



Comparison of methods: Micro-CT visualization method and epoxy cast-embedding reveal hidden details of bioerosion in the tube walls of Cretaceous polychaete worms

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ABSTRACT

Bioerosion in three serpulid tubes of the (sub-)genera *Cementula*, *Pyrgopolon* (*Septenaria*), and *Placostegus* from the Bohemian Cretaceous Basin was studied by a combination of micro-computed tomography and vacuum cast-embedding technique producing polymer resin casts. Results gained from both methods were evaluated and compared in terms of material usability, destructive force, quality of the resulting image, and hardware/software requirements. The advantage of the micro-CT methodology is its non-destructiveness and the ability to make three-dimensional images, animations, and serial sections through the object; this method is suitable for most examined materials, but it is limited by low quality of the resulting image and demanding hardware/software requirements. To ensure good visibility of the borings in the resulting X-ray reconstructions, a material contrast between hard substrate and borings is necessary. The advantage of the vacuum cast-embedding technique followed by SEM observation is high quality of the resulting image, but it is limited by destructive force and material composition. Vacuum casting is unusable for undissolvable silicified or pyritized substrates, as happened in our *Placostegus zbylavus* specimen, which suffered from incomplete dissolution in acid. However, epoxy casting provided detailed morphology of borings that were beyond the detection limit of the given spatial resolution of the micro-CT scanner, and helped with ichnotaxonomic identification. Tiny branching canals of *Iramena* in *Cementula* sp. and the finer apertural canals connecting the *Entobia* chambers to the substrate surface in *Pyrgopolon* (*Septenaria*) cf. *tricastata* and *Cementula* sp. were not detected using micro-CT, but were visible in SEM images of epoxy resin casts.

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Final citation: Heřmanová, Zuzana, Kočová Veselská, Martina, Kočí, Tomáš, Jäger, Manfred, Bruthansová, Jana, and Mikuláš, Radek. 2023. Comparison of methods: Micro-CT visualization method and epoxy cast-embedding reveal hidden details of bioerosion in the tube walls of Cretaceous polychaete worms. *Palaeontologia Electronica*, 26(2):a18.

<https://doi.org/10.26879/1255>

palaeo-electronica.org/content/2023/3585-comparison-of-methods-micro-ct-and-epoxy-cast-embedding

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Keywords: micro-computed tomography; vacuum casting; Serpulidae; boreholes; late Cretaceous

Submission: 23 November 2022. Acceptance: 5 May 2023.

INTRODUCTION

In recent decades, vacuum cast-embedding and following-up scanning electron microscopy visualization (SEM) became the most widely applied procedure for the study of recent and fossil bioerosive structures in carbonate substrates (e.g., Golubic et al., 1970; Wisshak, 2012; Seuss and Nützel, 2019). This method was introduced by Maaløe and Birch-Andersen (1956), followed by Glauert et al. (1956) for biomedical purposes. Since the 1970s, it has been applied in ichnology with several modifications (e.g., Golubic et al., 1970). Nevertheless, this method still required pressure impregnation and heating of embedding medium at about 60°C for at least 40 hours, and thus is not suitable for material sensitive to heat or pressure. Nielsen and Maiboe (2000), followed by Wisshak (2012), introduced less-time-consuming vacuum cast-embedding, based on filling bioerosional tunnels with a mixture of epoxy resin and hardener (Epofix) under vacuum conditions and dissolving the substrate with diluted hydrochloric acid. This procedure is used in the present paper.

Micro-computed tomography has recently experienced a boom in all branches of biology, paleontology, and geology. Micro-CT devices utilize X-rays to see inside an object, enabling virtual three-dimensional reconstruction of the specimen, and generation of videos using individual section images. It is intensively used for study of recent (Mees et al., 2003; Beuck et al., 2006; Schönberg and Shield, 2008; Lukeneder and Lukeneder, 2011; Lukeneder, 2012; Lukeneder et al., 2014; Amon et al., 2015; Färber et al., 2016; Silbiger et al., 2016) and fossil bioerosional traces (e.g., Benner et al., 2008; Beuck et al., 2008; Lee et al., 2017; Wisshak et al., 2017; Heřmanová et al., 2020). In our study,

we used a combination of micro-computed tomography and vacuum cast-embedding technique producing polymer resin casts followed by SEM observations to visualize bioerosion inside serpulid polychaete tubes. Serpulids are commonly represented in the fossil record by their calcitic or calcitic/aragonitic tubes (e.g., Jäger, 1983, 2005, 2012; Vinn et al., 2008a+b+c+d; ten Hove and Kupriyanova, 2009; Vinn and Mutvei, 2009; Ippolitov et al., 2014; Vinn 2020), but to date, only a few publications referred to domichnial traces inside serpulid tubes (Kočí et al., 2017; Heřmanová et al., 2020; Kočová Veselská et al., 2021; Mikuláš et al., 2022). The low bioerosion degree is strongly affected by the serpulid tube curvature and very tiny tube walls, ranging between 0.4–0.6 mm. However, in the case of rarity of any other suitable thick-shelled fauna at the locality, serpulids could be surprisingly highly infested, as shown in Kočová Veselská et al. (2021). The aim of this paper is:

- (1) to document bioeroded serpulid polychaetes from the late Cretaceous of the Bohemian Cretaceous Basin,
- (2) to evaluate results from micro-CT and vacuum cast-embedding – how the method used affects the display and subsequent description of boreholes in serpulid tubes, and
- (3) to compare advantages and limitations of both methods.

GEOLOGICAL AND GEOGRAPHICAL SETTING

The bioeroded serpulid tubes were collected from nearshore, shallow-water deposits at Velim, Kamajka, and Kaňk “Na Vrších”, situated along the southern and eastern margin of the Bohemian Cre-

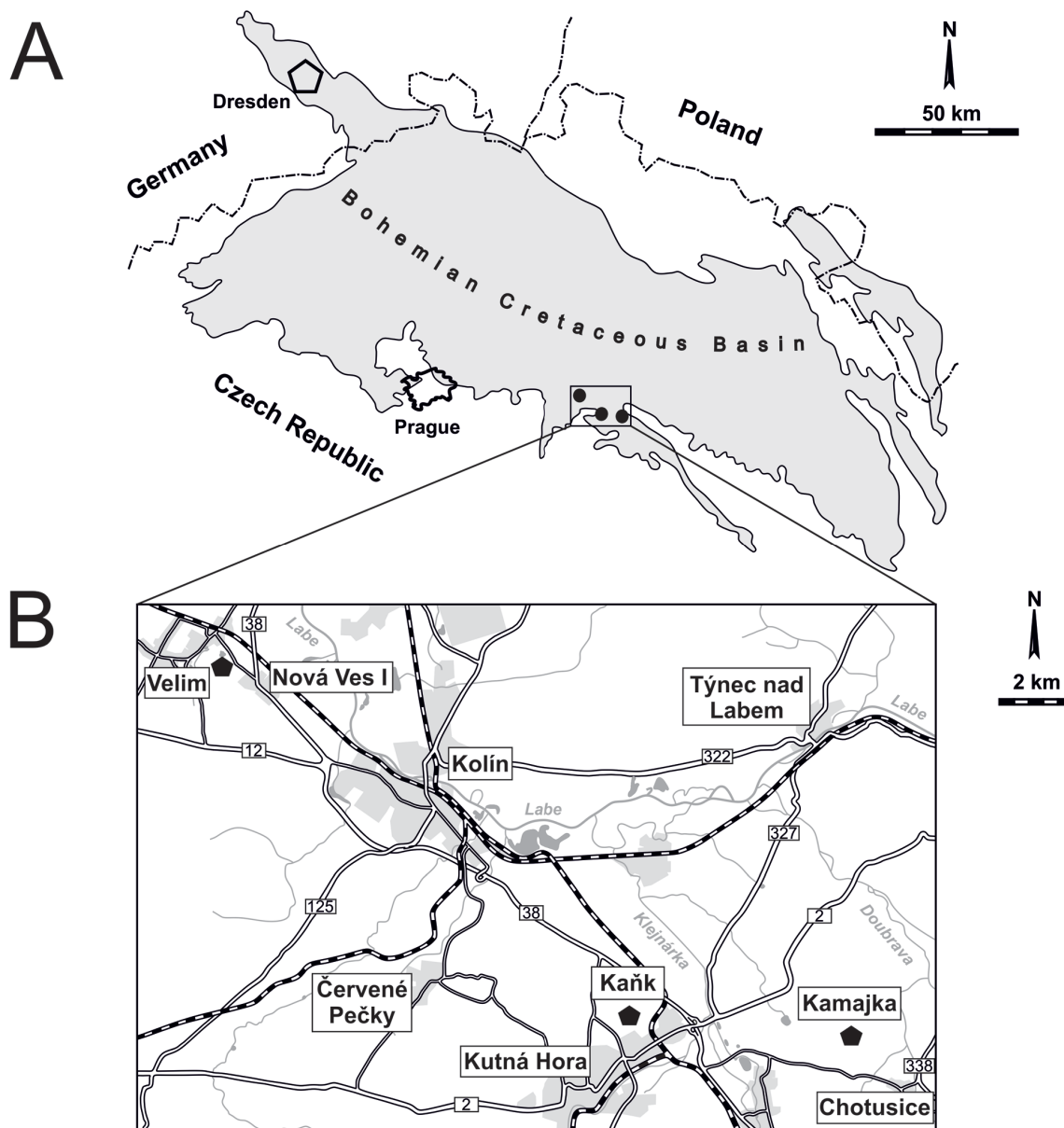


FIGURE 1. A. Simplified geographic map of Bohemian Cretaceous Basin indicating locations of the studied sites (in rectangle). B. Geographic position of nearshore deposits at Velim, Kaňk “Na Vrších” and Kamajka, where samples were taken (black pentangles).

taceous Basin (Figure 1A), which are interpreted to have been laid down under high-energy conditions (Žiřt et al., 1997). Localities are situated approximately 60–100 km east of Prague in the vicinity of Kolín and Kutná Hora (Figure 1B), where nearshore sediments are exposed in depressions of metamorphic rocks. The bioeroded serpulids from Kaňk and Kamajka treated in the present publication are early Turonian in age, whereas those from Velim are late Cenomanian (Figure 2). The exact

correlation among the localities is complicated by frequent non-sequences and redeposition of older faunas (Kořová Veselská et al., 2014). The sedimentation was also influenced by palaeohydrodynamics, palaeogeography, clast distribution, and character and abundance of detrital material (Žiřt and Nekvasilová, 1989, 1994, 1996; Žiřt 1992; Žiřt et al. 1998, 2006). Generally, the horizon yielding serpulid polychaetes is developed in characteristic facies, consisting of calcareous claystones at Kaňk

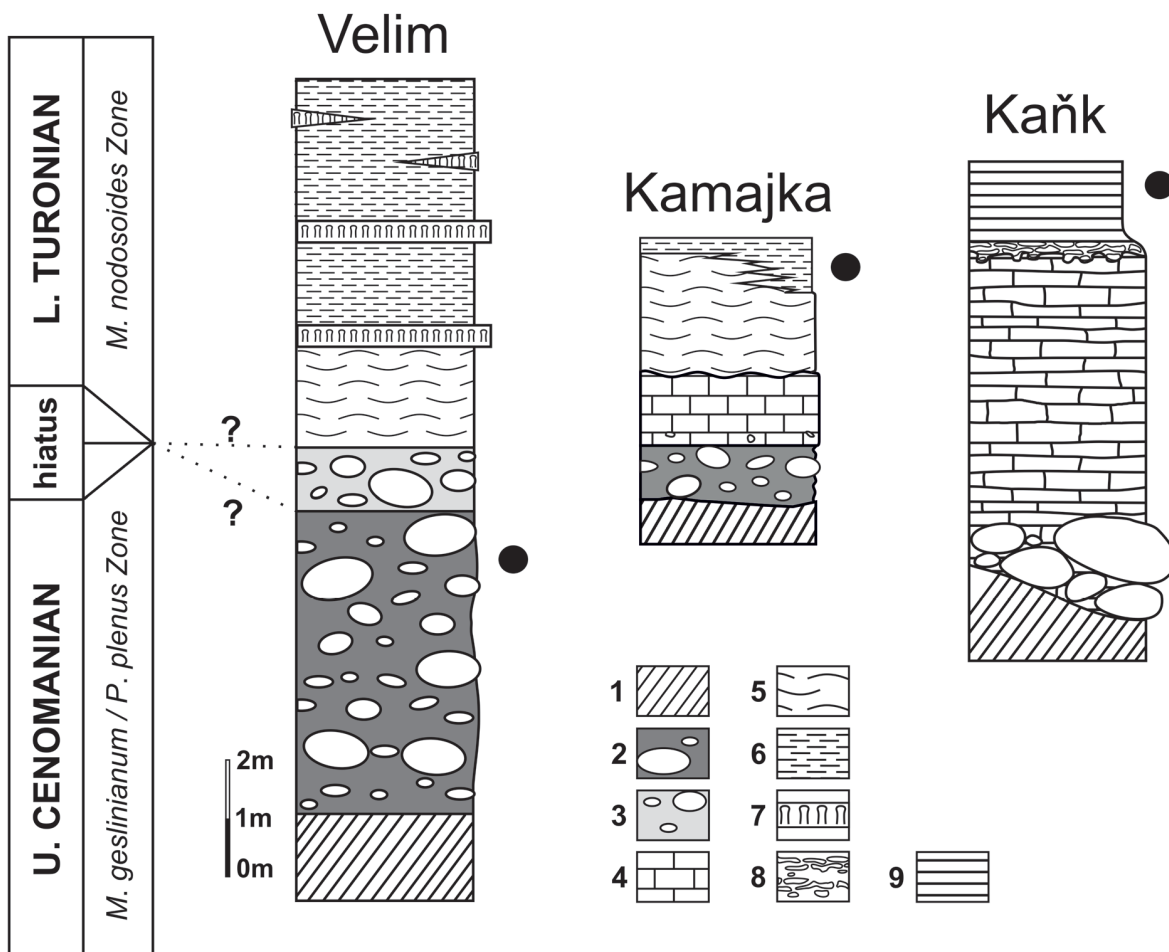


FIGURE 2. Stratigraphic provenance of serpulid tubes from Velim, Kamajka, and Kaňk. 1 - crystalline basement; 2 - basal Cenomanian conglomerate; 3 - redeposited Turonian conglomerate; 4 - bioclastic limestone with calcitic-clayey matrix; 5 - organodetritic clayey limestone; 6 - marly siltstone with intercalations of phosphatized horizon; 7 - sponge 'meadows'; 8 - limestone layer with nodule-like bodies; 9 - calcareous claystone (modified from Košťák et al., 2010; Kočí, 2012). Full filled circles indicate position of serpulid fauna.

“Na Vrších”, organodetritic clay limestones at Kamajka, and calcareous siltstones with abundant organodetritus at Velim and Kamajka (Figure 2). The rich fauna from Velim and Kamajka was studied and described by Žítt et al. (1997) and Košťák et al. (2010); fossil communities from Kaňk “Na Vrších” were examined by Vodrážka et al. (2013).

MATERIAL AND METHODS

Thousands of serpulid tubes collected by Tomáš Kočí at Velim, Kamajka, and Kaňk were visually examined for evidence of boreholes using an Olympus DP70 light microscope at the National Museum in Prague. Of these, three serpulid tubes exemplifying the range of bioerosional structures were selected for closer examination: one tube of *Cementula* sp. from Velim (CZ2), one fragment of

Pyrgopolon (Septenaria) cf. *tricolorata* (Goldfuss, 1841) from Kaňk “Na Vrších” (NM O8728), and one tube of *Placostegus zbylavus* (Ziegler, 1984) from Kamajka near Chotusice (NM O8727). All material is kept in the paleontological collection of the National Museum in Prague.

All selected specimens were cleaned in a 7% solution of hydrogen peroxide in an ultrasonic bath. For visualising and determining trace fossils left by bioeroders inside the serpulid tubes, X-ray microtomography, and a vacuum cast-embedding technique producing polymer resin casts were applied to all examined specimens.

The micro-computed tomography analysis was performed by using an X-ray micro-tomography SkyScan 1172 (Bruker) device at the Natural Museum in Prague (Czech Republic). The system

provides several X-ray “shadow” transmission images from many views in different angles while the object rotates. From these shadow images, cross-section images of the object were reconstructed using N-Recon Version: 2.0.0 Software, creating a complete three-dimensional representation of internal microstructure. Images were created by Avizo (Version: 2020.3) software. Revealed information was examined using the Volume Rendering and Ortho-slice mode of surface and/or sections. Isosurface visualization and segmentation were applied to effectively display the structures of the borings. The X-ray microfocuss tube was operated at 100–140 μ A and 70–100 kV. Filters used were Al (0.5 mm) and combined filter Al+Cu or Cu filter (1mm). Rotation Step was 0.2–0.4°, two frames for one-step were used, random movement was between 2 and 10, and 180° rotation was used. Average image pixel size was 1.96–13.54 μ m.

Subsequently, all serpulid tubes were cast in epoxy resin, using the technique of Wisshak (2012). Samples were placed in a small vacuum chamber for cast-embedding (Struers CitoVac) and infiltrated with a mixture of EpoFix low-viscosity epoxy resin and hardener in a ratio of 15/2 ml. After curing, the samples were longitudinally cut in half with a rock saw, decalcified by treatment with 10% HCl (except for silicified tube of NM O8727, whose solubility in acids was very low and did not dissolve even in 48% HF), rinsed in distilled water to prevent further etching and possible chemical precipitation, and dried. The polymer resin casts were then mounted on stubs before gold coating (NM O8727) and SEM analysis (Hitachi S-3700 N SEM at National Museum in Prague), to display the most delicate morphological features.

REMARKS ON BIOERODED SERPULID WORM TUBES

Many polychaete worms, many genera of Serpulidae and the sabellid *Glomerula*, differ from other polychaetes by forming a calcareous (calcitic or calcitic/aragonitic) two-layered tube (Vinn et al., 2008b). Since the Permian (Sanfilippo et al., 2018; Ramsdale, 2021), the vast majority of serpulids are typical encrusters in the marine realm, and today a single genus lives in the freshwater of karst caves. Their tubes are usually attached to hard substrates like rocks, pebbles, shells, and other solid skeletal remains of various marine animals, as well as, although less often, to algae and seagrasses. Few species become free-lying loosely on or sticking in soft ground (e.g., *Ditrupea*, some species of

Pyrgopolon). Serpulids and sabellids are also characteristic elements of the mesofauna in the rocky coast facies situated at the southwestern and southern margin of the Bohemian Cretaceous Basin (BCB), where thousands of specimens of a dozen genera have been collected to date. They have been documented in numerous publications (Reuss, 1845, 1846; Geinitz, 1875a, 1875b; Ziegler, 1984; Jäger and Kočí, 2007; Kočí, 2008, 2010, 2012; Jäger, 2014; Kočí and Jäger, 2015).

Despite such a large number of serpulids in the BCB, only a few specimens show signs of domichnial bioerosion, of which three tubes were selected for closer examination: one specimen each of *Cementula* sp. from Velim, *Placostegus zbylavus* (Ziegler, 1984) from Kamajka near Chotusice and *Pyrgopolon* (*Septenaria*) cf. *tricastata* (Goldfuss, 1841) from Kaňk Na Vrších. *Cementula* sp. (Figure 3) has a coiled tube with its outer whorls usually attached to the inner whorls; boundaries between whorls are smoothed by deposition of calcareous tube material. *Placostegus zbylavus* (Figure 4) is preserved as a 6 mm long fragment of the possibly curved or coiled attached posterior tube portion, followed by the long, nearly straight free anterior tube part, which rises almost vertically, is slightly twisted around its longitudinal axis, bears three keels, and is triangular in cross-section. *Pyrgopolon* (*Septenaria*) cf. *tricastata* (Figure 5) is represented by a 6 mm long horn-shaped tube with three prominent and wide longitudinal ridges subcircular in cross-section, laterally divided by furrows on the external surface.

Obviously the very thin wall of serpulid tubes, ranging between 0.4–0.6 mm, is the reason for the low bioerosion degree in such tubes. The near-shore deposits in the BCB yielded numerous thicker-shelled fauna (bivalves, brachiopods, echinoids) as well as basal conglomerates representing a more suitable substrate for domichnia (e.g., Žitt et al., 2006, 2015; Vodrážka et al., 2013). Moreover, all studied serpulids come from high-energy environment, where it is difficult for organisms to settle on substrates that are frequently buried and exhumed; the degree of bioerosion thus depends on the duration of exposure before burial in the sediment (El-Hedeny, 2007; Madani et al., 2022). Because serpulids were moved and rolled on the seafloor, they all are only fragmentarily preserved and strongly mechanically damaged. Besides fragmentation, tube surfaces show evidence of selective dissolution and abrasion generated by abiotic processes. The most detailed paper

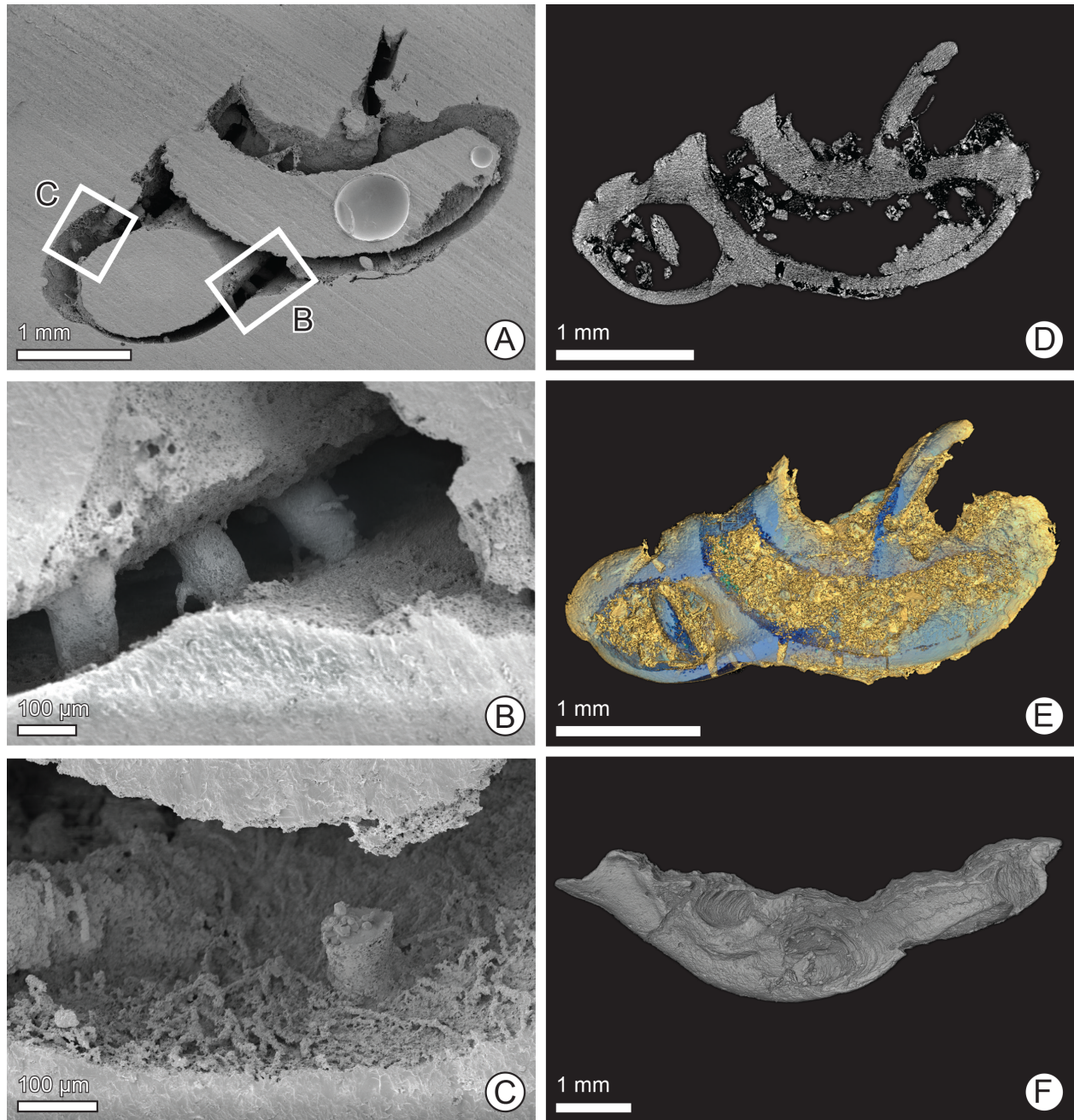


FIGURE 3. *Cementula* sp., a coiled tube, no. CZ2, from Velim. A–C. Scanning electron microscope images of resin cast, B–C insets in A showing microbial erosion beneath D–F. Micro-CT images. A, D and E. Identical views using different methods. B. SEM image of resin cast shows branching stolons of *Iramena* isp., below lower limit of micro-CT resolution. C. Detail showing microbial erosion beneath tube surface and shaft incompletely filled with epoxy resin. D. 2D section through both tubes. E. Semi-transparent rendering of 2D section. F. Volume reproduction image, 3D view to smooth inner surfaces of the tubes.

on sclerobionts associated with polychaetes from BCB is that of Kočí and Jäger (2015), concerning borings and encrusters on sabellid and serpulid worms from the rocky coast facies (late Cenomanian) at Předboj near Prague.

The surface of the investigated serpulids is highly pitted by circular to oval borehole apertures measuring 0.01–0.4 mm in diameter. Because the thickness of serpulid tube walls is not constant throughout the tube, but decreases towards the aperture and increases at the longitudinal ridges

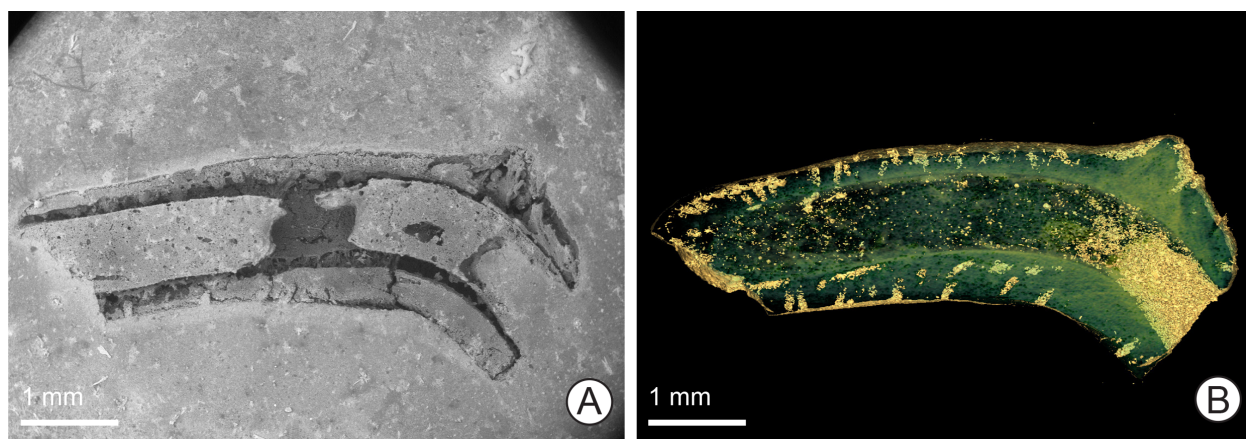


FIGURE 4. *Placostegus zbyslavus* (Ziegler, 1984), longitudinal section of a tube from Kamajka near Chotusice, no. NM O8727. A. SEM image of the resin cast showing a high degree of silicification that led to incomplete dissolution of the tube wall in HCl; image shows only indeterminate non-branching shafts. B. The same view of the specimen using micro-CT clearly shows relatively frequent *Maeandropolydora* isp. and shallow shafts of *Trypanites* isp.

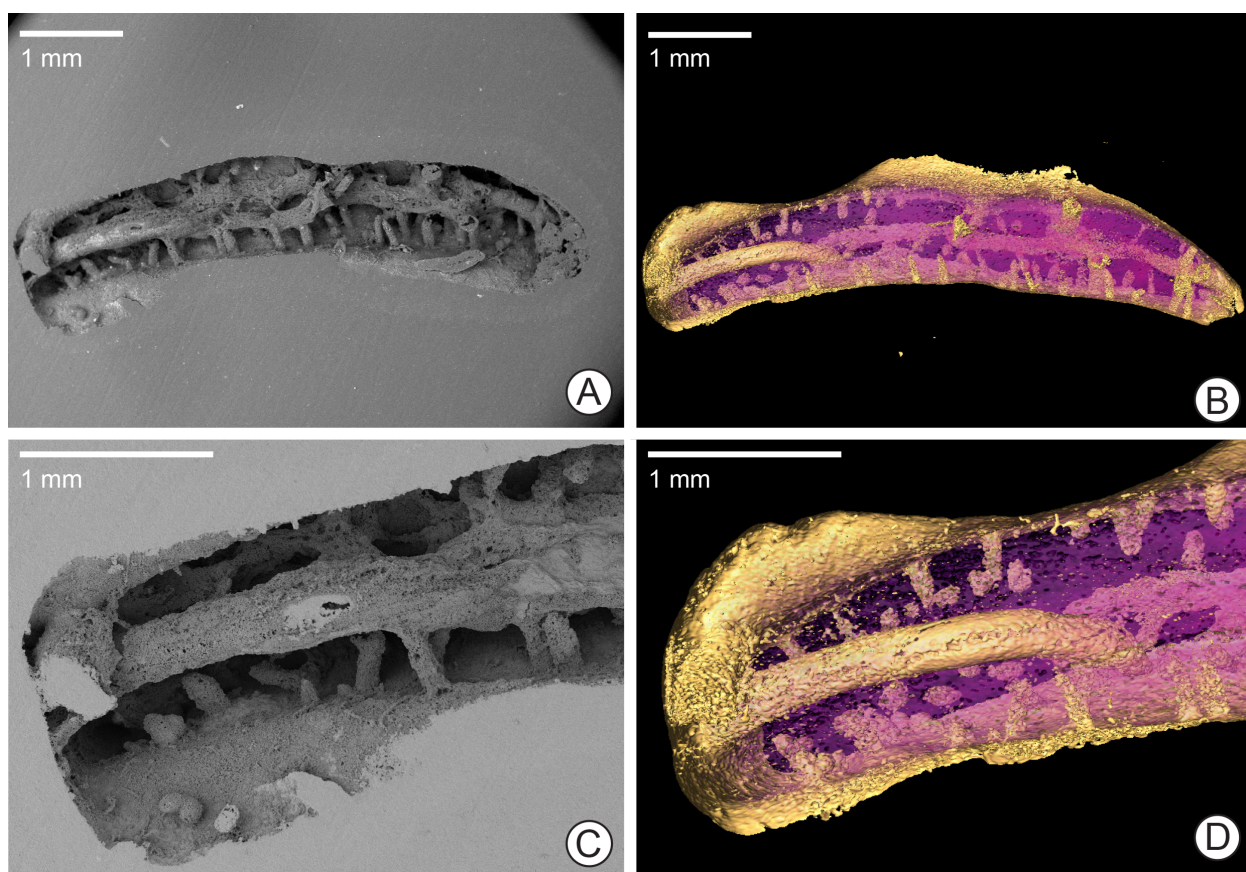


FIGURE 5. *Pyrgopolon (Septenaria) cf. tricostata* (Goldfuss, 1841), longitudinal section of a tube from Kaňk "Na Vrších", no. NM O8728. A. SEM image of the resulting cast providing a three-dimensional view of three bi-camerate specimens of *Entobia* isp. and numerous shafts of *Trypanites* isp. cut by the longitudinal boring *Maeandropolydora* isp.; galleries are duplicated, more or less parallel, partially touching each other. B. The same view to the specimen by using micro-CT. C. detail of the resin cast showing a pair of bi-camerate *Entobia* isp. D. micro-CT scan from the same view, details of *Entobia* chambers are below the lower limit of micro-CT resolution.

and keels, domichnia should be expected rather in the thicker parts of the tube wall. However, combining micro-CT and vacuum cast-embedding proved that boreholes are evenly distributed, regardless of the morphology of the host tubes, and revealed borings attributable to four ichnogenera: *Entobia* Bronn, 1837, *Trypanites* Mägdefrau, 1932, *Maeandropolydora* Voigt, 1965, and *Iramena* Boekschoten, 1970. Borings were analyzed separately in each serpulid tube to look for any specific associations or relationships, and their characteristics are described below:

Entobia has ovoid or bag-like chambers, connected to the surface by narrow tunnels. As the chambers occur very close to the surface of the serpulid tubes, because of the very thin tube walls, some connecting tunnels are very short (length ranges from 0.1 to 2 mm) and narrow (diameter varies from 0.01 to 0.4 mm). Because SEM images of the resulting casts have significantly better resolution than micro-CT, tiny chambers are clearly visible only on SEM images of epoxy casts. Specimen NM O8728 shows a trinity of two-camerate, small (up to 100 μm) spherical bodies, partially penetrating each other (Figure 5C). From the outside of the substrate, the spheres appear smooth at a particular resolution. Their surfaces correspond to the grain size (much smaller than the object being examined) of the mineralized filling (<10 μm). On the sediment-facing side, six “stems,” shorter than 10 μm , protrude from the two spherical bodies, corresponding to exploratory threads of entobians (Bromley and D’Alessandro, 1984). Another two *Entobia* specimens on sample NM O8728 show comparable morphology and dimensions, but are in the back of the epoxy cast and so not easily accessible for study (Figure 5A). A sole well-casted bag-like chamber was also found in sample CZ2 (Figure 3A).

Trypanites are represented by thin, short, smooth, and unbranched shafts, usually passing roughly vertically through the whole preserved wall of a serpulid tube. The diameter of individual shafts tends to be constant, ranging from 0.1 to 0.2 mm, oval in cross-section with rounded or gently oval openings. The usual length (depth) of the tubes is 0.25 to 0.4 mm, which corresponds to the thickness of the serpulid tube walls. Specimens NM O8727 and O8728 display a minimum of 30 short shafts of *Trypanites* borings, which includes about one-half of the original serpulid tube. Whereas *Trypanites* in O8728 are visible in micro-CT (Figure 5B, D) as well as in SEM images of polymer resin cast (Figure 5A, C), *Trypanites* in NM O8727 were

readily discernible only by using micro-CT reconstruction (Figure 4B). The epoxy casting was unsuccessful because of an incompletely dissolved tube, and the resulting image shows only signs of a few short shafts (Figure 4A). Specimen CZ2 has at least three incompletely preserved borings visible in micro-CT (Figure 3E) and SEM pictures (Figure 3A, B).

Maeandropolydora found in tube of *Pyrgopolon* (*Septenaria*) cf. *tricostata* (NM O8728) consists of long, cylindrical galleries with several apertures, running through the substrate in irregular, not too sharp turns. Some of the views of the epoxy cast show that the galleries are duplicated, partially touching each other; in the remaining part, they are somewhat less densely spaced but remain roughly parallel (Figure 5). The length of the individual boring is 5.8 mm, which corresponds to the overall length of the studied serpulid. The diameter of the galleries is somewhat variable but basically circular; it ranges from 0.3 to 0.5 mm. The surface of the galleries is smooth. Serpulid tube O8728 was filled with calcium carbonate and was redeposited in a high physical energy environment of the rocky shore. Then this cylindrical object was available for boring. The resulting configuration of borings is therefore quite different from the other studied specimens, which have the character of relatively thin-walled tubes.

Cementula sp. (specimen CZ2) micro CT and the epoxy cast revealed three short cylindrical to somewhat conical fillings of borings through the entire wall. In the micro-CT image, these borings resemble short shafts oriented perpendicular to the tube surface (Figure 3E). The axes of the bored cylinders/cones form an angle of 70–85° with the surface of the host tube, which is close to the values found for *Trypanites*. However, the SEM image of the epoxy cast shows “branching” of these main borings (Figure 3C). The branching stolons, below the lower limit of micro-CT resolution, are characterized by a significantly smaller and variable diameter (10–30 μm), compared to the main boring. The axes of the stolons form an angle of 30–60° with the axis of the main borings. The presence of tunnels of different diameters and their branching detected by SEM images of the epoxy cast resembles the ichnogenus *Iramena*.

DISCUSSION

Both micro-CT and epoxy vacuum cast-embedding followed by SEM observations are widely regarded as very helpful and necessary tools for studying and visualizing the external and

internal surfaces as well as the two-dimensional and three-dimensional (3D) architecture of tunnel systems left by bioeroders in various substrates (Tapanila, 2008, Wisshak, 2012) see Table 1. Nevertheless, both methods also have limitations, and using each method alone could make it difficult to diagnose and describe minute bioerosive structures as discussed below:

Material of hard substrate and borings filling. In general, most substrates can be examined using X-ray, so there are no restrictions for hard substrate in micro-CT device. The micro-CT can detect material of different densities if the contrast between the fossil material and the respective boring infilling is sufficient. For serpulid tubes and their infillings, the contrast was enhanced by using Al, Cu, or combined Al+Cu filters. A suitable sample size of specimens for micro-CT is approximately 0.5–10 cm. Significantly smaller samples are studied with nano-CT, and significantly larger samples are studied with common medical CT. However, these devices were not used in our study.

The vacuum embedding method is a fast and powerful tool for the study of bioerosion within calcareous substrates. The preparation of samples, including ultrasonic cleaning treatment as discussed above, usually precedes the resin infiltration and casting and subsequent chemical carbonate removal steps. This process is thus inaccessible for undissolvable silicified or pyritized substrates (Wisshak et al., 2017). This occurred with specimen NM O8727; it had a high degree of silicification, which led to incomplete dissolution of the walls in HCl and HF, thus making it unsuitable

for the epoxy-casting methodology. The borings were identified using micro-CT, because the SEM image shows only short indeterminate non-branching shafts (Figure 4A). Vacuum casting is also unsuitable for boreholes infilled by a dense matrix. If boreholes are not empty, the resulting quality of epoxy casts is highly dependent on adequate porosity and permeability of the boring fill, since it must be impregnated by epoxy resin. Especially if the deepest and thinnest parts of macroborings are filled with residual fine-grained sediment impermeable by epoxy resin, complete casting of all of the borings is often not successful (Tapanila, 2008). In specimens CZ2 and NM O8727, epoxy resin did not infill all boreholes available, compared to what is seen in micro-CT reconstruction (Figure 4A, B), or filled their proximal parts only (Figure 3B). Dense carbonate inside the deepest part of some boreholes in CZ2 did not allow the resin to infiltrate the borings completely, so these structures were partly destroyed by subsequent dissolving in diluted acid. Boreholes in NM O8727 were partly filled within soluble silicified matrix. Specimen NM O8728 proved to be the ideal sample for epoxy castings. Its tube was filled with impermeable, acid-soluble carbonate matrix that prevented resin intrusion, as boreholes were empty, ready for resin impregnation. After filling and hardening the resin, the tube was longitudinally cut in half, and together with tube filler was decalcified in HCl. After this procedure, SEM images of the resulting cast provided a detailed three-dimensional view of the ichnological processes inside the entire serpulid tube (Figure 5A, C).

TABLE 1. 1 Comparison of micro-computed tomography method and epoxy vacuum cast-embedding followed by SEM method in terms of material, cost and fees, destructiveness, image resolution, and possibility of three-dimensional animation.

Item of comparison	Micro-computed tomography	Epoxy vacuum-embedding followed by SEM
Material of hard substrate	Material contrast between hard substrate and borings is necessary.	Material must be soluble in hydrochloric acid.
Infilled borings	Boring should be filled with a contrasting (different) material from the hard substrate or empty.	Boring should be empty or filled with permeable sediment.
Destructiveness	Non-destructive. The sample will remain unaffected.	Destructive. The sample will be dissolved in acid.
Image resolution	Usually low.	Usually high.
Three-dimensional animation	Possible.	Not possible.
Equipment/hardware used	Micro-CT equipment (high purchase price, legal restrictions on use of X-ray technology).	Vacuum chamber for cast-embedding (low purchase price). Scanning electron microscope (high purchase price).
Software used	Expensive software with demanding requirements on both hardware and data storage space.	Inexpensive software, minimal demands on hardware.

External and internal morphology of hard substrate. Micro-CT is useful in non-invasively visualizing gross internal and external morphological features in the μm to mm size range. This facilitated the taxonomic identification of serpulid specimen *Cementula*. In outer morphology, the tubes of *Cementula* are very similar to those of *Spiraserpula*, but *Spiraserpula* possesses internal longitudinal ridges on the inner surface of the tube wall (internal tube structures, see Pillai, 1993; Pillai and ten Hove, 1994), while *Cementula* lacks them (Figure 3F).

Destructiveness. Because micro-CT allows non-invasive visual depicting of internal structures of various objects, it is suitable for valuable specimens, e.g., holotypes, paratype series, and other types that should be preserved for further studies (Abel et al., 2012). Non-destructivity is also needed for museum practice: once a sample enters a museum's database, it becomes a subject of often strict regulations. The raw data obtained from micro-CT can be processed and visualized as virtual sections in diverse planes, three-dimensional views, animations in a full 360° range, or segmentation. Micro-CT produces so-called "virtual fossils" (Abel et al., 2012; Sutton et al. 2014). Once the specimen is X-rayed, the images obtained are stored in the computer and can be worked with further. If fossils are then destroyed during vacuum cast-embedding, they are at least preserved and remain accessible as virtual fossils.

Image resolution and three-dimensional animation. The most serious limits of micro-CT are related to resolution. The ideal smallest resolution for micro-CT is about $5\ \mu\text{m}$, which may limit studies on microborings as well as tiny macroborings (Heřmanová et al., 2020). Domichnia in serpulid polychaetes are smaller than those in bivalves, brachiopods, or echinoderms, which suggests that serpulids were mainly occupied by small, dwarfed but adult tracemakers (Mikuláš et al., 2022). Because resolution of less than $10\ \mu\text{m}$ could not be effectively achieved for the serpulid samples, and we suspected that micro-CT did not fully detect possible details in the tube substrates, we decided for epoxy casting. Although vacuum cast-embedding producing polymer resin casts is a destructive technique, it is an excellent method to display the most delicate morphological features, because the SEM images of the resulting casts have significantly better resolution than micro-CT (Golubic et al., 1983; Wisshak, 2012; Heřmanová et al., 2020).

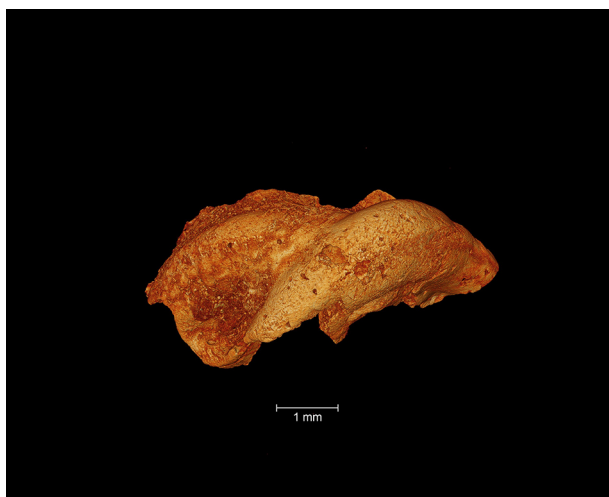
In specimen CZ2, both serpulid tube interiors were filled with epoxy resin and boreholes located

beneath the epoxy infilling are obscured. However, epoxy casting provided detailed morphology of borings that were beyond the detection limit of the given spatial resolution of the micro-CT scan, and helped with ichnotaxonomic identification. These are tiny branching canals of *Iramena* (compare Figure 3C and 3E) and the finer apertural canals connecting the *Entobia* chambers to the tube surface in specimens CZ2 and NM O8728. Epoxy casting also helped in displaying microbioerosion (about $10\ \mu\text{m}$ in diameter) just beneath the tube surface (Figure 3B). These structures were not detected using micro-CT, but are visible only in epoxy resin casts.

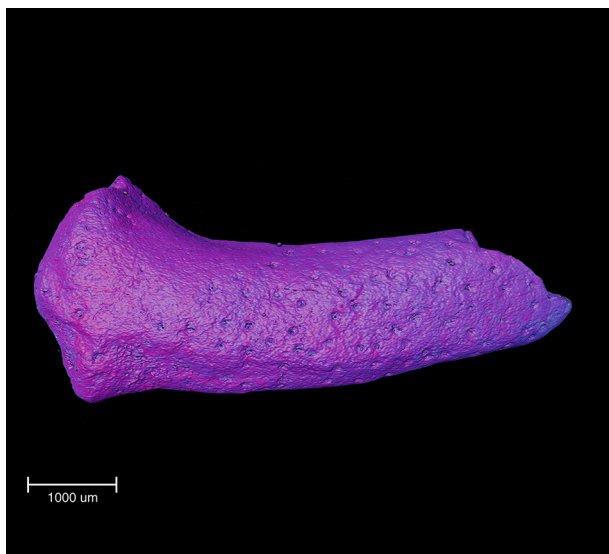
Three-dimensional animations are a standard output of the micro-CT methodology (see Animation 1 and Animation 2). This technology is not limited to a two-dimensional view, so short movies showing complete internal structures or surfaces from all sides are often generated. In three-dimensional animation it is possible to simultaneously view both the internal and external structures of the fossil, and make some parts of it semi-transparent.

Hardware and software. Micro-CT equipment (hardware) is an expensive device, the price is not quantified here. The price depends on the agreement of the buyer and the seller. Basically, the price of a micro-CT depends on the types of samples that need scanning and the results desired from the system. This determines the resolution needed for the appropriate micro-CT analysis, which determines the choice of X-ray source and detector. Micro-CT also makes serious demands on the attendant computer technology. Reconstruction systems must run on a powerful computer, and they produce very large datasets, bringing problems with long-term storage of such data (Paterson et al., 2014; Keklikoglou et al., 2019). In addition, there are legal restrictions on the operation of X-ray equipment in many countries.

On the other hand, epoxy vacuum-embedding equipment can be acquired orders of magnitude more cheaply than micro-CT. Epoxy vacuum-embedding is followed by SEM observation. The purchase price of scanning electron microscope equipment is relatively high, and as with micro-CT equipment, depends on the agreement between the seller and the buyer. However, the software requirements for the epoxy vacuum-embedding method are significantly lower. Data production is much smaller, and arranging for long-term storage of images is usually trivial. In many institutions, it is possible to rent micro-CT and SEM equipment.



ANIMATION 1. Three-dimensional animation of specimen *Cementula* sp., a coiled tube, no. CZ2, from Velim locality, the Czech Republic.



ANIMATION 2. Three-dimensional animation of specimen *Placostegus zbylavus* (Ziegler, 1984), from Kama-jka locality, the Czech Republic, no. NM O8727.

Prices per hour of use of the device vary. Documenting one sample (in our case sample CZ2) on the micro-CT equipment took 21 hours (net working time). Making resin casts is much more time-consuming. Curing, cutting, and decalcifying the samples took 5 days, and subsequent SEM analysis another 2 hours.

CONCLUSIONS

Studying serpulid tubes as a hard substrate for sclerobionts shows that different imaging techniques bias the diagnosis and description of bio-

erosion. The result of micro-CT compared to vacuum cast-embedding of the same specimen show the benefits and limits of both methods. The main advantage of SEM images of casted specimens is the depiction of fine details, which helped in the correct taxonomic determination: bifurcation of *Iramena* in CZ2 or *Entobia* chambers with the finer apertural canals in CZ2 and NM O8728. The image resolution is much better than those obtained by micro-CT. The disadvantages of the vacuum cast-embedding method are its destructiveness and the restrictions caused by insolubility of the fossil specimens. It proved unsuitable for specimen NM O8727, which is silicified and therefore insoluble in acid. Moreover, vacuum casting requires empty borings or at least porous and permeable boring fills. Distal parts of some boreholes in CZ2 were filled with a dense carbonate matrix; they could not be impregnated with epoxy resin and were dissolved in acid. The non-destructive nature is the greatest benefit of micro-CT. It is suitable for rare and valuable specimens which cannot be replaced. Micro-CT also helped with the taxonomic identification of *Cementula* sp. (specimen CZ2) by visualizing internal morphological structures. Micro-CT is also able to display boreholes filled with dense matrix, if sufficient contrast exists between boring filling and substrate. Micro-CT has an advantage in allowing the object to be viewed in full 3D, including the ability to make three-dimensional animations and serial sections through the object. SEM of casted specimens can also provide three-dimensional images of a borehole system inside serpulid tubes, but only when vacuum casting is applied to specimens with empty borings impregnated by epoxy resin, while the tube interior is filled with dense carbonate matrix and subsequently dissolved in acid (specimen NM O8728). The disadvantage of micro-CT, apart from storage requirements of gigabytes per specimen, is the inability to achieve the resolution of SEM images of casted specimens. Although macroborings are easily recognizable on maximally magnified micro-CT images (long, cylindrical galleries of *Maeandropolydora* and unbranched shafts of *Trypanites*), they lack details smaller than 10 μm in diameter, which are available from SEM images (meandering and tangled microborings just beneath the serpulid tube surface, *Entobia* chambers, and bifurcation of *Iramena*). In practice, micro-CT, although non-destructive, is not an optimal method for investigation of μm -sized objects in mm- to cm-sized serpulid tubes, and epoxy casting thus plays an important role in visualizing and identifying of such

tiny bioerosional structures. For pyritized or silicified substrates unsuitable for the cast-embedding technique, micro-CT is currently the only option for imaging their bioerosion structures. Our experience shows that both methods may complement and support each other.

2019–2023/2.l.e, National Museum, 00023272) and Centre for Geosphere Dynamics (UNCE/SCI/006). This study was financially supported by the Czech Science Foundation (GACR 18-05935S) and by the institutional projects of the Czech Academy of Sciences, Institute of Geology (RVO 67985831).

ACKNOWLEDGEMENTS

This work was financially supported by Ministry of Culture of the Czech Republic (DKRVO

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