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A conceptualisation of computed tomography outputs in entomological research by step by step displaying trough the CT-based visualization of a wood-boring larvae

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REVIEW ARTICLE

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ABSTRACT

The non-invasive diagnostic methods represent a new branch of insect diagnostics, which can provide novel information especially about insects with hidden lifestyle. Computed tomography (CT) is one of the most useful non-destructive techniques allowing for both qualitative and quantitative assessments. The aim of the present study was to attain entomological information through the implementation of CT imaging, hence contributing to the spread of non-invasive imaging in entomological research. Through monitoring the development of wood-dwelling cerambycid larvae in beech branches, we point out some outputs applicable in entomological studies, which originate from CT image post-processing. We present findings on the location, and size of specimens of some hidden arthropods, as well as cavities formed by them, stemming from the maximum and minimum intensity projections, windowing, 3D-reconstruction, or virtual endoscopy, as steps of the imaging. In summary, it is expected that our findings contribute to a wider recognition of the entomological information that can be gathered from these non-invasive imaging techniques.

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KEYWORDS

computed tomography, insect diagnostic processes, hidden lifestyle arthropod, non-invasive imaging, xylophagous insect

Hidden lifestyle in protected micro-habitats represent optimal living conditions for the development of a number of arthropods. The cavities offer favorable habitat and provide shelter against natural enemies or unfavourable climatic factors. In the majority of cases, only their mastication and emergence holes' hint at their presence (Kolk and Starzyk, 1996). The bio- and ecological studies focusing on these arthropods have been associated with traditional entomological methods, such as tissue dissection, or trapping methods (Jacobson, 1965; Himmi et al., 2018), for a long time. In the case of agricultural pests, plant protection diagnosis and the mapping of the degree of damage have also been based on such methods (Elliot et al., 1995; Underwood, 2000). In general, the main disadvantage of these methods is the disturbance of the target specimens, the injury of its natural environment, which in extreme cases, can be considered to be even irreversible.

A novel approach is represented by non-invasive imaging techniques, which are based on observation without disturbing the target organisms. The data originating from these processes can be deemed to be derived directly from natural environments, which reflect a "close to the real" conditions (Johnson et al., 2007; Bourne et al., 2019). These methods are facilitated by instruments such as x-ray and computed tomography (CT)-assisted visualisation, Magnetic Resonance (MR), Thermal Imaging, Confocal Laser Microscopy (CLSM), and Near Infrared Spectroscopy (NIRS) (Liu et al., 2017; Keszthelyi et al., 2020).

CT is one of the most useful non-destructive techniques ensuring both qualitative and quantitative results. It can visualise the texture and volume fractions of the examined objects. In addition, the density parameters can also be applied for statistical analysis throughout the 3D-volume (Goldman, 2007; Richards et al., 2012; Stadler et al., 2013). Nevertheless, there are little infiormation in connection with the observations of hidden lifestyle-pests (Himmi et al., 2018). The method has been used primarily in pest morphology and monitoring of insect development (Socha et al., 2007), as well as to illustrate the induced damage (Keszthelyi et al., 2021a,b). The first use of computed tomography (CT) imaging in entomology can be dated back to nearly 40 years, which provided data for forestry assays (Taylor et al., 1984). The subsequent scientific applications of these techniques in plant research have been rather scarce (Stadler et al., 2013).

The aim of our study was to draw attention to the possibilities of entomological data collection, provided by computed tomography as a non-invasive approach. Furthermore, we aim to emphasize the applicability of CT imaging in the research of hidden lifestyle insects, especially xylophagous arthropods, by which we hope to support the spread of non-invasive imaging in entomological studies. As a case study, the interdependent steps of this non-invasive approach were exemplified by an imaging process of a beech branch injured by a wood-boring larva was carried out.

DESCRIPTION OF THE PLANT-INSECT MATERIAL

In order to examine some specimens of hidden lifestyle arthropod species by computed tomography, fallen beech branches lying on the forest ground, in the vicinity of Szentlászló, Sasrét (Baranya County, Hungary), were collected (geographical coordinates: 46°13′11.36″N 17°47′59.97″E). Sample collecting was timed for the end of the annual vegetation season of the deciduous forest, when the xylophagous insects developing for years have already prepared for the overwintering inside their host, so they have been in the diapausing stage into the tissues of their host. In selecting the beech (*Fagus sylvatica*) branches to be studied, we have relied on the clearly visible holes and mastication signs triggered by the boring larvae of longhorn beetles (Col.: *Cerambycidae*) in the branches. 10 m long of arm-thick branches (10–12 cm diameter) were selected for our non-invasive investigation. These prepared plant materials were stored outdoor until the CT analysis was performed, to provide natural environmental conditions as closely, as possible.

DETAILS OF THE CT SET-UP FOR IMAGING ACQUISITION AND IMAGE RECONSTRUCTION FOR THE PLANT-INSECT MATERIAL

The image acquisition was performed by a Siemens Somatom Definition AS + CT scanner (Siemens Healthcare GmbH, Germany). The scanning parameters were set as follows: tube voltage 120 kV, current 192 mA, spiral data collection mode with pitch factor of 0.8 and collimation 128 × 0.6 mm. The overlapping scans were reconstructed by the iterative reconstruction algorithm (SAFIRE) implemented in the Somaris/7 syngo CT software (version VA48A). The reconstruction parameters were set in convolution kernel I50h, diameter 205 mm, with slice thickness of 0.4 mm (quasi-isotropic voxels). The images were archived in DICOM (Digital Imaging and Communications in Medicine) files.

POST-PROCESSING OF NATIVE IMAGES

The image post-processing was carried out by the above-mentioned Somaris/7 Syngo CT software programme (version VA48A) in case of 3D-rendering and virtual endoscopy. The 3D Slicer software was used for the cross-sectional images, Maximum and Minimum Intensity Projection. The mimicked endoscopy image was made by the Blender programme.

RESULTS

1st step. Evaluation of the cross sectional images

The CT-equipment reconstructs cross-sectional images from the collected radiation attenuation data. The field of view is a cylindrical volume 50 cm in diameter and 150 cm in length, but the bore size is 78 cm, so a slightly larger sample can be placed on the examination table. The weight of the sample must not exceed 200 kg.

It is possible to make a series of images of the entire plant, i.e. the whole volume, when performed with complete overlapping. In addition to the size of the pixels in the images, it has a depth that is characterised by the thickness of the slice, so the pixels correspond to spatial units called voxels. Voxel values are characterised by the average radiodensity of their volume on the Hounsfield scale (HU – Hounsfield Unit). A value of −1000 HU is associated with air, and the value of 0 HU is associated with the radiodensity of water. Accordingly, materials with a lower radiodensity than water are given a negative HU value, while those with a higher one are given a positive value. In the images used in radiology, the HU values are assigned to a grey scale, which shows the objects with different radiodensities. The start (black) and end (white) level of the grey scale (window) can be changed as appropriate, so it is possible to change the appearance of the picture to highlight particular structures. The brightness of the image is adjusted via the window level. The contrast is adjusted via the window width.

If the spatial extent of the voxels is chosen to be the same size (isotropic voxels), we have the ability to display and evaluate images of the same resolution in any desirable planes. This state is aimed to be attained in order to be able to determine measurable parameters (length, area, volume) on the images with the same measurement accuracy. The larvae of a wood boring species can be seen on Fig. 1 inside its cavity. This recording does not allow for a broader taxonomic origin to be determined. Thus, in the absence of essential racial identification keys, it is not possible to decide on the taxonomic family This recording does not allow for a broader taxonomic origin to be determined. Thus, in the absence of essential racial identification keys, it is not possible to decide on the taxonomic family. Therefore, only information about the basic physical parameters can be obtained, such as length of larvae (12 mm), size of formed cavity (221.43 mm^3).

2nd step. Advantages of the maximum intensity projection (MaxIP) and minimum intensity projection (MinIP) refined by grey-level mapping (windowing)

Depending on the density of the object to be assessed in the second step, one can typically decide on two types of 3D-rendering. If the formula to be displayed is high density, which can be e.g. larvae hiding inside a dry tree, the MaxIP display is appropriate. In this case, the higher density formulas are more clearly visible (larvae), while the lower ones are proposed to be

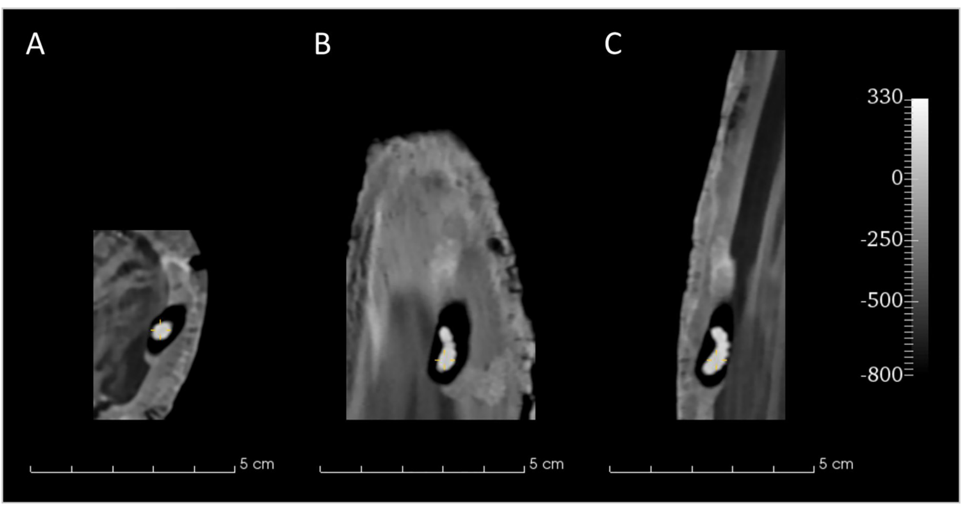

Fig. 1. Cross sectional images of a wood boring larvae in different planes (A: axial, B: coronal, C: sagittal)

employed in a transparent manner (Fig. 2). MinIP display aims to display low-density volumes, such as e.g. the cavities, passages (Fig. 3). These recordings already provide a deeper insight. A sharper image of the shape of the insect body, its segmentation as well as its exact location in the cavity can already contribute to a more exact identification and judgement of specific entomological characteristics. The shape of the larva clearly suggests an insect belonging to the buprestid and cerambycid families (broad thoracic, tapering abdominal segments). Irrespective of this, the visibility of the identification keys for family-level determination is still lacking (abdominal legs not visible).

3rd step. The tangible hiding. Imaging of thin slice 3D-reconstruction

The resolution of the studied areas can be significantly increased by repeated examination in more detail or by reconstruction with a smaller field of view (FoV) from the raw data in the existing spiral mode. The best resolution that can be reconstructed with CT devices used in human diagnostics can be achieved by selecting the smallest collimation and setting up spiral-mode data-acquisition with significant oversampling. In this case, submillimeter isotropic image sequences with appropriate signal-to-noise ratios can be generated from the raw data set (Fig. 4).

4th step. Volumetric displaying by means of the manipulation of 3D-rendered images

This display allows for separating different details by radiodensity ranges using colours. The parts that obscure the parts intended to be displayed can be virtual. This display permits the separation of different details by radiodensity ranges using colours. The parts that obscure the parts you want to display can be virtually cut off from deep-situating objects. This display

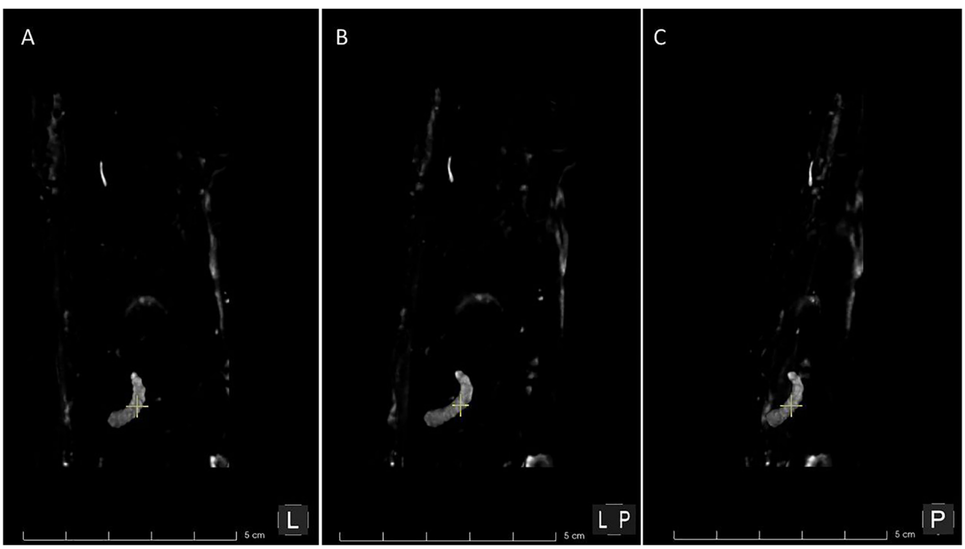

Fig. 2. Visibility of a wood boring larva by Maximum Intensity Projection from different perspectives. L: Left projection, LP: Left-posterior projection, P: Posterior projection (clockwise rotation around transversal axis)

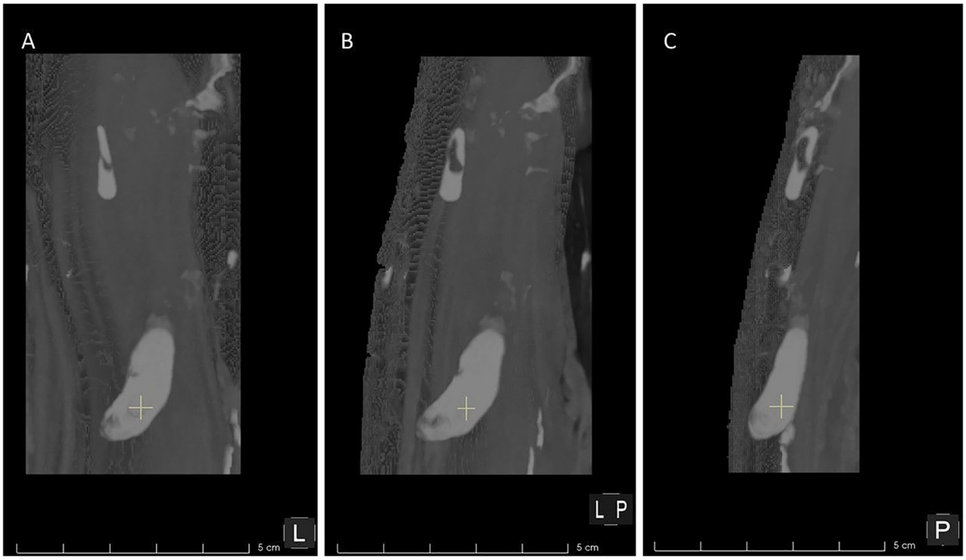

Fig. 3. Visibility of the cavity of insect wood boring larvae in beech branch by Minimum Intensity Projection from different perspectives. L: Left projection, LP: Left-posterior projection, P: Posterior projection (clockwise rotation around transversal axis)

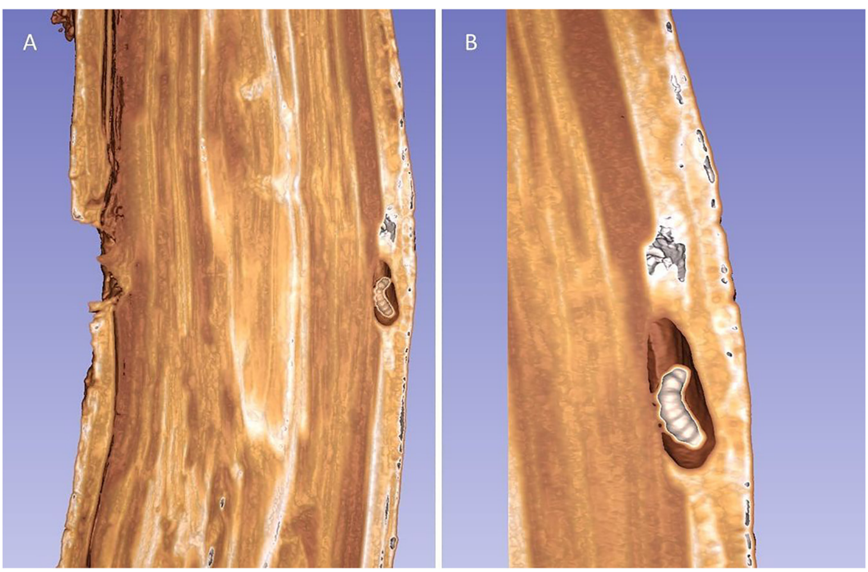

Fig. 4. 3D-rendered images of cermabycid larvae using 0.6 mm (A) and 0.1 mm (B) spatial resolution

allows you to separate different details by radiodensity ranges using colours. The parts that obscure the parts you want to display can be virtually cut off from deeply-hidden objects (Fig. 5). Similar to Fig. 4, the image processing of Fig. 5 provides a more detailed entomological insight. Thus, among other things, the abdominal legs of the wood borer larva can be seen, which confirms the fact that it belongs to cerambycids.

5th step. From the perspectives of the insects. Virtual endoscopy

Virtual endoscopy provides an opportunity to visualise from an internal perspective the pathways formed by the pest in the plant along its entire length. This can be accomplished by the virtual colonoscopy display technique (Fig. 6A) provided with the scanner, but using the data with a program that handles 3D-models can render it significantly more real (Fig. 6B).

DISCUSSION

The CT technique is a prospective method for the non-destructive examination of the lifestyle/life history of hidden pests (i.e. insects) (Keszthelyi et al., 2020). The surveys provide an

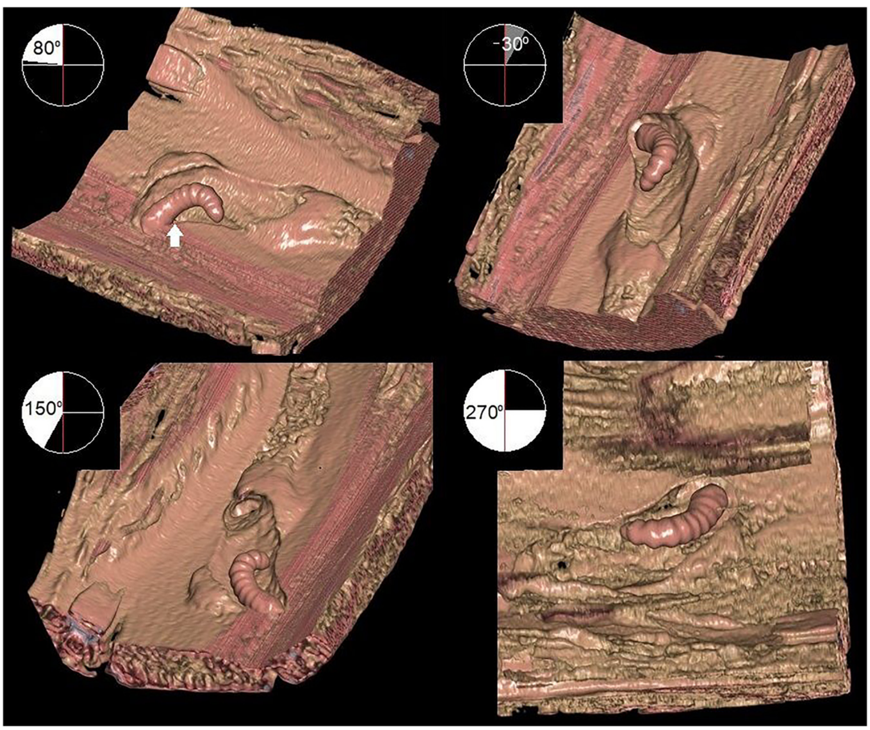

Fig. 5. 3D-rendered images of the cerambycid larva from different perspectives. Abdominal legs were indicated by white arrows

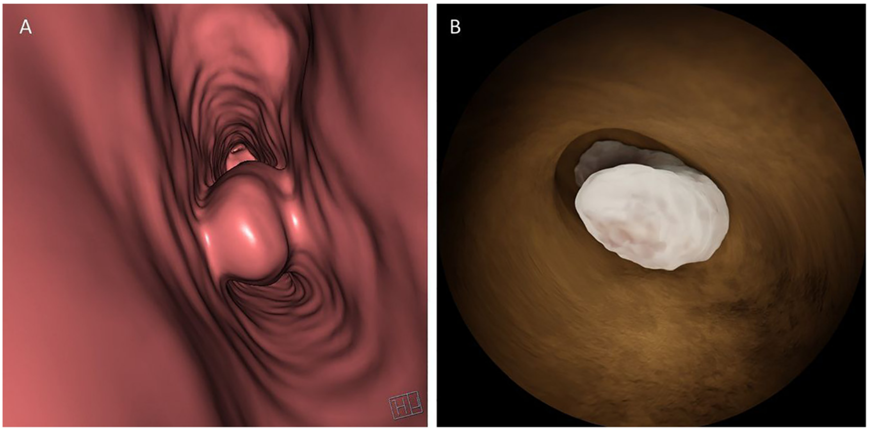

Fig. 6. Visualisation of a cerambyicid larva by virtual colonoscopy technique by Siemens (A) and an open-source 3D computed graphics programme (Blender) (B)

opportunity to perform measurements and maps that allow us to understand more about the lifestyle characteristics of insects (Fig. 7).

With the proposed methods, CT imaging is suitable for describing quantitatively the progress of the damage by insects and their temporal development by performing repeated

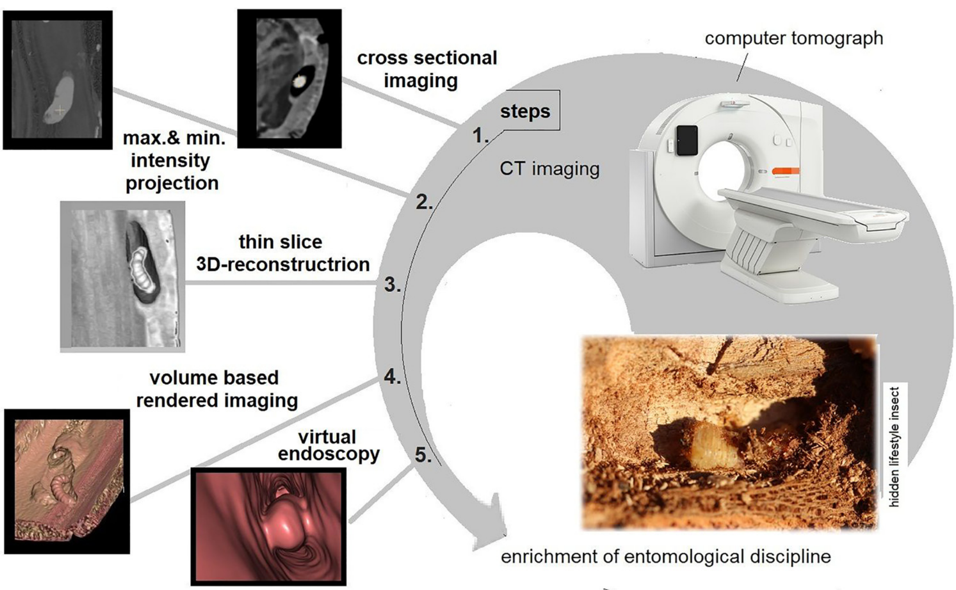

Fig. 7. Reviewing of the entomological outputs of some processing steps of the computed tomographical diagnostic

scanning. Both cross-sectional and 3D-rendered images can capture important data that would not be accessible by destructive examination alone. Quantitative data on modern 3D-image processing techniques can be collected on either the larvae or can serve to evaluate the extent of damage caused to the plant (Arbat et al., 2021).

The possibilities of the employment of human diagnostic CT equipment are described in this work emphasising both its entomological advantages and limitations when studying the example of a hidden lifestyle insect. However, it is known, that the μCT allows us to create additional, significantly higher – spatial resolution (below 10 μm) images. The μCT permits the mapping of micrometer-sized formulas, which points more towards the study of the morphology of insects (Mensa et al., 2022). Generally, due to the size limitation of micro CT and the significantly longer time required for applying their technique, diagnostic CT can remain the tool of choice for imaging larger organisms.

The technical knowledge of individual image processing steps can help to learn about some previously unknown entomological details. The cross sectional initial imaging output can provide lots of useful knowledge for both the basic and applied entomological researches, which obviously cannot be reached by traditional tissue dissection methods. Several data can be obtained from the evaluation of these images, such as positions related to each other of some developing stages, the spatial orientation of larvae, pupae, adults in plant tissue, or larvae dispersion after the period of the egg hatching. Similar results are reported by Martel and Belanger (1977), Crocker et al. (2014), and Orr et al. (2015). The feature of some ecological interactions (i.e. potential parasitism/predation) can be also clarified (Chudek et al., 1996). The features of the arthropod-plant interactions have been studied (Patra et al., 2010; Keszthelyi et al., 2021a,b), which supply data on the host such as the volume, location, and surface of the formed cavities or their position related to each other (Keszthelyi et al., 2018). Furthermore, the degree of organic matter destruction and the involvement of some tissues or organs can be objectively calculated from these results (Wei et al., 2011; McElrone et al., 2013). In summary, these data can greatly contribute to investigations aimed at dissecting the ethological and autecological features of the hidden lifestyle arthropods, which complement classic diagnostic tools.

The harmful effect of X-ray irradiation on the observable target organism is negligible. According to the radiation dose, the parameters of the CT exam were DLP (Dose Length Product) 709.9 mGy*cm. The radiation sensitivity factor (k-factor) of objectives (larvae in the wood) is unknown, that is why we choose to calculate the k-factor of the one most sensitive area (chest) of baby 0.099 (Romanyukha et al., 2016). In this case, the effective dose was 0.07 Gy. Keszthelyi et al. (2015) investigated the mortality of *Sitophilus granarius* L. using different doses. The irradiation did not cause higher mortality using 10 Gy compared to the control group. The CT examination has a hundred times smaller effective dose, so the deterministic effect is not expected, but the stochastic effect could only be established with long-term examinations.

The application of maximum and minimum intensity projections of the visual displaying can further support a better perceptibility of the animal tissues differing from the surrounding wood materials. It creates an opportunity for more exact detection of smaller animal bodies or profound information on the hidden lifestyle arthropod stages. Thus, the ecological, diagnostic data complex acquired via 2D-imaging is clarified by data obtained in this way.

Differences in the density range other than the above can be further refined by setting the "windowing" already mentioned during 2D-rendering, in the case of 3D-rendering as well.

The windowing allows the mapping of inner structures of wood giving shelter to the covertly developing species, as well as it can contribute to the cognition of other material and physical features of the plant tissues. From an entomological point of view, the importance of this step lies in obtaining supplementary information such as details of host plants and habitat choices in the case of some species.

3D-reconstruction can play a role also in the recognition of the morphological details (e.g. number of segments, some chaetotaxical characters) or deeper parameters of insect physiology. The very important advantage of the 3D-reconstruction is that such data can be obtained, which can be determinative factors in the species identification or taxonomical classification through the exactly measured longitudinal parameters or their shapes. In addition, the morphometrical mapping of some ontogenetic stages and the effect of abiotic factors on the phenological characters of the insect can also be investigated. In addition, the damage on cuticles or the presence of the ectoparasitoids could be assessed by means of evaluation of these images. Not only the target organism, but the location, orientation, volume, and shape of the cavities formed can also be studied by means of generating these data. The degree of plant impairments caused by some phytophagous insects can also be objectively determined.

The addition of surface features and colours to the 3D-formed arthropod bodies can contribute to knowing the role of olfactory and visual stimuli on the concealment processes. Furthermore, these added characters can help image the covert, biological regularities as well as to realise the visualisation of the scientific and educational excipients.

Virtual endoscopy offers a unique, hitherto unimaginable perspective, the potential applications of which other than human health are completely unknown in plant and entomological studies. Primarily, this method from the entomologists' perspective can give additional details to our knowledge on the physical and physiological processes through the mapping of covertly formed cavities and boring passages. The role of some organic matters derived from arthropods can be specified with the help of the evaluation of surface cavities such as pupal cradle of *Rhagium* spp. or the silk nets and tissues of lepidopterous pupae (*Bombyx mori*) and larvae (Tortricidae).

The displayed model could be unequivocally pointed out the presence of a hidden arthropod pest. In the next future developing industrial equipment building on this scheme can help the scouting of the invaders hiding among import packaging materials, which can contribute to the prevention of importation and escalation of the quarantine or other dangerous pests. The displayed model could be unequivocally pointed out the presence of a hidden arthropod pest. In the next future developing industrial equipment building on this scheme can help the scouting of the invaders hiding among import packaging materials, which can contribute to the prevention of importation and escalation of the quarantine or other dangerous pests.

In summary, the biology of hidden lifestyle insects as well as related plant physiological consequences, could be investigated by this non-invasive method. All this information can be obtained in such a way that the observed arthropods can remain, relatively undisturbed, in their natural environment, and through this circumstance, they are expected to behave as a component of their adequate ecosystem. The implementation of analysis on this undisputedly powerful instrument requires a high professional competence, and, though expensive, this special and expensive equipment provides in-depth information on hidden lifestyle insects. Undoubtedly, the disadvantage of the method is the x-ray irradiation load of the observed organisms. However, it can be stated that the high x-ray tolerance-ability of insects makes it possible to fulfil the criterion of *in situ* observability (Socha et al., 2007). Nevertheless, information and experimental data originating from this method could unequivocally contribute to the enrichment of the knowledge on the actual target organism.

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