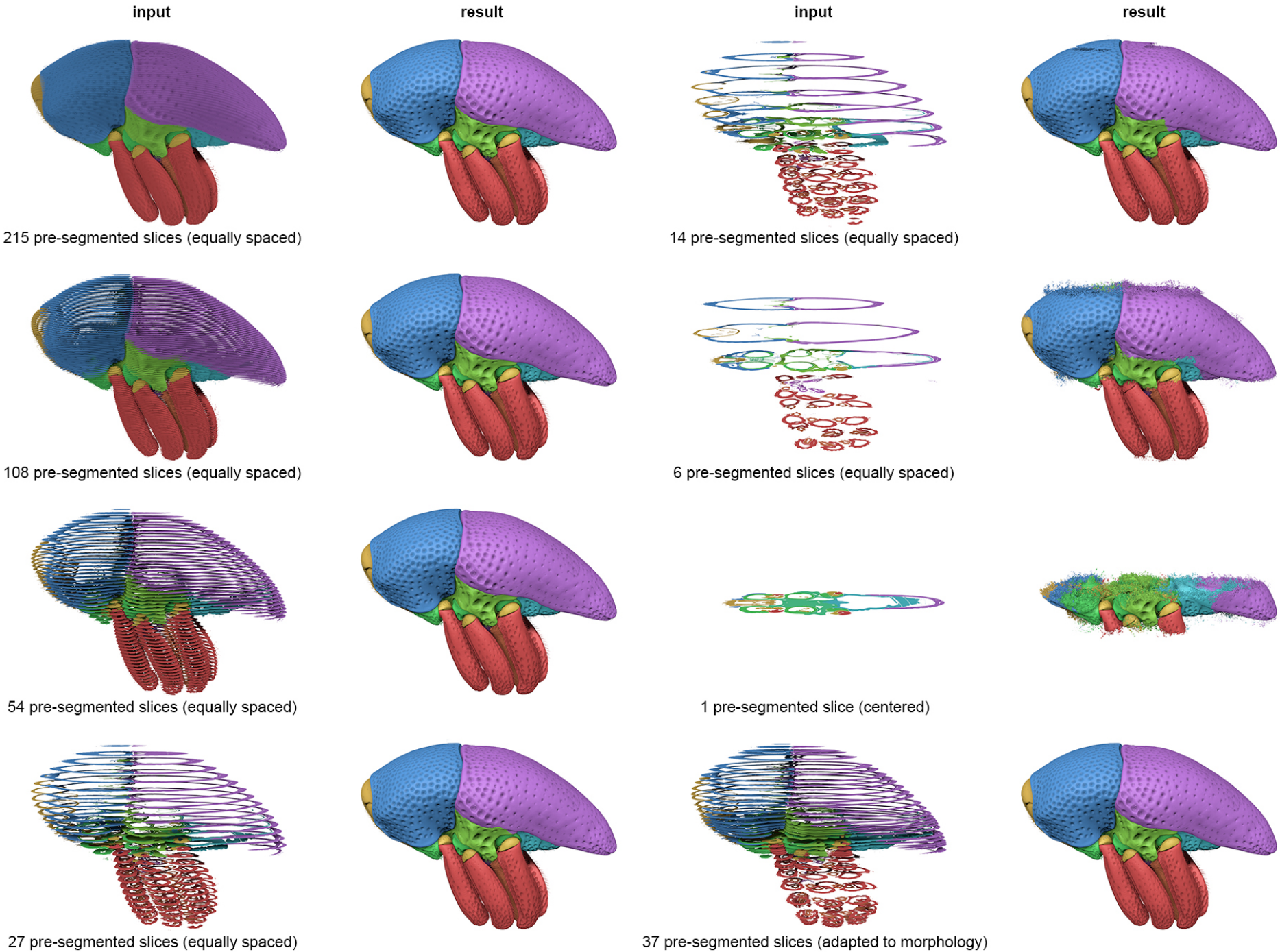
Processing time: months

- **BIOMEDISA algorithms developed in ASTOR & NOVA by Philipp Lösel (Uni Heidelberg)**
  - much faster
  - more accurate results

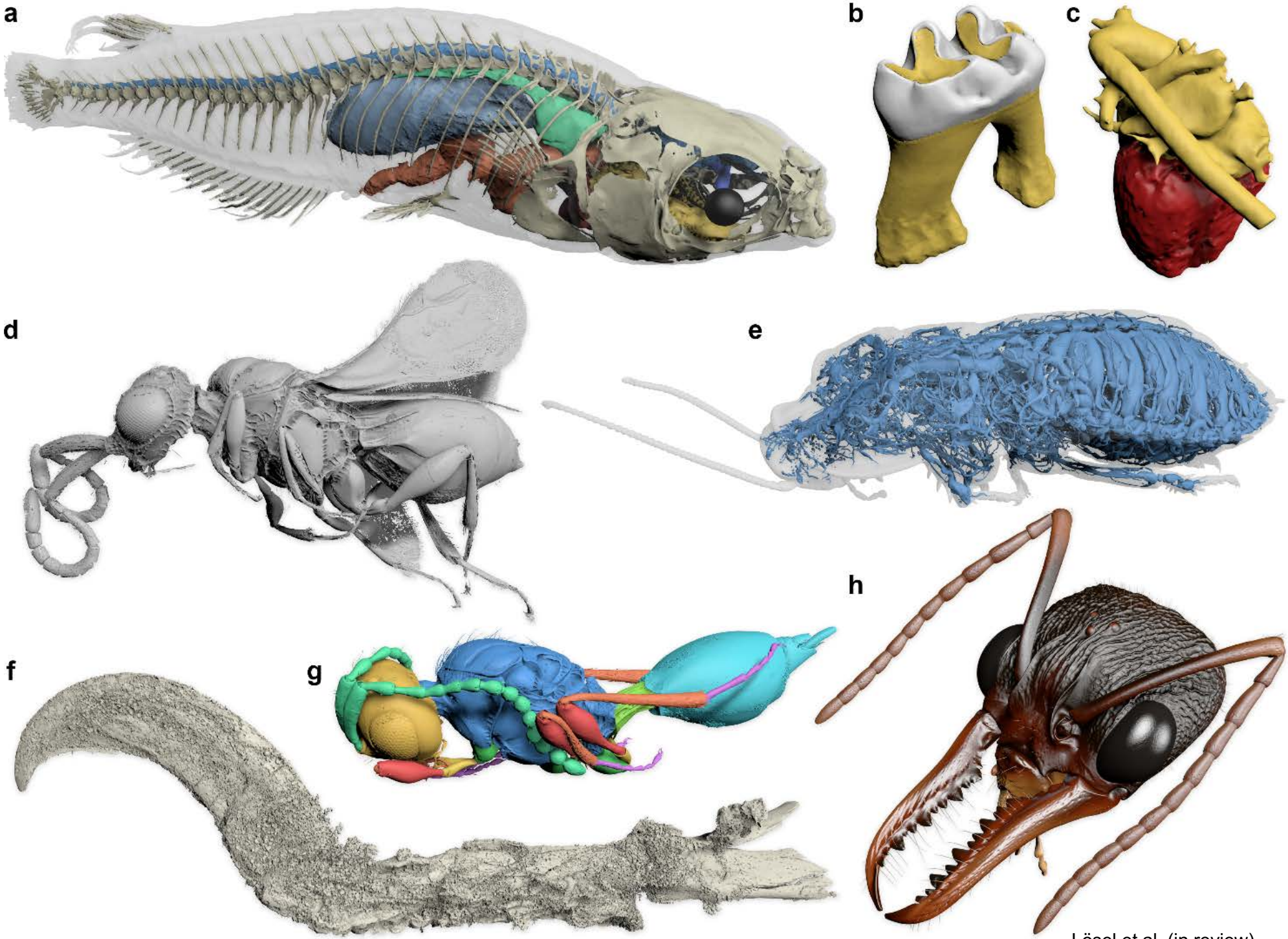
BIOMEDISA

https://biomedisa.de

Segmentation of a *Trigonopterus* weevil



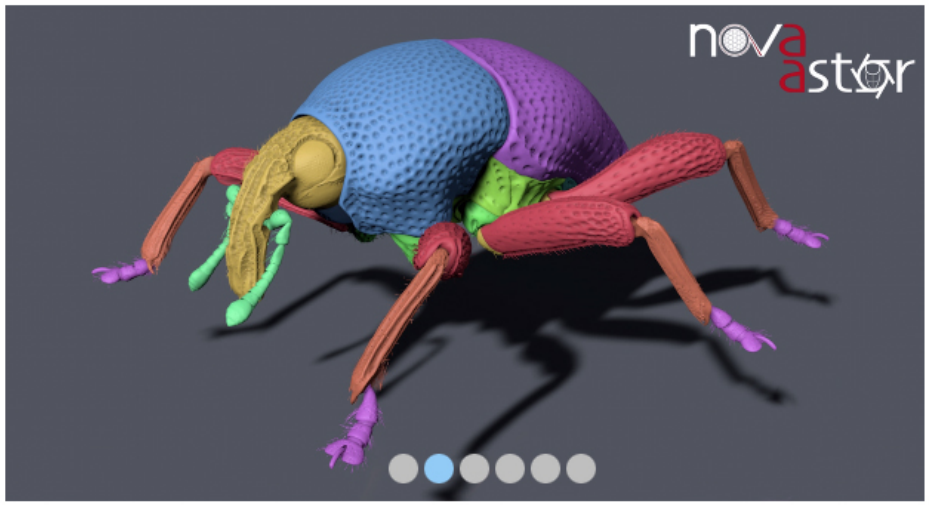
Lösel et al. (in review)



Lösel et al. (in review)

BIOMEDISA

The Biomedical Image Segmentation App



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About Biomedisa

The Biomedical Image Segmentation App (Biomedisa) is a **web application** for segmentation of computed tomography (CT) scans, magnetic resonance images (MRI) or any other volumetric images. Remaining one of the most challenging tasks in computer vision, image segmentation is a prerequisite for the investigation of morphological questions. Biomedisa can be used in addition to any segmentation tool like Amira, Fiji, ImageJ or MITK. The user-friendly segmentation process is hyper-parameter-free, eliminating the need for a complex and tedious configuration. The use of graphics processing units (GPUs) enables the evaluation of large image data (8 GB and more) in a short time. You can upload your image data and pre-labeled slices, run the segmentation process, and visualize the image data and results using 3D rendering software and a 2D slice viewer. Biomedisa finds its root in the projects ASTOR and NOVA financed by the Federal Ministry of Education and Research (BMBF), Germany. Biomedisa is free-of-charge.

novastor Bundesministerium für Bildung und Forschung

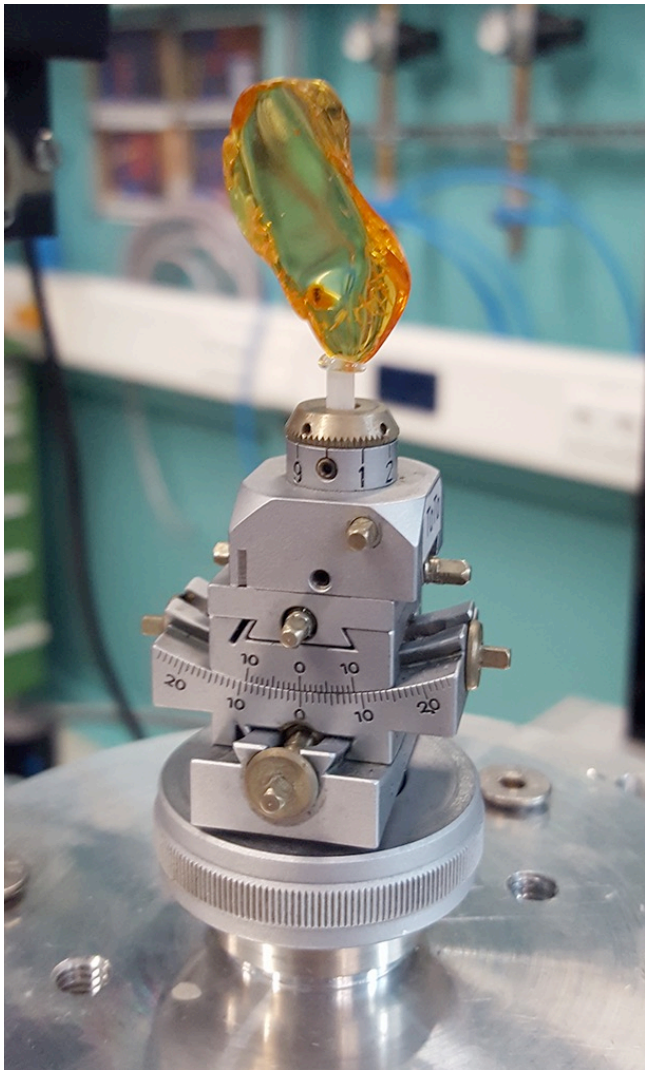
https://biomedisa.de

@biomedisa

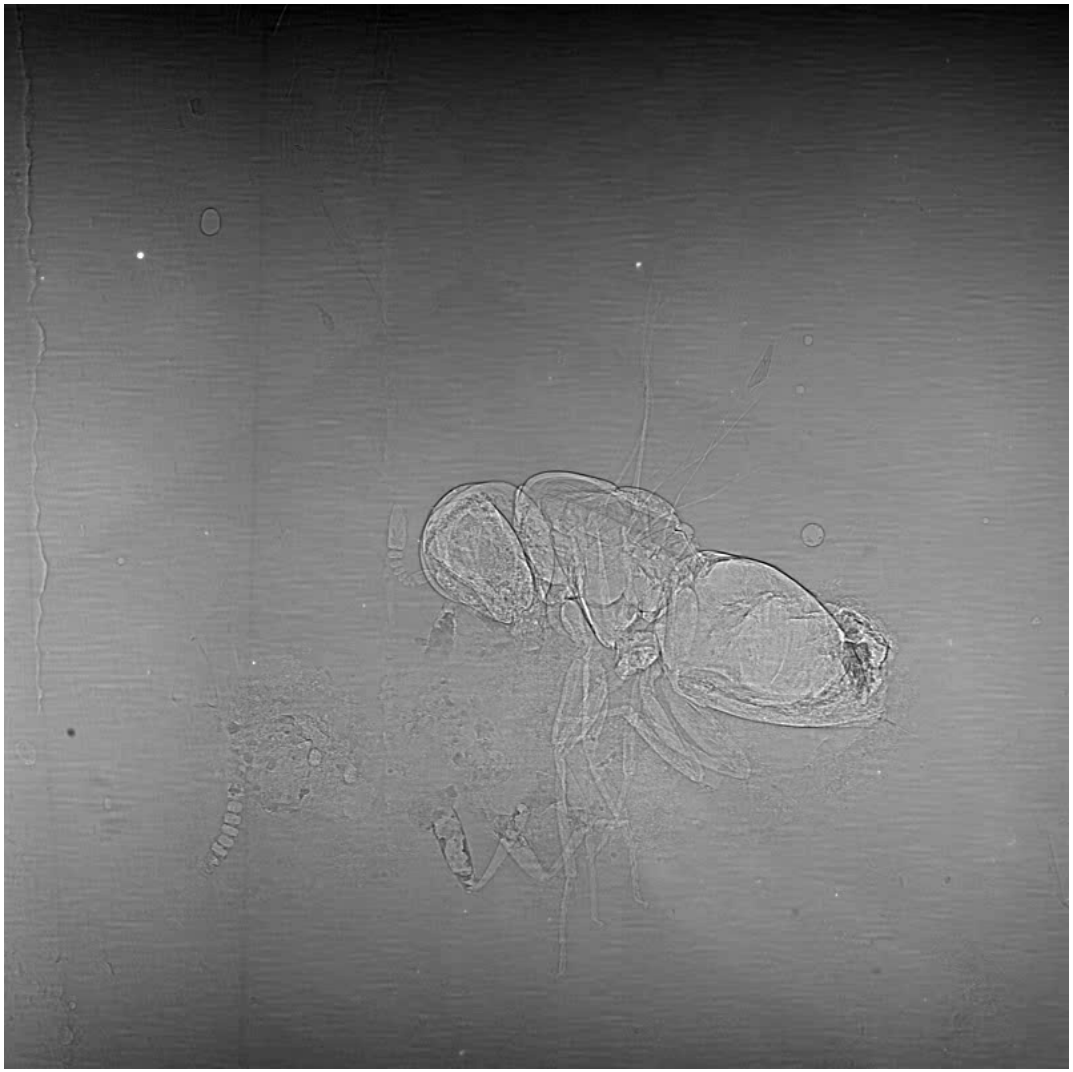
Imaging of fossil insects

Tomography of amber inclusions

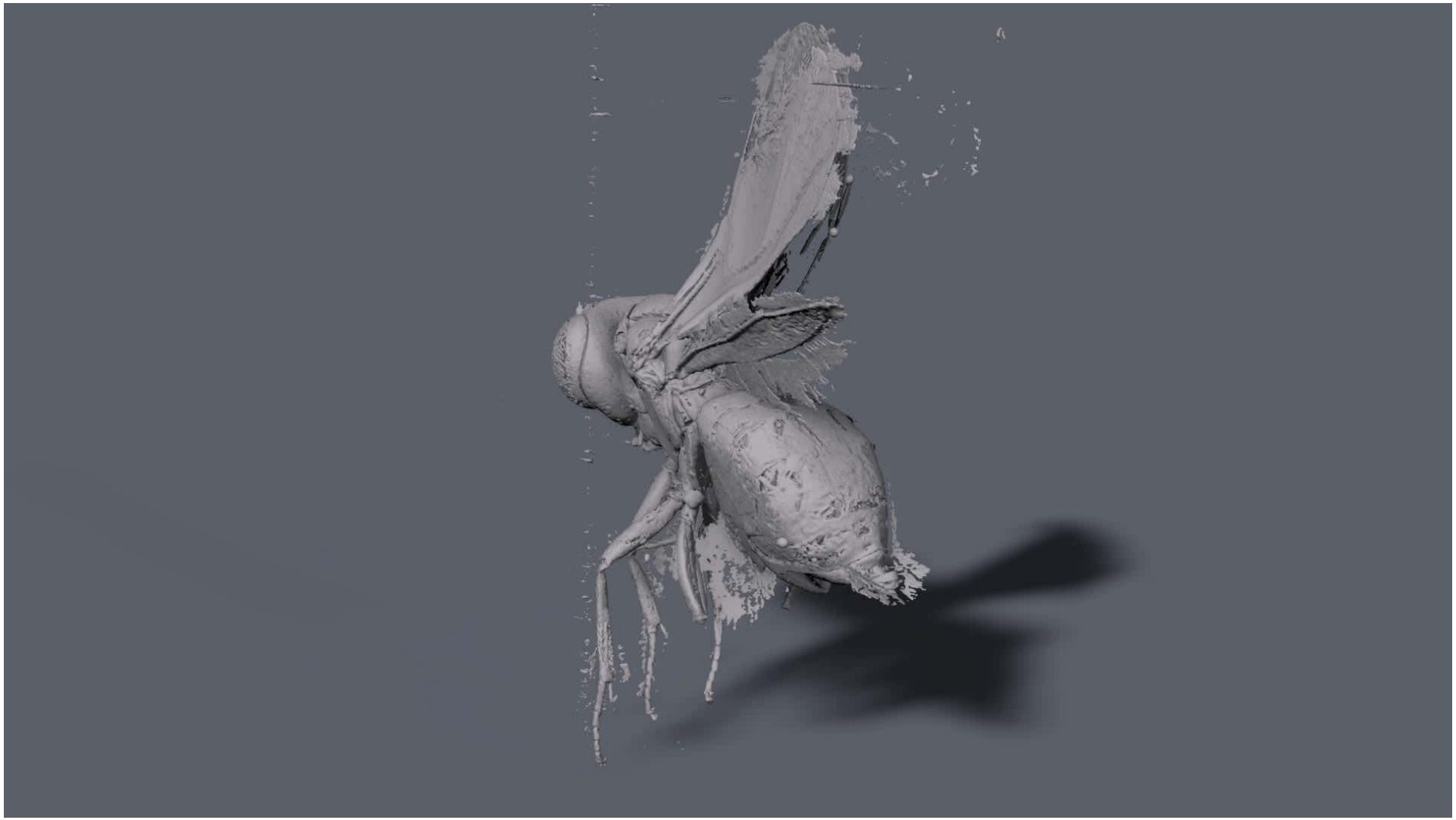
- Amber
  - Fossil resin from trees
  - Small organisms can be preserved as inclusions for millions of years
  - Studying amber inclusions brings light to the evolution of invertebrates
- Problems of manual examination
  - Often difficult due to optical artifacts
  - View can be blocked by other objects
  - Anatomical characters are hidden



Radiographs

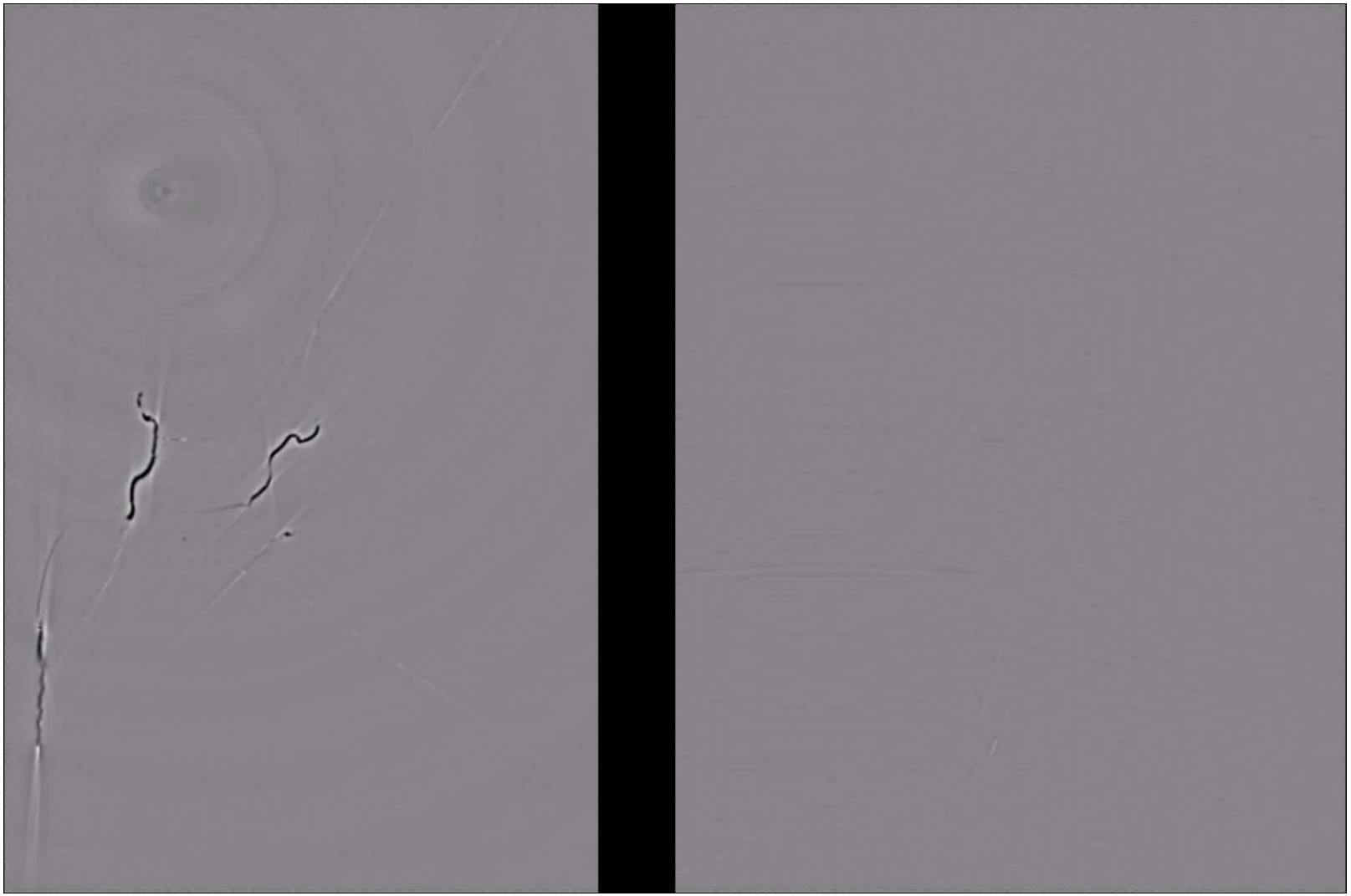


Greyscale-based surface model

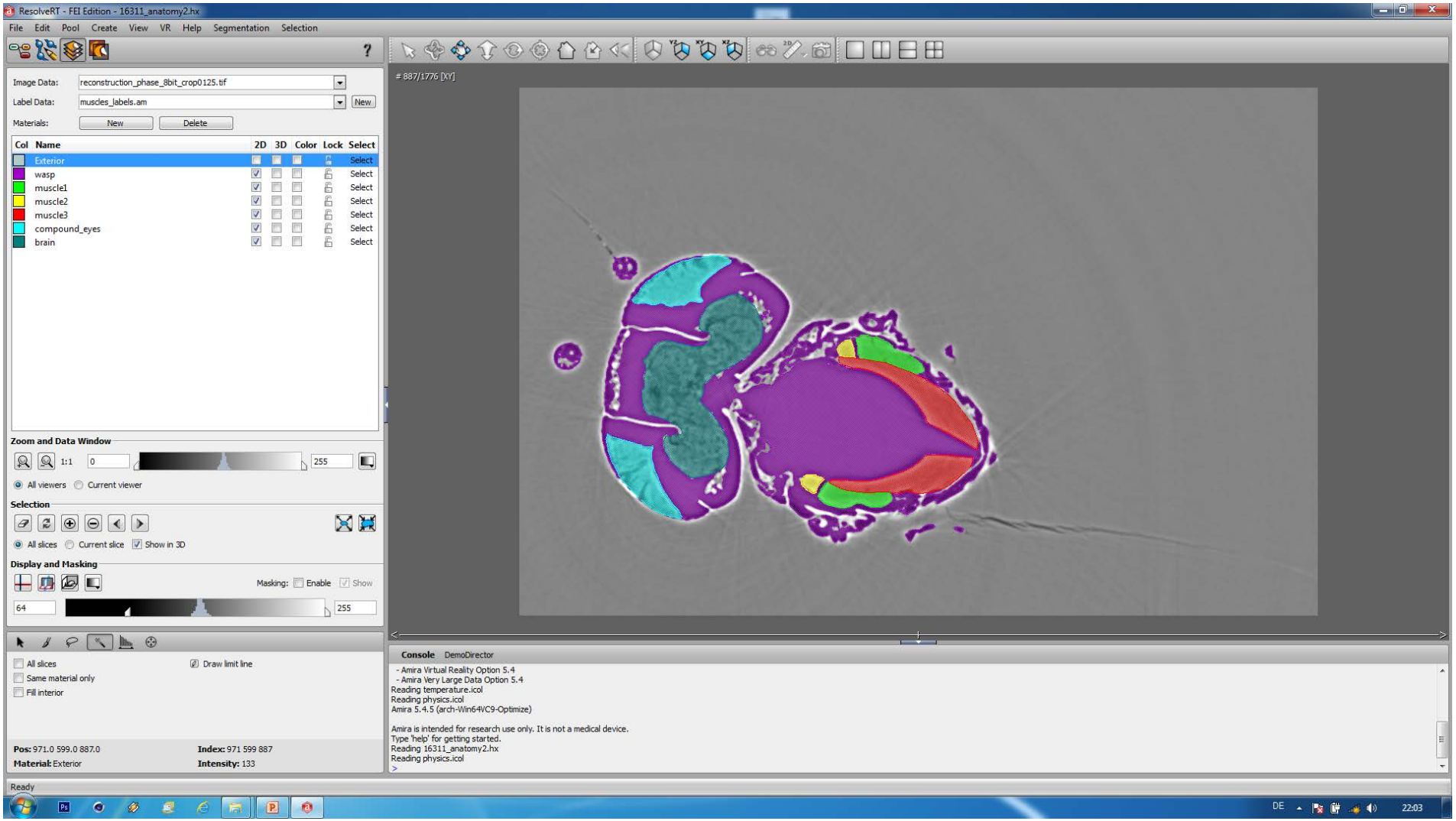


„cleaned“ surface model

Tomographic volume



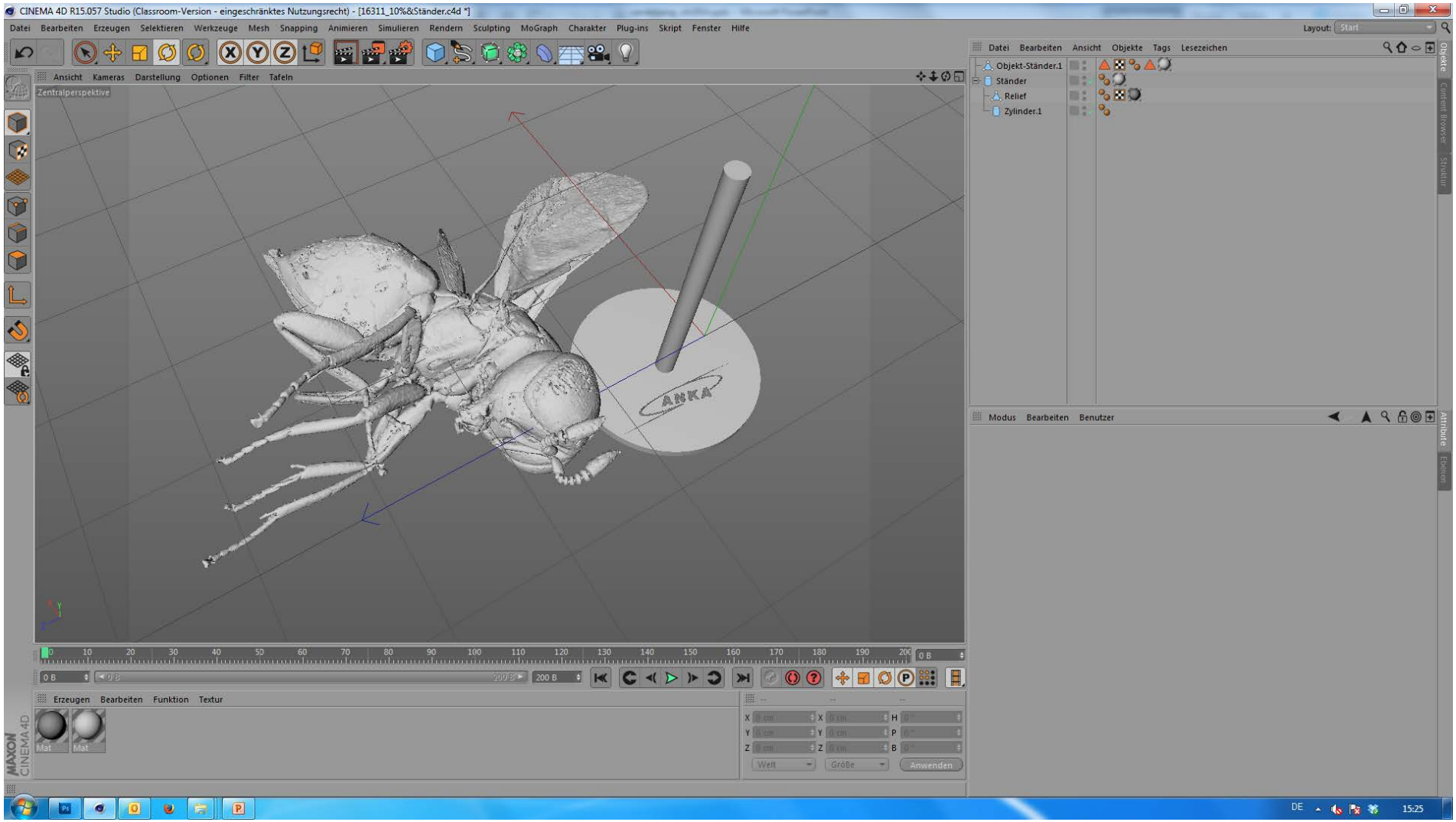
Segmentation



Volume rendering

Volume rendering & segmented muscles

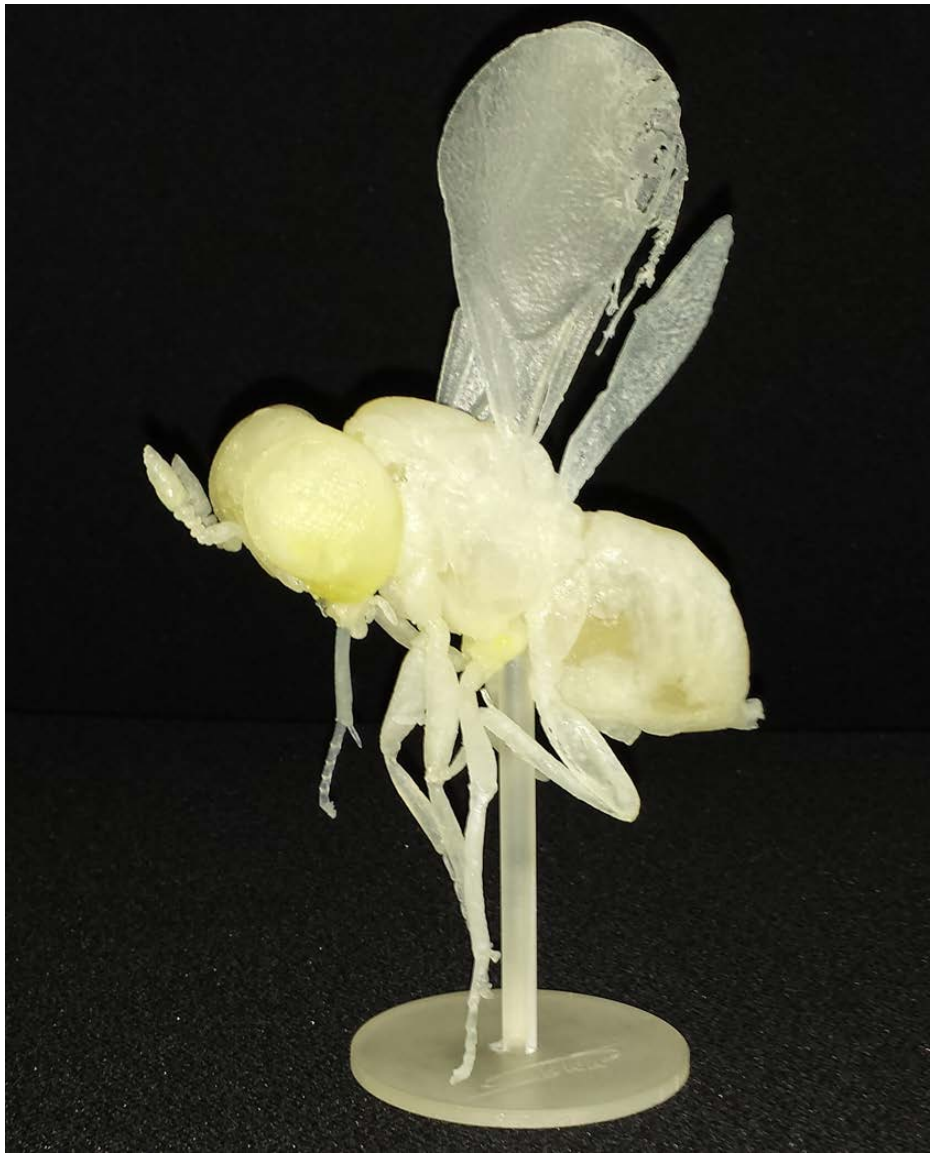
3D printing


CINEMA 4D R15.057 Studio (Classroom-Version - eingeschränktes Nutzungsrecht) - [16311_10%&Ständer.c4d *]
Datei Bearbeiten Erzeugen Selektieren Werkzeuge Mesh Snapping Animieren Simulieren Rendern Sculpting MoGraph Charakter Plug-ins Skript Fenster Hilfe
Layout: Start
Ansicht Kameras Darstellung Optionen Filter Tafeln
Zentralperspektive
Datei Bearbeiten Ansicht Objekte Tags Lesezeichen
Objekt-Ständer.1
Ständer
Relief
Zylinder.1
Modus Bearbeiten Benutzer
ANKA
Erzeugen Bearbeiten Funktion Textur
Mat
Mat
Anwenden
DE
15:25

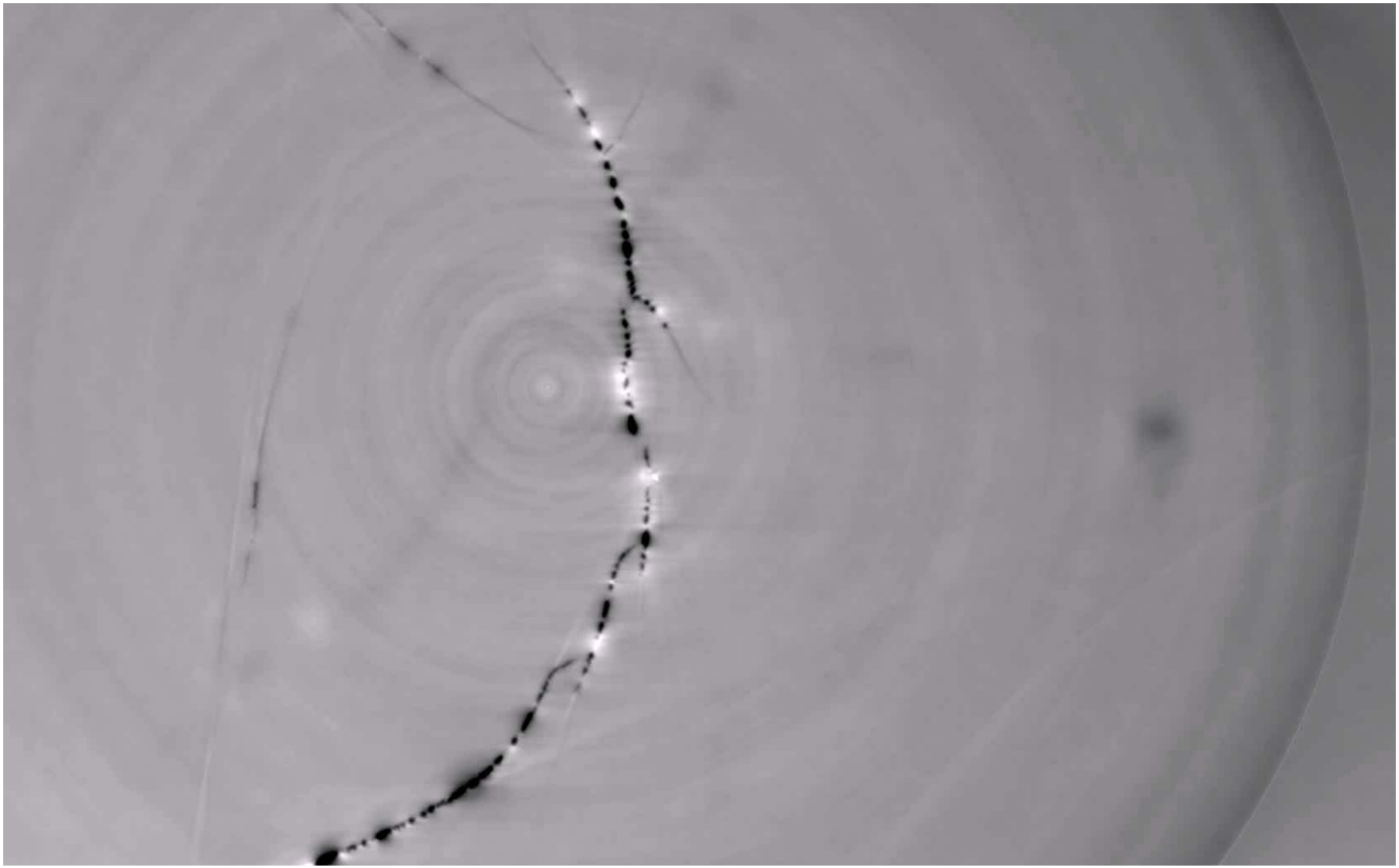
3D printing

40 h later…

3D printing



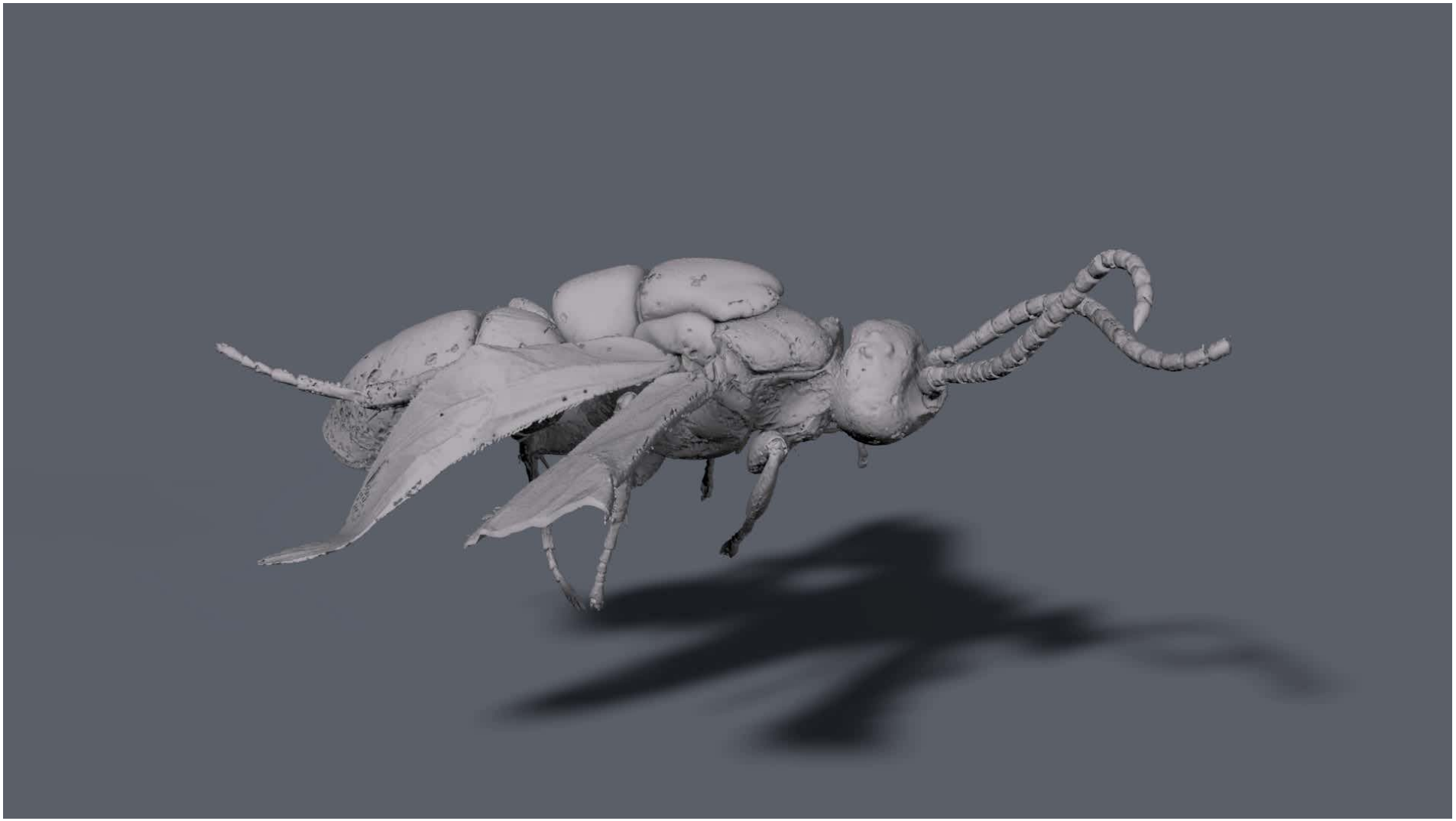
Chalcidoidea: Pteromalidae



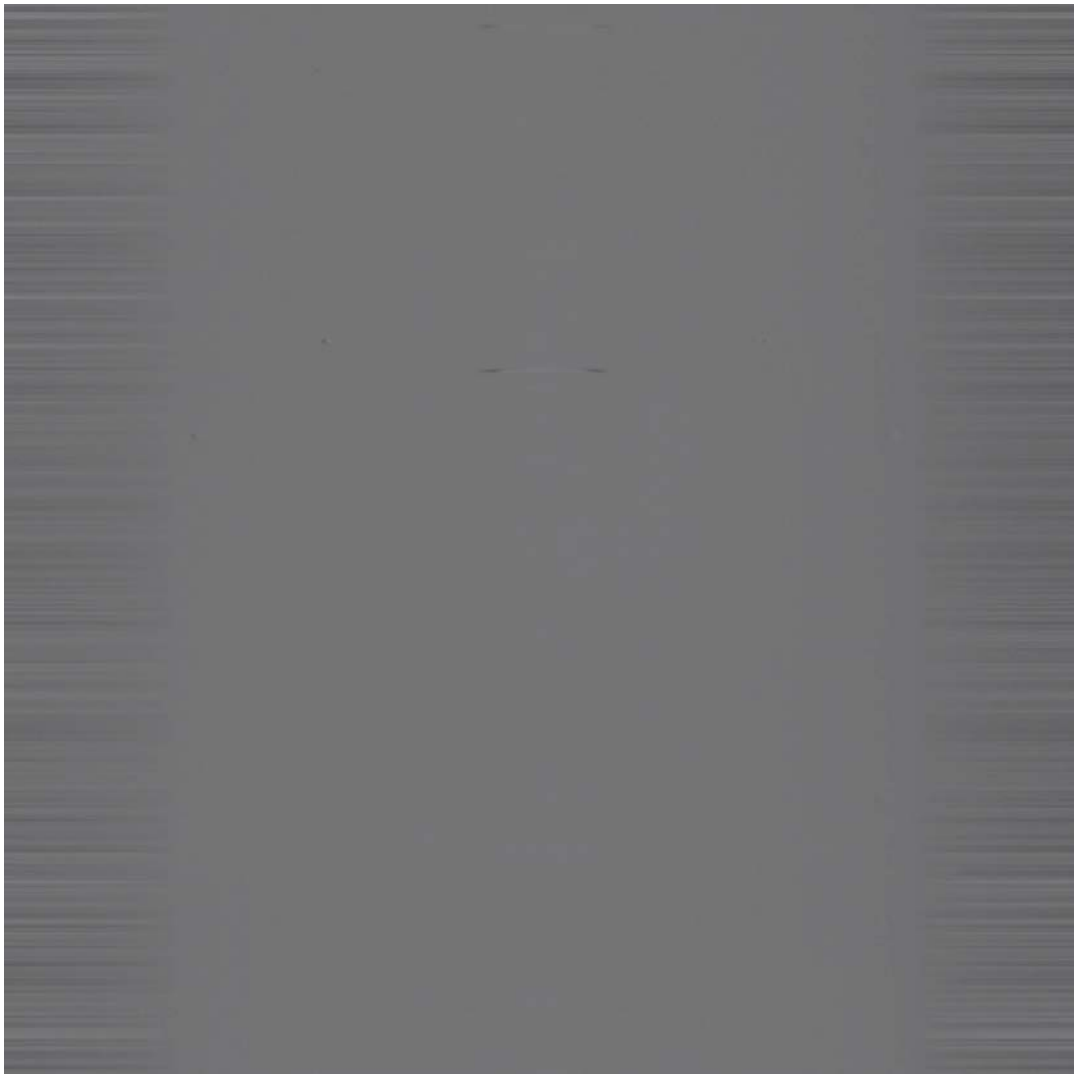
Chalcidoidea: Pteromalidae

Chalcidoidea: Pteromalidae

Ichneumonoidea: Braconidae

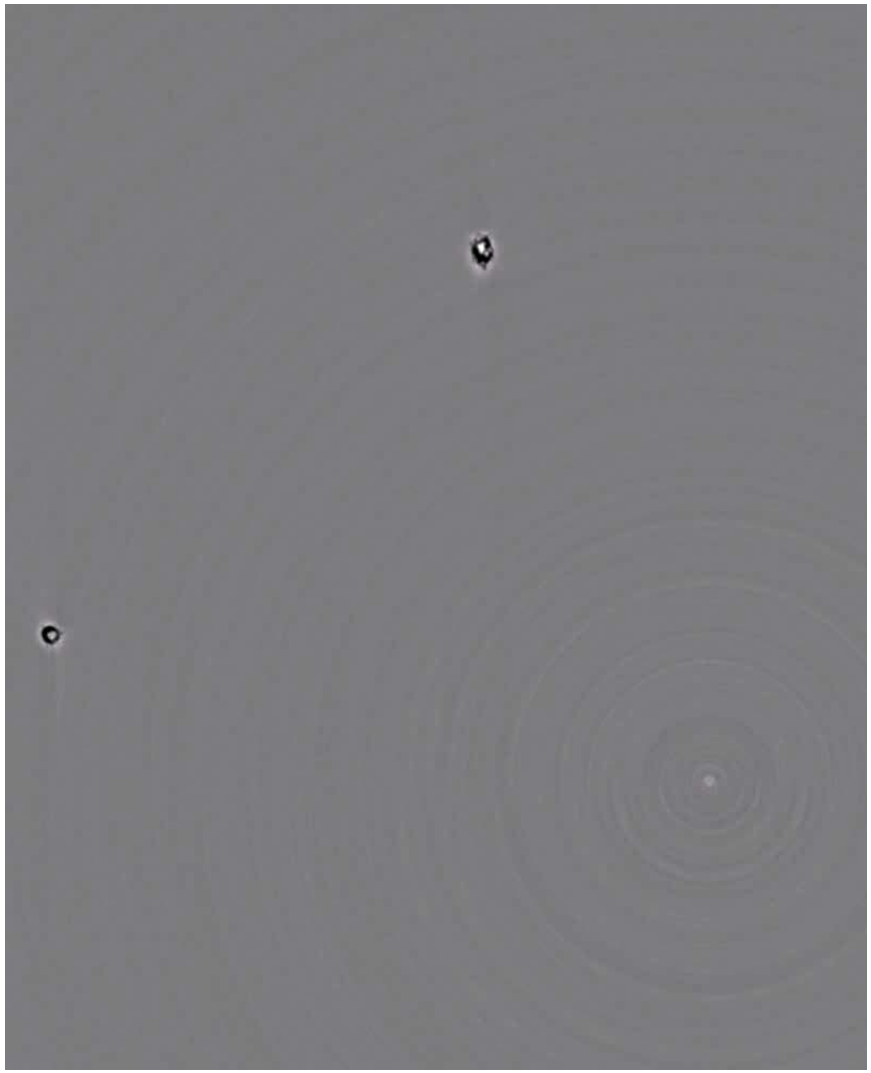


Ichneumonoidea: Braconidae



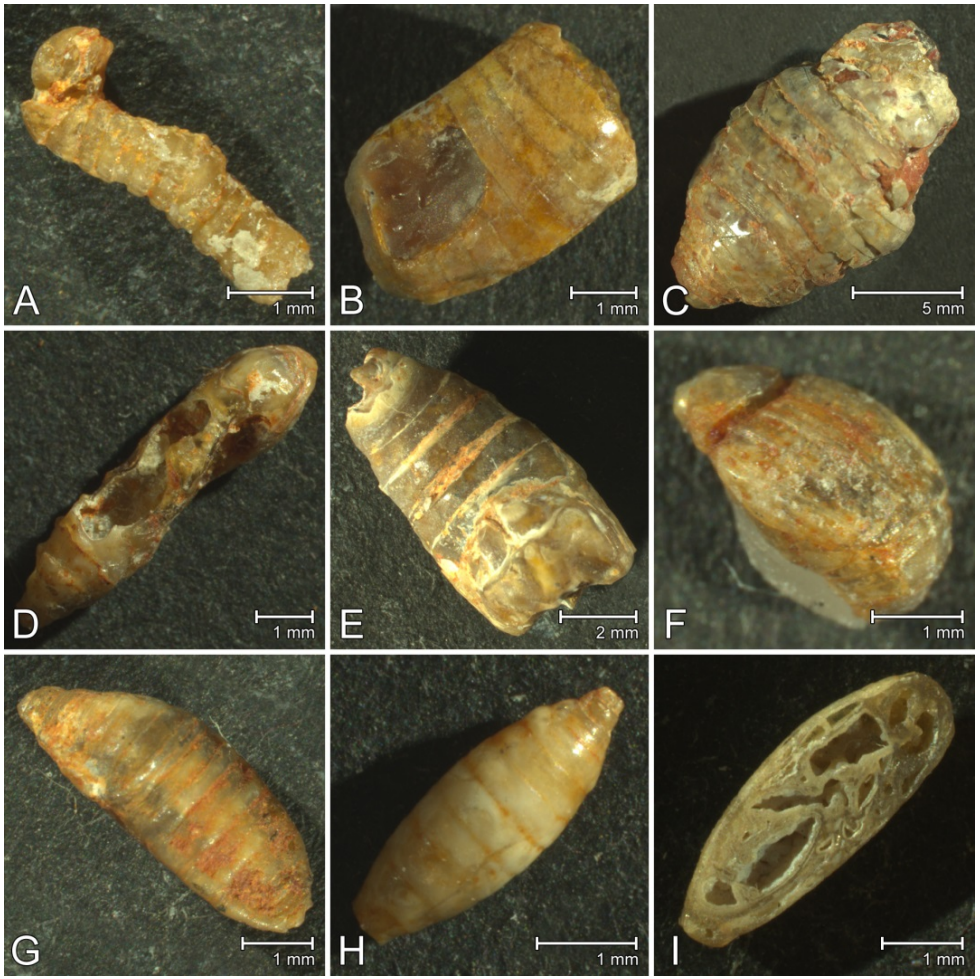
Ichneumonoidea: Braconidae

Braconidae



Fissure fillings

- Fast mineralization in phosphate-rich environment
- Locality: Quercy (FR)
  - ca. 30 myo (Oligocene)
- Little-known fossil type!



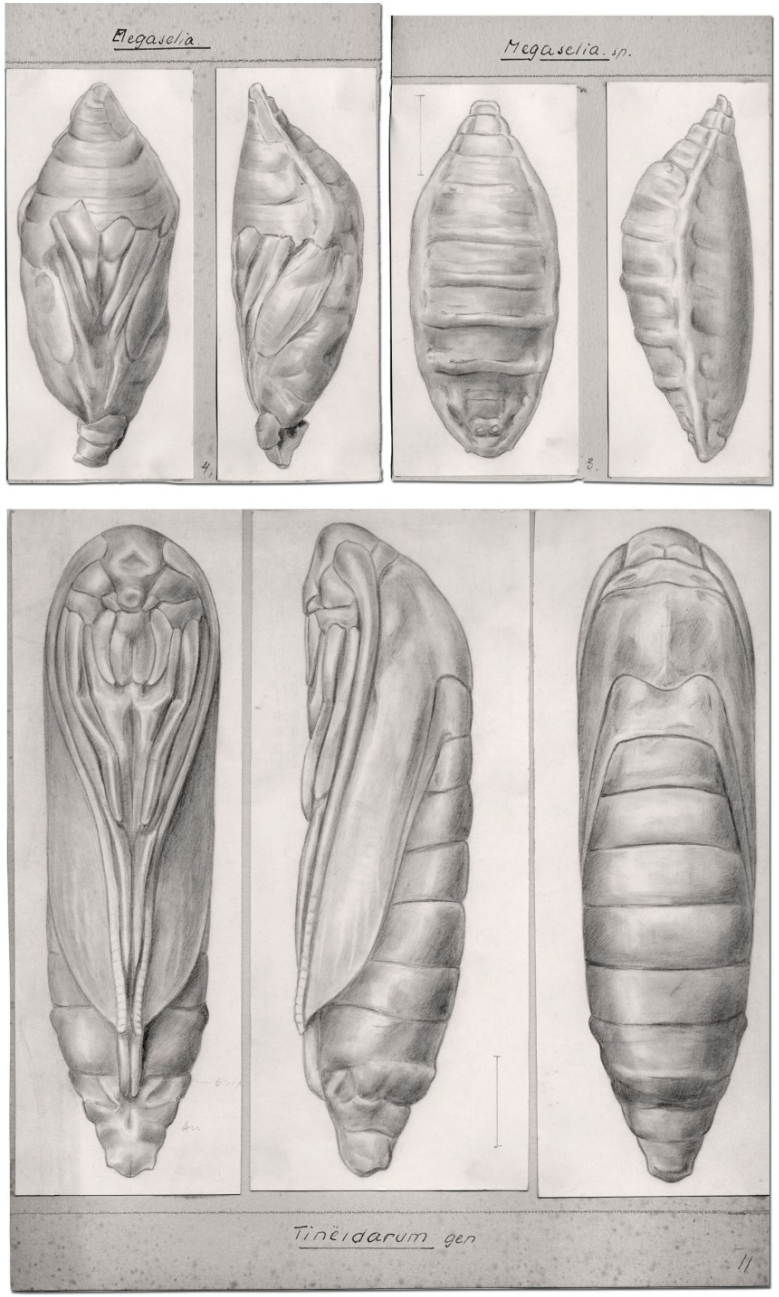
SCHWEIZERISCHE
PALAEONTOLOGISCHE ABHANDLUNGEN
Band 64

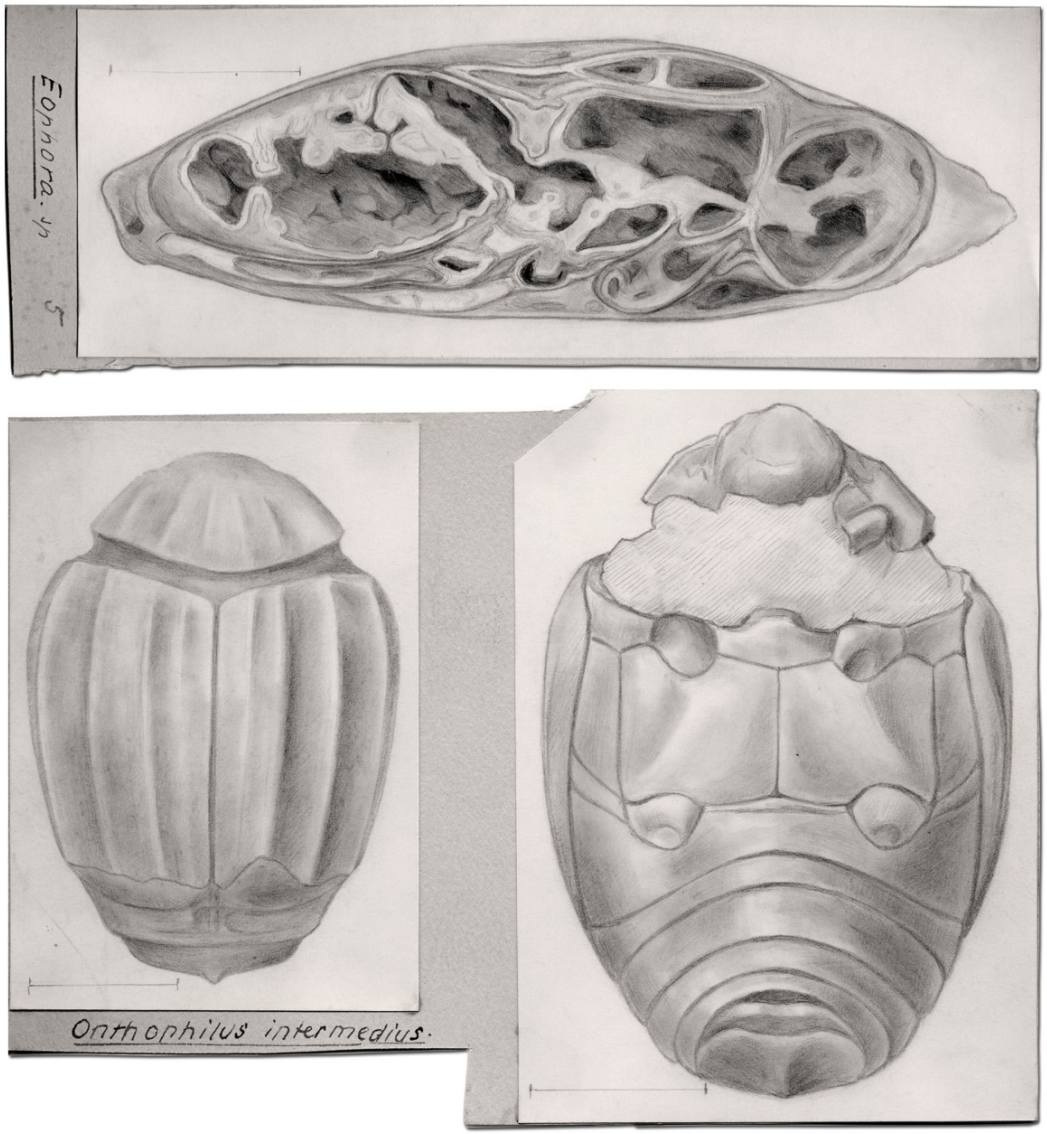
Insekten aus den Phosphoriten des Quercy

von
Eduard Handschin
Basel

Mit 3 Tafeln und 7 Figuren im Text

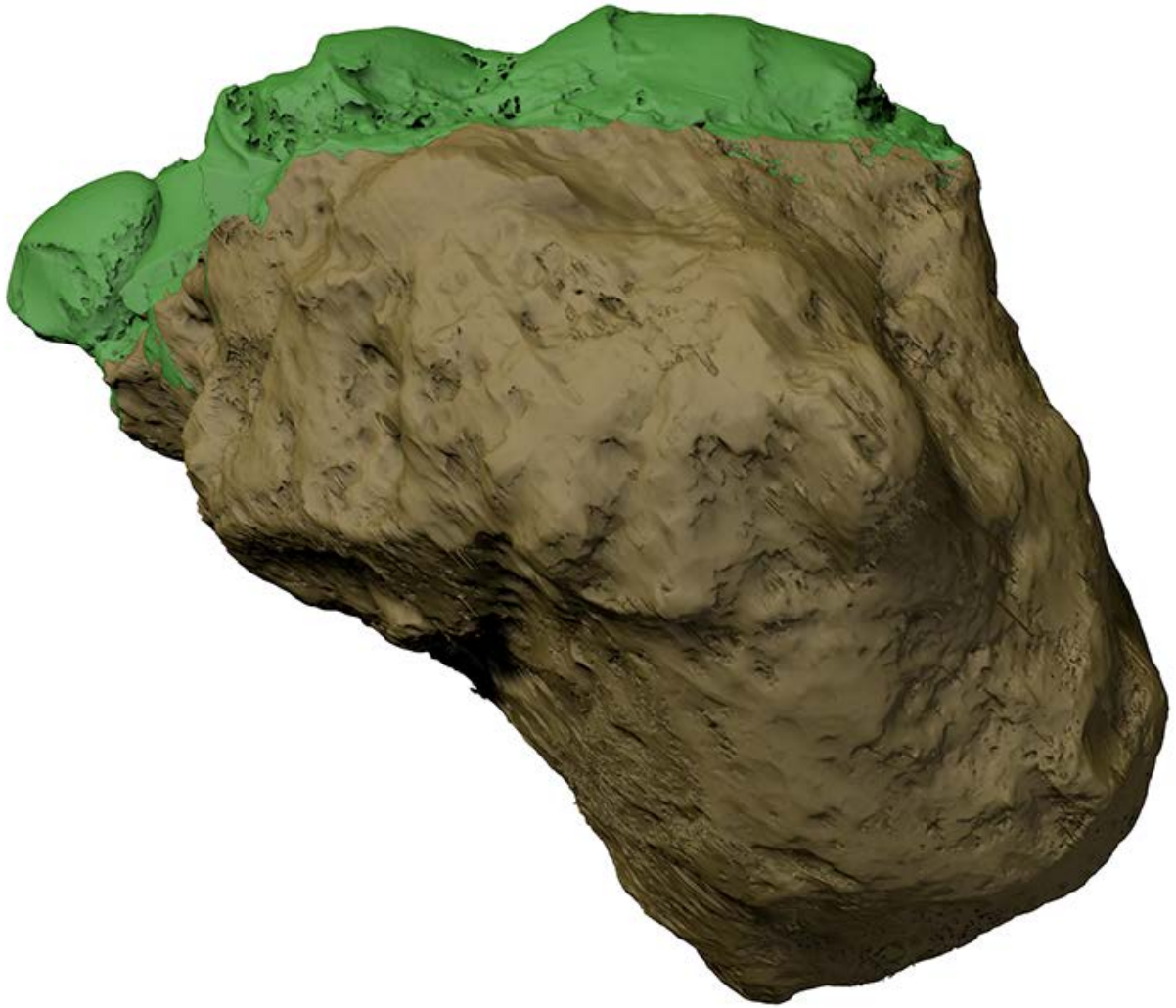
Ausgeführt und gedruckt mit Unterstützung
des Vergleichend-anatomischen Fonds
und der August Tobler-Stiftung
des Naturhistorischen Museums in Basel


Megaselia
Megaselia. sp.
Tineidarum gen


Onthophilus intermedius.

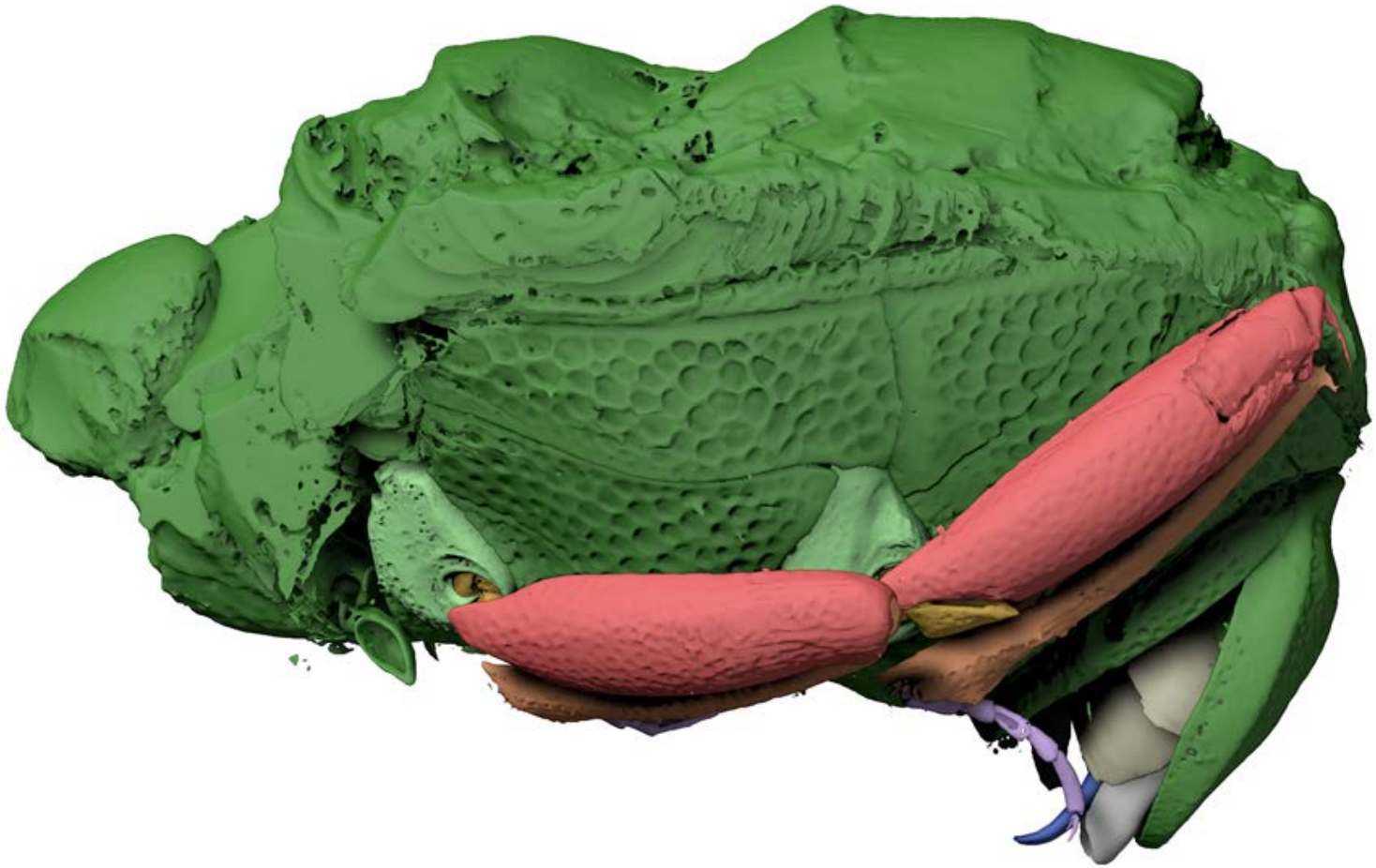
Fossil histerid beetle

Segmented model



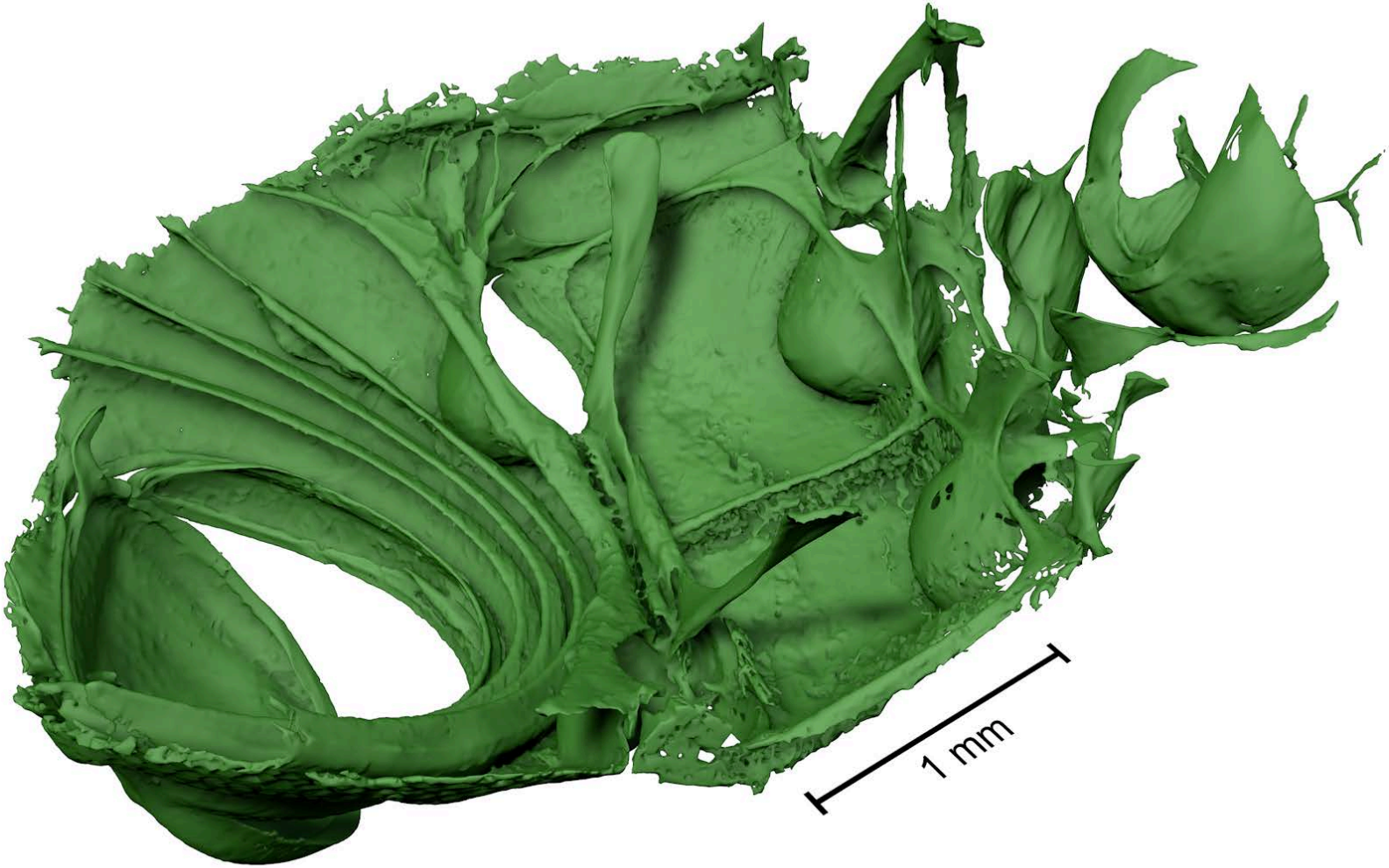
Schwermann et al. 2016: *eLife* 5: e12129

Segmented model



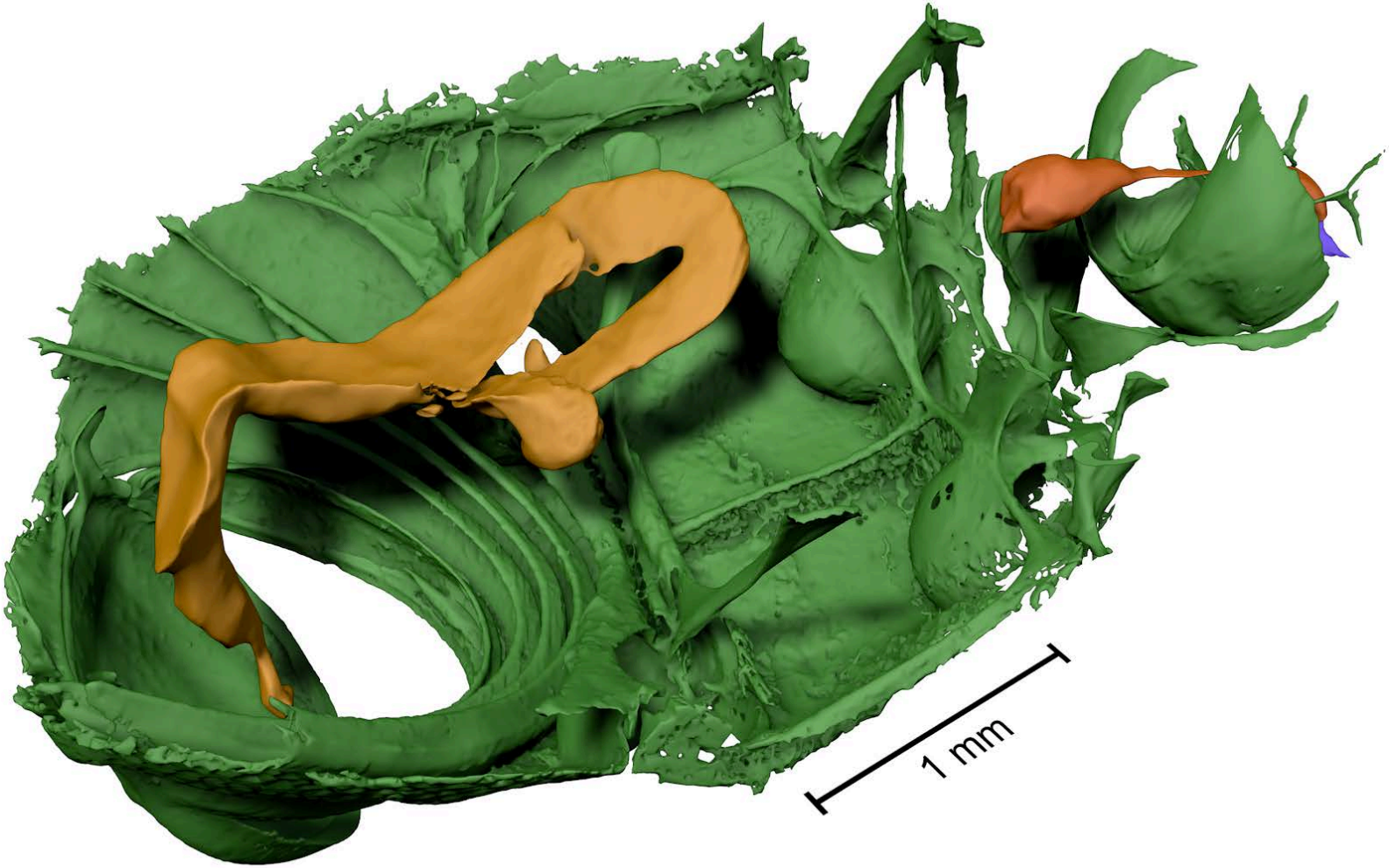
Schwermann et al. 2016: *eLife* 5: e12129

Segmented model



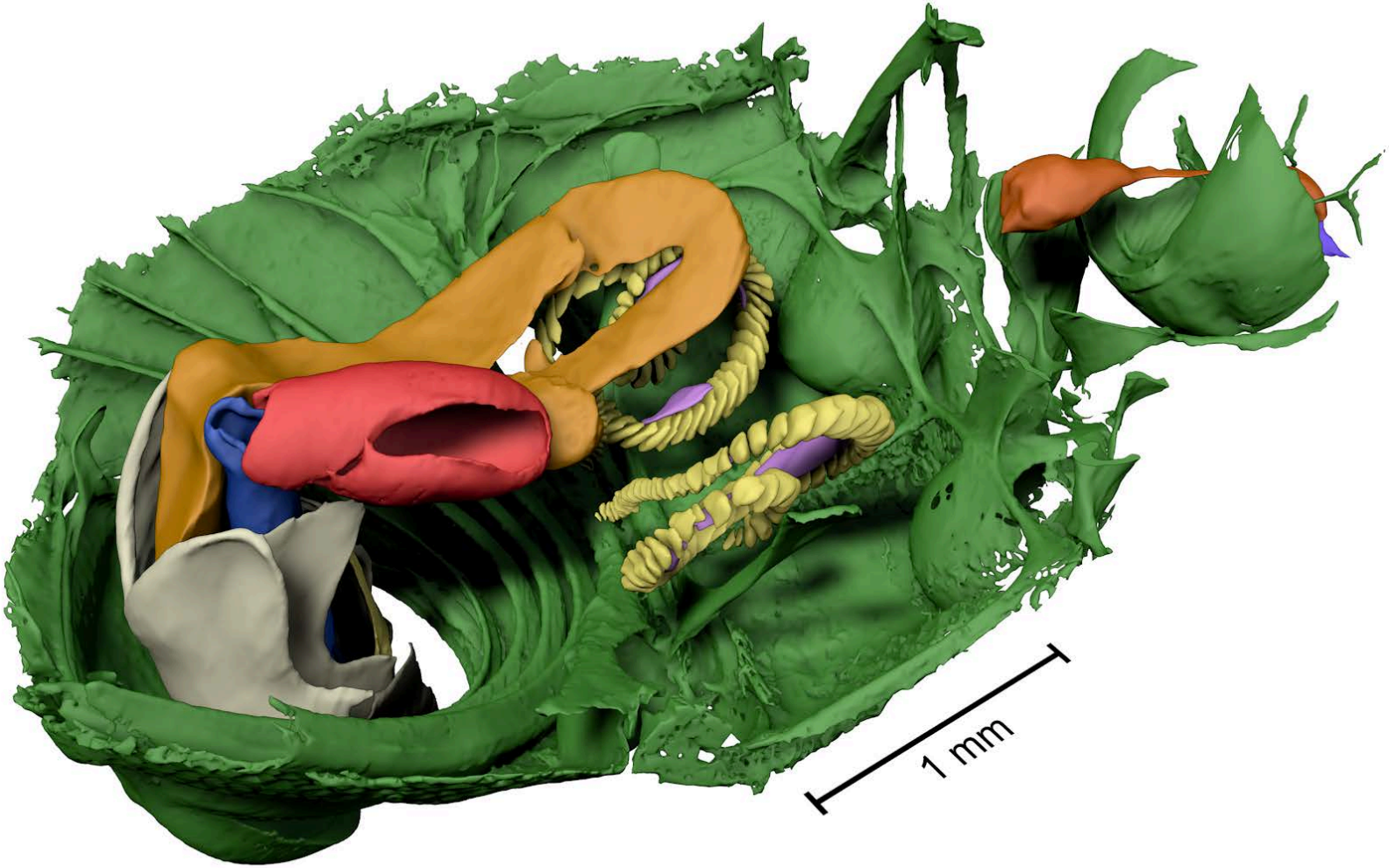
Schwermann et al. 2016: *eLife* 5: e12129

Segmented model



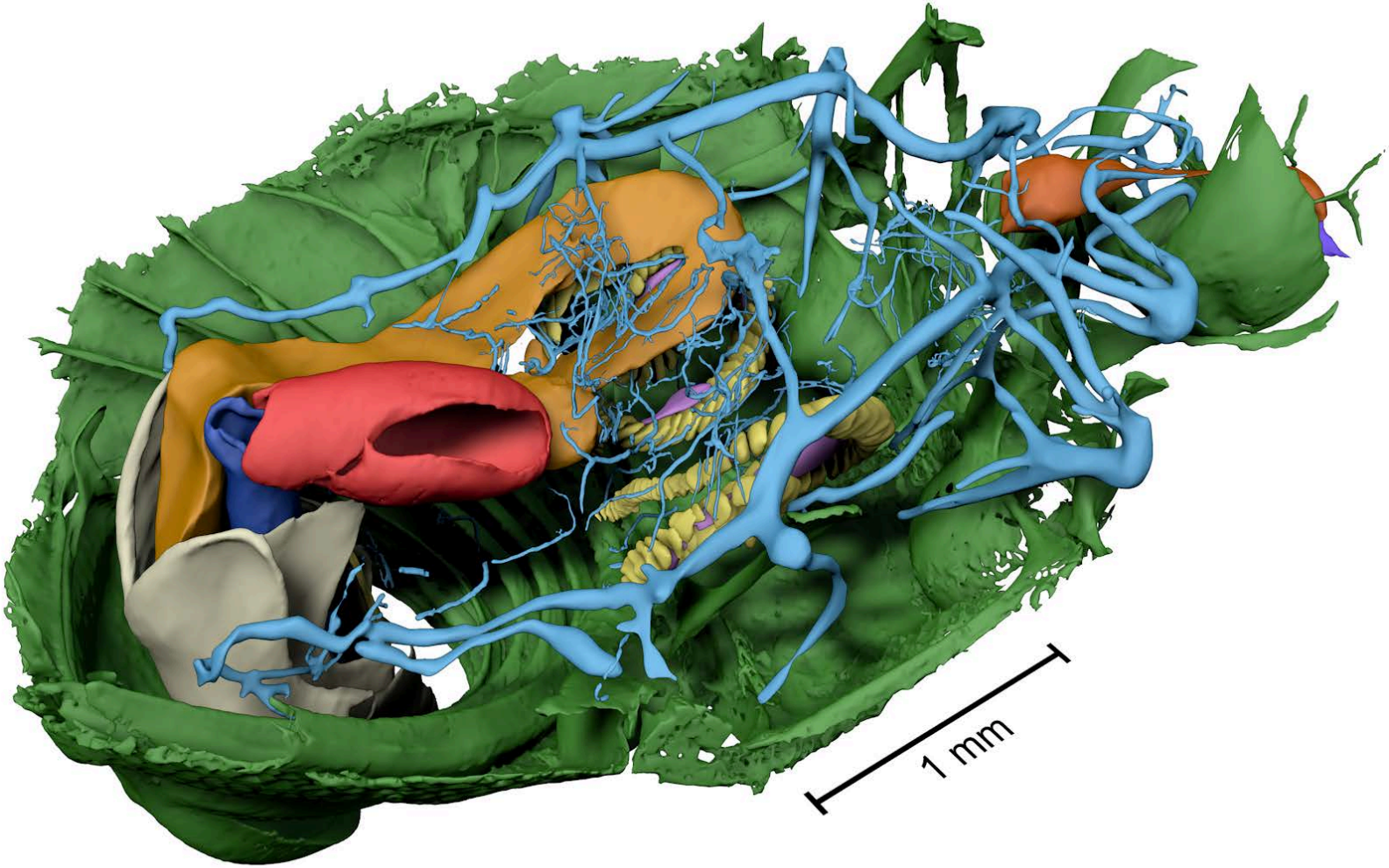
Schwermann et al. 2016: *eLife* 5: e12129

Segmented model



Schwermann et al. 2016: *eLife* 5: e12129

Segmented model



Schwermann et al. 2016: *eLife* 5: e12129


3 mm
29 fly puparia

van de Kamp et al. 2018: *Nature Communications* 9: 3325

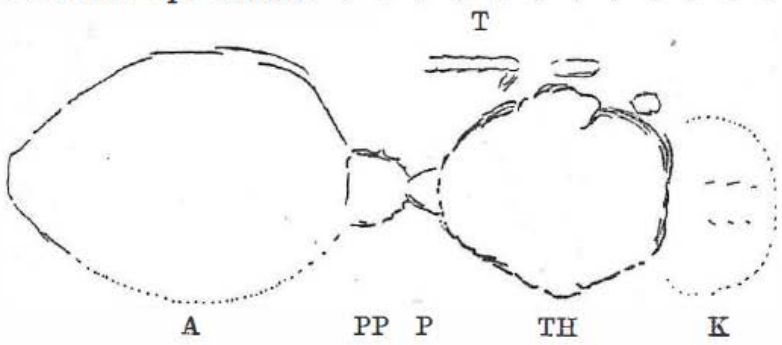
Parasitoid wasps

- Development on or inside a host, resulting in the death of the latter
- **10 to 20 % of all insects are parasitoid wasps**
- Extremely important for ecosystems
- Biological **pest control** agents
- Biology of most species completely unknown

Fossil parasitoids

- Known from other fossil types (e.g. amber)
- Direct evidence for endoparasitism missing (exception: Handschin!)

Fig. 13. *Braconidarum* sp. indet. 18



Horizontaler Längsschliff durch die Puppe von Megaselia, im Innern die Puppe einer Braconide zeigend.
Qu. 64; 25: 1.

K = Kopf. PP = Postpetiolus.
TH = Thorax. A = Abdomen.
P = Petiolus. T = Tibia d. 3. Beines.


1510 fly puparia

Data (four days of beamtime)

- Screening
  - 1,500 projections: 12.1 GB
  - tomogram (32 bit): 30.5 GB
  - tomogram (8 bit): 7.63 GB
  - sum: 50.23 GB
  - x 1,510: **75.85 TB**
- High resolution scans
  - 3,000 projektions: 25.8 GB
  - tomogramm (32 bit): 30.5 GB
  - tomogramm (8 bit): 7.63 GB
  - sum: 63.93 GB
  - x 182: **11.64 TB**
- **Total amount of data: 87.49 TB**

Data (four days of beamtime)

- Screening
  - 1,500 projections: 12.1 GB
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van de Kamp et al. 2018: *Nature Communications* 9: 3325

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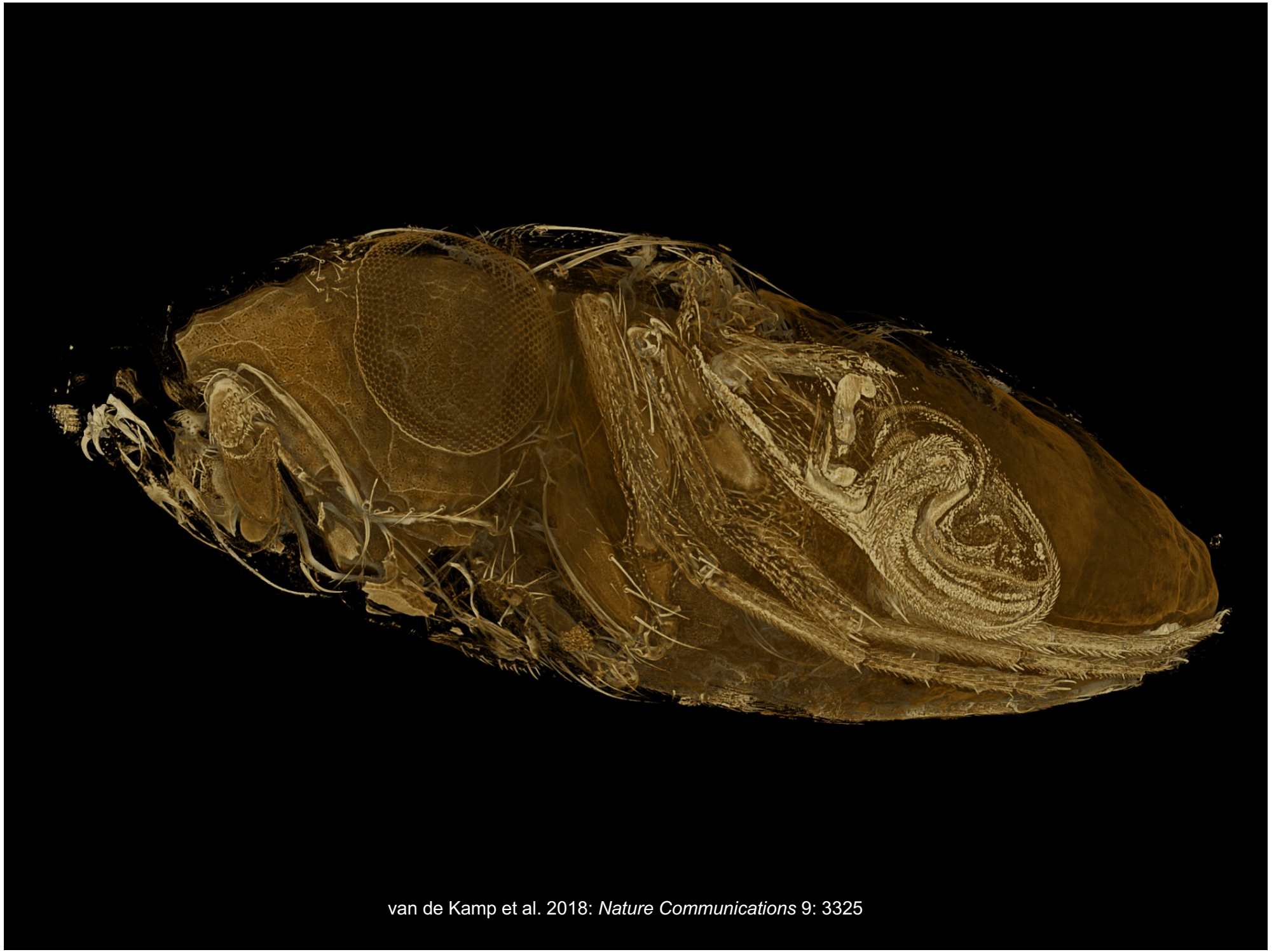
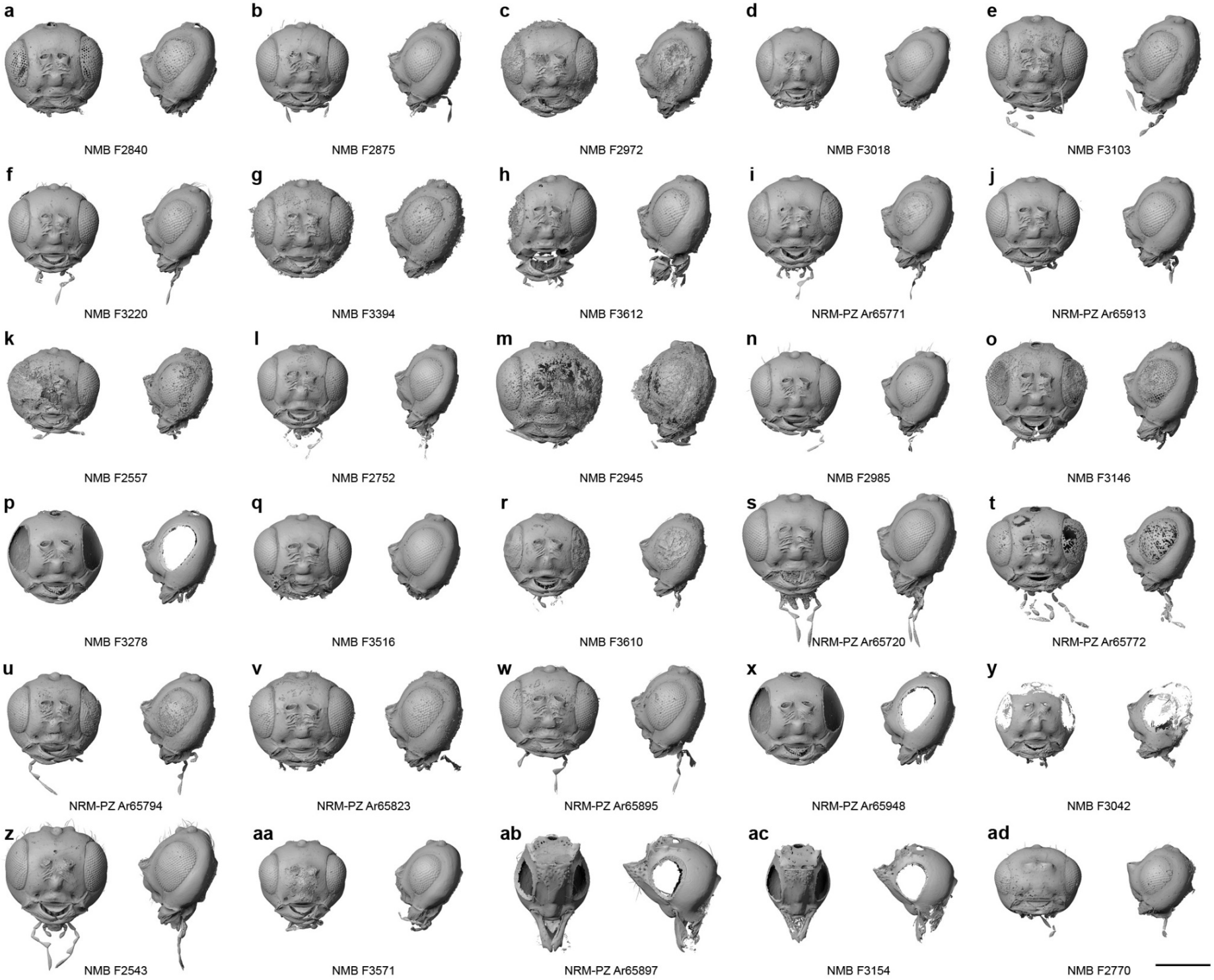


van de Kamp et al. 2018: *Nature Communications* 9: 3325


a
NMB F2840
b
NMB F2875
c
NMB F2972
d
NMB F3018
e
NMB F3103
f
NMB F3220
g
NMB F3394
h
NMB F3612
i
NRM-PZ Ar65771
j
NRM-PZ Ar65913
k
NMB F2557
l
NMB F2752
m
NMB F2945
n
NMB F2985
o
NMB F3146
p
NMB F3278
q
NMB F3516
r
NMB F3610
s
NRM-PZ Ar65720
t
NRM-PZ Ar65772
u
NRM-PZ Ar65794
v
NRM-PZ Ar65823
w
NRM-PZ Ar65895
x
NRM-PZ Ar65948
y
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ac
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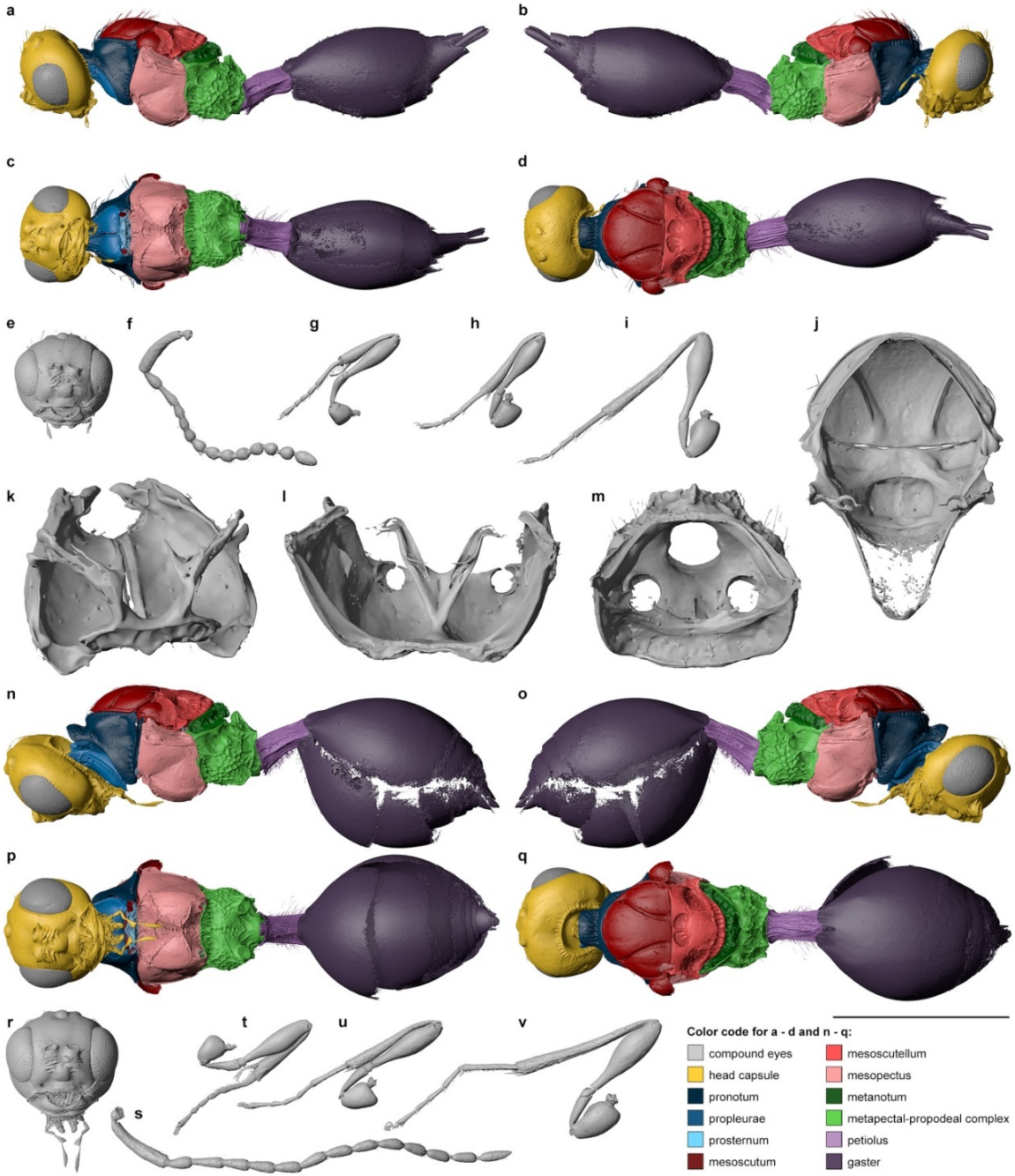
ad
NMB F2770

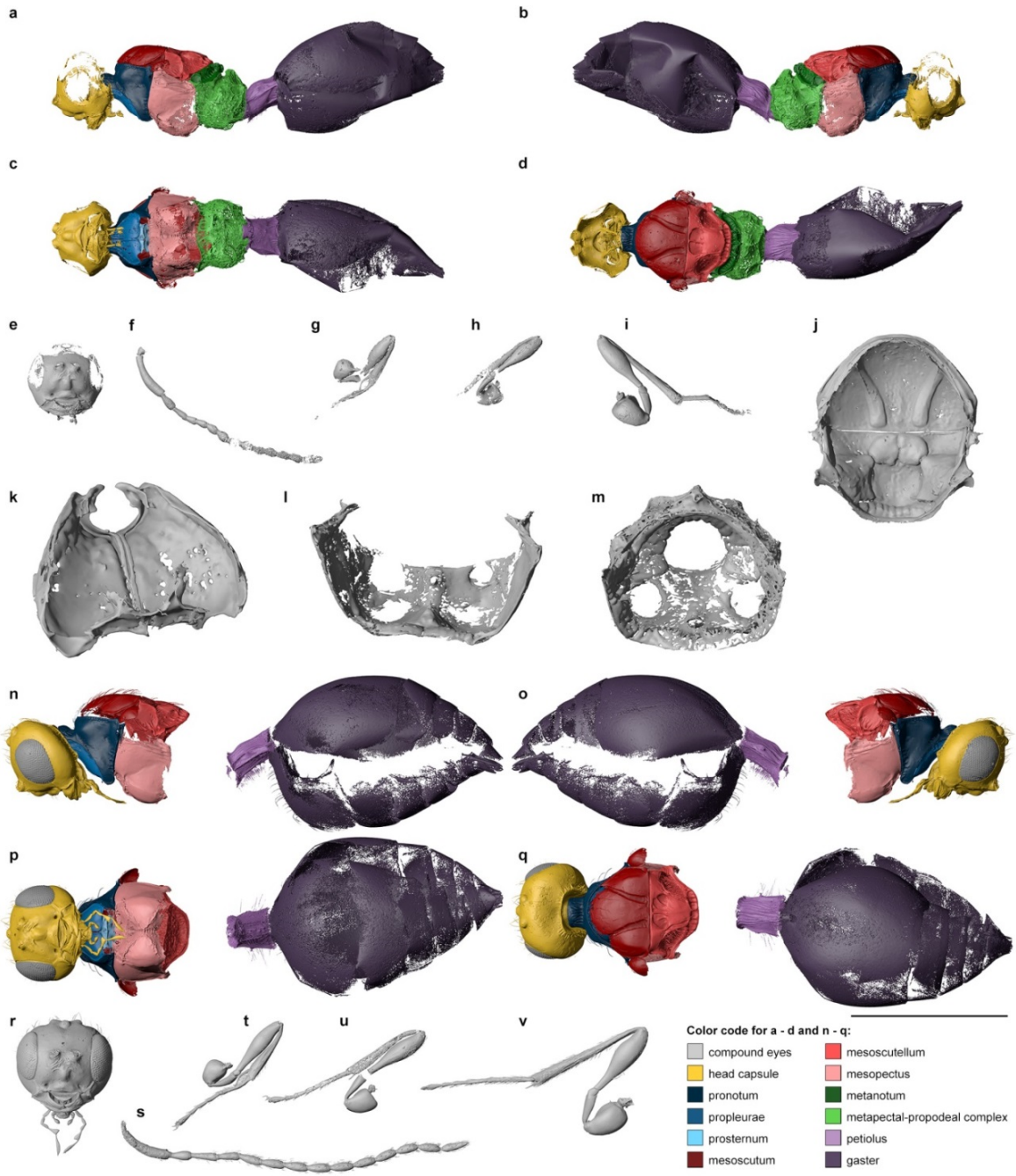


Xenomorphia resurrecta

Diapriidae

Diapriinae

Spilomicrini

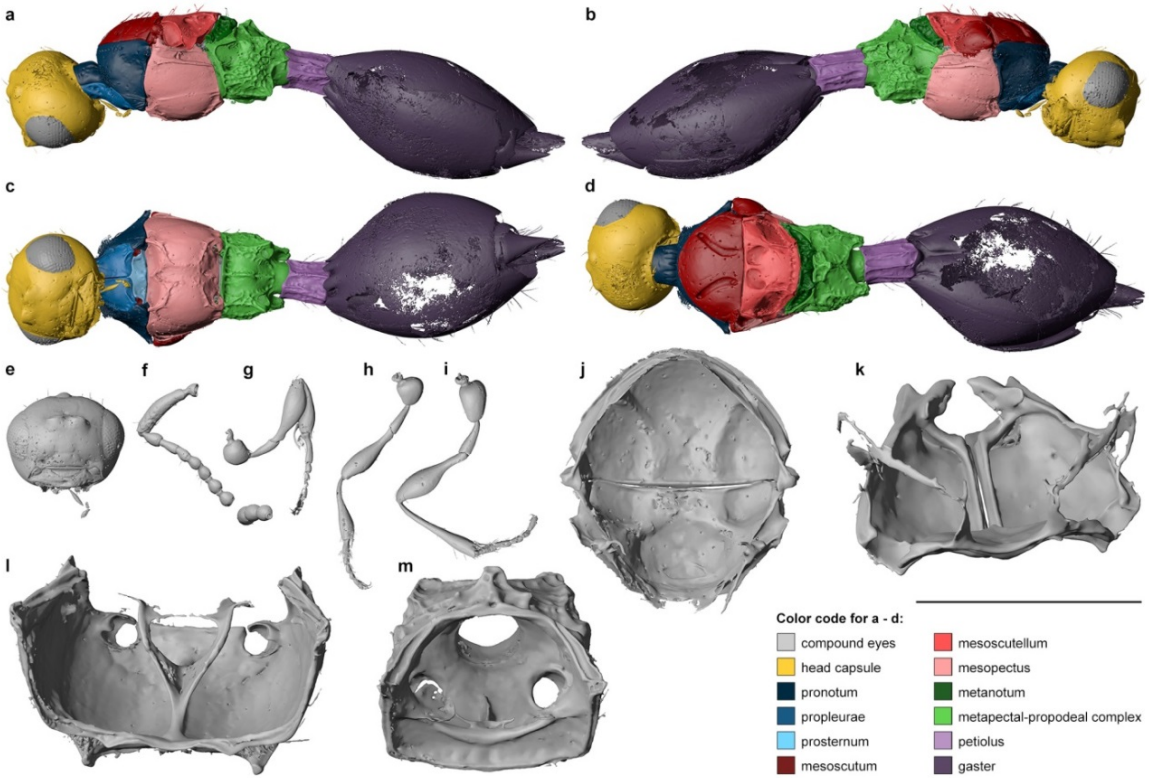


Xenomorphia handschini

Diapriidae

Diapriinae

Spilomicrini

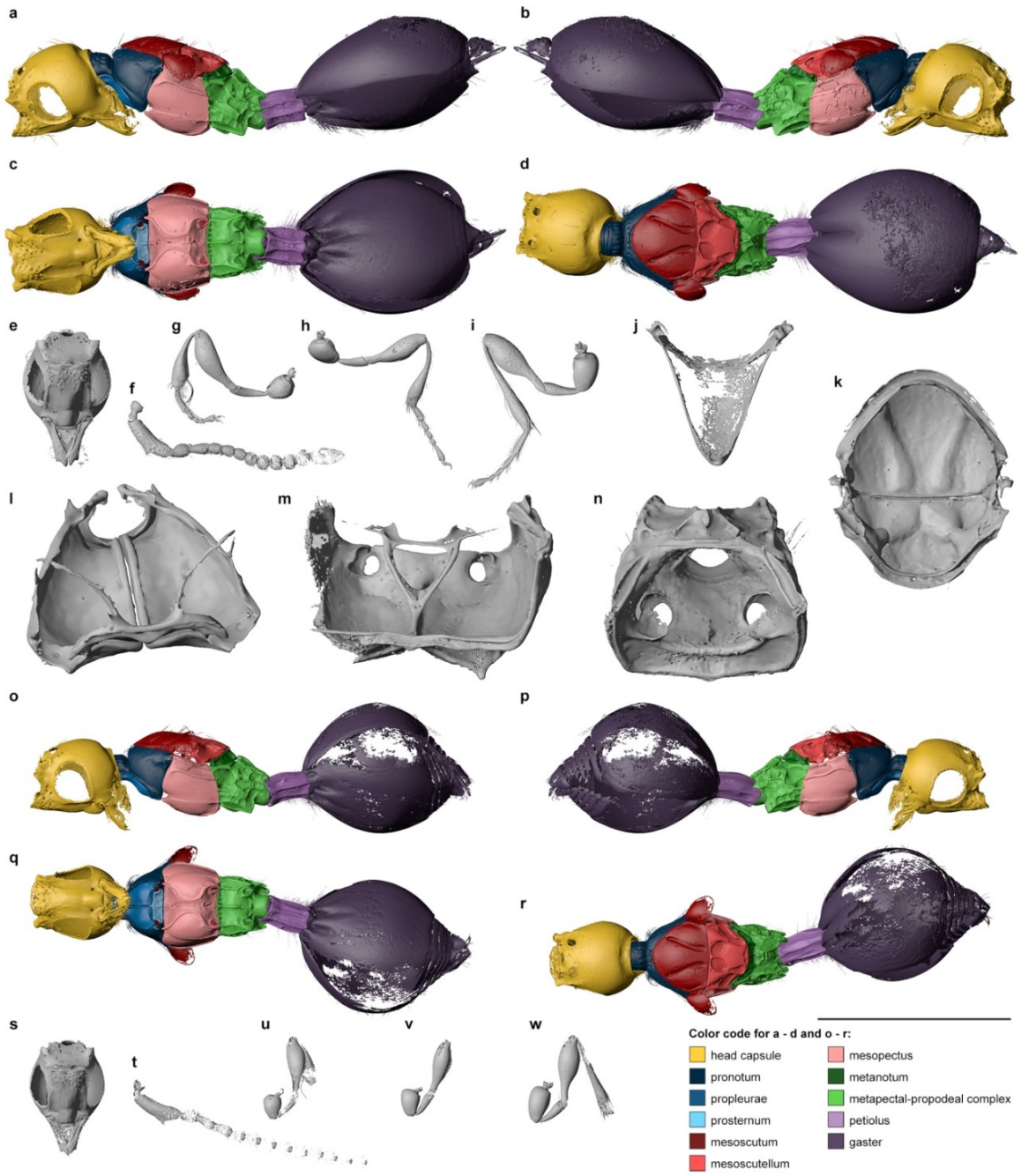


Palaeortona quercyensis

Diapriidae

Diapriinae

Psilini



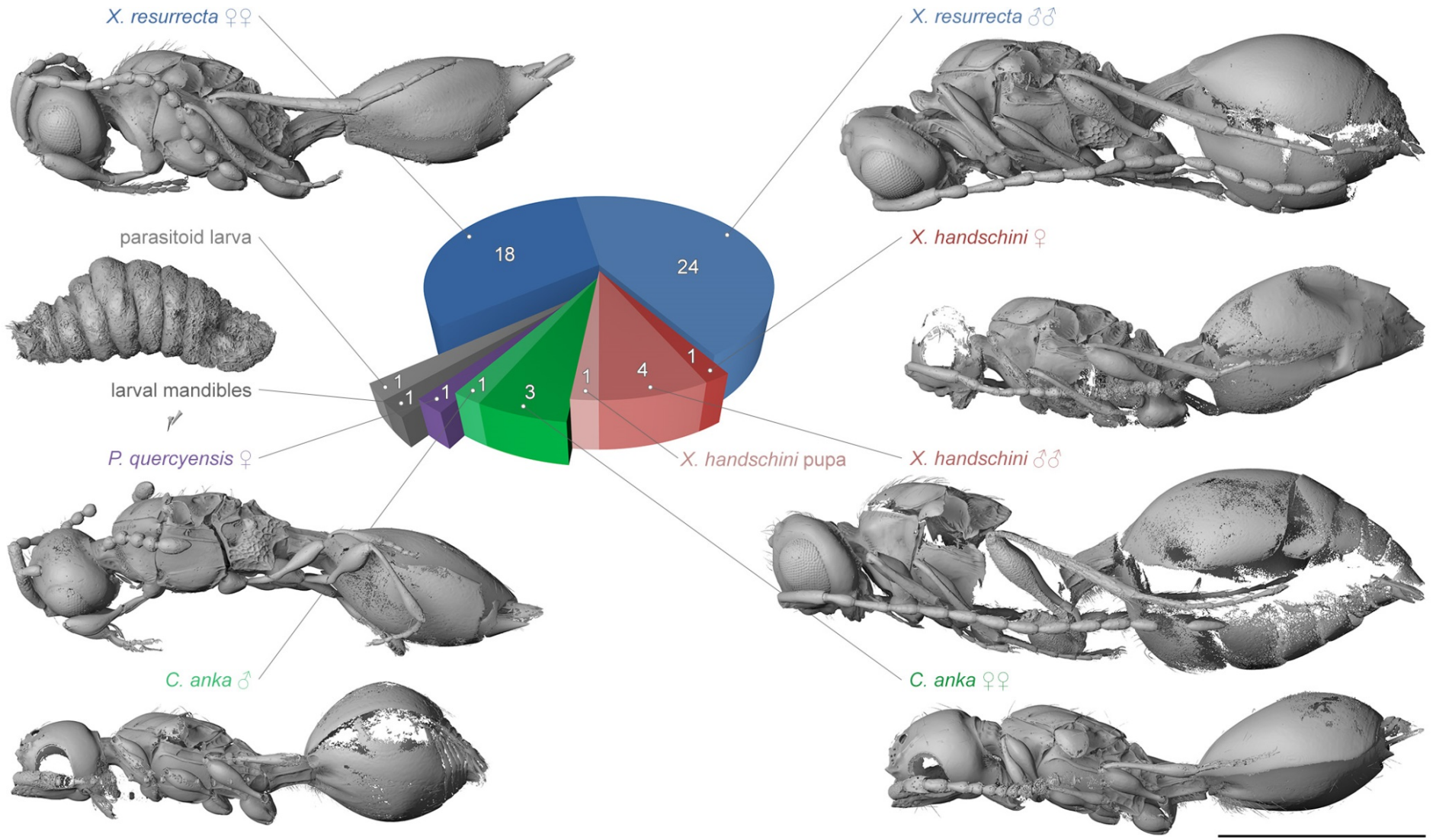
Karlsruhe Institute of Technology

Coptera anka

Diapriidae

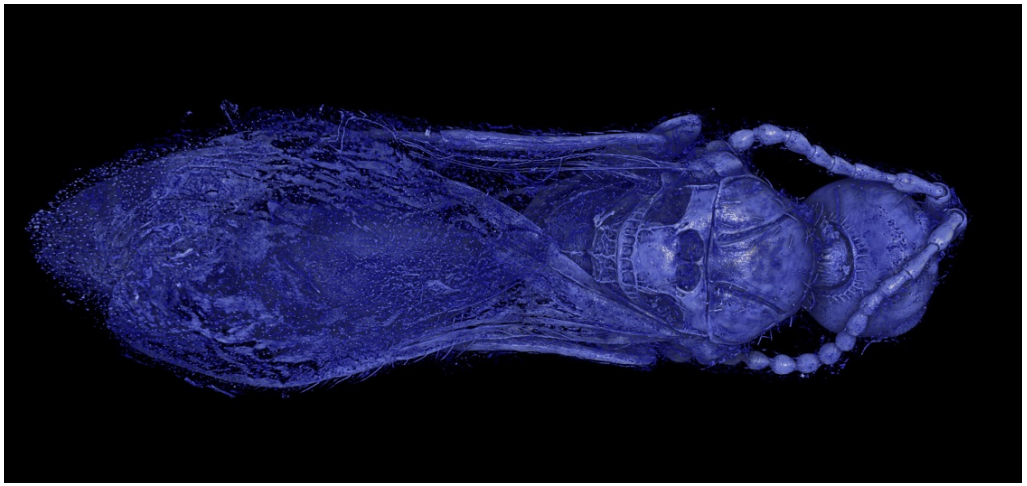
Diapriinae

Psilini


X. resurrecta ♀♀
X. resurrecta ♂♂
parasitoid larva
X. handschini ♀
larval mandibles
P. quercyensis ♀
X. handschini pupa
X. handschini ♂♂
C. anka ♂
C. anka ♀♀
18
24
1
4
1
3
1
1
1
1

Results

- Four species, three preserved with both sexes
- One unknown parasitoid larva
- One complete fly and many separate parts like legs, eyes etc.
- Ecological information
  - Sex ratio
  - Size variation
  - Parasitism rate
  - Type of parasitism
  - Hosts

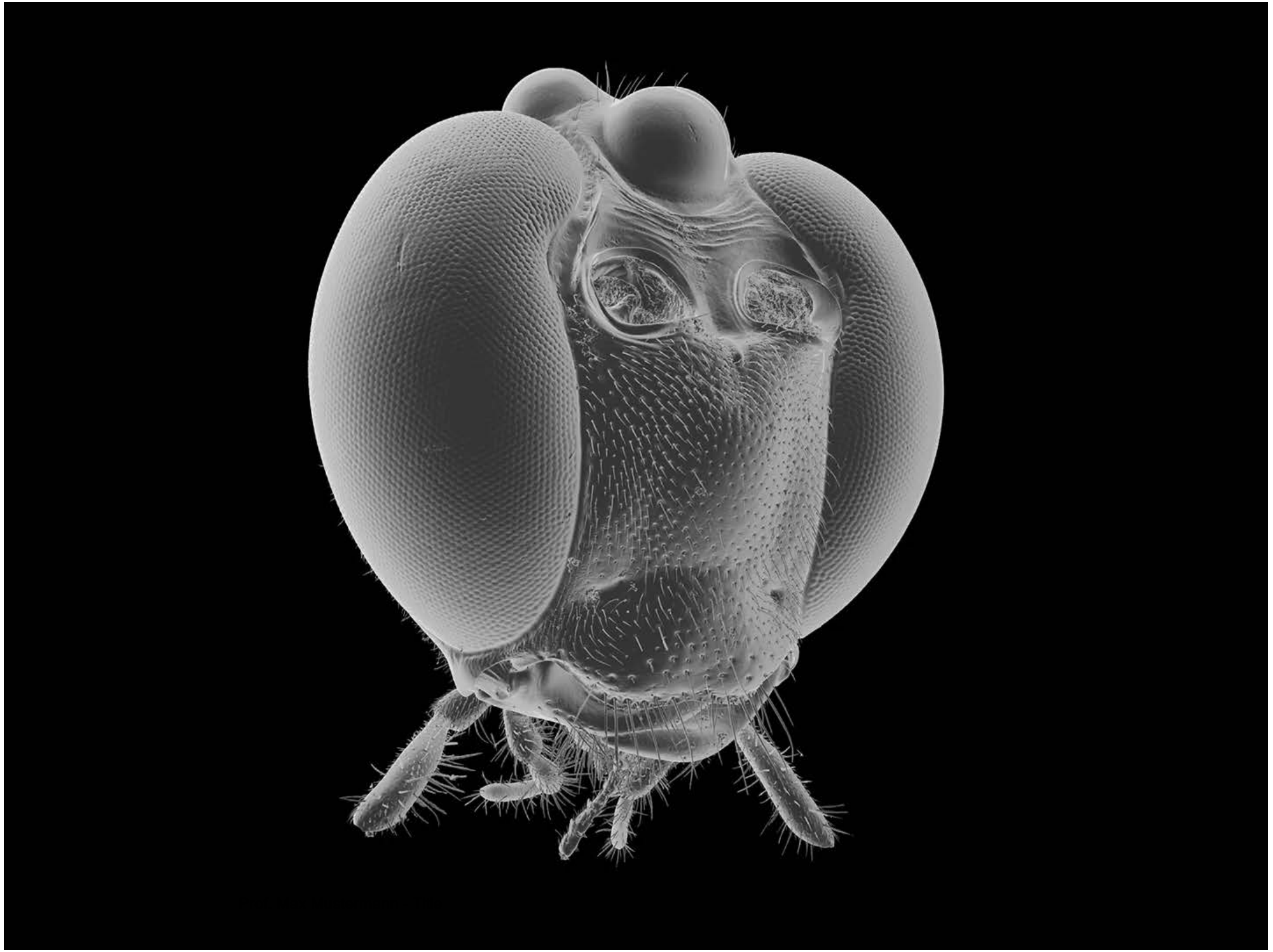


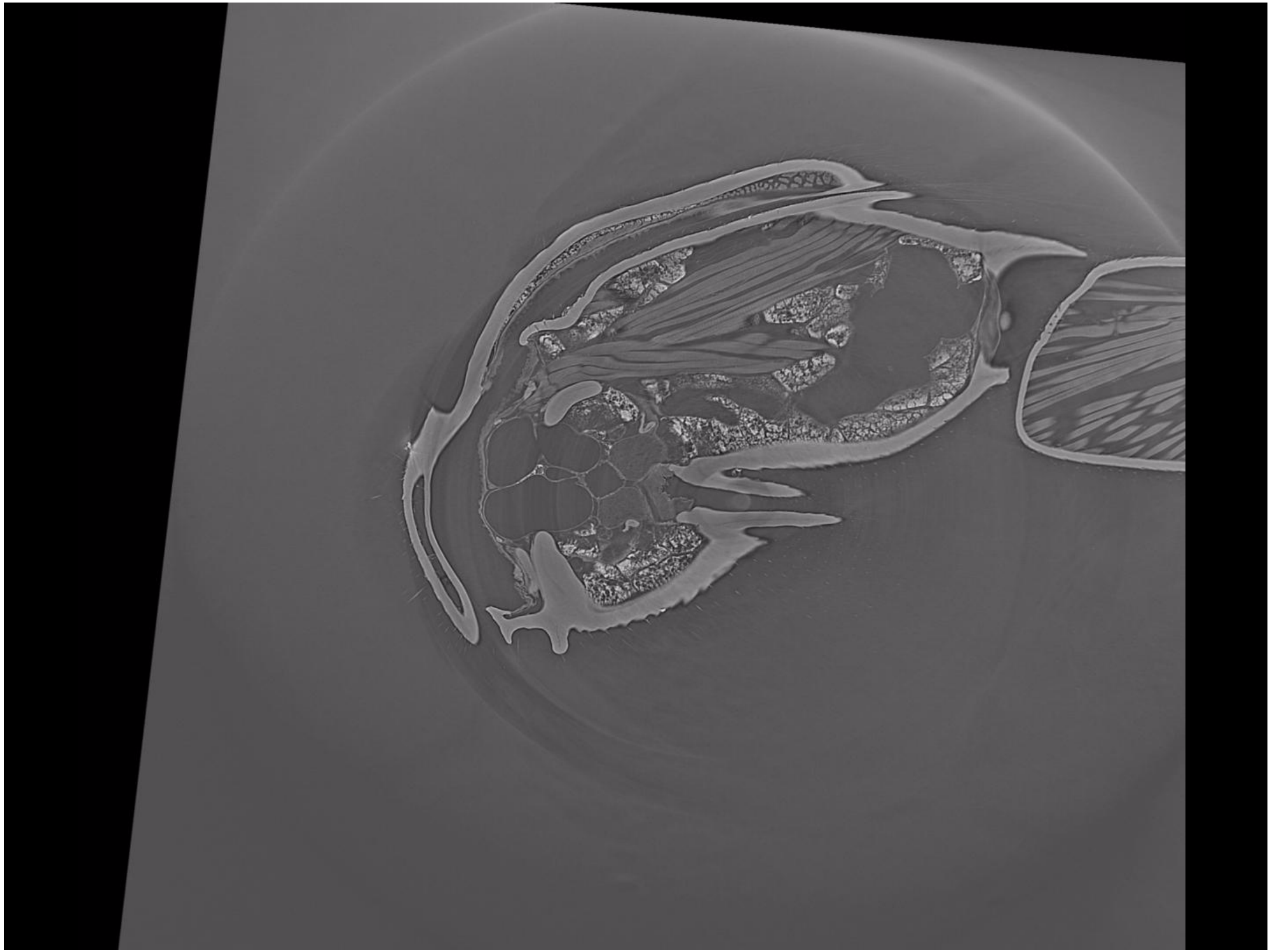
Illustration

Perspectives

Perspectives

- Digitization of morphological data becomes increasingly important
- State-of-the-art digital 3D imaging techniques facilitate entirely new insights into small animals
- Huge potential for scientific discoveries
  - morphology, biomimetics, palaeontology, biodiversity etc.
- Automation of data analysis is crucial for processing large amounts of specimens





Acknowledgements

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Matthias Vogelgesang
Marcus Zuber

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- **ASTOR: A**rthropod **S**tructure Revealed by Ultra-fast **T**omography and **O**nline **R**econstruction
- **NOVA: N**etwork for **O**nline **V**isualization and synergistic **A**nalysis of tomographic data

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