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Two novel approaches to study arthropod anatomy by using dualbeam FIB/SEM

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ABSTRACT

Transmission Electron Microscopy (TEM) has always been the conventional method to study arthropod ultrastructure, while the use of Scanning Electron Microscopy (SEM) was mainly devoted to the examination of the external cuticular structures by secondary electrons. The new generation field emission SEMs are capable to generate images at sub-cellular level, comparable to TEM images employing backscattered electrons. The potential of this kind of acquisition becomes very powerful in the dual beam FIB/SEM where the SEM column is combined with a Focused Ion Beam (FIB) column. FIB uses ions as a nano-scalpel to slice samples fixed and embedded in resin, replacing traditional ultramicrotomy. We here present two novel methods, which optimize the use of FIB/SEM for studying arthropod anatomy.

1. Introduction

Traditionally, anatomical studies of arthropod fine structures have always been conducted using Scanning Electron Microscopy (SEM) combined with Transmission Electron Microscopy (TEM). In particular, minute external structures (such as pores, sensilla, scales, ommatidia) have been thoroughly described thanks to the use of SEM while TEM has been widely used to investigate the internal anatomy of the animal analysing ultrastructural features.

The presence of a hard exoskeleton facilitates the study of arthropod external morphology reducing the risk of artefacts and making easier to observe small cuticular structures employing SEM. However, the hardness of the integument and its thickness may represent an obstacle for the processing of arthropod samples; specifically, the chitinous nature of the exoskeleton (sclerotized and often mineralized) can cause not only several difficulties regarding the penetration of fixatives but it makes also very challenging to obtain satisfactory thin and ultrathin sections during the ultramicrotomy process.

These problems have affected the progresses of the anatomical studies of arthropods leading to a lack of balance between the analysis of external and internal features favouring fine morphological SEM studies over ultrastructural analyses of tissues and cells.

Over the past twenty years microscope technology has developed new instruments especially for physics and engineering applications (like scanning probe microscopy, focused ion beam microscopy, ultrasonic microscopy, X-ray microscopy), some of them combining more technology in a single complex system. However, biological studies rarely profited of these new technologies being more tied to the use of the classical microscopy techniques like optical, confocal, scanning or transmission microscopy.

In the last few years, as highlighted by Friedrich et al. (2014), entomologists showed a renewed interest in insect morphology and anatomy partly thanks to the possibility to combine complementary techniques ranging from the traditional approaches (dissections, histology and maceration) to the use of modern and advanced instruments like Confocal Laser Scanning Microscopy (CLSM), Micro Computed Tomography (μ-CT) or FIB/SEM. In particular, the possibilities offered by these new techniques allowed researchers to thoroughly analyse anatomical features that otherwise would be very hard or almost impossible to inspect. For example CLSM permits to three-dimensionally visualize the insect morphology and even the internal anatomy of small and delicate samples that are very troublesome to dissect and/or extremely hard to prepare for a successful histological analysis (Grzywacz et al., 2014; Smolla et al., 2014). Micro-CT instead makes it possible to acquire a complete series of virtual thin sections that are almost free of artefacts, perfectly aligned and obtained in a very short time compared to lengthy and often unsuccessful acquisition of histological sections (Martín-Vega et al., 2017). Other than providing a three dimensional data of both internal and external structures this analysis is non-destructive, so it can be performed on rare material (Simonsen and Kitching, 2014) and can be used even on ancient or fossil samples embedded in amber (Tafforeau et al., 2006; Pohl et al., 2010).

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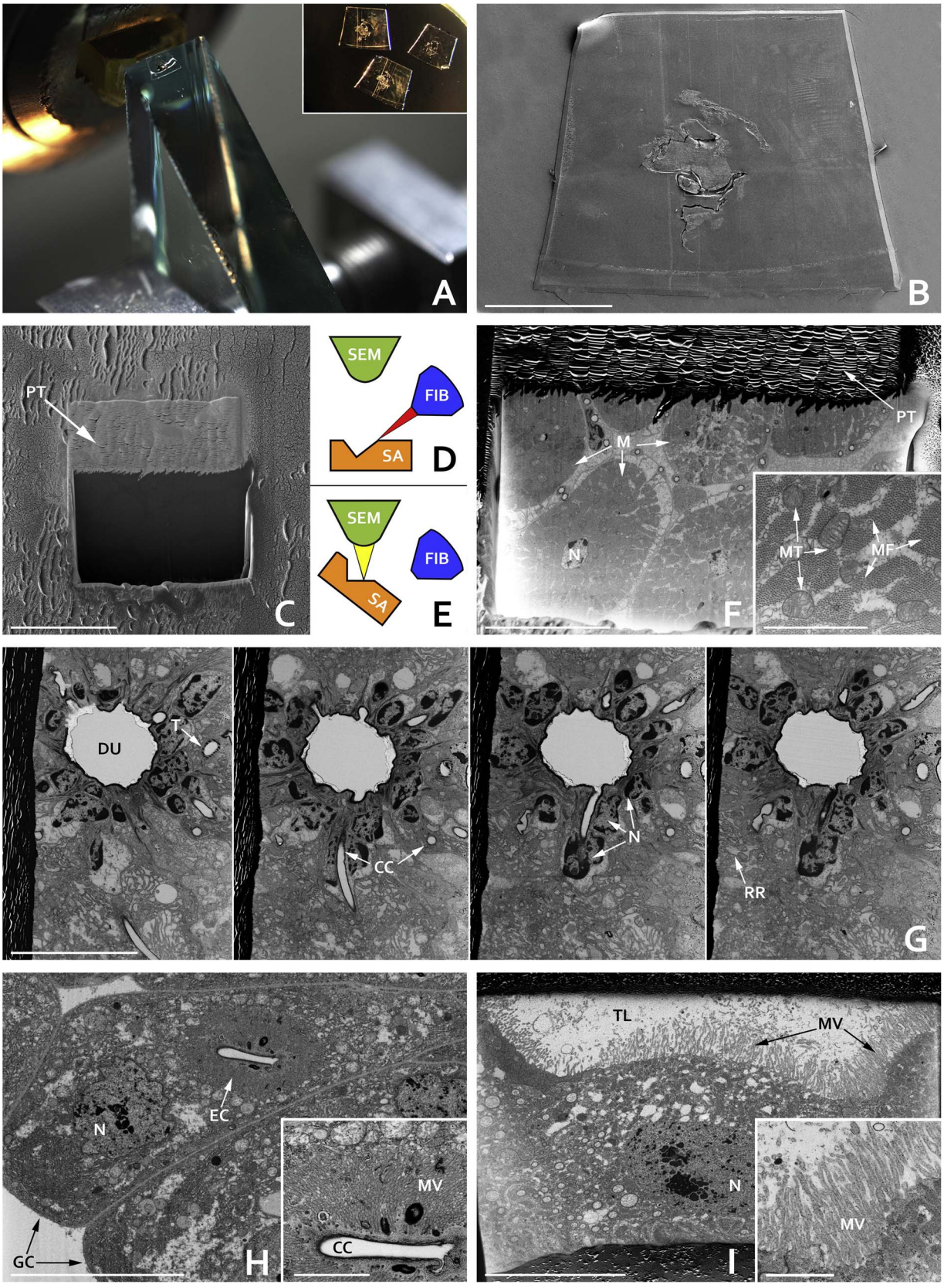

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