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MicroCT 3D reconstruction of three described braconid species (Hymenoptera: Braconidae)

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ABSTRACT. Traditionally, entomologists have used destructive methods especially dissection in order to investigate the important taxonomic characters of specimens. New technologies for imaging and analyzing in taxonomy, offer opportunities to deposit three-dimensional (3D) data to proposed for rare and valuable type materials in museums and collections. Micro-computed tomography, as a non-destructive imaging technique, has become an emerging and progressive technology in insect science. However, this technology is rarely used in entomology compared to in medical and industrial applications. In this study, MicroCT imaging protocols are explained in detail using three species of braconid wasps: *Aleiodes arnoldii* Tobias, 1976 (Braconidae: Rogadinae), *Hormius moniliatus* Nee, 1811 (Braconidae: Hormiinae) and *Macrocentrus bicolor* Curtis, 1833 (Braconidae: Macrocentrinae). MicroCT scan data of three braconid wasp species from Iran, depicted main identification of skeletal body parts. A brief step-by-step is provided on image acquisition, 3D reconstruction and mesh editing to create a virtual model of the species utilized for morphological and morphometric studies. As a result, the use of micro-computed tomography as a non-invasive virtual examination tool was explored. The complete datasets containing the raw TIFF MicroCT data, 3D models and 3D rotation videos available for download at http://www.morphosource.org/Detail/ProjectDetail/Show/project_id/822

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Introduction

Micro-computed tomography (micro-CT or μCT) is an emerging technology in taxonomic science that enables to construct a high-resolution 3D reconstruction of specimens (Friedrich & Beutel, 2008; Greco et al., 2008). These 3D reconstructions can be virtually rotated, measured and dissected. The success of this imaging technique lies in its non-destructive

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dissection and fast imaging (Metscher, 2009). Conventionally, entomologists use destructive dissection techniques which often damage specimens and expose the type materials (Friedrich et al., 2014; Friedrich & Beutel, 2008; Iwan et al., 2015). Godfray (2007) suggested the idea to create virtual types with easy accessible correct and permanent three-dimensional reconstructions of type material to scientists and non-specials. After that, Faulwetter et al. (2013) introduced the definition of "Cybertypes" and proposed a workflow to create such virtual collections. Advancements of computed tomographic technology and data processing software (e.g., FEI Amira, Mimics innovation suite, VG Studio Max and SPIERS) and the increasing availability of MicroCT facilities, is enabling scientists to use 3D reconstructed images and cybertypes (Akkari et al., 2015). In recent years, X-ray based methods have been extensively applied in the analysis of insect sciences including cephalic morphology (Hörnschemeyer et al., 2002), functional morphology (Beutel et al., 2008, 2010; Lipke et al., 2014; Pasandideh Saqalaksari et al., 2020; Wojcieszek et al., 2012; Zimmermann et al., 2011) forensic entomology (Richards et al., 2012), developmental biology (Metscher, 2009) and insect taxonomy (Fischer et al., 2016; Garcia et al., 2017; Sarnat et al., 2017; Simonsen & Kitching, 2014; van de Kamp et al., 2018).

Braconidae is the second largest family of Hymenoptera with over 21000 described species worldwide (Yu et al., 2016). This family is one of the largest groups of parasitoids mainly in the larval stages of insect pests (Quicke, 2015; Shaw & Huddleston, 1991). Taxonomy of Braconidae is thoroughly founded on the morphology of the females (Sharkey & Wharton, 1997; Wharton et al., 1992). Due to the female morphology, the majority of studies use basic characters such as setation, surface sculpture, character's size, differences in shape of few body parts and wing venation (Wharton et al., 1992). Correct identifications require careful and precise examinations. When literature-based descriptions are insufficient, the only option is to loan and examine physical type materials. Loaning type material from natural history museum that spread globally is a costly and time-consuming process.

In this study, the MicroCT imaging properties are described stepwise, from preparation to data analysis, using three braconid wasp species. The dataset presented was created with the purpose to visually support descriptive taxonomic studies of braconid wasps especially Iranian braconids. The 3D raw data are made available here for public and can also be used for morphological examinations and educational goals. Up to now, this is the first publicly-available MicroCT dataset of Braconidae that includes scans of three described species of Braconidae from northern Iran. Although virtual representation of a specimen does not replace of physical examination of museum type materials, the MicroCT dataset presented will serve as an extra taxonomic tool that may be sufficient in species identifications. By making the dataset publicly accessible, we allow taxonomists potentially to spend less time and cost for specimen examination and character comparisons.

Material and methods

Scans of three species from three different subfamilies of Braconidae are presented: *Aleiodes arnoldii* Tobias, 1976 (Rogadinae), *Macrocentrus bicolor* Curtis, 1833 (Macrocentrinae) and *Hormius moniliatus* Nees, 1811 (Hormiinae) (see Tobias, 1976; Curtis, 1833; Nees von Esenbeck, 1811; Farahani et al., 2012, 2015; Ameri et al., 2016). All specimens were collected in northern Iran and the materials are deposited at Tarbiat Modares University Entomological Collection (TMUC), Tehran, Iran. An overview of the specimen information is provided in Table 1.

Table 1. Data summary of MicroCT scanning for the three species of braconid parasitoid wasps (Hym.: Braconidae).

Species	Subfamily	Body part scanned	Voxel size	Magnific-ation	Scintill-ator	Filter	Frame per second	Scan duration
Aleiodes arnoldii	Rogadinae	Full body	6.11 µm	2x	50 µm LuAG	500 µm Aluminium	120	25 sec
Hormius moniliatus	Hormiinae	Full body	2.44 µm	5x	12 µm LSO:Tb	200 µm Aluminium	80	37.5 sec
Macrocentrus bicolor	Macrocentr-inae	Full body	6.11 µm	2x	50 µm LuAG	500 µm Aluminium	120	25 sec

The Synchrotron X-ray microtomography (SR- MicroCT) was performed at the UFO imaging station of the KIT light source (Greco et al., 2008; Richards et al., 2012; Garcia et al., 2017). A parallel polychromatic X-ray beam was spectrally filtered to obtain a peak at about 15 keV. The detector consisted of a scintillator, optically coupled via a Nikon Nikkor 85/1.4 photo-lens to a pco.dimax camera with a pixel matrix of 2008×2008 pixels. 3,000 radiographic projections were acquired for each scan. Tomographic reconstruction was performed with the GPU-accelerated filtered back projection algorithm implemented in the software framework UFO. *Macrocentrus bicolor* & *Hormius moniliatus* were scanned in two, *Aleiodes arnoldii* in one step. The projections have resolutions of 2008×2008 pixels and the original file size was 5784 MB for all scans.

Post processing of raw data was performed with FEI Amira (version 6.0). Volume rendering of all post-processed datasets was performed in Amira 6.0 by using the "volume rendering" function. The desired volume rendering was generated by thresholding that the color space range adjust the visibility of the exterior surface of specimens. Screenshot of surface display volume renderings were made with "snapshot" function. 3D surface models were converted into polygon meshes by using the "Isosurface" function. The meshes were exported as STL files and reassembled in Meshlab (Version 2016.12) which was employed for polygon reduction (Hita Garcia et al. 2017). The data were saved in PLY, imported into PDF3D ReportGen (version 2.9) and converted into PDF documents.

Results

First MicroCT database of Iranian Braconidae includes three species from northern Iran including *Aleiodes arnoldii* (Rogadinae), *Hormius moniliatus* (Hormiinae) and *Macrocentrus bicolor* (Macrocentrinae) are provided.

Aleiodes arnoldii Tobias (Braconidae: Rogadinae) (Figs 1, 4, 5, 6)

http://www.morphosource.org/Detail/MediaDetail/Show/media_id/46163
The cybertype dataset consists all volumetric raw data in TIFF format (1481 slices), 3D pdfs and 3D rotation videos of the full body. We also provide a freely accessible 3D surface model of the materials at Sketchfab (https://sketchfab.com/3d-models/aleiodes-arnoldii-6d6f9b8910b44944bf62e9d03b6d2976).

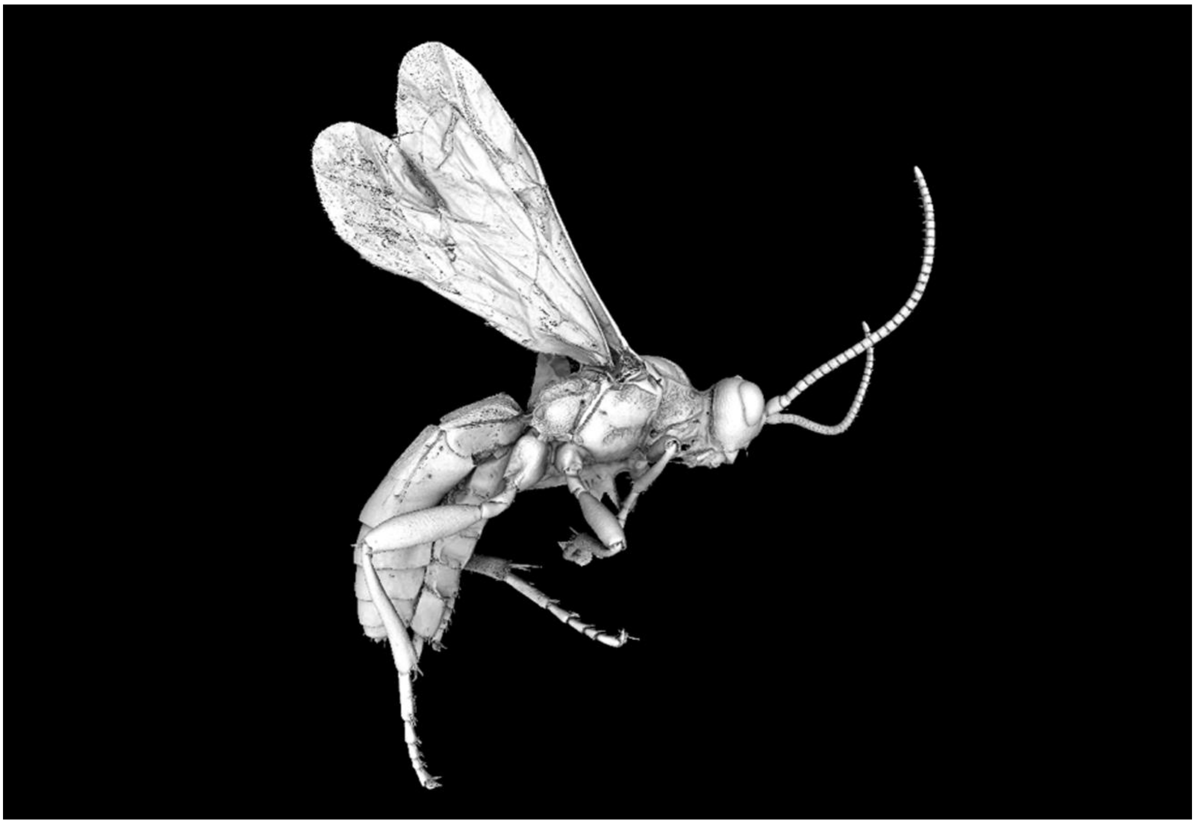

Figure 1. MicroCT volume reconstructions (three-dimensional) of *Aleiodes arnoldii* (Hym.: Braconidae: Rogadinae).

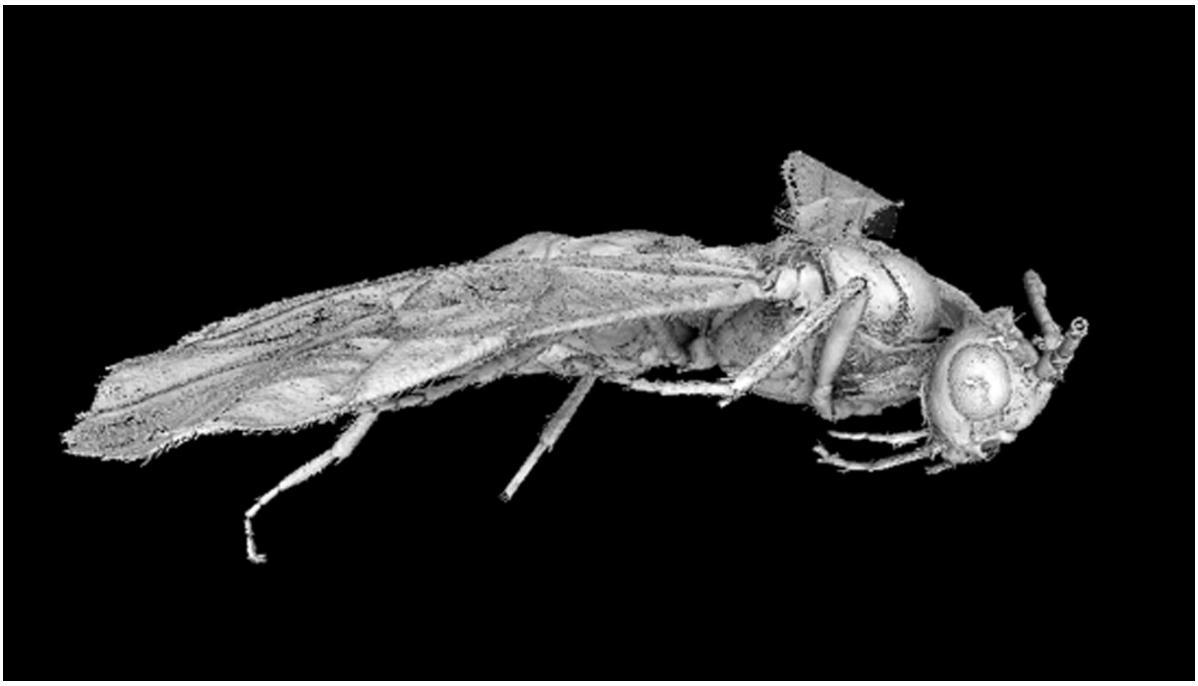

Figure 2. MicroCT volume reconstructions (three-dimensional) of *Hormius moniliatus* (Hym.: Braconidae: Hormiinae).

Hormius moniliatus **Nees (Braconidae: Hormiinae) (Figs 2, 4)**
https://www.morphosource.org/Detail/MediaDetail/Show/media_id/46164
The cybertype dataset consists all volumetric raw data in TIFF format (1481 slices), 3D pdfs and 3D rotation videos of the full body. We also provide a freely accessible 3D surface model of the materials at Sketchfab (https://sketchfab.com/3d-models/hormius-moniliatus-d99fd0d2ed9943b7b466802546b1bd1f).

Macrocentrus bicolor **Curtis (Braconidae: Hormiinae) (Figs 3, 4)**
https://www.morphosource.org/Detail/MediaDetail/Show/media_id/46168
The cybertype dataset consists all volumetric raw data in TIFF format (1481 slices), 3D pdfs and 3D rotation videos of the full body. We also provide a freely accessible 3D surface model of the materials at Sketchfab (https://sketchfab.com/3d-models/macrocentrus-bicolor-4408e246a72e450b98eedca59f19cb71).

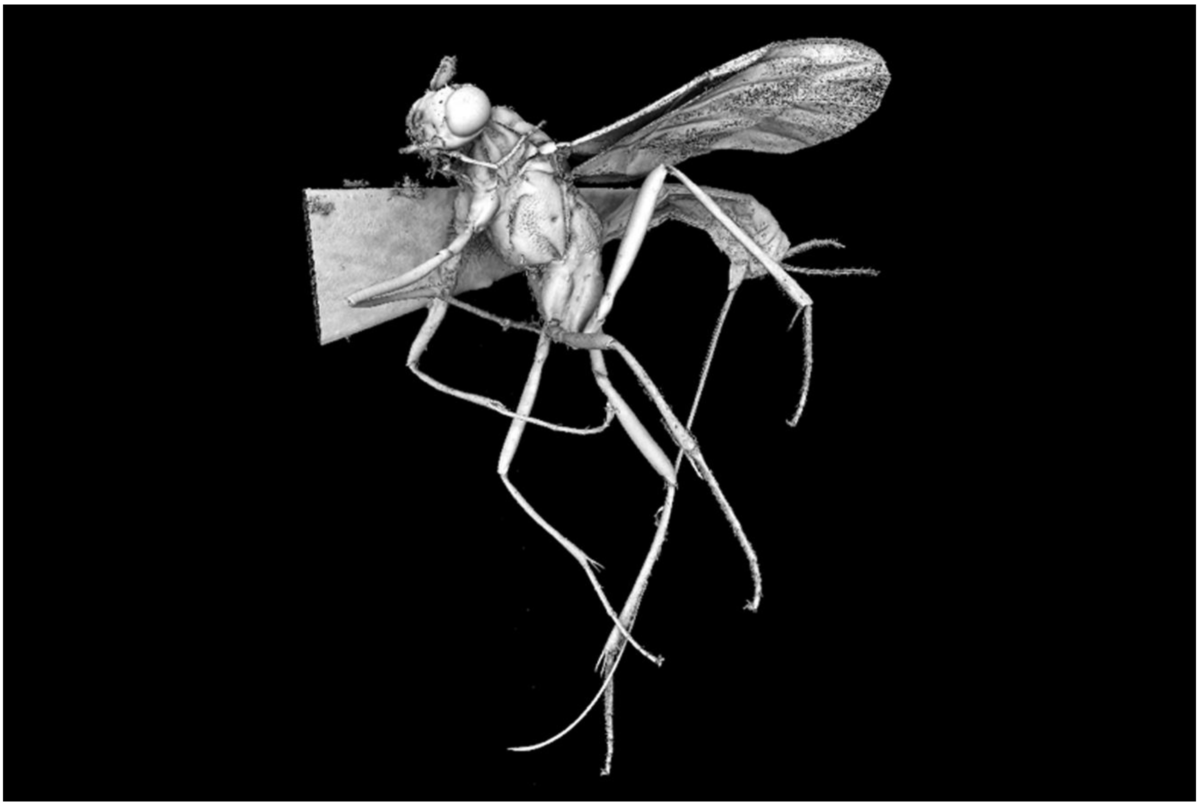

Figure 3. MicroCT volume reconstructions (three-dimensional) of *Macrocentrus bicolor* (Hym.: Braconidae: Macrocentrinae).

Discussion

The dataset presented can be utilized as an example of taxonomic studies generally; however the scans of the braconid wasps can also use to study the external morphology and it provides adequate structure visualization for characterization. As was the case for ants (Garcia et al., 2017) external morphological characters such as general shape, surface sculpture, body size and dimensions are important for identification and taxonomic discrimination and MicroCT scans are as precise as physical specimens in representing these features (Fig. 4).

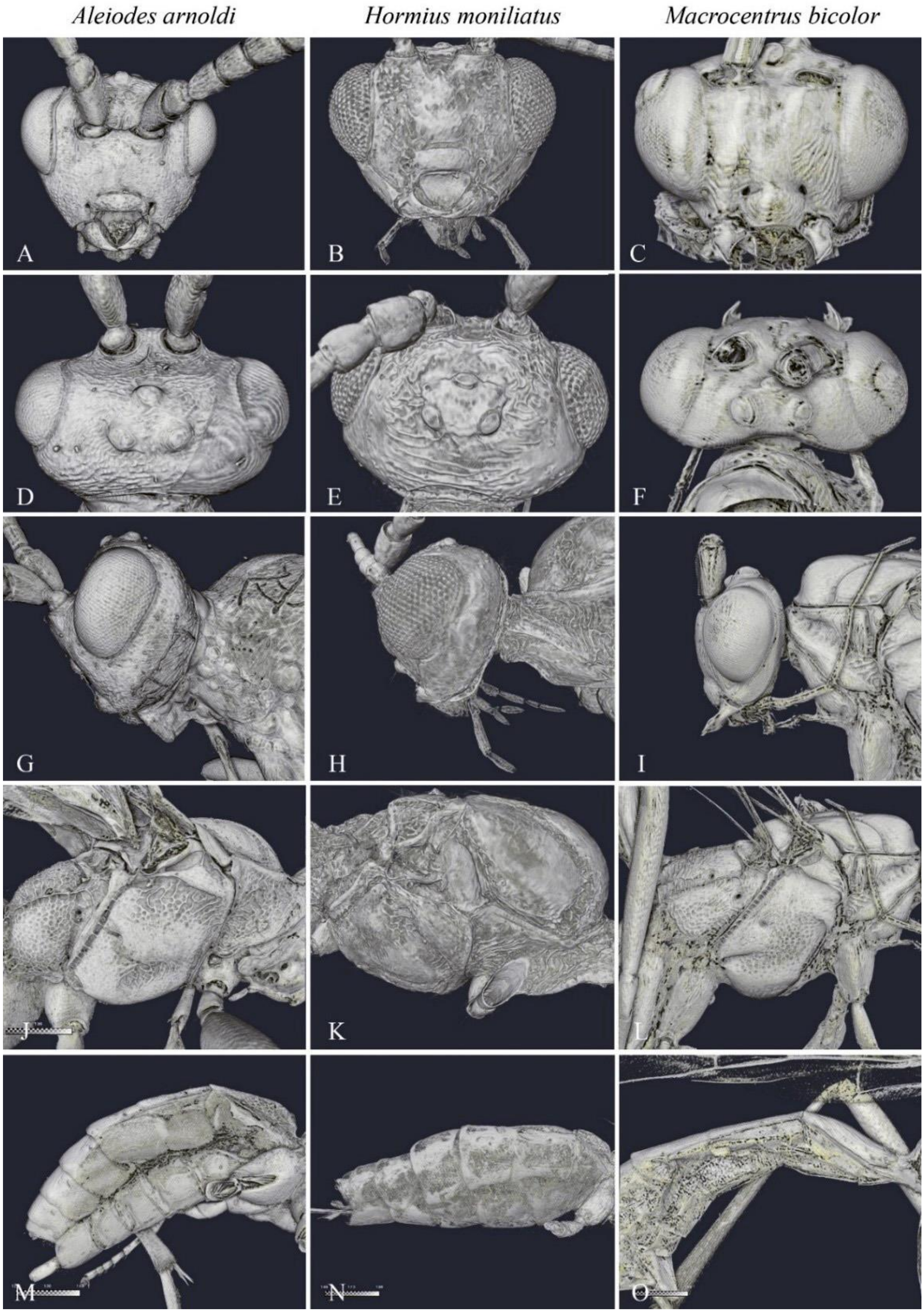

Figure 4. Volumetric rendering based on MicroCT images of *Aleiodes arnoldii* (left column) (Hym.: Braconidae, Rogadinae), *Hormius moniliatus* (middle column) (Hym.: Braconidae, Hormiinae) and *Macrocentrus bicolor* (right column) (Hym.: Braconidae, Macrocentrinae). **A, B, C.** Head, frontal view; **D, E, F.** Head, dorsal view; **G, H, I.** Head, lateral view; **J, K, L.** Mesosoma, lateral view; **M, N, O.** Metasoma, lateral view.

Although internal structures and features are not universally important in parasitoid taxonomy, the study of these structures can be widely used in physiological and morphological researches. Internal anatomical features are distinctive in all scans. Muscular structure, nervous system, tentorium, genitalia and the digestive system are visible in the volumetric data (Fig.5, Fig. 6). However, in comparison with a live and fresh specimen, the dry mount specimens do not provide enough internal information due to shrinking and desiccation of the structures (Garcia et al., 2017; Zimmermann et al., 2011). For this reason, alive, freshly killed and ethanol-preserved specimen have been required (Greco et al., 2008). The non-destructive virtual dissection of female genitalia and ovipositor is another application that not explored in this study, which traditionally done by dissection. Dissection is a hard and time-consuming process and requires proficient expert (Boring et al., 2009; Rahman et al., 1998). The application of 3D reconstruction of wasp's ovipositor can be used to study the functional morphology of this organ and taxonomic facilitation in species-level identification.

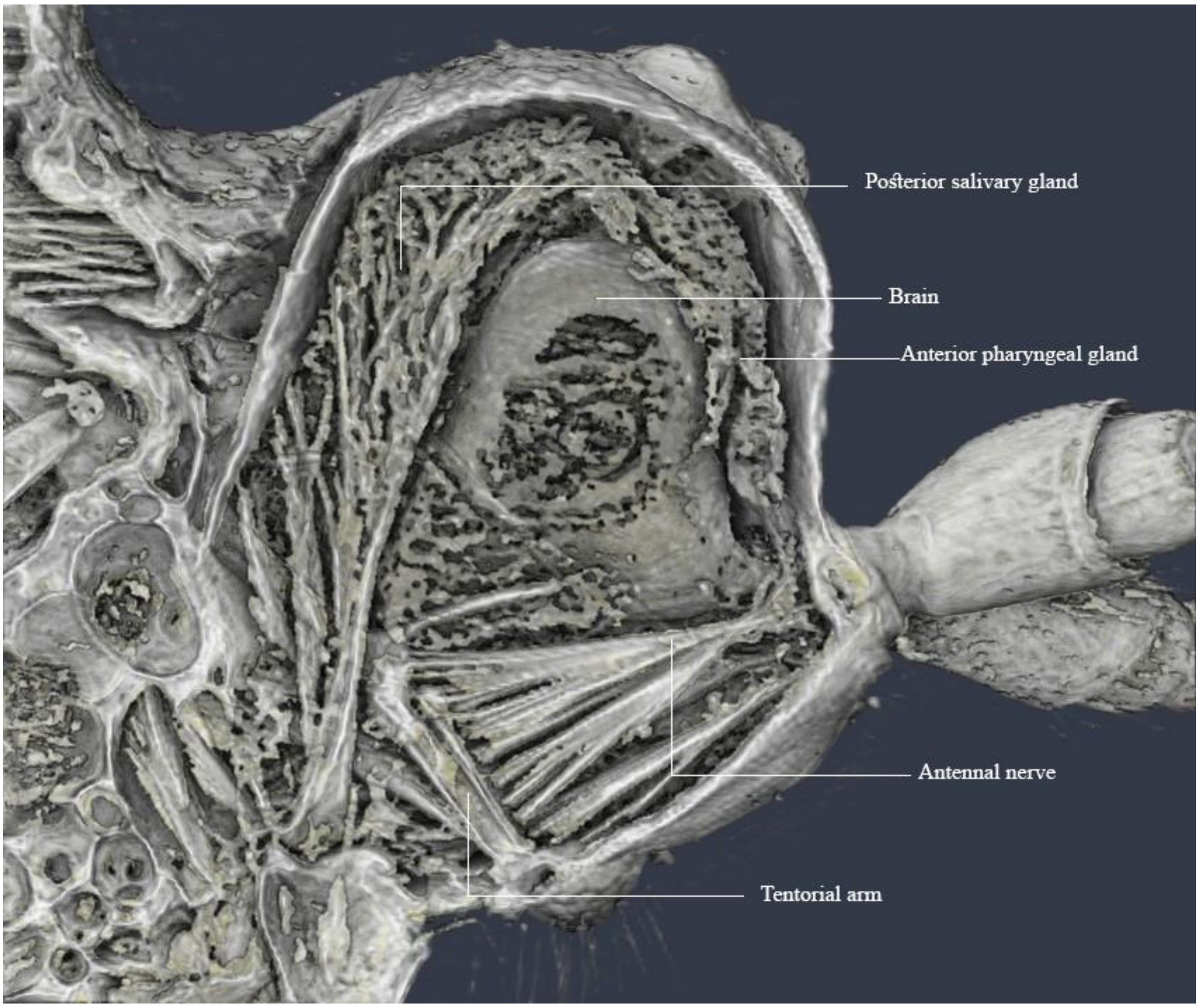

Figure 5. Volume rendering of the *Aleiodes arnoldii* (Hym.: Beaconidae, Rogadinae); head in a lateral view, showing the external and internal anatomical structures.

The value of the presented scans is in the potential of downloading freely a 3D virtual reconstructed specimen that otherwise would have to be loaned (Akkari et al., 2018). Loaning specimens requires cost and effort, in addition to the potential risk of loss or even destruction of a specimen. This dataset is freely accessible for download and at the same time can be examined by infinite researchers virtually.

The presented MicroCT dataset is deposited in the Morphosource Database Repository (http://morphosource.org). For the dataset, each scan contains the reconstructed stack slice images as 16-bit tiff stacks. 3D rendered video of each scan is provided for rapid overview (Suppl. materials 1, 2, 3). The 3D interactive PDF file is also provided that can be opened by Acrobat Reader. The online 3D web player of the meshes are also viewable to give users a direct summary of the data.

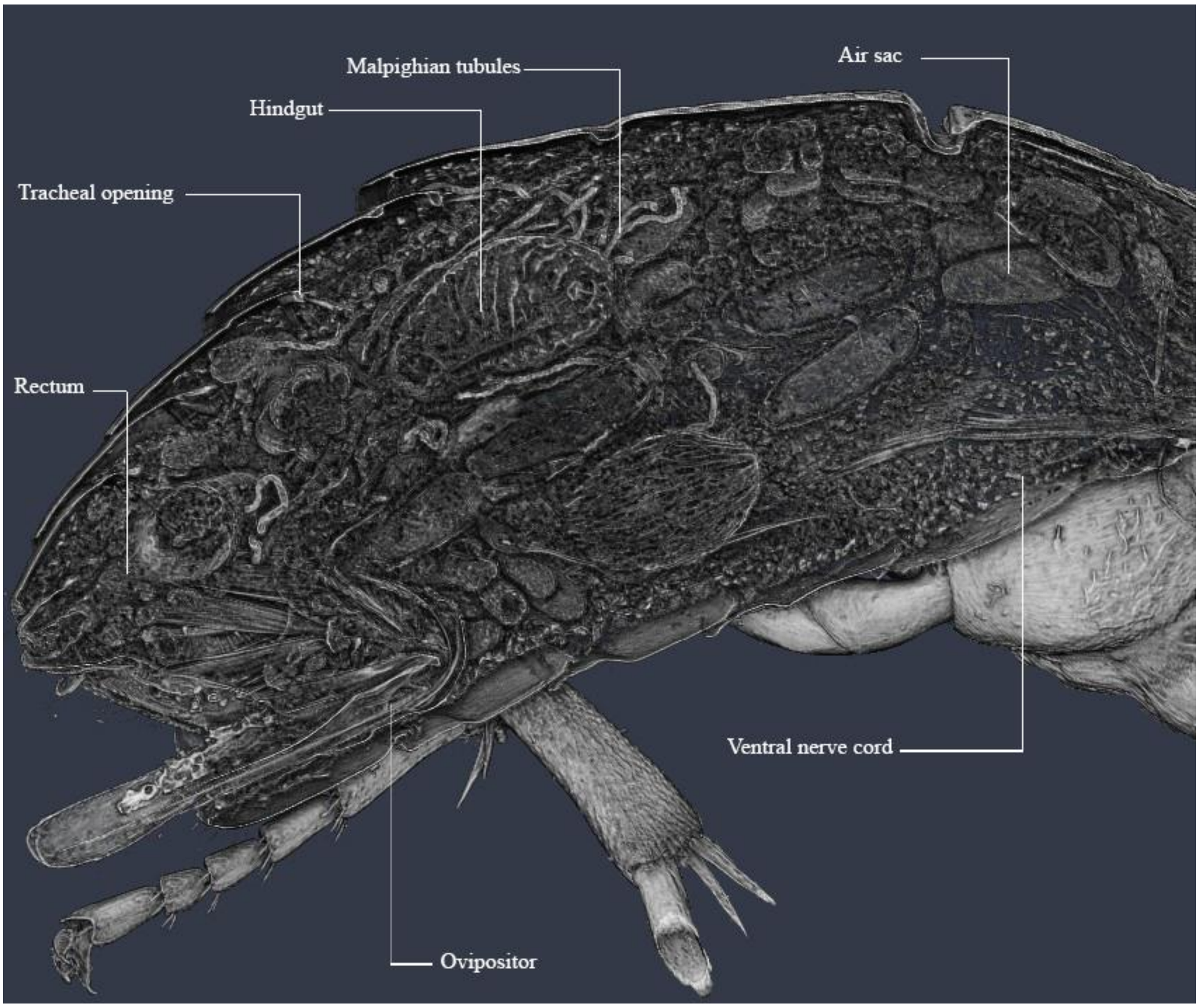

Figure 6. Volume rendering of the *Aleiodes arnoldii* (Hym.: Braconidae, Rogadinae); internal view of the lateral abdominal region showing the internal organs.

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Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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بازسازی سه بعدی ریزبرش‌نگاری رایانه‌ای سه گونهٔ توصیف شده از زنبورهای براکونید (Hymenoptera: Braconidae)

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چکیــده: به طور سنتی، حشره‌شناسان به منظور بررسی ویژگی‌های مهم تاکسونومی نمونه‌ها، از روش‌های مخرب بویژه تشریح استفاده می‌کنند. فناوری‌های نوین در تصویربرداری و تجزیه و تحلیل‌های تاکسونومی، فرصت‌هایی برای ذخیره‌سازی داده‌های سه بعدی جهت ارایه نمونه‌های نادر و با ارزش در موزه‌ها و کلکسیون‌ها فراهم می‌کند. ریزبرش‌نگاری رایانه‌ای، به عنوان یک تکنیک تصویربرداری غیرمخرب، به یک فناوری نوظهور و مترقی در علم حشره‌شناسی تبدیل شده است. با این حال، این فناوری در مقایسه با کاربردهای پزشکی و صنعتی، به ندرت در حشره‌شناسی مورد استفاده قرار گرفته است. در این پژوهش، تصویربرداری ریزبرش‌نگاری رایانه‌ای با استفاده از سه گونه از زنبورهای براکنید شامل *Aleiodes arnoldii* Tobias, 1976 (Braconidae: Rogadinae)، *Hormius moniliatus* Nee, 1811 (Braconidae: Hormiinae) و *Macrocentrus bicolor* Curtis, 1833 (Braconidae: Macrocentrinae) به تفصیل توضیح داده شده است. داده‌های اسکن ریزبرش‌نگاری رایانه‌ای سه گونه زنبور خانواده Braconidae از ایران، ویژگی‌های اجزای اصلی اسکلت بدن را نشان داد. یک شرح قدم به قدم از تهیه تصاویر، بازسازی سه بعدی و ویرایش مش‌ها تا ساخت مدل‌های مجازی گونه ها جهت استفاده در مطالعات مرفولوژیک و مرفومتریک ارائه شده است. به عنوان نتیجه، استفاده از فناوری ریزبرش‌نگاری رایانه‌ای به عنوان یک ابزار بررسی مجازی غیرتهاجمی، مورد بررسی قرار گرفت. مجموعه کامل داده‌ها شامل داده‌های TIFF ریزبرش‌نگاری رایانه‌ای، مدل‌های سه بعدی و فیلم‌های چرخشی سه بعدی به صورت آنلاین در آدرس http://www.morphosource.org/Detail/ProjectDetail/Show/project_id/822 در دسترس قرار گرفته است.

واژگــان کلیدی: MicroCT، تصویربرداری سه بعدی، سایبرتایپ، Braconidae، تاکسونومی، پارازیتوئید