

NEW METHODS TO DOCUMENT FOSSILS FROM LITHOGRAPHIC LIMESTONES OF SOUTHERN GERMANY AND LEBANON

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ABSTRACT

We present different documentation methods tested on fossil specimens from Solnhofen-type lithographic limestones (Upper Jurassic, southern Germany) and from the related deposits from the Upper Cretaceous of Lebanon. One of the principles is composite imaging. This combines image fusion, i.e., coalescing several images of the same area but at different focal planes, resulting in a single image of high depth of field, and image stitching, i.e., combining fused images of several areas to a high resolution image of the complete specimen. The basis for the composite images can be normal light images, but most fossils from Solnhofen-type lithographic limestones are autofluorescent under UV light such that UV-fluorescence images can be equally well applied. In this context, we report a new fluorescence type for specimens not showing good UV fluorescence, i.e., those from the Zandt lagerstätte or some from Lebanon. These specimens fluoresce orange when exposed to green light. Specimens from Lebanon exhibiting green-orange fluorescence have been documented under a confocal laser scanning microscope (cLSM). Fossils showing a relatively high relief can be documented with stereo images; based on these surfaces, 3D models can be produced. A large specimen preserved uncompressed has been documented using a medical X-ray computer tomography scanner. All these methods facilitate the high-resolution documentation of complete specimens (= "virtual specimens"). Specimens from both Solnhofen-type lithographic limestones and Lebanon have further been examined for their elemental composition using energy dispersive X-ray spectroscopy (EDX). The fossils differ significantly from the surrounding matrix by containing 6–14% phosphorus.

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INTRODUCTION

Fossils from the Solnhofen Lithographic Limestones and similar deposits of the Upper Jurassic of southern Germany are famous for their extraordinary preservation (we will unite these lagerstätten under the term ‘Solnhofen-type’ in this study). The crustacean fossils in particular yield significant information for reconstructing phylogenies (Schram and Dixon 2004), and it is one of the few fossil deposits where larval specimens have been found (Polz 1972, 1973, 1984, 1995; Haug et al. 2008). Comparable faunal composition and similarly-appearing preservation is known from fossils of the Upper Cretaceous of Lebanon (e.g., Dames 1886; Roger 1946; Garassino 1994).

Although the preservation of these fossils is rather good, extraction of all details from the specimens and the documentation of these in an appropriate way are still mainly based on macrophotography (e.g., Garassino and Schweigert 2006). An important enhancement for fossils from Solnhofen-type lithographic limestones is to expose them to UV light, as most of the fossils show autofluorescence at this wavelength (about 358 nm). This enhances the contrast between the fossil and the matrix enormously and can be used to visualize complete specimens that are weakly defined under normal light (e.g., Polz 1995; Garassino and Schweigert 2006) or to make unseen details visible (e.g., Polz 1993; for an extensive review on the use of UV light on fossils see Tischlinger 2002). For fossils from Lebanon, this contrast-enhancing method is usually not applied (Schram et al. 1999; Lange et al. 2001; Ah Yong et al. 2007, but see Fuchs et al. 2009; Pasini and Garassino 2009).

Initial attempts to document whole specimens under high resolution (Haug et al. 2008) have demonstrated that even small specimens, at first glance appearing to exhibit no details, can yield significant information. Therefore, we have further developed different methods related to the one described by Haug et al. (2008) for documenting specimens in two dimensions, and we also made

the first attempts to extract three-dimensional (3D) information from fossils with higher relief.

MATERIAL AND METHODS

The following specimens were used for testing the methods described below; for stratigraphy of the lithographic limestones of southern Germany see Schweigert (2007):

- Two specimens, possibly representing juveniles of *Cancrinus claviger* Münster, 1839 (Figures 1.1–3, 4.1–4); found near Öchselsberg (Solnhofen Lithographic Limestones, Upper Kimmeridgian, Beckeri Zone, Ulmense Subzone) resp. Langenthalheim (Solnhofen Lithographic Limestones, Lower Tithonian, Hybonotum Zone, Rueppellianus Subzone); private collection of Matthias Wulf, Rödelsee;
- One specimen of *Scalda spinosa* Kunth, 1870, preserved with part and counterpart (Figure 1.4–6); found in the Nusplingen Lithographic Limestone (Upper Kimmeridgian, Beckeri Zone, Ulmense Subzone); collection of the Staatliches Museum für Naturkunde, Stuttgart (SMNS 63997/1);
- One specimen of ?*Scalda* sp. (Figure 2) from the Upper Cretaceous (Cenomanian) of Hadjoula, Lebanon; private collection of two of the authors (CH & JTH);
- One specimen of *Scalda pennata* Münster, 1840 (Figure 3); found near Zandt (Solnhofen Lithographic Limestones, Lower Tithonian, Hybonotum Zone, Riedense Subzone); collection of the Staatliches Museum für Naturkunde, Stuttgart (SMNS 67505);
- One specimen of *Antrimpos* sp. (Figure 4.6–8); found near Solnhofen (Solnhofen Lithographic Limestones, Lower Tithonian, Hybonotum Zone, Rueppellianus Subzone); private collection of one of the authors (RF);
- One specimen of an undetermined caridean shrimp; found near Eichstätt (Solnhofen Lithographic Limestones, Lower Tithonian,

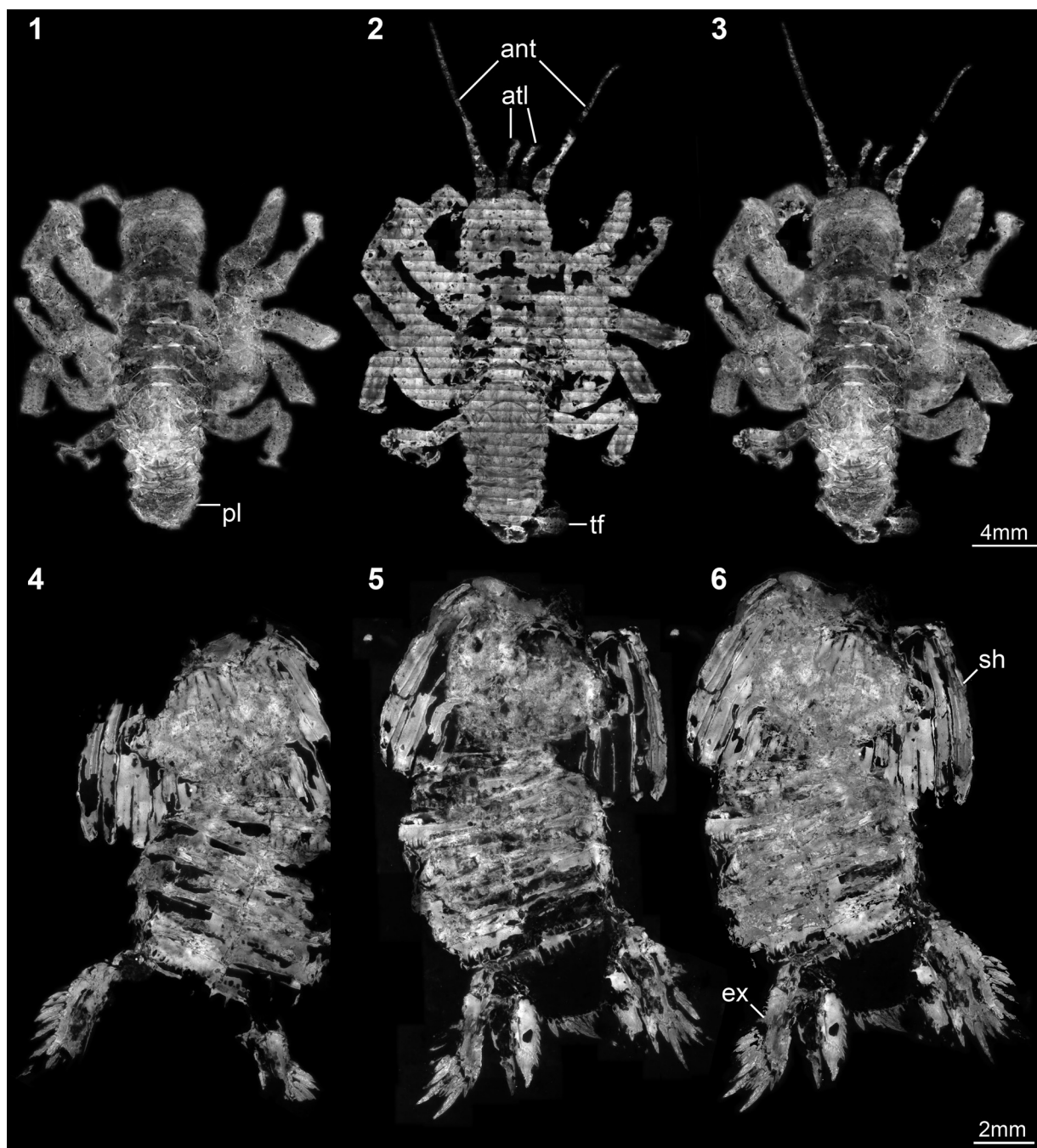


FIGURE 1. Composite images of fossil specimens from Solnhofen-type lithographic limestones. All parts of the images not displaying fossil substance were virtually removed, except for image 1.5. **1.1–3.** Composites of the smaller juvenile of *Cancrinos claviger* MÜNSTER, 1839 (Öchselberg, Solnhofen Lithographic Limestones, Upper Kimmeridgian, Beckeri Zone, Ulmense Subzone); private collection of Matthias Wulf, Rödelsee. **1.1.** Composite image with normal light. Image inverted. The pleon (pl) appears three-dimensional. **1.2.** Composite with UV light. Note the antennulae (atl), antennae (ant) and tail fan (tf), which were not visible under normal light. **1.3.** Combined image 1.1 projected on 1.2. **1.4–6.** UV-fluorescence composite of the specimen of *Sculda spinosa* Kunth, 1870 (Nusplingen Lithographic Limestone, Upper Kimmeridgian, Beckeri Zone, Ulmense Subzone); collection of the Staatliches Museum für Naturkunde, Stuttgart (SMNS 63997/1). **1.4.** Counterpart. **1.5.** Part. Note the clear contrast between fossil substance and matrix, which allows virtual removal of the matrix. **1.6.** Combination of 1.4 projected on 1.5. Note the amended shield (sh) and the uropodal exopod (ex).



FIGURE 2. Composite images of a specimen of ?*Sculda* sp. (Hadjoula, Lebanon, Upper Cretaceous, Cenomanian); private collection of two of the authors (CH & JTH). **2.1.** Green-orange-fluorescence composite. Note the visible flagella of the antennula (atl), and the movable spines on the outer margin of the uropodal exopod (ex). Other abbreviations than before: mxp2: second maxilliped. **2.2.** Composite under normal light of the same specimen as in 2.1. Note the missing details on antennulae (atl) and uropodal exopod (ex).

Hybonotum Zone, Riedense Subzone); private collection of one of the authors (RF).

Composite imaging:

General settings: Specimens were documented exhaustively under high resolution. As the specimen is larger than the field of view, several overlapping images are taken to document the complete specimen in an x- and y-axis. Each single area was documented not with a single image, but with a stack of images, i.e., a number of images with a shifting plane of focus in the z-axis. The images of each stack were fused with the freely available image fusion software CombineZM. These resulting images were then combined in the x- and y-axis, partially automated via the photomerge function and/or by hand in Photoshop and the freely available software program GIMP. The result is a high-resolution composite image. The

exact number of images for each specimen and applied light setting are given in Table 1.

Specifically, we used three different types of light:

Normal light: Two possible specimens of *Cancrinos claviger* and one of ?*Sculda* sp. were documented under a Leica stereomicroscope with a mounted DCM 500 ocular camera. For homogeneous lighting a ring lamp was used. To produce stacks the camera was set to take an image every two seconds, while the focus was progressively shifted manually.

Ultraviolet: The smaller of the two specimens of *Cancrinos claviger* and additionally a specimen of *Sculda spinosa* (part and counterpart) were documented under UV light (358 nm) on an Axio Scope 2 with a mounted Axiocam.

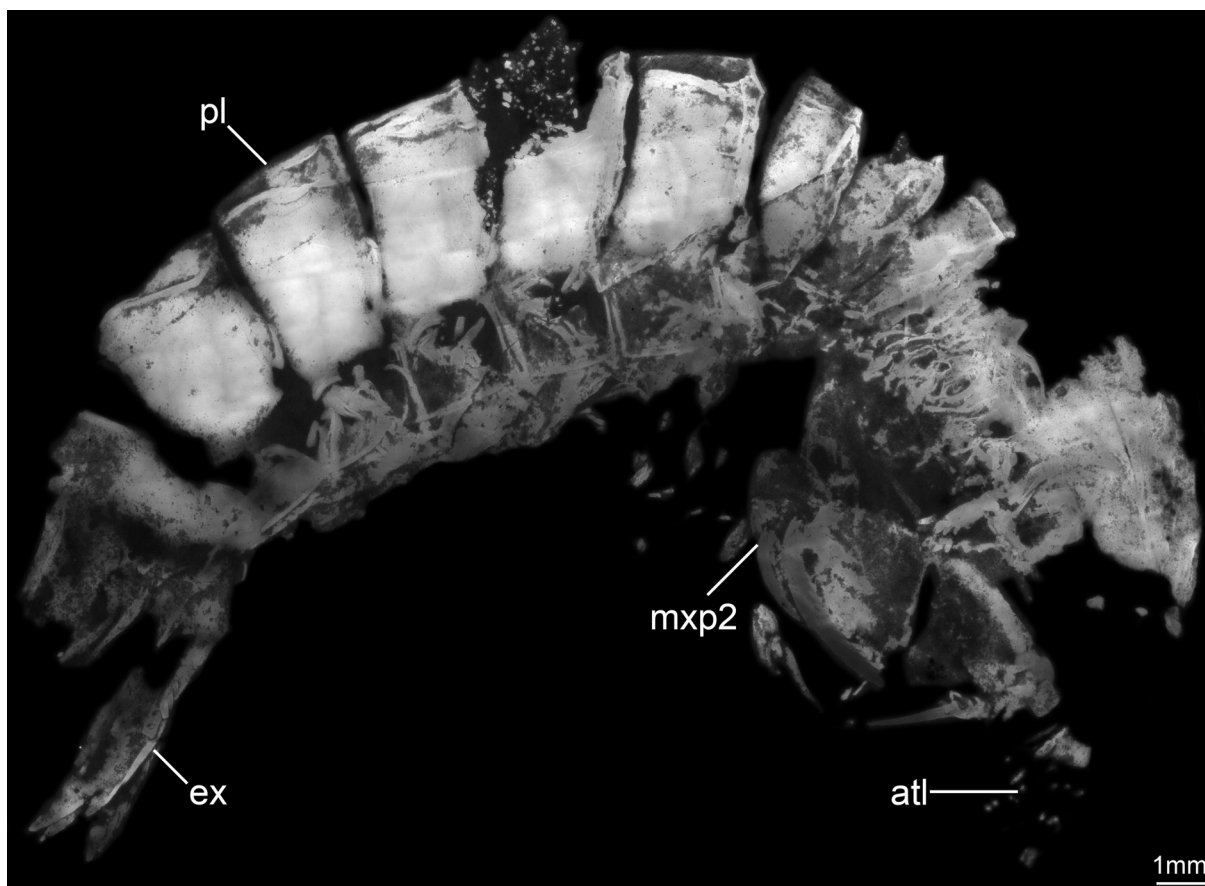


FIGURE 3. Green-orange-fluorescence composite of a specimen of *Sculda pennata* MÜNSTER, 1840 (Zandt, Solnhofen Lithographic Limestones, Lower Tithonian, Hybonotum Zone, Riedense Subzone); collection of the Staatliches Museum für Naturkunde, Stuttgart (SMNS 67505); preserved in lateral aspect. Outer part of the matrix virtually removed. Abbreviations as before.

Green light: A specimen of *Sculda pennata* and a specimen of ?*Sculda* sp. were both documented under green light (546 nm). All other settings were the same as for the documentation with UV light.

Combining UV-fluorescence images of part and counterpart: Part and counterpart of the specimen of *Sculda spinosa* (see above) were digitally combined. All black areas on the counterpart were set as transparent using the magic wand tool in Adobe Photoshop (CS3). The composite image of the counterpart was then mirrored and placed above the composite image of the part (terms 'part' and 'counterpart' are exchangeable in this special specimen as it is extremely flattened and both parts contain a lot of substance of the fossil; in general it would be useful to leave the part with more substance unaltered while processing the other one with transparency).

Combining normal light images with a UV-fluorescence image of the same specimen: The composite images of the smaller of the two speci-

mens of *Cancrinus claviger*, one under normal light, one under UV light, were combined into a single image. For this purpose, the image under normal light was inverted, and all parts not assignable to the fossil were set transparent. The resulting image was placed on the UV image.

3D attempts:

Confocal laser scanning microscopy (cLSM): Details of the specimen of ?*Sculda* sp. were documented under a Leica cLSM. The excitation wavelength was set to a range of 488–543 nm. In total the stack was made up of 43 images. The resulting stack of images was further processed using the freely available software program ImageJ, where the 'find edges' function was used to enhance contrast. The resulting stack was 3D-projected (Maximum Intensity Projection, MIP) using the freely available DICOM-viewer OsiriX.

Stereo images and 3D models via Structure from Motion (SFM): The pleon of the larger possi-

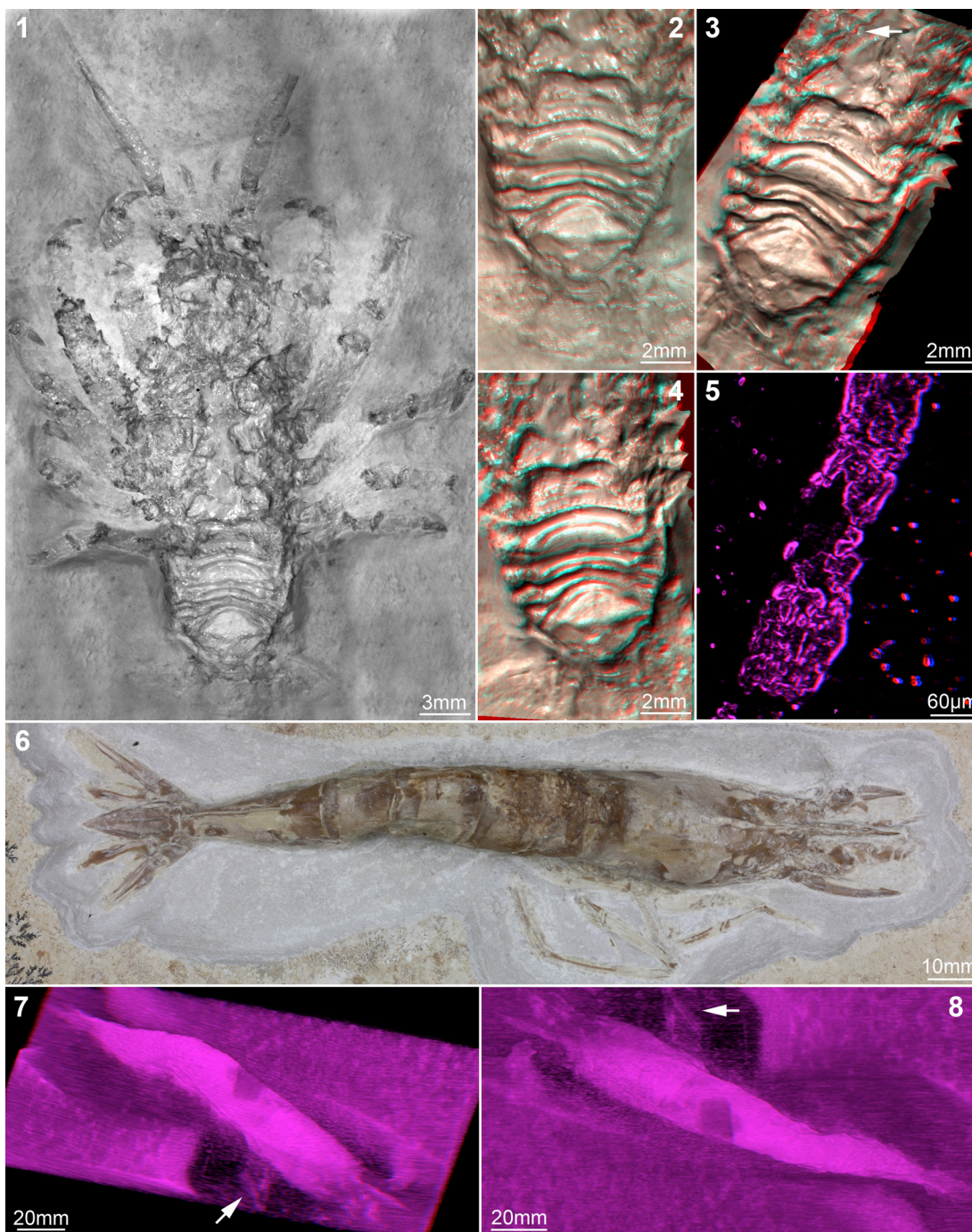


FIGURE 4. Attempts to extract 3D information from fossils of the lithographic limestones. **4.1–4.** A larger juvenile of *Cancrinus claviger* MÜNSTER, 1839 (Langentaltheim, Solnhofen Lithographic Limestones, Lower Tithonian, Hybonotum Zone, Rueppellianus Subzone); private collection of Matthias Wulf, Rödelsee. **4.1.** Composite with normal light. **4.2.** Red-cyan stereo image of the pleon of the real specimen in top view. **4.3.** Red-cyan stereo image of the 3D-surface model based on the stereo image (4.2), oblique view. Arrow pointing to one distortion, an artificial hump. **4.4.** As 4.3, but in top view. **4.5.** Red-blue anaglyph of some annuli of the antennula of a specimen of ?*Scalda* sp. (cf. Figure 2) under cLSM. **4.6–8.** An uncompressed *Antrimpos* sp. (Solnhofen, Solnhofen Lithographic Limestones, Lower Tithonian, Hybonotum Zone, Rueppellianus Subzone); private collection of one of the authors (RF). **4.6.** Macrophotography in almost dorsal view. **4.7–8.** Blue-red stereo image of a volume rendering of the CT data. Arrows mark slight indications of the pereopods. **4.7.** In oblique view, anterior to the right. **4.8.** In top view, anterior to the left.

TABLE 1. Numbers of images taken in total, number of image stacks and maximum number of images taken per stack for every specimen documented. If a specimen had been documented with different light settings, the numbers are given for the different settings separately, as well as for part and counterpart in one specimen of *Sculda spinosa*.

Specimens	Settings	total number of images	number of image stacks	max. number of images per stack
<i>Cancrinos claviger</i> (smaller specimen)	normal light	40	7	6
<i>Cancrinos claviger</i> (smaller specimen)	UV light (358 nm)	> 5000	> 400	33
<i>Cancrinos claviger</i> (larger specimen)	normal light	360	33	24
<i>Sculda spinosa</i> (part)	UV light (358 nm)	1894	104	40
<i>Sculda spinosa</i> (counterpart)	UV light (358 nm)	1812	100	38
<i>Sculda pennata</i>	green light (546 nm)	1146	168	10
? <i>Sculda</i> sp.	normal light	28	6	5
? <i>Sculda</i> sp.	green light (546 nm)	1199	107	19

ble specimen of *Cancrinos claviger* was documented as a stereo image, produced with a tiltable Zeiss Stemi 1000 stereomicroscope with a mounted DCM 500 ocular camera (tilting of about 6°). The images were then loaded into the freely available test version software “Structure from Motion” (MeeSoft) (for principles of this method see Dellaert et al. 2000). Five reference points were assigned, i.e., corresponding structures on the two single images of the stereo image were marked. Based on this, a 3D model was calculated.

Computer tomography (CT) scans: A large uncompressed specimen of *Antrimpos* sp. was scanned in a medical CT-scanner (Philips Brilliance iCT 256) under the setting for small structures (resolution of 0.667 mm) for very heavy patients to enhance the emitted energy. The resulting stack of 402 images was processed using the freely available DICOM-viewer OsiriX (volume rendering).

SEM-EDX analysis:

The specimen of ?*Sculda* sp. and the specimen of an undetermined caridean shrimp were put into the SEM (ZEISS DSM 962) and their elemental composition analysed using a mounted energy dispersive X-ray spectroscopy (EDX) unit. The acceleration voltage used was 25 kV.

RESULTS AND DISCUSSION

Composite imaging:

The method of composite imaging was adopted successfully from palaeobotany (Bomfleur et al. 2007) for documenting fossils from Solnhofen-type lithographic limestones (Haug et al. 2008). As the specimen is documented at high resolution under a microscope, small details, not always resolved via macrophotography, can be visualised, and information from small specimens becomes available (Haug et al. 2008). Because the specimen is usually larger than the field of view, several overlapping images are taken to document the complete specimen in the x- and y-axis in order not to omit any possible details of interest. For moving the specimen in the x- and y-axis, placing the specimen on a movable platform (present in every microscope) is advantageous, as the later process of combining the images is much faster if one need not rotate the images, but only move them. Additional to moving the specimen in the x- and y-axis, it is necessary also to take several images in the z-axis, i.e., to produce an image stack, as under the high resolution the flattened fossils of the lithographic limestones also show substantial relief and, therefore, are not completely sharp in one focal plane.

Stacks of images can easily be produced by hand using a typical mounted camera (Haug et al.

2009), but it is much faster with an automated stage, where one only has to define the upper and lower border, while the microscope automatically takes images in defined distance steps in z-axis within this defined range. An alternative for accelerating the process of producing stacks without having an automated stage is using a camera that is capable to take one image after a defined time. We used a DCM 500 ocular camera, which was set to take an image every two seconds, while the focus was shifted after each image by hand. Compared to the automated stage this is much more labour-intensive, but compared to taking each image by hand using a usual mounted camera (see Haug et al. 2009) the method with a time setting is much faster. The resulting fused images have a very high depth of field and are fully in focus.

The fused images then have to be combined in the x- and y-axis. Adobe Photoshop provides an automated tool for this purpose called photomerge. This tool is more reliable in newer versions (CS3) compared to older ones, but still is not able to faithfully combine many images produced for one specimen. Nevertheless, it can be used to combine images to stripes of up to about a dozen images. These stripes then have to be combined further by hand. Alternatively, the whole process can be done by hand also in Photoshop or freely available alternatives, e.g., GIMP.

The result is more or less a virtual specimen, which can be studied in detail later. Also detail images for publications can be extracted directly from the resulting composite image. This method is, therefore, extremely useful for material on loan, especially if obtained from private collections, or for providing direct virtual access, i.e., via databases.

Under normal light, i.e., using a stereomicroscope, it is important to provide homogenous lighting. A ring lamp proved to be the best choice for this purpose. Normal lighting has an advantage over the fluorescence methods because it gives a better impression of 3D structures. Specimens with a high relief are best documented under these settings (cf. Figure 4.1); such specimens are usually too large to be put under the fluorescence microscope anyway. For smaller specimens the use of fluorescence for contrast enhancement is very important. Small details as, for example, the flagellimeres of the antennulae, can only be seen under fluorescence (Haug et al. 2008) (Figure 1.1–2).

We were pleased to discover that specimens that were known to have an indistinct or weak fluorescence under UV, e.g., specimens from the Zandt lagerstätte close to Solnhofen and from Leb-

anon, show fluorescence under green light (Figures 2.1, 3). The specimens glow orange when exposed to green light of 546 nm wavelength. The contrast is slightly weaker than in specimens demonstrating UV fluorescence. When UV fluorescence can be applied, the matrix appears black, whereas in specimens exhibiting green-orange fluorescence the matrix appears only dark grey (Figures 2.1, 3). Nevertheless, it enhances the possibilities to spot tiny details, and especially for fossils from Lebanon, the enormous difference between normal light and green-orange fluorescence is apparent. For the specimen of ?*Sculda* sp. the exopod of the uropod is seen under normal light only as a simple stripe, whereas under green-orange fluorescence the teeth on the outer margin become visible (Figure 2). An advantage of using green-orange fluorescence is that, in contrast to UV light, minute dust particles always present on fossils do not glow under green light. In cases when the specimen exhibits fluorescence both under UV light and green light, the use of green light may be preferable for this reason.

Combination of fluorescence images of the part and counterpart can easily be applied, as the contrast to the matrix allows the virtual extraction of the substance of the counterpart of the specimen and the addition of this substance to the part. In the case of the *Sculda spinosa* specimen mainly the shield and the exopod of the left uropod become much more complete compared to the single images (Figure 1.4–6).

The combination of different composite images further enhances the visualisation of all information from a specimen. As already stated, normal light settings provide a better impression of 3D structures, whereas UV fluorescence makes small structures visible. The combined image of the small specimens probably representing a juvenile of *Cancrinus claviger* compared to the exclusively normal light image and the exclusively UV-fluorescence images demonstrates that the combined image apparently mediates the best impression of the whole information of the specimen (Figure 1.1–3).

3D attempts:

Confocal laser scanning microscopy has become a very important tool for inferring morphologies in Recent animals (Zupo and Buttino 2001; Buttino et al. 2003; Michels 2007) and has been successfully applied to fossil material (Chi et al. 2006; Chen et al. 2007). The antennulae of the ?*Sculda* sp. specimen were documented under the

cLSM. The available cLSM could not perform UV light, therefore only orange-green-fluorescing specimens could be tested. The second candidate, the specimen of *Sculda pennata* from Zandt, is very flat, as is typical for fossils from this area, and contains no 3D information. Thus, the ?*Sculda* sp. was the only suitable specimen for applying this method. Most structures were simply too large and only some annuli of the antennulae could be documented. The three-dimensional information of these structures is rather limited, thus the result does not provide significant additional information (Figure 4.5).

A simple way for documenting 3D information is stereo imaging. We further processed these stereo images with programs (MeeSoft, Structure from Motion (SFM), see above) that are freely available and easily applicable. SFM is again a step towards producing "virtual specimens." The first advantage is that the depth impression can be varied. When comparing the original stereo image to that of the SFM-model in the same position, the stereo image of the SFM-model appears to have a much deeper impression. This can be used to improve stereo images, which had been documented under too small an angle (Figures 4.2, 4.4). It is, in principle, possible to rotate the calculated 3D model. When the original image is rendered directly onto the surface of the model, the impression is quite satisfying (Figure 4.3). When looking closer, smaller program-based distortions can be recognised (Figure 4.3). Thus, the algorithm appears to be as yet imperfect, but is adequate for specimens like those documented here. As the method is simple to apply using a stereomicroscope with a mounted camera and as the software is freely available, this method has the potential to become widespread for producing fast and cheap "virtual specimens."

CT scanning has become a popular method for investigating fossils, in some cases with very impressive results (Donoghue et al. 2006; Tafforeau et al. 2006). The CT scans of our fossil did not produce satisfying results. This was mainly due to the low resolution. Although we were allowed to use one of the most modern medical CT scanners worldwide, the resolution was not high enough to resolve the small structures of interest. As the specimen is embedded in a very large slab (Figure 4.6), it was not possible to place it into a μ CT-scanner, which can resolve structures of less than 1 μ m in size. Such scanners have provided interesting information on fossil specimens from related Lagerstätten of the Crato Formation (Grimaldi and Engel 2005). The results at least

show the potential to extract 3D information from lithographic limestone fossils with the aid of CT scans, as the fossil itself can clearly be distinguished from the matrix, for example one of the legs is recognizable (Figure 4.7–8, marked by arrow). CT-scanners with higher resolutions facilitating the investigation of such large specimens will be tested in the future.

Elemental composition analysis:

EDX analysis was performed mainly as a by-product. The original idea was to test whether the method of enhancing the contrast of fossils with SEM using back-scattered electrons described by Orr et al. (2002) for organically preserved fossil ostracods could be applied to fossils from the lithographic limestones. The result was not successful.

EDX analysis showed significant differences in element composition between the fossils and the matrix. While both the matrices and the fossils contained oxygen, carbon and calcium, both fossils, but not their matrices, contained phosphorus, about 6% in the Solnhofen specimen and about 14% in the Lebanese specimen (Figure 5). This interesting initial result has to be investigated further. It is not clear whether this finding can explain the fluorescence capacities of the fossils, especially as the ?*Sculda* sp. shows orange-green fluorescence, while the undetermined caridean shrimp shows UV fluorescence. Tischlinger (2002) already pointed to the fluorescence capacities of calcium phosphate, but he referred to bones and did not discuss the composition of fossil arthropod cuticle.

CONCLUSIONS

Composite imaging enhances the documentation possibilities for lithographic limestone fossils. Composite imaging can be applied under normal light and fluorescence settings. UV fluorescence exhibits the best contrast between the fossil and its matrix. Fossils not showing good UV fluorescence may show green-orange fluorescence. This finding is new and holds true for fossils from Zandt and from Lebanon. Especially for fossils from Lebanon, green-orange fluorescence makes structures visible that are completely invisible under normal light. Composite images can be further combined to optimise the visualisation of specimens, e.g., by combining several lighting methods or combining the part and counterpart.

3D information from specimens from the lithographic limestones can be documented, too. Stereo images and further processing via Structure from Motion appears to be promising. Fluorescing

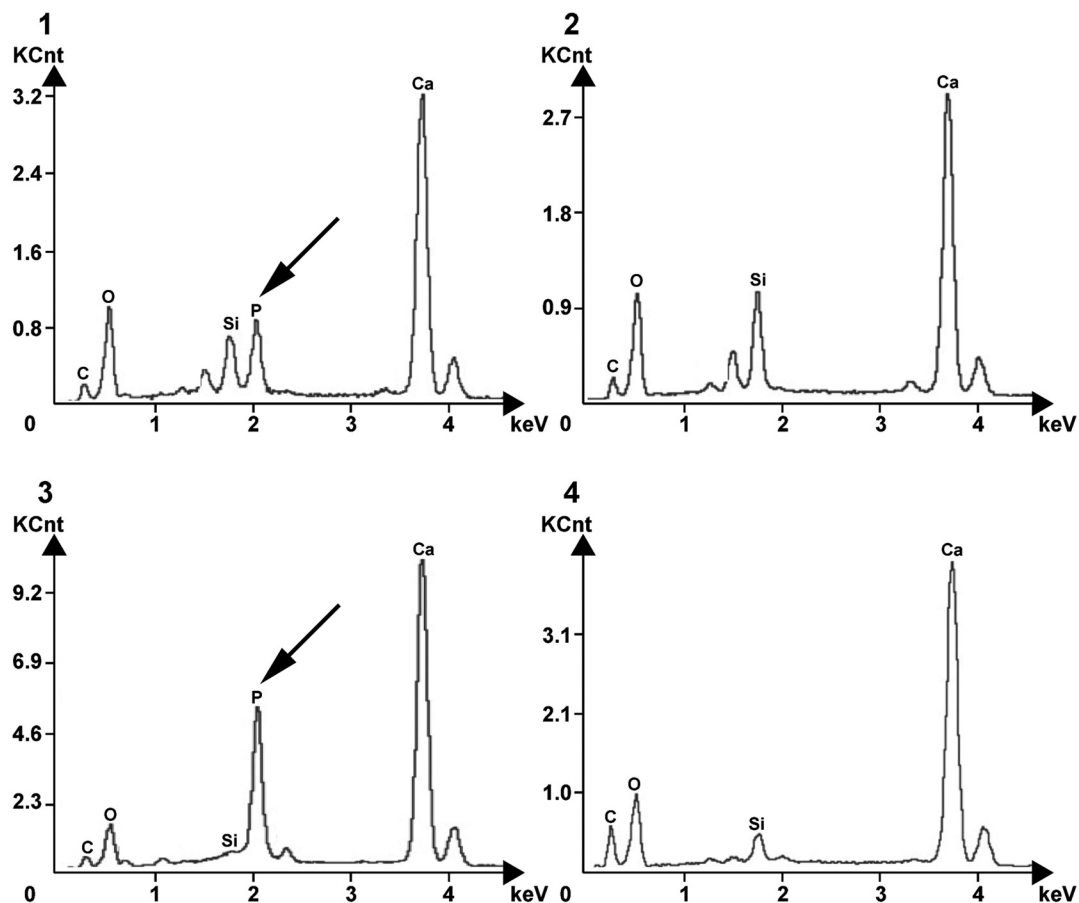


FIGURE 5. EDX spectra of an undetermined caridean shrimp (Eichstätt, Solnhofen Lithographic Limestones, Lower Tithonian, Hybonotum Zone, Riedense Subzone) (5.1) and its matrix (5.2), as well as of ?*Sculda* sp. (Hadjoula, Lebanon, Upper Cretaceous, Cenomanian) (5.3) and its matrix (5.4). X-axis shows the energy of the electron beam in keV, y-axis shows the counts of thousands of gamma-photons (kCnts). Beam times were for 5.1 = 45 sec., 5.2 = 36 sec., 5.3 = 100 sec. and 5.4 = 65 sec. Abbreviations for the elements: C = carbon, Ca = calcium, O = oxygen, P = phosphorus, Si = silicon. Note the peak of the phosphorus in 5.1 and 5.3, missing in 5.2 and 5.4.

specimens can be documented, or at least small details from them, with cLSM. X-ray tomography appears to be possible in principle, but resolution is at the moment still limited.

EDX element analysis indicates that fossils contain phosphorus; whether this is correlated to the fluorescence capacities of the fossil remains unclear at the moment. The presented method will be used to further investigate small specimens in particular from the lithographic limestones.

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