APPLICATION NOTE

A1 HD25/A1R HD25/AX/AX R Confocal Microscope

Ecology of a fossilized cockroach in amber was revealed by confocal microscopy and thin sectioning technology

- How did organisms of the past perceive the world?

Insect sensory organs play an essential role in detecting information about their surrounding environment. Despite their small size and few sensory neurons, they have excellent abilities to process information, comparable to those of vertebrates. This is considered one of the primary reasons for the great success of insects, which account for 70% of all animal species. It is therefore important to investigate the sensory organs in evolutionary paleontological studies on insects.

Ryo Taniguchi and Associate Professor Yasuhiro Iba of the Graduate School of Science, Hokkaido University, Assistant Professor Hiroshi Nishino of the Research Institute of Electronic Science, Hokkaido University, Dr. Shûhei Yamamoto of the Hokkaido University Museum, and Associate Professor Hidehiro Watanabe of the Department of Earth System Science, Fukuoka University, reported a method for removing the amber substrate from a male fossilized cockroach in amber, *Huablattula hui*, as much as possible, and creating thin section specimens with the sensory organs still enclosed. The results of confocal microscopy observation of the specimen show that analysis of micro sensory organs is extremely effective in reconstructing detailed lifestyles of fossilized insects. In this application note, we introduce an example of the contribution of the laser scanning confocal microscope to the results of this research.

Keywords: transmitted light image, laser scanning confocal microscope, fossils, insects, sensory organs

Overview

The origin of cockroaches can be traced back about 300 million years, and they adapt to dark habitats such as forest floors, caves, and night environments. Cockroaches have sensory organs that reflect the ecology, such as relatively small compound eyes and developed antennae. Therefore, they are model organisms for the evolutionary history and diversity of insect sensory organs.

Fossils are the only direct evidence for tracing the evolution and diversity of sensory systems, but they are rarely preserved as fossils because of their small size and fragility. The exception to this is amber, which preserves even micro sensory organs, but observation with an electron microscope used in the study of modern species is extremely difficult because it is necessary to remove the surrounding amber to expose the sensory organs. In addition, it is not possible to visualize the sensory organs in detail with X-ray CT or stereomicroscope because the resolution is insufficient for observation.

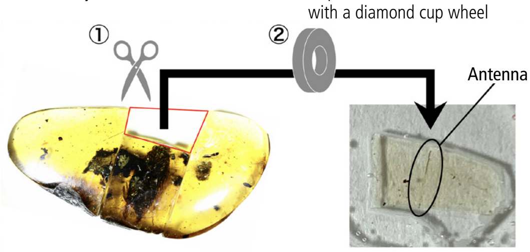


Fig. 1: Procedure for making an amber specimen

The authors prepared an amber specimen by cutting the right antenna together with amber and then polishing it to a thickness of 200 µm using a diamond cup wheel, with the cooperation of Kosuke Nakamura, a technical expert of the Thin Section Lab, Faculty of Science, Hokkaido University (Fig.1). A transmitted light image of the prepared slide was acquired using a laser scanning confocal microscope at high magnification, and the micro sensory organs, which cannot be visualized by non-destructive analysis, were observed and classified.

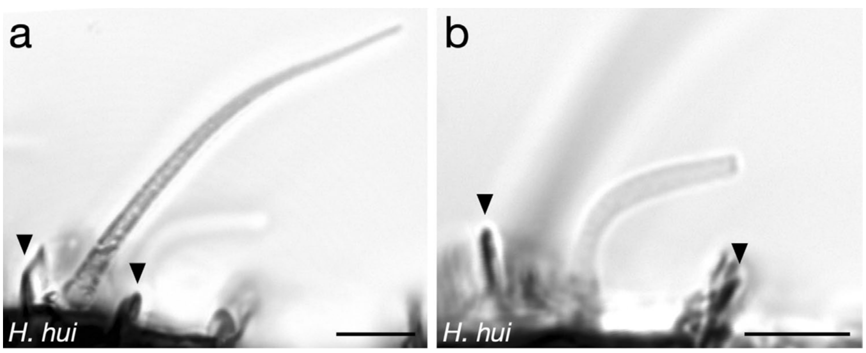


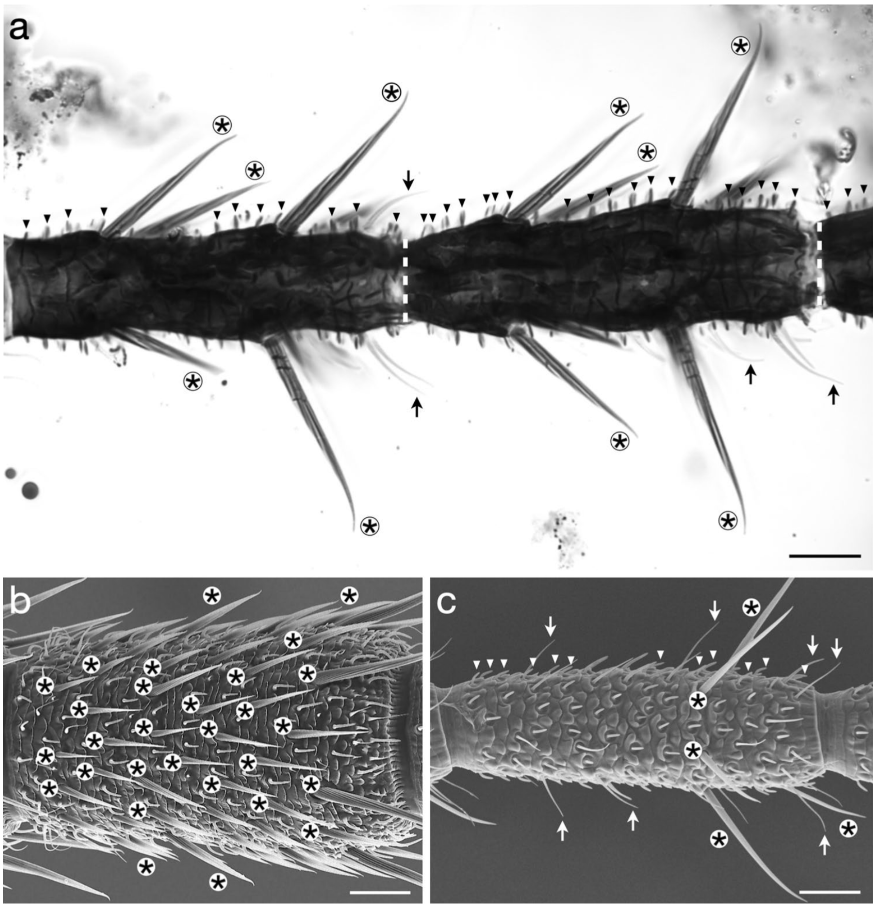
Fig. 2: Enlarged transmitted light images of trichoid sensilla (a) and perforated basiconic sensilla (b) of *H. hui*

When irradiated with various laser wavelengths, it was found that contrast was highest and clear observation was possible with a 640 nm laser.

Since the exoskeleton structure of insect fossils is well-preserved in amber, the degree of darkness in sensillum appearance is considered a reflection of the thickness of the cuticle walls. Therefore, it can be suggested that these sensilla are more transparent than the grooved basiconic sensilla (▼) and have a thin outer wall.

Scale bar: 5 µm

Objective: CFI Apochromat Lambda S 60X Oil
Wavelength: 640 nm
Scan Zoom: 7.246X



Imaging condition of (a)
Objective: CFI Apochromat Lambda S 60X Oil
Wavelength: 640 nm
Scan Zoom: 1.0X

Fig. 3: Transmitted light image of distal antennal segments of *H. hui* in amber specimen captured with LSCM (a) and SEM images of extant male cockroach *Periplaneta americana* (b) and mantis *Tenodera aridifolia* (c)

Various types of sensilla such as chaetic sensilla (★), trichoid sensilla (→) and grooved basiconic sensilla (▼), are preserved in detail on the antenna of *H. hui*. The nocturnal cockroach and diurnal mantis have significantly different distributions of chaetic sensilla, and *H. hui* has a similar sensillum pattern to the mantis.
Scale bar: 20 µm (a), 50 µm (b, c)

Summary

From a comparison of laser wavelengths, it was found that this sample can be observed with the optimal contrast when irradiated at 640 nm. Transmitted light images using a 640 nm laser allowed observation of a large number of finely preserved sensilla on the antennal surface of the fossilized insect in amber. In addition, the sensilla preserved the fine surface structures, making detailed morphological comparison with extant species possible. In this study, it was found that a cockroach having a sensillum pattern (= sensory system) similar to extant mantises existed in the Cretaceous period. For example, the abundance of grooved basiconic sensilla in mantid males results from using sex pheromones, suggesting that *H. hui* may have likewise used mantis-like intersexual communication.

This study established a method for detailed analysis of the micro sensory organs of insect fossils preserved in amber and reconstruction of their function and owners' ecology in high resolution. This is expected to reveal the sensory systems of more fossilized insects and lead to the development of neuroscientific research in paleontology, with these fossils as new models.

References

The Science of Nature (2021) 108:45
https://doi.org/10.1007/s00114-021-01755-9
Reconstructing the ecology of a Cretaceous cockroach: destructive and high-resolution imaging of its micro sensory organs
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Product Information

AX R Confocal Microscope

Supports high-speed, high-resolution, large field-of-view confocal imaging, with reduced phototoxicity to living cells and photobleaching.

- High speed: Up to 720 fps (resonant at 2048 x 16 pixels)
- High resolution: Up to 8K (galvano) / 2K (resonant)
- High throughput: Ultra-wide field of view of 25 mm

CFI Apochromat Lambda S 60X Oil

High NA objective optimal for multicolor confocal imaging, correcting chromatic aberrations over a wide range from violet to near-IR.

- NA: 1.40
- WD: 0.14