Comparing micro-CT results of insects with classical anatomical studies: The European honey bee (*Apis mellifera* Linnaeus, 1758) as a benchmark (Insecta: Hymenoptera, Apidae)

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Introduction

In many papers are mentioned the advantages of the micro-CT because it does not damage the samples, and because the images are comparable with others we can get with scanning.

*Figure 1.* Picture composition of volume rendering reconstructions of honey bee workers in their daily activities: foraging and sipping nectar on a daisy flower (*Aster* sp.). To point out the internal structures the insect and the flower have been “virtually” cut by using the free Skyscan’s software CT Vox. Colors were obtained varying the color transfer function curves, conjunction with the lighting and shadowing options within CTVox’s software, as described by [1].
Figure 2. Mounting and fixation of samples to the sample holder for the scanning process. A prepared honey worker bee, on an excavated piece of Basotect®, inside a plastic container (a, b, d); e: garden daisy flower (Aster sp.), fresh-fixed with plasticine; c: detail of a NRecon’s view of a specimen to show how transparent is the Basotect® material to x-ray.

electron microscopy (SEM). There is no discussion about it, and there is a general consensus on this respect [2]–[5]. In many of them obtained rendering images are of high quality, permitting to defend that micro-CT is more than a complementary technique [4], [6], [7]. Previously we have been publishing different studies of small animals anatomy pointing out the incredible results that can be obtained not only in the field of the anatomy [5], [8]–[18], but
Figure 3. Volume rendering of a honey worker bee. External anatomy in lateral view (a); sagittal cut showing the internal structures and organs (b).
Figure 4. Volume rendering of a honey worker bee showing the external anatomy of the head in a lateral (a) and a frontal view (b), with mouth parts composing the sucking proboscis, and details of the very reduced maxillary pal (see red arrow in a, and c). Ventral view of the tip of the tarsus of first leg (f). Original figures (d, e,) from Snodgrass (1910)’s are included to be able to compare.
Figure 5. Volume renderings of the wings (a) of a honey worker bee showing in b details of the hamuli (a row of hook on the fore margin of the hind wing. These fix and attach it to the fore wing, permitting to synchronize the flight movements). Original figure by Snodgrass (1910) it is included to be able to compare (c).
Figure 6. Volume renderings of the head in a lateral view, showing the external (a) and internal (b) anatomical structures.
Figure 7. Volume renderings of internal anatomy of the head in a lateral view, progressive cuts (c, d) show the internal anatomical structures with a high definition. Observe the correspondence with the original Snodgrass (1910)'s figures (a, b).
Figure 8. Volume renderings of head in a frontal view. In the colored lower picture the external surface has been slightly cut permitting to evidence the fore glandular complex.
Figure 9. Volume rendering superficial section of head in a frontal view permitting to see the internal structures, and especially the anterior pharyngeal glands. Original Snodgrass (1910)'s figure (b), is included to be able to compare.
Figure 10. Volume rendering section of the head in an anterior view, evidencing the brain and its main nerves, as well as the eyes structure.
Figure 11. Volume rendering section of the head in a posterior view showing the internal structures.
Figure 12. Volume rendering of a front-posterior section of the head showing the detailed structure of the compound eye (b), permitting to compare it with the Philip’s original figure reproduced the classic study by Snodgrass (1910) (a). For details about the use of micro-CT to study the insect’s eye structure see [8].
Figure 13. Volume renderings of the internal organs of a honey worker bee in ventro-dorsal (b) and dorso-ventral (c) views, respectively and the comparison with the original figure by Snodgrass (1910). To be able to get these images it was used the sphere cutting in the cutting/clipping option within CTVox’s menu. Colors are artificial and represent the opacity of structures to x-Ray, according with the scale.
Figure 14. Volume rendering of an internal view of the dorsal abdominal region of the honey bee’s worker showing the dorsal vase (heart) and it winged shaped muscles, as well other structures (b). It is included an original figure by Snodgrass (1910) to compare. To be able to get this image it was used the sphere cutting options in the cutting/clipping option within CTVox’s menu. Colors are artificial and represent the opacity of structures to x-Ray, according with the scale in figure 13.
Figure 15. Volume rendering of a mid-section of the hind abdominal part of the honey worker bee (b) and its comparison with the original figure by Snodgrass (1910) (a)
Figure 16. Volume renderings of sections of the hind abdominal part of the honey worker bee, in a lateral (b) and a dorso-ventral view (c), showing the detailed anatomy of the sting and its accessory structures. For comparison the original figures by Snodgrass (1910)- in a lateral (a) and a dorso-ventral (b) views- have been included.
Figure 17. Volume renderings of section, in dorso-ventral views, of the internal anatomy of the honey worker bee. Most of the internal structures have been removed with software to be able to see the nervous system: the brain in the head, and the thoracic (b), and abdominal (c) ventral ganglionic nerve cord, permitting to compare it with a similar view drawn by Snodgrass (1910) (a)
also resulted of relevant importance to elucidate adaptive survival strategies of insects [19], [20].

**Aims**

We already tested the quality of the images obtained with micro-CT [12]–[14], [17], [18], [21]–[25], with the advantage that the images dataset can be visualized as many times as needed with the possibility to change the observation perspective and the original sample can be returned to the collections or museum where it is deposed. Now that it seems that there are no doubts about the importance of micro-CT as a modern research tool, as it is demonstrated in the increasing of papers using it. Because we are involved in a project studying bees, recently we published a general micro-CT study of a mason bee (*Osmia rufa*) [9], and we decided to undertake a detailed micro-CT study of the European honey bee (*Apis mellifera* Linnaeus, 1758), species well known for the general public and from which has been publish much literature. However, a classical anatomical study was published in the beginning of the twentieth century [26], still it is the more complete compendium about the anatomy of the honey bee and in general it represents some sort of bible about the knowledge of the insect anatomy. Recently have been publish different papers in which using modern microscopic techniques, or even micro-CT, are studied different body structures, but mainly the brain (i.e. [27]–[34]). We decided in this paper to show details of the external and internal anatomy of the whole body of worker bees, comparing the micro-CT volume renderings images with the Snodgrass’ drawings as the most classic representative study.

**Method**

Workers of the the European honey bee (*Apis mellifera* Linnaeus, 1758) were collected alive when foraging in the Sierra Nevada montains, southern Spain (11-12-2016, 2-I-2017, Pinos Genil, Granada 980 m. a.s.l.). Specimens were killed and preserved in 70% ethanol for 5 days and then transferred to a 1% solution of Iodine in 100% ethanol during 24 hours, after that they were submerged in Hexamethyldisilazane for 9-24 hours and overnight air dried. The sample was mounted in a piece of BASOTECT® (melamine resin foam, created by the Chemical Company BASF), inside a plastic container to avoid any movement provoked by the air refrigerating current or forced air during the scan process (see fig. 2). The Basotect’s material results of very low density, and therefore very transparent to X-Ray (see fig. 2c), and therefore it can be easily eliminated during the segmentation procedure.

A SkyScan 1172 high resolution microtomograph, upgraded to have a Hamamatsu 100/250 source and a SHT 11Mp camera was used. The scanning parameters were setup as it follows: Isotropic voxel size = 4.45 µm per pixel; Source Voltage=53-55KV, Source Current=124-127µA, and image. Rotation step=0.5°, 360° of rotation scan. The daisy flower (*Aster* sp.) used for the artistic composition of fig.18, was cut from the garden of the faculty campus, immediately fixed with plasticine to the sample holder, and fresh scanned the following setting parameters: Isotropic voxel size = 23.5 µm per pixel; Source Voltage=54KV, Source Current=43µA, and image. Rotation step=0.5°, 180° of rotation scan.

For primary reconstructions and the “cleaning” process to obtain the datasets of crossection images (“slices”) it was used the up-to-dated last versions of the free Bruker micro-CT’s Skyscan ([www.skyscan.be](http://www.skyscan.be)) software (NRecon, DataViewer, CTAnalyser,). Volume renderings images were obtained with FEI’s Amira software v.6.2 [35]. The free Skyscan’s software CTVox was used to get color volume rendering images in figures: 1, 13 and 14. Colors were obtained varying the color transfer function curves, conjunction with the lighting and shadowing options. For more detailed explanation of the process see the previous paper [1]. It is important to point out that Amira’s volume renderings were obtained directly by loading image datasets obtained with the Bruker-microCT Skyscan’s, resulting mirrored inverted images.
Results and discussion
In figures 3 to 17 are illustrated the results obtained of this study on the anatomy of the honey bee and the respective comparison of the structures as were drawn by Snodgrass in his classic paper of the first decade of the twentieth century [26]. These demonstrate the high precision with which the old authors did the anatomical studies, because after a thorough comparison can be observed only small differences in shapes. In general Snodgrass drawings are somewhat more elongated than real, but even when looking at very delicate small structures as the brain and its nerves agree with what we could enhance by using the modern technique of micro-tomography.

Conclusion
As other authors found and we observed in previous papers, micro-CT is not only valid but incredibly reliable for anatomical studies, permitting to study the animals or structures scanned as many times as desired, virtually moving the sample and exploring it from any angle/perspective. Making this extremely valuable not only for actual, but for future new studies based on the same stored images dataset. Thereafter, we consider very important to start to organize an international depository where to upload images datasets that could help the researchers in a similar way as genetic database depositories already are being used by researchers around the world to share their discoverings.

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