

Readme

This README file includes the Online supplementary files for the manuscript "Moulting and development in a freshwater prawn from the Late Cretaceous of Morocco" by

Sinéad Lynch, Pierre Gueriau, Harriet B. Drage, Didier B. Dutheil, Sylvain Charbonnier, Nora Corthésy, Javier Luque, Cristina Martin-Olmos, Allison C. Daley

The Online Supplementary Material is organised as follows:

The Online Supplementary Material 1 contains: Accession number, measurements, moult/carcass assignment, moult evidence type, and developmental stage assignment for each specimen studied

The Online Supplementary Material 2 contains: The R-code analysing the measurements, moult/carcass assignment, moult evidence type, and developmental stage assignment

The Online Supplementary Material 3 contains: The raw images used in the figure of this paper, as well as the multispectral images in TIFF format

The Online Supplementary Material 4 contains: The spectral data

The Online Supplementary Material 5 contains: The R script used to analyse the spectral data

Programs used:

Online Supplementary Material 1: Microsoft Excel (Microsoft 365, Version 2505, Build 16.0.18827.20102)

Online Supplementary Material 2: R (version 4.2.1); ggplot2 package (version 3.4.4)

Online Supplementary Material 4: Microsoft Excel (Microsoft 365, Version 2505, Build 16.0.18827.20102)

Online Supplementary Material 5: R (version 4.2.1)

File Formats and Compatible Software:

Online Supplementary Material 1: .xlsx (excel; version used for R-script) and .csv (plain text format that can be opened with free spreadsheet software or any text editor)

Online Supplementary Material 2: .R (R-studio or other text editor)

Online Supplementary Material 3: PNG, JPG and TIFF (any standard photo viewer or image editing software); multispectral TIFF (ImageJ)

Online Supplementary Material 4: .xls (excel; version used for R-script) and .csv (plain text format that can be opened with free spreadsheet software or any text editor)

Online Supplementary Material 5: .R (R-studio or other text editor)

File names and organisation in Online Supplementary Material 3:

Each figure in the manuscript has a corresponding folder including the figure and its raw images.

Figure 3: - Files are organised into folders based on the type of imaging used (Multispectral, UV, Visible). Within each of these folders, subfolders are named according to the corresponding figure panel letter and the specimen's accession number.

- These contain raw PNG or JPG. For visible light (Filename = Accession number_Imaging type). For UV light (Filename = Accession number_Imaging Type_Number Of Views_Camera Setting).

- The multispectral contains a PNG of the false-colour RGB overlays and the TIFF file containing the raw grayscale images used for this false-colour RGB overlays (Filename = "R"_RedCombinationxIlluminationWavelengthFFilterNumber-exposuretime_"G"_GreenCombinationxIlluminationWavelengthFFilterNumber-exposuretime_"B"_BlueCombinationxIlluminationWavelengthFFilterNumber-exposuretime.TIFF)

Figure 5: - These folders are named according to the corresponding figure panel letter and the specimen's accession number.

- These all contain BSE raw TIFF, the same image is either with the legend included (Filename = Accession number_"BSE"_"legend"), without (Filename = Accession number_"BSE"), or both.

Figure 7 and 8: - These folders are named according to the corresponding figure panel letter and the specimen's accession number; If an image shows a morphological detail captured than this is mentioned in the title (Filename = Panel Letter, accession number, and body part); If the image was taken using an imaging method other than standard visible light (e.g., SEM, Keyence, UV), the technique is indicated in the filename (Filename = Panel Letter, Accession number, body part, and imaging technique when necessary)

- These are contains raw PNG, JPG or raw TIFF (Filename = Accession number_Imaging type)

- In BES raw TIFF, the image is either with legend included (Filename = Accession number_"BSE"_"legend"), without (Filename = Accession number_"BSE"), or both. One is a calcium-eds map, which is specified in the filename and folder with ("BSE-EDS calcium map").

Figure production:

- Adobe Photoshop (22.5.1); Adobe Illustrator (25.4.1); Affinity Designer (2.6.3); Affinity photo (2.5.3); ImageJ (1.53t)

Data collection:

Online Supplementary 1: The measurements were made on scaled digital images of specimens using Adobe Photoshop 22.5.1.

Online Supplementary 4: UV–vis–NIR emission spectroscopy was measured on the cuticle of moults and the cuticle and muscles of carcasses, using a Specbos 1211UV spectroradiometer (JETI). UV illumination was provided by a U1c 6W 365 nm UV LED flashlight (JAXMAN), and a long-pass filter (cutoff wavelength: 410 nm) was placed in front of the detector to remove diffuse reflection of the excitation by the sample. Spectra (up to 1000 nm) were collected from ~1 mm² area pinpointed using the spectroradiometer's internal target spot laser.

Imaging details:

- UV and Visible: Specimens were photographed under visible and UV light using a Canon camera (EOS-800D) equipped with a macro lens (EFS 60 mm 1:2.8) and the EOS Utility photo software. UV light was applied with a 365 nm UV LED flashlight (JAXMAN U1c, 6W).
- Keyence: Digital microscopy images were taken with a Keyence microscope (VHX-7000) fitted with a dual objective zoom lens (VH-ZST), using the ZS-20 macro lens (20-200x).
- Backscattered electron micrographs (BSE): Images of the cuticle macrostructure were captured using a Zeiss Gemini 500 scanning electron microscope (SEM) in variable pressure (environmental) mode on uncoated specimens. The SEM was operated with the backscattered electron detector (BSD), at an electron high tension voltage (EHT) set to 15 kV, an aperture of 60 µm and the chamber pressure maintained at 40 Pa during imaging.
- The BSE-EDS calcium map: was generated using the same settings as described above, with the Oxford AZtec Microanalysis System (Oxford X-max 150 detector, software version 4.2 SP1; Oxford Instruments, High Wycombe, UK)
- Multispectral: Using a broad spectrum of light wavelengths from UV-A to the near-infrared (NIR) to enhance contrast within the fossil remains. Each selected carcass (MHNK-KK-OT 75a, 60a, 78a, UC 102-1) and moult (MHNK.F.A88748, MHNK-KK-OT 82b, 64b, UC 102-2, UC 135-2) was imaged under the same conditions (i.e. same distance from the camera, same LED intensity, same exposure time for three illumination-emission wavelength combinations : 385 nm / 472±15 nm (luminescence), 660 nm / 719±30 nm (reflectance + luminescence) and 460 nm / 472±15 nm (reflectance), which each produce a grayscale image. For each specimen, the three resulting grayscale images were combined into false-colour RGB overlays (red: illumination 385 nm / detection 472±15 nm, green: 660 nm / 719±30 nm (green) and blue, 460 nm / 472±15 nm)