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博士学位論文

Neuroanatomy of insects in amber

(琥珀内昆虫化石の神経解剖学的研究)

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Abstract

Insects contain 1 million species, accounting for more than 50% of all species. These most diverse organisms on Earth originated over 400 million years ago, and they have pioneered and adapted to the new environments until the present. The unique sensory system of insects is one of the key adaptations that have supported their ongoing evolutionary success. Insect sensory organs are miniaturized, while they have chitinous exoskeletons and high fossilization potential, especially in amber, the best preservational medium. Previous studies, unfortunately, have missed these notable minute sensors due to a lack of viewpoints and low-resolution methodology. Here, I revealed the exceptionally well-preserved sensory organs of insects in Cretaceous amber while developing novel visualization methods. Their unique morphology enabled the direct comparison with extant counterparts and functional reconstruction in ecological contexts. This study focused on the three insect taxa used as traditional models of modern neurobiology: cockroaches, ants, and mantises. The eyes and antennae of the 100-million-year-old cockroach suggested that the species had a different ecology from extant relatives in active time and sexual communication. The earliest ants showed antennal sensory properties shared with those of extant species, indicating possession of advanced pheromone communication and sociality. The well-developed vision with high visual resolution and stereoscopic prey recognition was revealed in the Cretaceous mantis species, which is the closest out-group of all modern mantodeans. The advanced visual system of mantises has likely supported their principal predatory niches for 90 million years. The miniaturized and sophisticated peripheral nervous systems of these insects tell us about their ancient behavioral ecology, including social and predatory adaptations, and their evolutionary timelines based on direct sensory evidence.

General introduction

Insects are the most diverse group of organisms, which include 1 million living species and account for half of all species on Earth (1, 2). They occupy various ecological niches and play essential roles in shaping ecosystem structure and function. For instance, insect activities account for 30% of the global carbon flux from deadwood and mediate pollination of 80% of plant species (3, 4). The ecological importance of insects has likely affected the development of terrestrial ecosystems and biological interactions throughout their geologically long evolutionary history for 400 million years (5, 6). They pioneered new environments and expanded the niches in some evolutionary events; e.g., they were the first animals to achieve terrestrial colonization and active flight (7, 8). The key evolutionary innovation that has supported such insect reign is their uniquely adapted nervous system. The body of insects is relatively small (mm~cm scale), and thus, their nervous system is strongly restricted in size. Insect brains comprise significantly smaller number of neurons ($10^5\sim10^6$) than well-developed vertebrate brains ($10^9\sim10^{11}$) (9, 10). Nonetheless, the miniature nervous system of insects adaptively overcomes the size restriction and enables complicated information processing and behavior, such as flying at high speed, determining to attack/escape from other animals, and memorizing and communicating complex information (11–15). Peripheral sensory organs of insects (e.g., compound eyes, antennae, maxillary and labial palpi, cerci) work on the primary input of various surrounding information and provide crucial criteria for consequent responses (16–20). Insect sensory neurons, occupying relatively much of the total neurons compared to vertebrates, pre-screen specific sensory information and send it to the central nervous system (9, 10, 20). This peripheral information screening system of insects compensates for small brains with limited processing capacity and often causes the execution of stereotyped behaviors (9, 10, 20–22). Although these sensors are miniaturized in μm scale, like other neural components, they comprise chitinous exoskeletons with a high potential to be fossilized. Insect sensory organs can be direct evidence to reconstruct the ecological function of the notable nervous system in extinct insects and their deep-time evolution.

Among the preservational media of insect fossils, the exceptional condition of inclusions in amber, fossilized resin, is distinguished. The insect carcasses encapsulated in amber have been protected from external agents, such as scavengers and decomposers, and can be maintained without collapse or degradation (23–25). Although insect remains in amber are usually restricted to the cuticle due to autolysis, such cuticular exoskeletal structures are three-dimensionally

preserved, even microstructures (26–28). The study of amber inclusions potentially sheds light on the fossilized chitinous sensory organs of insects. There are numerous studies of insect fossils in amber; however, most of them have focused only on macro-scale morphology for taxonomic description, which is observed via non-destructive techniques (macrophotography and X-ray computed tomography) (29–31). This traditional perspective seems to cause the limitation of accessible anatomical features and restrict the contribution of studies to principally taxonomic and some biogeographic contexts. Previous studies have overlooked the importance of insect micro-sensory systems and have yet to reveal their detailed function, ecology, and evolution.

In this study, I developed novel methods to analyze insect sensory organs, especially compound eyes (light detectors) and antennae (multimodal signal detectors, such as for odors and physical contact) preserved in the oldest-class fossiliferous amber from the Cretaceous period. Based on the observed sensor morphologies, I reconstructed their ecological function and discussed their evolutionary importance. In Chapter 1, the eyes and antennae of a cockroach were analyzed by combining destructive and non-destructive approaches. I suggested the more diverse ecology and behavior of extinct cockroaches than extant species, the traditional models in neurobiology. The techniques developed here were applied and updated in the following chapters. In Chapter 2, I updated the destructive method to visualize the distribution of micro-sensory protrusions on the antennae, which detect various pheromones. This new method revealed that Cretaceous ants were equipped with unique antennal micro sensors for alarm signaling and nestmate recognition, sharing distinctive features with extant ants. Here, I reconstructed the complex chemical communication and advanced sociality of the earliest ants. Chapter 3 evaluated the visual system of an extinct mantis species, the closest out-group of all extant relatives, based on the three-dimensional morphology. This primitive tiny mantis was likely an adaptive predator with a high-resolution visual system and effective binocular stereopsis. Through these studies, I established the methodology for analyzing the micro sensory organs of fossilized insects in amber. Furthermore, I reconstructed the function of peripheral nervous systems and evolutionally advanced behavior in fossil counterparts of notable neurobiological models.

References

1. B. R. Scheffers, L. N. Joppa, S. L. Pimm, W. F. Laurance, What we know and don't know about Earth's missing biodiversity. *Trends Ecol. Evol.* **27**, 501–510 (2012).
2. N. E. Stork, How many species of insects and other terrestrial arthropods are there on Earth? *Annu. Rev. Entomol.* **63**, 31–45 (2018).
3. S. Seibold, W. Rammer, T. Hothorn, R. Seidl, M. D. Ulyshen, J. Lorz, M. W. Cadotte, D. B. Lindenmayer, Y. P. Adhikari, R. Aragón, S. Bae, P. Baldrian, H. Barimani Varandi, J. Barlow, C. Bässler, J. Beauchêne, E. Berenguer, R. S. Bergamin, T. Birkemoe, G. Boros, R. Brandl, H. Brustel, P. J. Burton, Y. T. Cakpo-Tossou, J. Castro, E. Cateau, T. P. Cobb, N. Farwig, R. D. Fernández, J. Firn, K. S. Gan, G. González, M. M. Gossner, J. C. Habel, C. Hébert, C. Heibl, O. Heikkala, A. Hemp, C. Hemp, J. Hjältén, S. Hotes, J. Kouki, T. Lachat, J. Liu, Y. Liu, Y.-H. Luo, D. M. Macandog, P. E. Martina, S. A. Mukul, B. Nachin, K. Nisbet, J. O'Halloran, A. Oxbrough, J. N. Pandey, T. Pavlíček, S. M. Pawson, J. S. Rakotondranary, J.-B. Ramanamanjato, L. Rossi, J. Schmidl, M. Schulze, S. Seaton, M. J. Stone, N. E. Stork, B. Suran, A. Sverdrup-Thygeson, S. Thorn, G. Thyagarajan, T. J. Wardlaw, W. W. Weisser, S. Yoon, N. Zhang, J. Müller, The contribution of insects to global forest deadwood decomposition. *Nature* **597**, 77–81 (2021).
4. S. Chowdhury, M. P. Zalucki, J. O. Hanson, S. Tiatragul, D. Green, J. E. M. Watson, R. A. Fuller, Three-quarters of insect species are insufficiently represented by protected areas. *One Earth* **6**, 139–146 (2023).
5. D. A. Grimaldi, M. S. Engel, *Evolution of the Insects* (Cambridge Univ. Press, Cambridge, Repr., 2006).
6. B. Misof, S. Liu, K. Meusemann, R. S. Peters, A. Donath, C. Mayer, P. B. Frandsen, J. Ware, T. Flouri, R. G. Beutel, O. Niehuis, M. Petersen, F. Izquierdo-Carrasco, T. Wappler, J. Rust, A. J. Aberer, U. Aspöck, H. Aspöck, D. Bartel, A. Blanke, S. Berger, A. Böhm, T. R. Buckley, B. Calcott, J. Chen, F. Friedrich, M. Fukui, M. Fujita, C. Greve, P. Grobe, S. Gu, Y. Huang, L. S. Jermiin, A. Y. Kawahara, L. Krogmann, M. Kubiak, R. Lanfear, H. Letsch, Y. Li, Z. Li, J. Li, H. Lu, R. Machida, Y. Mashimo, P. Kapli, D. D. McKenna, G. Meng, Y. Nakagaki, J. L. Navarrete-Heredia, M. Ott, Y. Ou, G. Pass, L. Podsiadlowski, H. Pohl, B. M. Von Reumont, K. Schütte, K. Sekiya, S. Shimizu, A. Slipinski, A. Stamatakis, W. Song, X. Su, N. U. Szucsich, M. Tan, X. Tan, M. Tang, J. Tang, G. Timelthaler, S. Tomizuka, M. Trautwein, X.

- Tong, T. Uchifune, M. G. Walz, B. M. Wiegmann, J. Wilbrandt, B. Wipfler, T. K. F. Wong, Q. Wu, G. Wu, Y. Xie, S. Yang, Q. Yang, D. K. Yeates, K. Yoshizawa, Q. Zhang, R. Zhang, W. Zhang, Y. Zhang, J. Zhao, C. Zhou, L. Zhou, T. Ziesmann, S. Zou, Y. Li, X. Xu, Y. Zhang, H. Yang, J. Wang, J. Wang, K. M. Kjer, X. Zhou, Phylogenomics resolves the timing and pattern of insect evolution. *Science* **346**, 763–767 (2014).
7. J. A. Dunlop, R. J. Garwood, Terrestrial invertebrates in the Rhynie chert ecosystem. *Philos. Trans. R. Soc. B Biol. Sci.* **373**, 20160493 (2018).
 8. A. Nel, J. Prokop, M. Pecharová, M. S. Engel, R. Garrouste, Palaeozoic giant dragonflies were hawked predators. *Sci. Rep.* **8**, 12141 (2018).
 9. M. Mizunami, F. Yokohari, M. Takahata, Exploration into the adaptive design of the arthropod “microbrain.” *Zoolog. Sci.* **16**, 703–709 (1999).
 10. M. Mizunami, F. Yokohari, M. Takahata, Further exploration into the adaptive design of the arthropod “microbrain”: I. Sensory and memory-processing systems. *Zoolog. Sci.* **21**, 1141–1151 (2004).
 11. Y. Yamawaki, Defence behaviours of the praying mantis *Tenodera aridifolia* in response to looming objects. *J. Insect Physiol.* **57**, 1510–1517 (2011).
 12. T. J. Wardill, S. T. Fabian, A. C. Pettigrew, D. G. Stavenga, K. Nordström, P. T. Gonzalez-Bellido, A Novel Interception Strategy in a Miniature Robber Fly with Extreme Visual Acuity. *Curr. Biol.* **27**, 854–859 (2017).
 13. M. Domae, M. Iwasaki, M. Mizunami, H. Nishino, Functional unification of sex pheromone-receptive glomeruli in the invasive Turkestan cockroach derived from the genus *Periplaneta*. *Neurosci. Lett.* **708**, 134320 (2019).
 14. S. Dong, T. Lin, J. C. Nieh, K. Tan, Social signal learning of the waggle dance in honey bees. *Science* **379**, 1015–1018 (2023).
 15. K. Kiuchi, H. Shidara, Y. Iwatani, H. Ogawa, Motor state changes escape behavior of crickets. *iScience* **26**, 107345 (2023).
 16. M. Ozaki, A. Wada-Katsumata, K. Fujikawa, M. Iwasaki, F. Yokohari, Y. Satoji, T. Nisimura, R. Yamaoka, Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. *Science* **309**, 311–314 (2005).

17. F. R. Prete, J. L. Komito, S. Dominguez, G. Svenson, L. Y. López, A. Guillen, N. Bogdanivich, Visual stimuli that elicit appetitive behaviors in three morphologically distinct species of praying mantis. *J. Comp. Physiol. A* **197**, 877–894 (2011).
18. A. Couto, A. Mitra, D. Thiéry, F. Marion-Poll, J.-C. Sandoz, Hornets have it: a conserved olfactory subsystem for social recognition in Hymenoptera? *Front. Neuroanat.* **11**, 48 (2017).
19. C. Amat, F. Marion-Poll, M. A. Navarro-Roldán, C. Gemenio, Gustatory function of sensilla chaetica on the labial palps and antennae of three tortricid moths (Lepidoptera: Tortricidae). *Sci. Rep.* **12**, 18882 (2022).
20. H. Watanabe, S. Ogata, N. Nodomi, K. Tateishi, H. Nishino, R. Matsubara, M. Ozaki, F. Yokohari, Cuticular hydrocarbon reception by sensory neurons in basiconic sensilla of the Japanese carpenter ant. *Front. Cell. Neurosci.* **17**, 1084803 (2023).
21. D. D. Yager, G. J. Svenson, Patterns of praying mantis auditory system evolution based on morphological, molecular, neurophysiological, and behavioural data. *Biol. J. Linn. Soc.* **94**, 541–568 (2008).
22. D. D. Yager, Predator detection and evasion by flying insects. *Curr. Opin. Neurobiol.* **22**, 201–207 (2012).
23. X. Martínez-Delclòs, D. E. G. Briggs, E. Peñalver, Taphonomy of insects in carbonates and amber. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **203**, 19–64 (2004).
24. C. C. Labandeira, Amber. *Paleontol. Soc. Pap.* **20**, 163–216 (2014).
25. L. J. Seyfullah, C. Beimforde, J. Dal Corso, V. Perrichot, J. Rikkinen, A. R. Schmidt, Production and preservation of resins – past and present. *Biol. Rev.* **93**, 1684–1714 (2018).
26. D. Grimaldi, A revision of Cretaceous mantises and their relationships, including new taxa (Insecta: Dictyoptera: Mantodea). *Am. Mus. Novit.* **3412**, 1–47 (2003).
27. W. Gilka, E. A. Makarchenko, M. K. Pankowski, M. Zakrzewska, Myanmaro primus gen. et sp. nov., the first orthoclad (Diptera: Chironomidae) from Cretaceous Burmese amber. *Zootaxa* **4565** (2019).
28. P. Vršanský, H. Sendi, J. Hinkelman, M. Hain, *Alienopterix* Mlynský et al., 2018 complex in North Myanmar amber supports Umenocoleoidea/ae status. *Biologia (Bratisl.)* **76**, 2207–2224 (2021).

29. M. Perreau, P. Tafforeau, Virtual dissection using phase-contrast X-ray synchrotron microtomography: reducing the gap between fossils and extant species. *Syst. Entomol.* **36**, 573–580 (2011).
30. X. Lin, C. C. Labandeira, C. Shih, C. L. Hotton, D. Ren, Life habits and evolutionary biology of new two-winged long-proboscid scorpionflies from mid-Cretaceous Myanmar amber. *Nat. Commun.* **10**, 1235 (2019).
31. H. Sendi, P. Vršanský, L. Podstrelená, J. Hinkelman, T. Kúdelová, M. Kúdela, Ľ. Vidlička, X. Ren, D. L. J. Quicke, Nocticolid cockroaches are the only known dinosaur age cave survivors. *Gondwana Res.* **82**, 288–298 (2020).

Chapter 1.

Prototyping visualization of fossilized micro sensory organs and ecological reconstruction of a Cretaceous cockroach

Abstract

Animals depend highly on their sensory organs to detect information about their surrounding environment. Among animal sensory organs, those of insects have a notable ability to detect information despite their small size, which might be, therefore, one of the reasons for the evolutionary success of insects. However, insect sensory organs are seldom fossilized in sediments due to their small size and fragility. A potential solution to this problem is the study of exceptionally well-preserved fossil material from amber. Unfortunately, the resolution of existing non-destructive analysis is insufficient to observe details of these micro sensory organs even with amber preservation. Here, we focus on the analysis of the micro sensory organs of an extinct male cockroach (*Huablattula hui* Qiu et al., 2019) in Cretaceous amber by combining destructive and non-destructive methods. Compared to extant species inhabiting dark environments, *H. hui* has relatively large compound eyes, and all the antennal sensilla for detecting multimodal information observed here are fewer or smaller. The characteristics of these sensory organs support the diurnality of the bright habitats of *H. hui* in contrast to many extant cockroaches. Like extant male mantises, grooved basiconic-type sensilla exist abundantly on the antenna of the fossilized specimen. The abundance of grooved basiconic sensilla in mantid males results from using sex pheromones, and therefore, *H. hui* may have likewise used mantis-like intersexual communication. These lines of evidence suggest that the ecology and behavior of Cretaceous cockroaches were more diverse than those of related extant species.

1.1. Introduction

The sensory organs of animals play an essential role in detecting food, habitats, predators, and mating partners. These organs, therefore, strongly control the survival, selection, ecology, and evolution of the animals. Insects, which account for more than 70% of all animal species (1, 2), are the most diverse organisms on Earth. Although their sensory organs are miniaturized and equipped with a small number of sensory neurons, the organs have a notable ability to detect complicated information (3–5). Given this adaptive overcoming of the size restriction and the extremely high diversity of insects in the “kingdom” Animalia, their sensory organs can be regarded as one of the most successful evolutionary adaptations. Fossils are the only direct evidence for tracing the diversity and evolution of these remarkable sensory organs in the *ca.* 400 million years of the ongoing evolution of insects (6). However, insect sensory organs are rarely preserved in sediments because of their small size and fragility. Even if they are eventually fossilized, details like their three-dimensional morphology and microstructure are not preserved (7, 8), although there are very few exceptions (9, 10). In contrast, these micro sensory organs can be exceptionally well preserved in amber (11–15). Therefore, amber and its fossil insect inclusions are the most suitable material for understanding the perception systems and ecology of past insects.

The term “cockroaches” (order Blattodea) includes *ca.* 4600 extant species (16). Many of them adapt to dark habitats such as forest floors, caves, or night environments (17); their sensory organs accurately reflect this mode of life. They have relatively small compound eyes and well-developed long filiform or moniliform antennae. Cockroaches have existed since the Late Carboniferous and maintained their conservative body plan in their relatively long evolutionary history (18, 19). They can therefore be considered model organisms of sensory organ evolution in the geological record.

In this study, the compound eyes and antennae of a fossilized cockroach (*Huablattula hui* Qiu et al. 2019) in mid-Cretaceous amber from Myanmar (*ca.* 100 Ma) have been analyzed in detail and compared with extant species. Compound eyes are the primary visual organs for detecting light, composed of ommatidia. The antennae detect multimodal information such as odors, physical contact, and temperature via morphologically specialized protrusions called sensilla. These are, therefore, especially crucial sensory organs for insects. Furthermore, we reconstructed the sensory-receptive system and ecology of an extinct cockroach in high-resolution based on the micro sensory organs.

1.2. Material and methods

The amber piece ($27.5 \times 13.5 \times 3.0$ mm, 0.8 g), containing one fossilized cockroach (Fig. 1.1), derives from the Hukawng Valley, Kachin State, in northern Myanmar (20, 21). Kachin amber, previously known as Burmese amber, includes very diverse fossils from the Cretaceous period (22, 23). The deposit has been conservatively dated as 98.79 ± 0.62 Ma (Early Cenomanian) based on U–Pb dating of zircons from a volcanoclastic matrix (24). A slightly older age, the late Albian to early Cenomanian period, was suggested by biostratigraphic approaches (25). Considering its age and the excellent preservation of insect fossils, Kachin amber currently offers the most appropriate fossil material for understanding the sensory organs of fossilized insects and cockroaches.

The fossil specimen was photographed by a Canon EOS 5DS digital camera with a Canon MP-E 65 mm macro lens (F2.8, 1–5x). We also used a Canon Extender EF 2 × III for higher-magnified images. The focus of the obtained images was blended with Helicon Focus 7.6.3 (Helicon Soft). Diameters of the ommatidia on the compound eyes were measured from these images. The specimen was scanned with a micro-focus X-ray CT system (XT H225 ST; Nikon) at Tomakomai Industrial Technology Center (Hokkaido, Japan), and it was analyzed three-dimensionally (220 kV of acceleration voltage, $10.67 \mu\text{m}/\text{voxel}$). The CT data were visualized with Amira 2019.3 (Thermo Fisher Scientific). The surface area of the compound eyes was measured by using the 3D model on Amira. We estimated the number of ommatidia composing the compound eyes based on the measured eye size.

We applied thin sectioning, a petrological method, to observe sensilla because they were too small to be visualized by non-destructive methods, which would have left the amber intact. In this thin sectioning process, the right antenna was cut off with the adjoining amber. It was then ground and polished down to a 200- μm thickness using a diamond cup wheel (NIPPON DIAMOND Co. Ltd.). The thin-section images were obtained with a Nikon A1R HD25 laser scanning confocal microscopy (LSCM) system in the Nikon Imaging Center at Hokkaido Univ. The focus of images was also blended with Helicon Focus. The main body and thin section specimens are deposited in the American Museum of Natural History under the assigned number AMNH Bu-SY28. We obtained antennal sensillum images of an extant male American cockroach (*Periplaneta americana* (Linnaeus, 1758)) and praying mantis (*Tenodera aridifolia* Stoll, 1813) with an LSCM (LSM-510; Carl Zeiss) and a scanning electron microscopy (SEM) system (S-4800; Hitachi). Mantids (order Mantodea) are the most related sister taxon to Blattodea (16, 26).

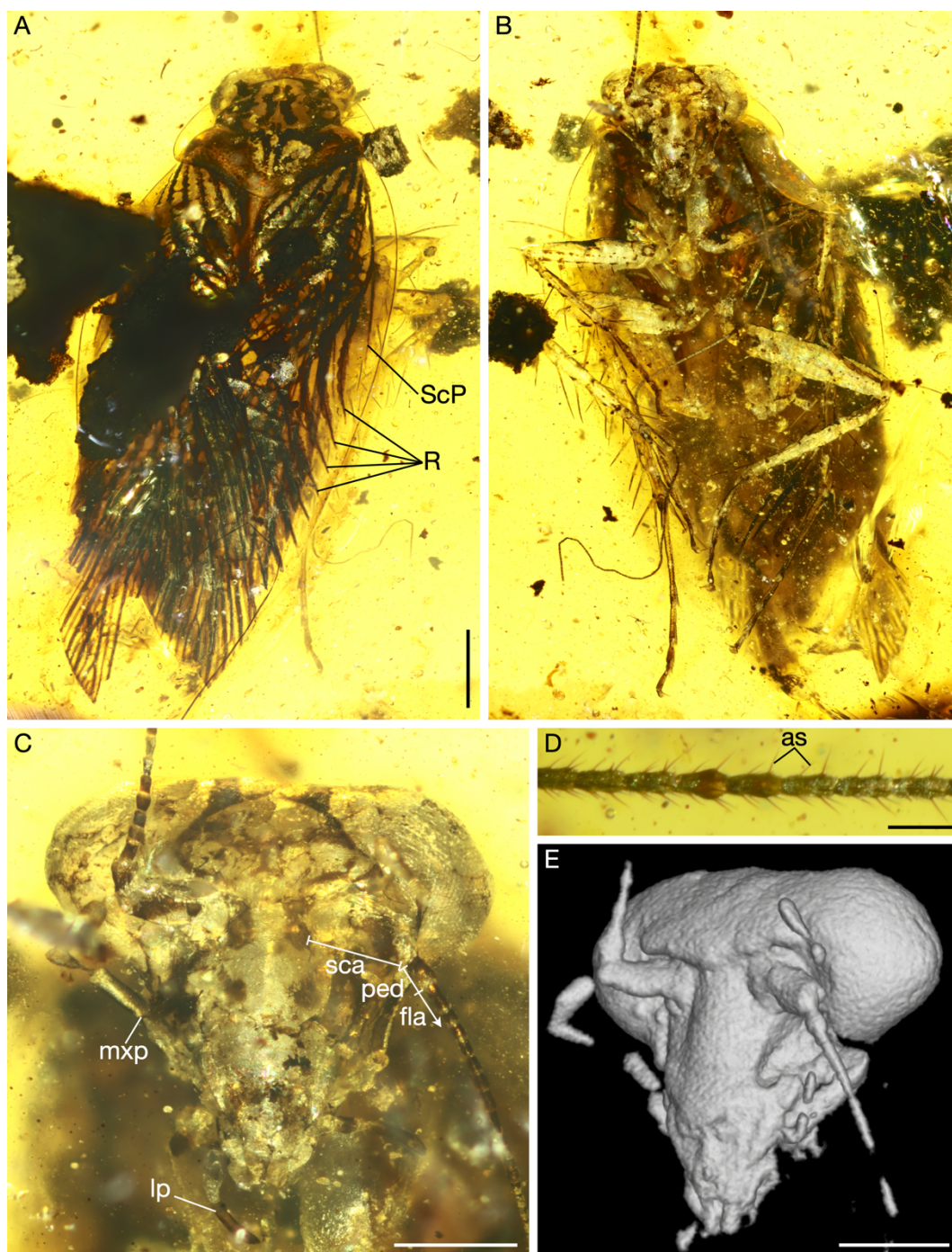


Fig. 1.1 Photographs of the fossilized cockroach, *Huablattula hui* (AMNH Bu-SY28) in amber (A–D) and a three-dimensional model of the head from CT data (E). (A) Dorsal view. Simple ScP and sub-parallel R veins are visible. (B) Ventral view. (C) Head. (D) Middle part of right antenna. (E) Micro-CT image of left oblique view. Abbreviations: scape (sca), pedicel (ped), flagellum (fla), maxillary palp (mxp), labial palp (lp), antennal sensilla (as). Scale bars: 1 mm (A, B); 0.5 mm (C, E); 0.1 mm (D)

1.3. Results

The cockroach specimen is exceptionally well preserved, showing many morphological features in detail. The specimen has a preserved triangular head with large compound eyes, symmetrical dark patterns with some light-colored spots, characteristically arranged on the pronotum, and long simple ScP and sub-parallel branched R veins on the forewings that are partially damaged (Fig. 1.1a, b). These morphological features indicate that the specimen is identified as an adult of *H. hui* Qiu et al., 2019 included in the extinct family Blattulidae (Qiu et al. 2019). The lack of an external ovipositor and an 8.58-mm body length indicate that the sex is male (Fig. 1.1a, b).

The large and rounded compound eyes are located at the distal corners of the head (Fig. 1.1a–c). Many ommatidia are visible on the surface of the eyes (Fig. 1.1c). The diameter of each corneal lens is *ca.* 20 μm . We regarded each lens as a regular hexagon and calculated the area as $2.60 \times 10^{-4} \text{ mm}^2$. The total surface area of both compound eyes was measured at *ca.* 1.7 mm^2 . From these data, we estimated the compound eyes to be composed of *ca.* 6000 ommatidia.

The specimen shows well-developed long antennae; a scape, pedicel, and flagellum (comprised of many flagellomeres) are preserved on each antenna (Fig. 1.1c). The lengths of the scape and pedicel are 0.46 mm and 0.12 mm, respectively. The flagellomeres are longer toward the antennal tip and are 0.07–0.12 mm long. The length-to-width ratio of flagellomeres is larger than the extant *P. americana* and extinct *Piniblattella vitimica* (Vishnyakova, 1964) from the Cretaceous period (27, 28). The left antenna is about 9.43 mm long with 90 antennomeres. A large number of sensilla is observed on the flagellum (Fig. 1.1d). These sensilla were scrutinized via the thin section observation and classified based on their morphological characteristics following the classification of the extant *P. americana* (29, 30) (Figs. 1.2 and 1.3). Chaetic sensilla are large spines with 0.8- μm wide longitudinal grooves, and trichoid sensilla have a thin hair-like shape and are sharply tapered (Figs. 1.2a and 1.3a). Perforated basiconic (p-bas) sensilla are short transparent hairs with rounded tips (Fig. 1.3b). We considered that the degree of darkness in sensillum appearance reflects the thickness of cuticular walls because insect fossils in amber well preserve the exoskeletal structure (22, 31). Putative grooved basiconic (g-bas) sensilla (thick, double-walled in *P. americana*) were therefore designated for dark serrate structures, which are short and stand on the antenna surface (Figs. 1.2a and 1.3a, b).

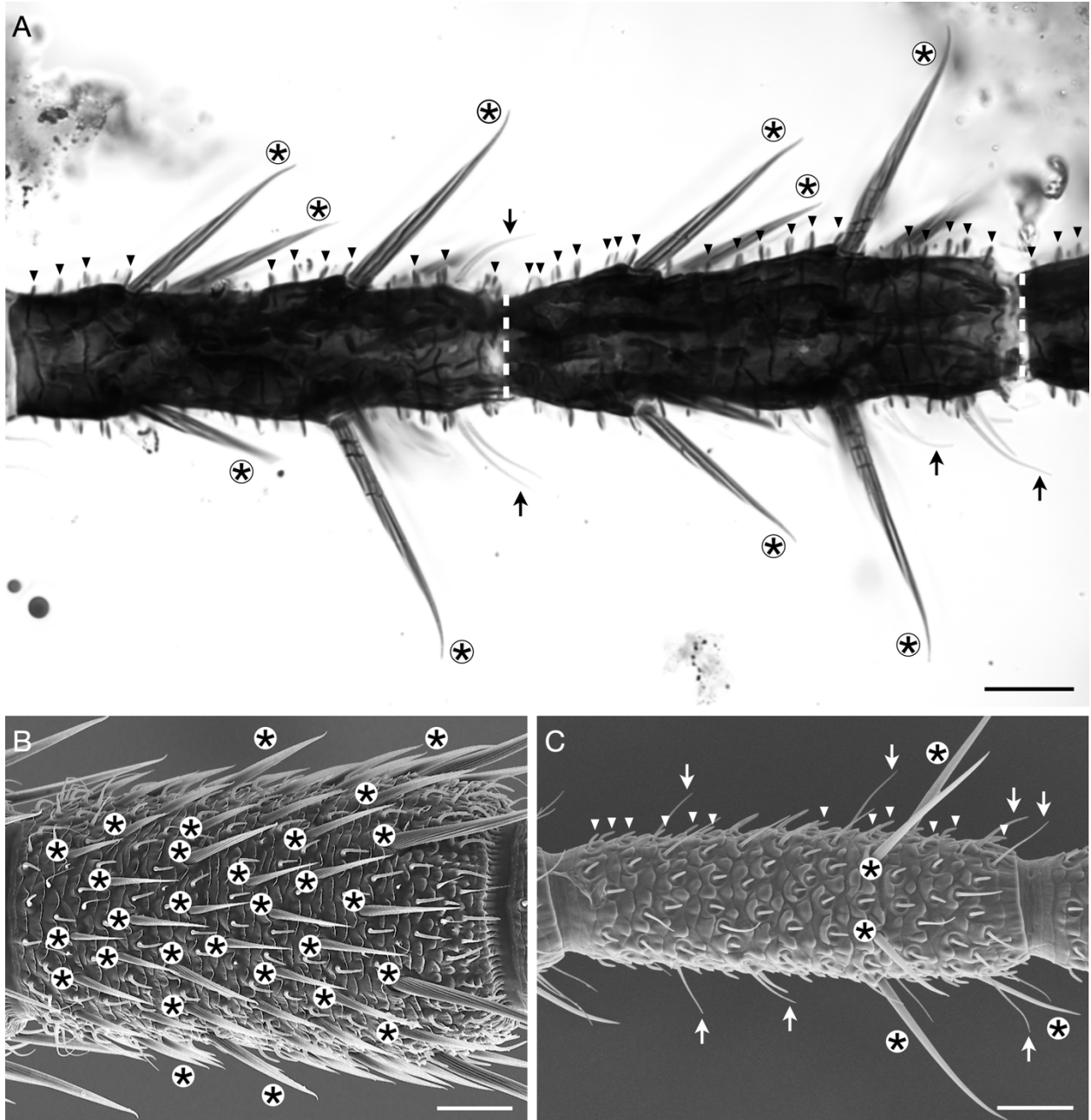


Fig. 1.2 Distal antennal images of *Huablattula hui* (AMNH Bu-SY28) via LSCM (A), extant male cockroach *Periplaneta americana* (B), and mantis *Tenodera aridifolia* via SEM (C). White dot lines show the boundaries between segments in (A). Chaetic sensilla (marked by asterisks) in *H. hui* (A) and *T. aridifolia* (C) are markedly fewer than *P. americana* (B). Trichoid and grooved basiconic (called grooved peg in *T. aridifolia*) sensilla are marked by arrows and triangles, respectively (A, C). Scale bars: 20 μ m (A); 50 μ m (B, C)

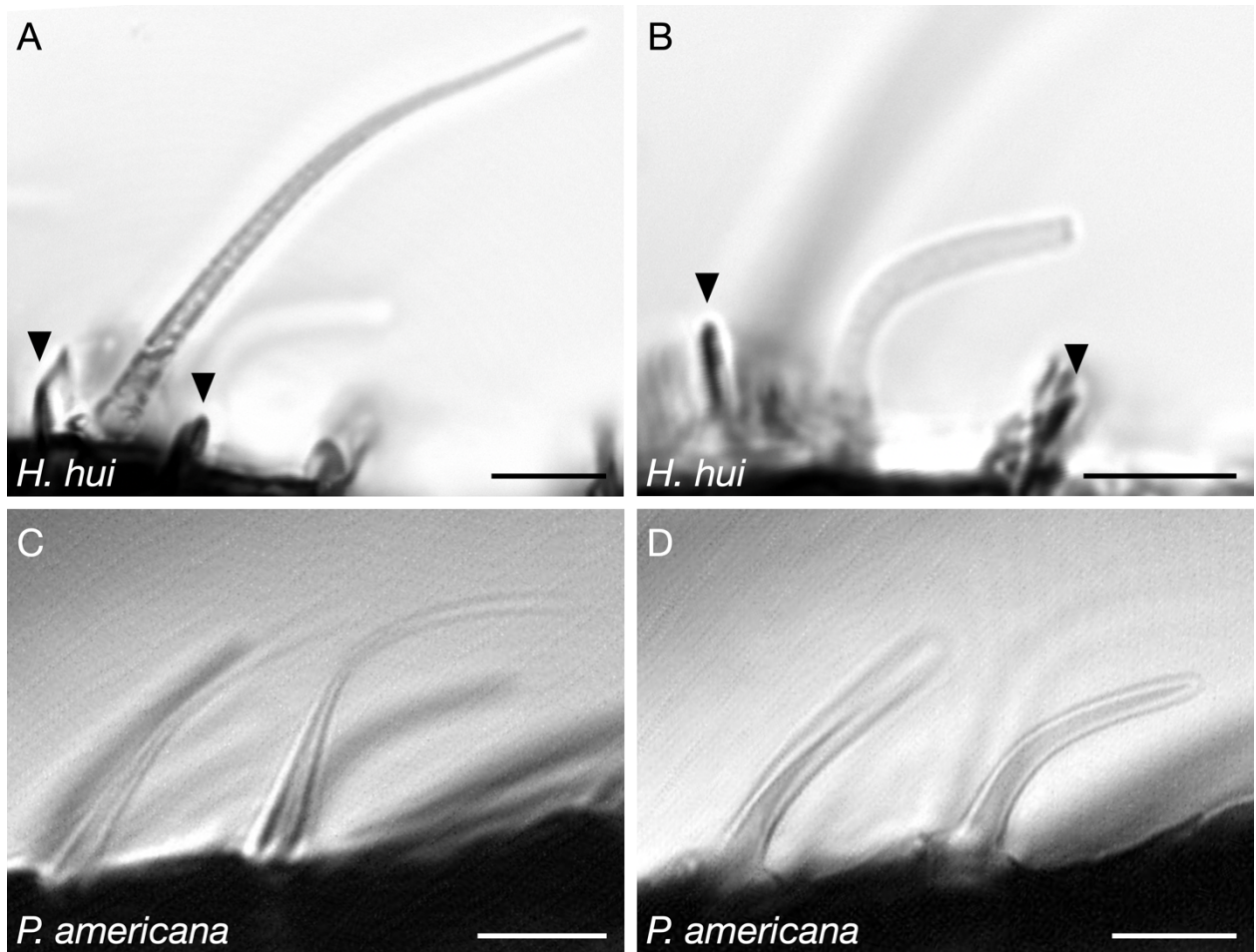


Fig. 1.3 Antennal sensillum images of *Huablattula hui* (AMNH Bu-SY28) (A, B) and *Periplaneta americana* via LSCM (C, D). (A, C) Trichoid sensilla. (B, D) Perforated basiconic sensilla. Grooved basiconic sensilla are marked by triangles in (A, B). Scale bars: 5 μm (A, B); 10 μm (C, D)

1.4. Discussion

The sensory organs of insects are minute and fragile, and their fossilized counterparts are unfortunately poorly preserved in sediments, with a few exceptions (7–10, 19). Studies based on non-destructive techniques (stereo microscopic imaging, synchrotron X-ray CT) have provided images of specimens without many observable microstructures of the body surface, even if the specimens are from exceptionally preserved insects in amber (32–34). For the first time, the present study focused on new details of insect sensory organs from *ca.* 100 million years ago. Based on the holistic characteristics of sensory organs, which are crucial for survival and reproduction, the perception systems and the ecology of extinct insects can be reconstructed with unprecedented resolution.

The compound eyes of insects consist of ommatidia. Each ommatidium has a rhabdom functioning as a retina under the corneal lens (35). Insects recognize objects as images subdivided via ommatidia; thus, a large number of ommatidia achieves a high spatial resolution (35). The spatial resolution is restricted by the body size, limiting the number of ommatidia (36). The number of ommatidia in the *H. hui* specimen was estimated at *ca.* 6000. This number is 1.2–1.5 times larger than that counted in extant nocturnal cockroaches, e.g., *P. americana* and *Gromphadorhina portentosa* (Schaum, 1853) (37, 38). Both species have about five times larger body sizes than *H. hui*. The extant wood-inhabiting (*Panesthia angustipennis* (Illiger, 1801), *Salganea taiwanensis* Roth, 1979) and cavernicolous species (*Alluaudellina cavernicola* (Shelford, 1910), *Helmablatta louisrothi* (Vidlička et al., 2017), *Paratemnopteryx stonei* Roth, 1990) also possess small or highly reduced eyes (17, 39–41). Compared to these species occupying dark habitats, *H. hui* had well-developed eyes and probably relied on a visual system to detect information about the environment.

The studied cockroach specimen also shows well-preserved micro sensilla in great detail. This observation allowed us to closely compare their morphology with extant taxa (Figs. 1.2 and 1.3). Our findings suggest the morphological stability of cockroach sensilla on a geological time scale over 100 Myr. The physiological properties of morphological types of antennal sensilla have been well defined in different species of cockroaches (28, 39, 42), and the sensillum function is also generally homologous in many other taxa: Diptera, Lepidoptera, Hymenoptera, Hemiptera, Orthoptera, Mantodea, Phasmatodea, Archaeognatha, Zygentoma (43–47). The function of chaetic sensilla is mechanoreception, and the ones of trichoid, p-bas, and g-bas sensilla are all different types of olfaction for each other. Although other types of sensilla also exist on insect antennae,

such as proprioceptive sensilla (43, 46, 48), we focus on the exteroceptive ones in this study. For *H. hui*, the ratios of sensillum length to body size are smaller than cave-inhabiting *Paratemnopteryx stonei* and *P. howarthi* Roth, 1990 (39). The number of chaetic sensilla in *H. hui* is markedly smaller than *P. stonei*, *P. howarthi*, nocturnal *P. americana* (Fig. 1.2b), *Blaberus craniifer* Burmeister, 1838, *Blattella germanica* (Linnaeus, 1767), and *Rhyparobia maderae* (Fabricius, 1781) (39, 49–51). A small number of antennal chaetic sensilla is also observed in praying mantises that are the most related sister taxon to cockroaches and heavily rely on vision for predation, such as *T. aridifolia* (Fig. 1.2c), *Hierodula majuscula* Tindale, 1923, *Mantis religiosa* (Linnaeus, 1758), *Oxythoespis maroccana* Bolivar, 1908, and *Pseudomantis albofimbriata* Stal, 1860 (52–55). The low number of antennal sensilla and the large compound eyes indicate that *H. hui* was active in bright and open environments during the daytime. This is in contrast to many extant cockroaches living in dark habitats such as forest floors, caves, or night environments (17). Cretaceous cockroaches may have occupied a much larger variety of ecological niches than their extant counterparts.

We identified the specimen as an adult male based on the body size and absence of an external ovipositor. The g-bas sensilla are remarkably more common than other types of sensilla on the antenna, similar to extant male mantises (Fig. 1.2a, c). Various insects indicate sexual dimorphism by the number of particular antennal sensilla. The dimorphism of sensillum patterns has been interpreted as a result of using sex pheromones (46, 54, 56–59). Especially in praying mantises (*H. majuscula*, *M. religiosa*, *P. albofimbriata*, *T. aridifolia*), apparent sexual dimorphism is mirrored by the more significant number of double-walled type sensilla in males, which are homologous to g-bas sensilla (46, 52–55). Although information about the female antenna is absent, the abundance and distributional patterns of g-bas sensilla in male *H. hui* suggest that they might have utilized the sensilla for intersexual communication as in mantises. It is usually difficult to precisely reconstruct ecological modes of sexual cues in extinct animals because such specific sensory organs and sex are seldom fossilized (7, 60). Our study shows that destructive and highly detailed visualization of sensory systems in amber allows for obtaining useful information for evaluating the behavior and other ecological parameters of extinct organisms.

References

1. B. R. Scheffers, L. N. Joppa, S. L. Pimm, W. F. Laurance, What we know and don't know about Earth's missing biodiversity. *Trends Ecol. Evol.* **27**, 501–510 (2012).
2. N. E. Stork, How many species of insects and other terrestrial arthropods are there on Earth? *Annu. Rev. Entomol.* **63**, 31–45 (2018).
3. J. H. Van Hateren, M. V. Srinivasan, P. B. Wait, Pattern recognition in bees: orientation discrimination. *J. Comp. Physiol. A* **167**, 649–654 (1990).
4. M. Mizunami, F. Yokohari, M. Takahata, Exploration into the adaptive design of the arthropod “microbrain.” *Zoolog. Sci.* **16**, 703–709 (1999).
5. T. Fujiwara, T. Kazawa, T. Sakurai, R. Fukushima, K. Uchino, T. Yamagata, S. Namiki, S. S. Haupt, R. Kanzaki, Odorant concentration differentiator for intermittent olfactory signals. *J. Neurosci.* **34**, 16581–16593 (2014).
6. M. S. Engel, D. A. Grimaldi, New light shed on the oldest insect. *Nature* **427**, 627–630 (2004).
7. T. Gao, C. Shih, C. C. Labandeira, J. A. Santiago-Blay, Y. Yao, D. Ren, Convergent evolution of ramified antennae in insect lineages from the Early Cretaceous of Northeastern China. *Proc. R. Soc. B Biol. Sci.* **283**, 20161448 (2016).
8. J. Lindgren, D.-E. Nilsson, P. Sjövall, M. Jarenmark, S. Ito, K. Wakamatsu, B. P. Kear, B. P. Schultz, R. L. Sylvestersen, H. Madsen, J. R. LaFountain, C. Alwmark, M. E. Eriksson, S. A. Hall, P. Lindgren, I. Rodríguez-Meizoso, P. Ahlberg, Fossil insect eyes shed light on trilobite optics and the arthropod pigment screen. *Nature* **573**, 122–125 (2019).
9. P. Vršanský, D. L. J. Quicke, A. P. Rasnitsyn, H. H. Basibuyuk, A. Ross, M. Fitton, L. Vidlička, The oldest fossil insect sensilla. *Publ. AMBAB21013ABSD*, 1–8 (2001).
10. P. Vršanský, Umenocoleoidea – an amazing lineage of aberrant insects (Insecta, Blattaria). *AMBA Proj.* **7**, 1–32 (2003).
11. G. Tanaka, A. R. Parker, D. J. Siveter, H. Maeda, M. Furutani, An exceptionally well-preserved Eocene dolichopodid fly eye: function and evolutionary significance. *Proc. R. Soc. B Biol. Sci.* **276**, 1015–1019 (2009).
12. P. Vršanský, G. Bechly, New predatory cockroaches (Insecta: Blattaria: Manipulatoridae fam.n.) from the Upper Cretaceous Myanmar amber. *Geol. Carpathica* **66**, 133–138 (2015).

13. H. Sendi, P. Vršanský, L. Podstrelená, J. Hinkelman, T. Kúdelová, M. Kúdela, Ľ. Vidlička, X. Ren, D. L. J. Quicke, Nocticolid cockroaches are the only known dinosaur age cave survivors. *Gondwana Res.* **82**, 288–298 (2020).
14. H. Sendi, J. Hinkelman, L. Vršanská, T. Kúdelová, M. Kúdela, M. Zuber, T. Van De Kamp, P. Vršanský, Roach nectarivory, gymnosperm and earliest flower pollination evidence from Cretaceous ambers. *Biologia (Bratisl.)* **75**, 1613–1630 (2020).
15. P. Vršanský, H. Sendi, J. Hinkelman, M. Hain, *Alienopterix* Mlynský et al., 2018 complex in North Myanmar amber supports Umenocoleoidea/ae status. *Biologia (Bratisl.)* **76**, 2207–2224 (2021).
16. G. Beccaloni, P. Eggleton, Order Blattodea. *Zootaxa* **3703**, 46–48 (2013).
17. W. J. Bell, L. M. Roth, C. A. Nalepa, *Cockroaches: Ecology, Behavior, and Natural History* (The Johns Hopkins University Press, Baltimore, 2007).
18. P. Vršanský, R. Oruřinský, D. Aristov, D.-D. Wei, Ľ. Vidlička, D. Ren, Temporary deleterious mass mutations relate to originations of cockroach families. *Biologia (Bratisl.)* **72**, 886–912 (2017).
19. M. Hörnig, C. Haug, J. Schneider, J. Haug, Evolution of reproductive strategies in dictyopteran insects – clues from ovipositor morphology of extinct roachoids. *Acta Palaeontol. Pol.* **63** (2018).
20. R. D. Cruickshank, K. Ko, Geology of an amber locality in the Hukawng Valley, Northern Myanmar. *J. Asian Earth Sci.* **21**, 441–455 (2003).
21. T. Yu, U. Thomson, L. Mu, A. Ross, J. Kennedy, P. Broly, F. Xia, H. Zhang, B. Wang, D. Dilcher, An ammonite trapped in Burmese amber. *Proc. Natl. Acad. Sci.* **116**, 11345–11350 (2019).
22. C. C. Labandeira, Amber. *Paleontol. Soc. Pap.* **20**, 163–216 (2014).
23. M. Guo, L. Xing, B. Wang, W. Zhang, S. Wang, A. Shi, M. Bai, A catalogue of Burmite inclusions. *Zool. Syst.* **42**, 249–379 (2017).
24. G. Shi, D. A. Grimaldi, G. E. Harlow, J. Wang, J. Wang, M. Yang, W. Lei, Q. Li, X. Li, Age constraint on Burmese amber based on U–Pb dating of zircons. *Cretac. Res.* **37**, 155–163 (2012).

25. Y. Mao, K. Liang, Y. Su, J. Li, X. Rao, H. Zhang, F. Xia, Y. Fu, C. Cai, D. Huang, Various amberground marine animals on Burmese amber with discussions on its age. *Palaeoentomology* **1**, 91–103 (2018).
26. D. A. Evangelista, B. Wipfler, O. Béthoux, A. Donath, M. Fujita, M. K. Kohli, F. Legendre, S. Liu, R. Machida, B. Misof, R. S. Peters, L. Podsiadlowski, J. Rust, K. Schuette, W. Tollenaar, J. L. Ware, T. Wappler, X. Zhou, K. Meusemann, S. Simon, An integrative phylogenomic approach illuminates the evolutionary history of cockroaches and termites (Blattodea). *Proc. R. Soc. B Biol. Sci.* **286**, 20182076 (2019).
27. P. Vršanský, *Piniblattella* gen. nov. the most ancient genus of the family Blattellidae (Blattodea) from the Lower Cretaceous of Siberia. *Entomol. Probl.* **28**, 67–79 (1997).
28. H. Watanabe, Y. Koike, K. Tateishi, M. Domae, H. Nishino, F. Yokohari, Two types of sensory proliferation patterns underlie the formation of spatially tuned olfactory receptive fields in the cockroach *Periplaneta americana*. *J. Comp. Neurol.* **526**, 2683–2705 (2018).
29. Y. Toh, Fine structure of antennal sense organs of the male cockroach, *Periplaneta americana*. *J. Ultrastruct. Res.* **60**, 373–394 (1977).
30. H. Watanabe, S. S. Haupt, H. Nishino, M. Nishikawa, F. Yokohari, Sensillum-specific, topographic projection patterns of olfactory receptor neurons in the antennal lobe of the cockroach *Periplaneta americana*. *J. Comp. Neurol.* **520**, 1687–1701 (2012).
31. X. Martínez-Delclòs, D. E. G. Briggs, E. Peñalver, Taphonomy of insects in carbonates and amber. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **203**, 19–64 (2004).
32. H. Pohl, B. Wipfler, D. Grimaldi, F. Beckmann, R. G. Beutel, Reconstructing the anatomy of the 42-million-year-old fossil †*Mengea tertiaria* (Insecta, Strepsiptera). *Naturwissenschaften* **97**, 855–859 (2010).
33. M. Perreau, P. Tafforeau, Virtual dissection using phase-contrast X-ray synchrotron microtomography: reducing the gap between fossils and extant species. *Syst. Entomol.* **36**, 573–580 (2011).
34. P. Barden, H. W. Herhold, D. A. Grimaldi, A new genus of hell ants from the Cretaceous (Hymenoptera: Formicidae: Haidomyrmecini) with a novel head structure. *Syst. Entomol.* **42**, 837–846 (2017).

35. E. K. Buschbeck, M. Friedrich, Evolution of insect eyes: tales of ancient heritage, deconstruction, reconstruction, remodeling, and recycling. *Evol. Educ. Outreach* **1**, 448–462 (2008).
36. M. Streinzer, W. Huber, J. Spaethe, Body size limits dim-light foraging activity in stingless bees (Apidae: Meliponini). *J. Comp. Physiol. A* **202**, 643–655 (2016).
37. R. Butler, The anatomy of the compound eye of *Periplaneta americana* L. - 2. Fine Structure. *J. Comp. Physiol.* **83**, 239–262.
38. M. Mishra, V. B. Meyer-Rochow, Fine structural description of the compound eye of the Madagascar ‘hissing cockroach’ *Gromphadorhina portentosa* (Dictyoptera: Blaberidae). *Insect Sci.* **15**, 179–192 (2008).
39. R. G. Bland, D. P. Slaney, P. Weinstein, Antennal sensilla on cave species of Australian *Paratemnopteryx* cockroaches (Blattaria : Blattellidae). *Int. J. Insect Morphol. Embryol.* **27**, 83–93 (1998).
40. C. A. Nalepa, K. Maekawa, K. Shimada, Y. Saito, C. Arellano, T. Matsumoto, Altricial development in subsocial wood-feeding cockroaches. *Zoolog. Sci.* **25**, 1190–1198 (2008).
41. Ľ. Vidlička, P. Vršanský, T. Kúdelová, M. Kúdela, L. Deharveng, M. Hain, New genus and species of cavernicolous cockroach (Blattaria, Nocticolidae) from Vietnam. *Zootaxa* **4232**, 361–375 (2017).
42. L. Schaller, Structural and functional classification of antennal sensilla of the cockroach, *Leucophaea maderae*. *Cell Tissue Res.* **225**, 129–142 (1982).
43. T. A. Keil, Functional morphology of insect mechanoreceptors. *Microsc. Res. Tech.* **39**, 506–531 (1997).
44. J. R. Riesgo-Escovar, W. B. Piekos, J. R. Carlson, The *Drosophila* antenna: ultrastructural and physiological studies in wild-type and lozenge mutants. *J. Comp. Physiol. [A]* **180**, 151–160 (1997).
45. H. Nishino, M. Nishikawa, M. Mizunami, F. Yokohari, Functional and topographic segregation of glomeruli revealed by local staining of antennal sensory neurons in the honeybee *Apis mellifera*. *J. Comp. Neurol.* **515**, 161–180 (2009).
46. T. Carle, Y. Toh, Y. Yamawaki, H. Watanabe, F. Yokohari, The antennal sensilla of the praying mantis *Tenodera aridifolia*: a new flagellar partition based on the antennal macro-, micro- and ultrastructures. *Arthropod Struct. Dev.* **43**, 103–116 (2014).

47. C. Missbach, H. K. Dweck, H. Vogel, A. Vilcinskas, M. C. Stensmyr, B. S. Hansson, E. Grosse-Wilde, Evolution of insect olfactory receptors. *eLife* **3**, e02115 (2014).
48. B. Ehmer, W. Gronenberg, Proprioceptors and fast antennal reflexes in the ant *Odontomachus* (Formicidae, Ponerinae). *Cell Tissue Res.* **290**, 153–165 (1997).
49. S. Ishii, Structure and function of the antenna of the German cockroach, *Blattella germanica* (L.) (Orthoptera: Blattellidae). *Appl. Entomol. Zool.* **6**, 192–197 (1971).
50. R. Schafer, Antennal sense organs of the cockroach, *Leucophaea maderae*. *J. Morphol.* **134**, 91–103 (1971).
51. M. Lambin, Les sensilles de l'antenne chez quelques blattes et en particulier chez *Blaberus craniifer* (Burm.). *Z. Für Zellforsch. Mikrosk. Anat.* **143**, 183–206 (1973).
52. G. I. Holwell, K. L. Barry, M. E. Herberstein, Mate location, antennal morphology, and ecology in two praying mantids (Insecta: Mantodea). *Biol. J. Linn. Soc.* **91**, 307–313 (2007).
53. M. J. Faucheux, Antennal sensilla of the male praying mantid *Oxyothespis maroccana* Bolivar, 1908 (Insecta: Mantodea: Mantidae): distribution and functional implications. *Bull. L'Institut Sci. Sect. Sci. Vie* **30**, 29–36 (2008).
54. M. J. Faucheux, Sensory and glandular structures on the antennae of *Mantis religiosa*, *Iris aratoria* and *Rivetina baetica*: sexual dimorphism, physiological implications (Mantodea: Mantidae). *Bull. Inst. R. Sci. Nat. Belg.* **79**, 231–242 (2009).
55. L. E. Allen, K. L. Barry, G. I. Holwell, Mate location and antennal morphology in the praying mantid *Hierodula majuscula*. *Aust. J. Entomol.* **51**, 133–140 (2012).
56. H. Sass, Production, release and effectiveness of two female sex pheromone components of *Periplaneta americana*. *J. Comp. Physiol. A* **152**, 309–317 (1983).
57. R. A. Steinbrecht, M. Laue, G. Ziegelberger, Immunolocalization of pheromone-binding protein and general odorant-binding protein in olfactory sensilla of the silk moths *Antheraea* and *Bombyx*. *Cell Tissue Res.* **282**, 203–217 (1995).
58. K. Fukuda, A. Yanagawa, M. Tuda, G. Sakurai, S. Kamitani, N. Furuya, Sexual difference in antennal sensilla abundance, density and size in *Callosobruchus rhodesianus* (Coleoptera: Chrysomelidae: Bruchinae). *Appl. Entomol. Zool.* **51**, 641–651 (2016).
59. J. K. Yuvaraj, M. N. Andersson, O. Anderbrant, C. Löfstedt, Diversity of olfactory structures: a comparative study of antennal sensilla in Trichoptera and Lepidoptera. *Micron* **111**, 9–18 (2018).

60. L. Krogmann, M. S. Engel, G. Bechly, A. Nel, Lower Cretaceous origin of long-distance mate finding behaviour in Hymenoptera (Insecta). *J. Syst. Palaeontol.* **11**, 83–89 (2013).

Chapter 2.

Complex chemical communication and advanced sociality in early ants

Abstract

Advanced social behavior, or eusociality, has been evolutionarily profound, allowing colonies of ants, termites, social wasps, and bees to dominate competitively over solitary species throughout the Cenozoic. Advanced sociality requires not just nestmate cooperation and specialization but refined coordination and communication. Here, we provide independent evidence that 100-million-year-old Cretaceous ants in amber were social, based on chemosensory adaptations. Previous studies inferred fossil ant sociality from individual ants preserved adjacent to others. We analyzed several fossil ants for their antennal sensilla, using original rotation imaging of amber microinclusions, and found an array of antennal sensilla, specifically for alarm pheromone detection and nestmate recognition, sharing distinctive features with extant ants. Although Cretaceous ants were stem groups, the fossilized sensilla confirm hypotheses of their complex sociality.

2.1. Introduction

Sociality is the most complex and advanced biological form of cooperation because it requires communication with other individuals. The evolution of sociality brought altruism to a selfish world, and thus, it has been a key innovation in the history of life, spawning the large discipline of sociobiology (1–3). Among many social organisms, which are largely arthropods, the importance of ants (Hymenoptera: Formicidae) is reflected in their abundance as well as species and ecological diversity (e.g., herbivores, predators, and fungivores, with diverse symbionts). It is estimated that the world's ant population is 20 quadrillion (20×10^{15}) individuals, a biomass comparable to that of humans globally (4, 5). Such pervasiveness makes ants an essential group in terrestrial ecosystems (4–6), all supported by sophisticated social systems such as complex communication and a division of labor with sterile workers (7, 8), which have been investigated with behavioral experiments, genomics, physiology, and phylogenetic analyses (8–12). Ants, all living species of which are eusocial, probably split from their solitary wasp ancestors in the Early Cretaceous (13–15). The oldest definitive records can be traced to amber from the mid-Cretaceous of France and Myanmar [~ 105 to 100 million years (Ma) ago] (16, 17). Their sociality has been inferred on the basis of adjacent preserved conspecifics (17–19), as well as the possession of a metapleural gland (8), making it uncertain if or how much they interacted cooperatively. Most recently, an adult and a pupal ant were found in the same piece of Myanmar amber and described as the same species (19), the authors interpreting this as evidence of eusocial brood care.

Rather than relying on statistical encounters of inclusions in amber pieces, we focused on a comparative approach of features intrinsic to the social communication systems of ants. Ants communicate with nestmates by various pheromones, such as for alarm, defense, recruitment, and trails (20). Unfortunately, preservation of pheromones in amber is extremely unlikely because they are low-molecular weight volatiles (e.g., terpenoid aldehydes, alcohols, alkanes, and ketones), and the amber itself is a terpene polymer (20–22). An ant fossil in Myanmar amber has been found to preserve internal organs putatively for social communication, but functions are ambiguous of the partially degraded remains of pheromonal glands and gross brain structure (23). An alternative approach involves the sensory organs that receive the communication signals. Ant pheromones are detected by antennal sensilla, which are micrometer-scale sensory cuticle hairs with neuronal innervation (9, 24–26). Each sensillum type is morphologically and functionally distinctive (27, 28). Sensory organs on the exoskeleton of insects, although microscopic, are externally chitinous

and thus can be well fossilized in amber, possibly the only direct evidence for ant social communication. However, the resolution of traditional visualization [e.g., photomicrography and x-ray computed tomography (CT) (0.5- μ m resolution now, although not necessarily in amber)] is insufficient for these minute sensory organs because of light scattering and low-density differences. For this reason, previous studies have not examined the sensilla of fossilized ants (17–19).

Here, we analyzed the antennae of one of the earliest ants, *Gerontoformica gracilis* (Barden and Grimaldi, 2014) (29), preserved in 100-million-year-old Cretaceous amber from Kachin State in northern Myanmar with a partially destructive method. This involved developing an original rotating visualization technique for amber to perform high-resolution imaging from multiple angles via confocal laser scanning microscopy (CLSM), reducing the optical aberrations. This method allowed us to visualize the microstructure and distribution of the antennal sensilla, thus reconstructing the communication systems.

2.2. Material and methods

The two focal amber pieces derive from Kachin State in northern Myanmar. Both pieces are deposited in the American Museum of Natural History with the collection numbers AMNH JZC Bu109 and AMNH Bu-KL B1-21, selected for partial destructive analysis from among a large series of specimens for the species. JZC Bu109 contains one fossilized ant, and Bu-KL B1-21 contains two fossilized ants (specimens A and B) (Fig. 2.1). The lack of wings and the presence of abdominal stings indicate that they are adult females. These fossils were identified as *G. gracilis* (Barden and Grimaldi, 2014) based on the taxonomic systematics in a previous study (19, 30) (genus diagnosis: bidentate mandible fitting against clypeus at rest, and clypeus with setae and without lobate process; species diagnosis: mesonotum without transverse ridge, abdominal segment IV without constriction, petiole longer than tall, from Kachin amber, mesosoma lumpy, and mesometanotal and metanotopropodeal sulci anteroposteriorly broad) (Fig. 2.2). The age of Kachin amber is almost as old as the earliest fossil records of ants, and *Gerontoformica* is the oldest ant genus (16–18). These specimens are, therefore, ideal for reconstructing the communication systems of ants in their early evolutionary stages.

Macrophotos of the fossils were taken using a Canon EOS 5DS digital camera with a Canon MP-E 65 mm macro lens (F2.8, 1 $\times$ to 5 $\times$) and a ZEISS LED Cold Light Source Dual Pipe Light System. The amber pieces were immersed in clove oil (Wako Pure Chemical Industries) to cover

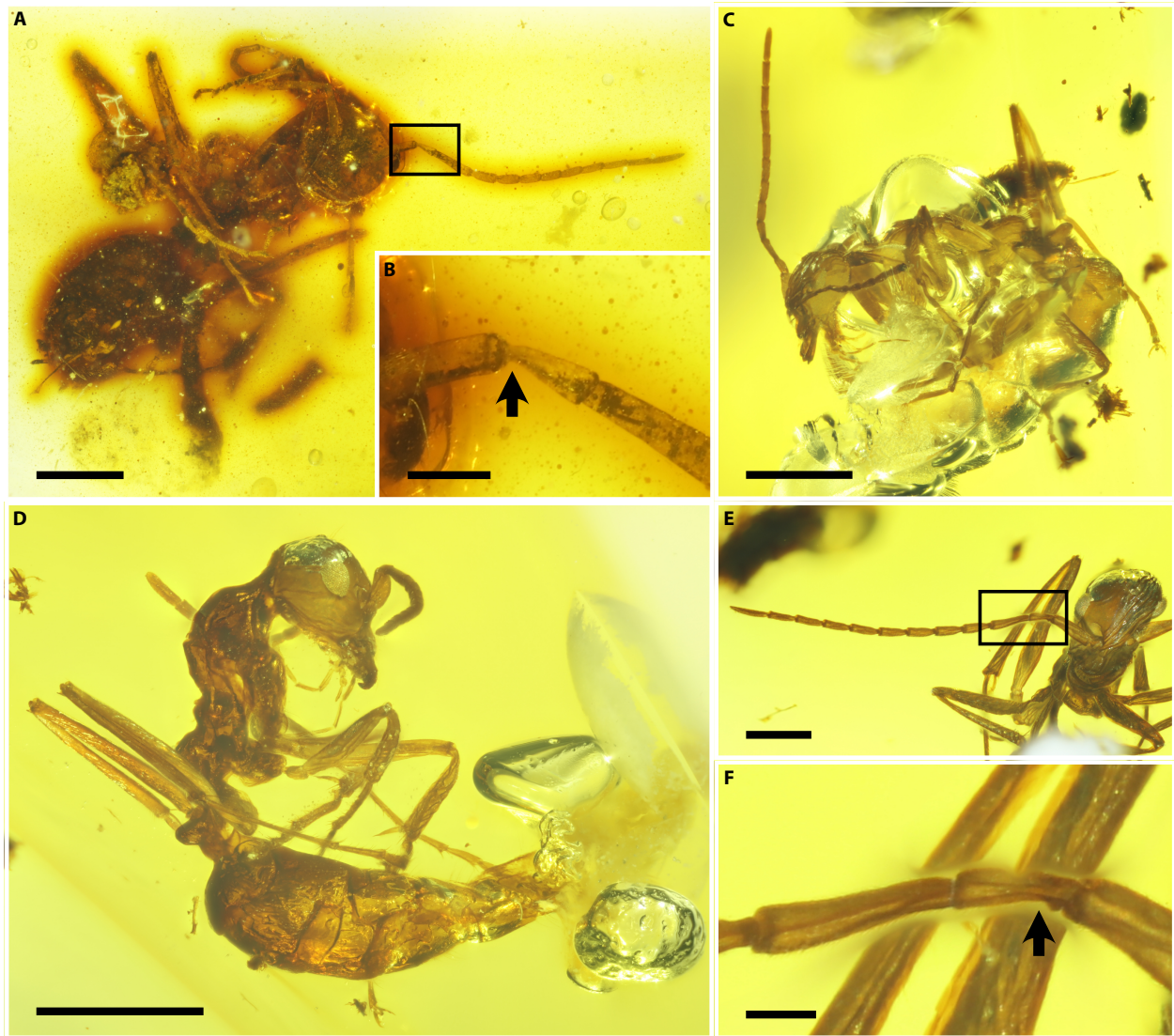


Fig. 2.1 (A) AMNH JZC Bu109, ventral view. (B) Enlarged image of the framed area in (A). (C) Specimen A in AMNH Bu-KL B1-21, lateral view. (D) Specimen B in AMNH Bu-KL B1-21, right lateral view. (E) The head of specimen B, frontal view. (F) Enlarged image of the framed area in (E). Specimen B shows the well-preserved forward-projecting right antenna (E). Pedicel constrictions indicating the inner lateral sides of antennae are marked by arrows in (B) and (F). Scale bars: 1 mm (A, C, D); 0.2 mm (B); 0.5 mm (E); 0.1 mm (F)

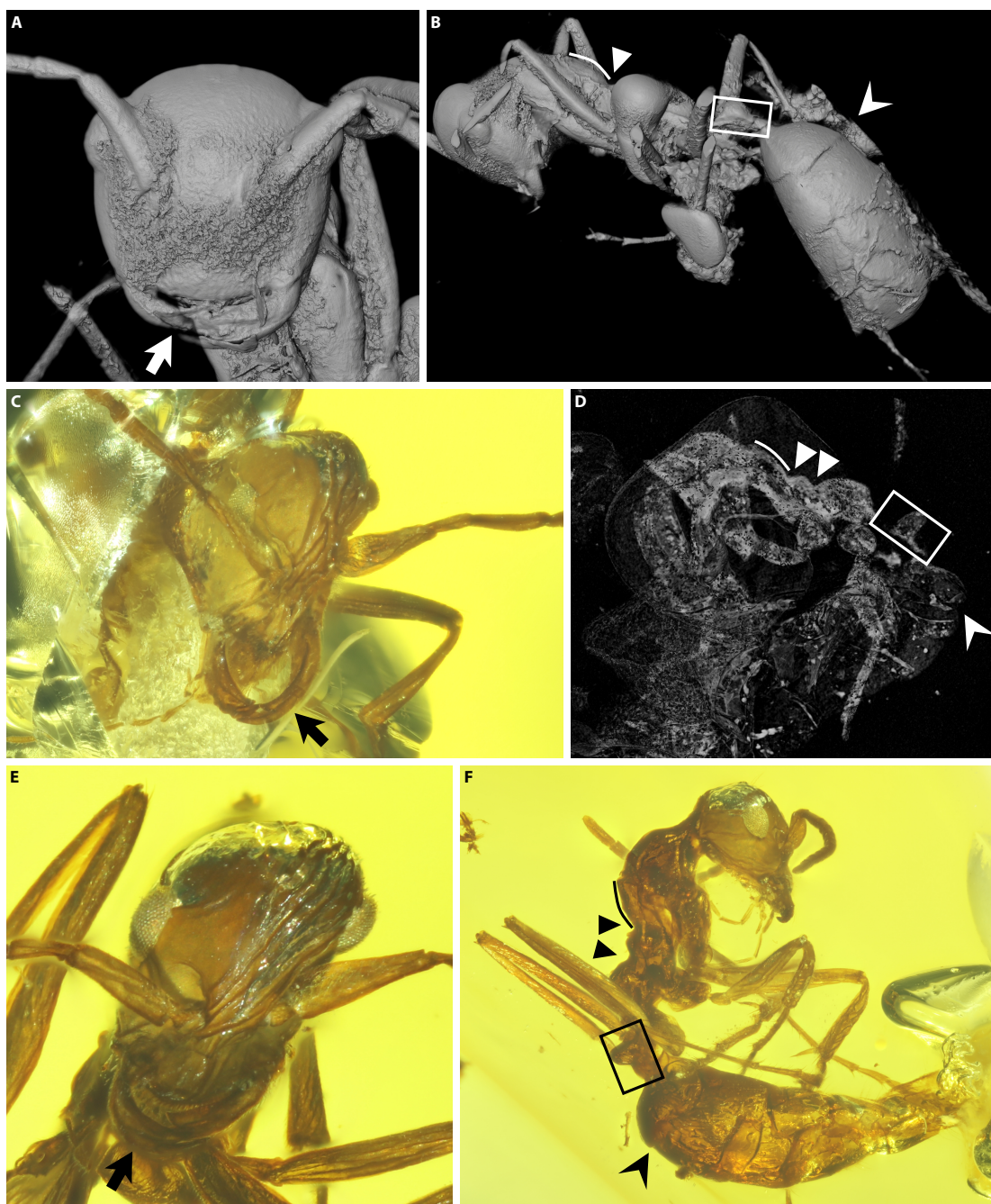


Fig. 2.2 (A, B) AMNH JZC Bu109. (C, D) Specimen A in AMNH Bu-KL B1-21 (E, F) Specimen B in AMNH Bu-KL B1-21. Bidentate mandibles fitting to each clypeus and clypei with setae and without lobate process are indicated by arrows in (A), (C), and (E). Smooth mesonota without apparent ridges are indicated by lines, not constricted fourth abdominal segments are indicated by arrowheads, petioles longer than tall are indicated by framed boxes, and broad mesometanotal and metanotopropodeal sulci are indicated by triangles in (B), (D), and (F). In JZC Bu109 (B), the metanotopropodeal sulcus is hidden by a debris inclusion.

the surface imperfections and improve optical resolution. We used Helicon Focus 8.1.2 (Helicon Soft) to focus-stack the images that we obtained. The specimens were scanned via a microfocus x-ray CT system (60 kV; Bruker SKYSCAN 2214) at Tomakomai Industrial Technology Center (Hokkaido, Japan). The voxel sizes are 4.2 μm^3 in JZC Bu109 and 3.5 and 3.0 μm^3 in specimens A and B in Bu-KL B1-21, respectively. The CT data were rendered with Amira 3D 2022.2 (Thermo Fisher Scientific).

We applied the amber thin-sectioning technique developed by Taniguchi *et al.* (31) to visualize antennal microsensilla and their distribution pattern. One antenna of each ant fossil was cut off with its amber matrix using a printed circuit board cutter (HOZAN TOOL INDUSTRIAL Co. Ltd.) and a diamond wire saw (Pepaless Co. Ltd.); the cut pieces were then ground and polished to $\sim 100\text{-}\mu\text{m}$ thickness using a diamond cup wheel (NIPPON DIAMOND Co. Ltd.). The antenna specimens in thinned amber pieces were visualized with a Nikon A1-Rsi CLSM system in the Nikon Imaging Center at Hokkaido University (using a Nikon Apo LWD 40 $\times$ WI λ S DIC N2 lens with 636.1-nm laser for JZC Bu109 and a Nikon Plan Apo VC 60 $\times$ WI DIC N2 lens with 487.4-nm laser for Bu-KL B1-21). After being observed and imaged in one position, the specimens were rotated 90° and ground/polished again to be observed and imaged from different angles (Fig. 2.3). This original rotation imaging of amber inclusions enabled us to visualize the entire distribution of micro sensilla on the antennal surface. A second view was also prepared with the A1-Rsi CLSM system. Images were stacked with Helicon Focus 8.2.3.

To compare the sensillum distribution patterns with fossils, we also observed antennae of six extant ant species belonging to four subfamilies: *Camponotus quadrinotatus* Forel, 1886; *Formica sanguinea* Latreille, 1798 (Formicinae); *Aphaenogaster famelica* (Smith, 1874); *Tetramorium tsushimae* Emery, 1925 (Myrmicinae); *Dolichoderus sibiricus* Emery, 1889 (Dolichoderinae); and *Pachycondyla chinensis* (Emery, 1895) (Ponerinae). These ants were ultrasonically cleaned in 50% acetone, fixed in 4% OsO₄ in 50% acetone, dehydrated gradually by changing acetone concentrations, and air-dried. We then cut off their heads with antennae and coated them with platinum-palladium using a Hitachi E-1030 ion sputter. The prepared antennae were observed via a Hitachi S-4800 SEM system (magnification, $\times 180$ to $\times 2000$). All figures are adjusted and compiled via Serif Affinity Designer 1.10.8.

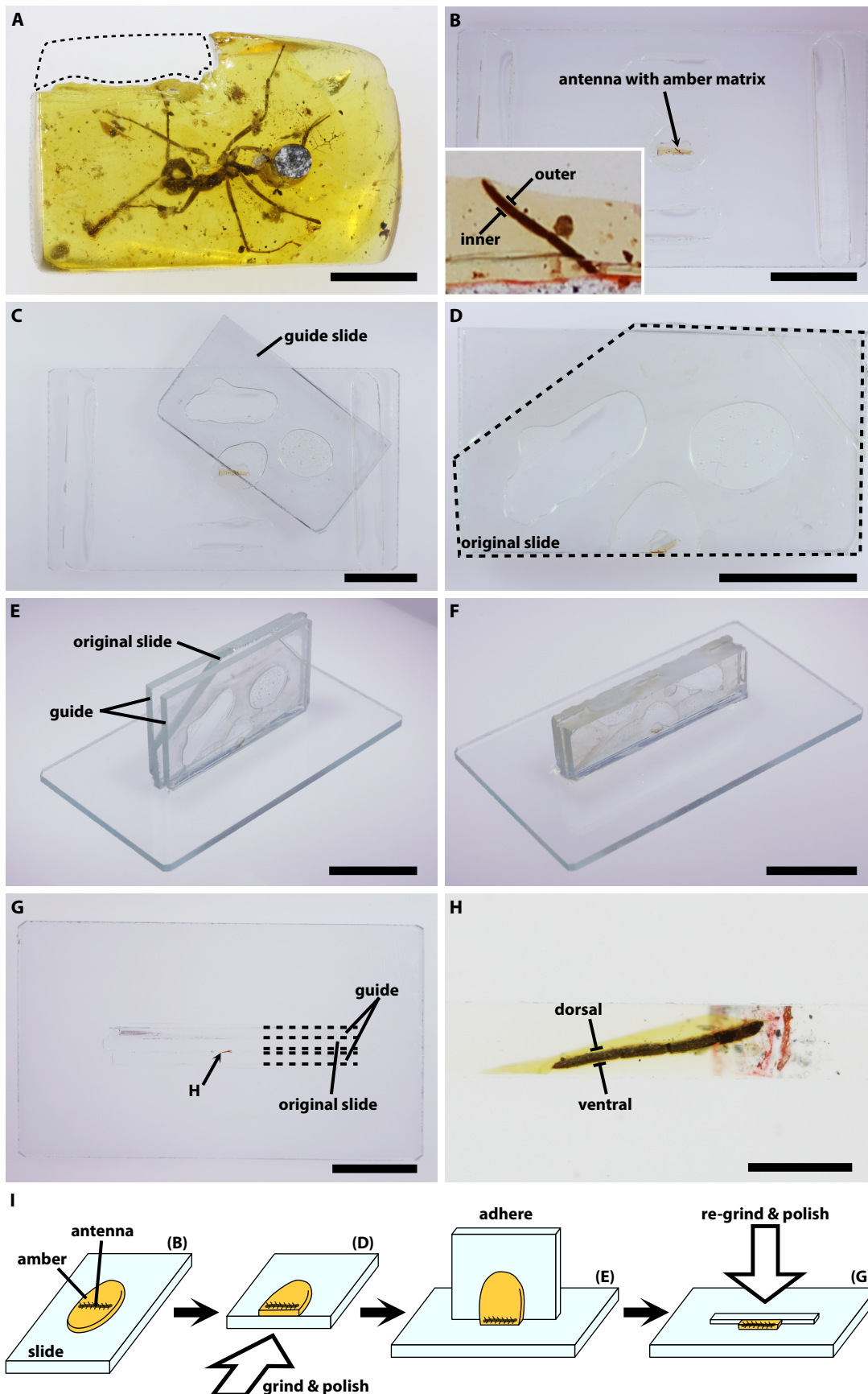


Fig. 2.3 (A) A fossilized ant in an amber piece (ventral view). The amber piece with the left antenna was cut off and thin-sectioned via a method developed by Taniguchi et al. (29) (surrounded area with a dotted line). (B) Thinned amber containing the antenna on the slide glass and a higher magnification image of the center area. (C) Specimen sandwiched between glass slides for guide. The guide slides were adhered along the antenna with an epoxy resin adhesive, indicating the vertical plane. (D) Specimen with the guide slides (cut, ground, and polished from [C]). The original slide is shown by a dotted line. (E) Specimen adhered orthogonally on a new slide. (F) Specimen ground in approximately half. (G) Completely re-thinned specimen. (H) Higher magnification image of the center area of (G). The antenna was rotated 90 degrees from the initial thin section in (B). (I) Simplified overall procedure for the re-thin sectioning. Each step corresponds to (B), (D), (E), and (G), respectively. Scale bars: 2 mm (A); 10 mm (B–G); 0.5 mm (H)

2.3. Results

Macroscale characters of the antennae ($>50\ \mu\text{m}$) were examined using macrophotography of three fossilized female *G. gracilis* ants preserved in Cretaceous amber [one ant in amber pieces AMNH JZC Bu109 and two ants (specimens A and B) in AMNH Bu-KL B1-21] (Fig. 2.1 and 2.2). The specimens, selected from a series of conspecific specimens, had well-preserved antennae projected away from the body (Fig. 2.1). Each antenna comprises 12 segments (a scape, a pedicel, and a flagellum with 10 flagellomeres), as in extant adult females (cf. 13 segments with 11 flagellomeres in males); it is geniculate between the scape and pedicel. The pedicel is constricted on one side as in extant ants (Fig. 2.1B, F) (27), allowing a landmark to the antennal inner lateral surface. Here, only the right antenna of specimen B in AMNH Bu-KL B1-21 could be accurately defined for the dorsal, ventral, inner, and outer surfaces because it was preserved linearly in the same plane (Fig. 2.1E). This specimen, therefore, was used for mapping the sensilla distribution patterns on the antenna.

The microsensilla of all three fossilized antennae (the left antenna from AMNH JZC Bu109 and each right antenna of specimens A and B from AMNH Bu-KL B1-21) were visualized via the thin-sectioning and CLSM technique developed by Taniguchi *et al.* (30) (Figs. 2.4A–G, 2.5A, B). All sensilla were classified into four morphotypes and identified as distinct sensillum types in extant ant taxa (27, 32, 33). Previous studies used different terminology for sensillum types, so we followed Ramirez-Esquivel *et al.* (28) because they compiled terminology from many previous studies. Sensilla basiconica are thumb-shaped pegs; sensilla trichodea are minute hairs tilted considerably toward the proximal direction of the antenna compared to the other sensilla (Fig. 2.4C, E–H). Sensilla trichodea curvata are curved, sword-like pegs (Fig. 2.4C–E, G, H). The most abundant slender hairs are thought to be either sensilla chaetica or trichoid II (Fig. 2.4C–H). In extant material, these two sensillum types are distinguished only based on ultrastructure observed with electron microscopy. These observations are too difficult for fossils in amber because the inclusions cannot be exposed and surface-scanned due to their fragility. Such diagnostic ultrastructure cannot be visualized even via our method; thus, we have left the possibility of two types here.

Because laser transmission does not penetrate the fossilized antennal cuticle (Fig. 2.4A, B), to visualize and map sensillum distributions, after one surface was analyzed via CLSM, the thinned amber piece, including the antenna, was rotated 90° and ground/polished again, so that we could

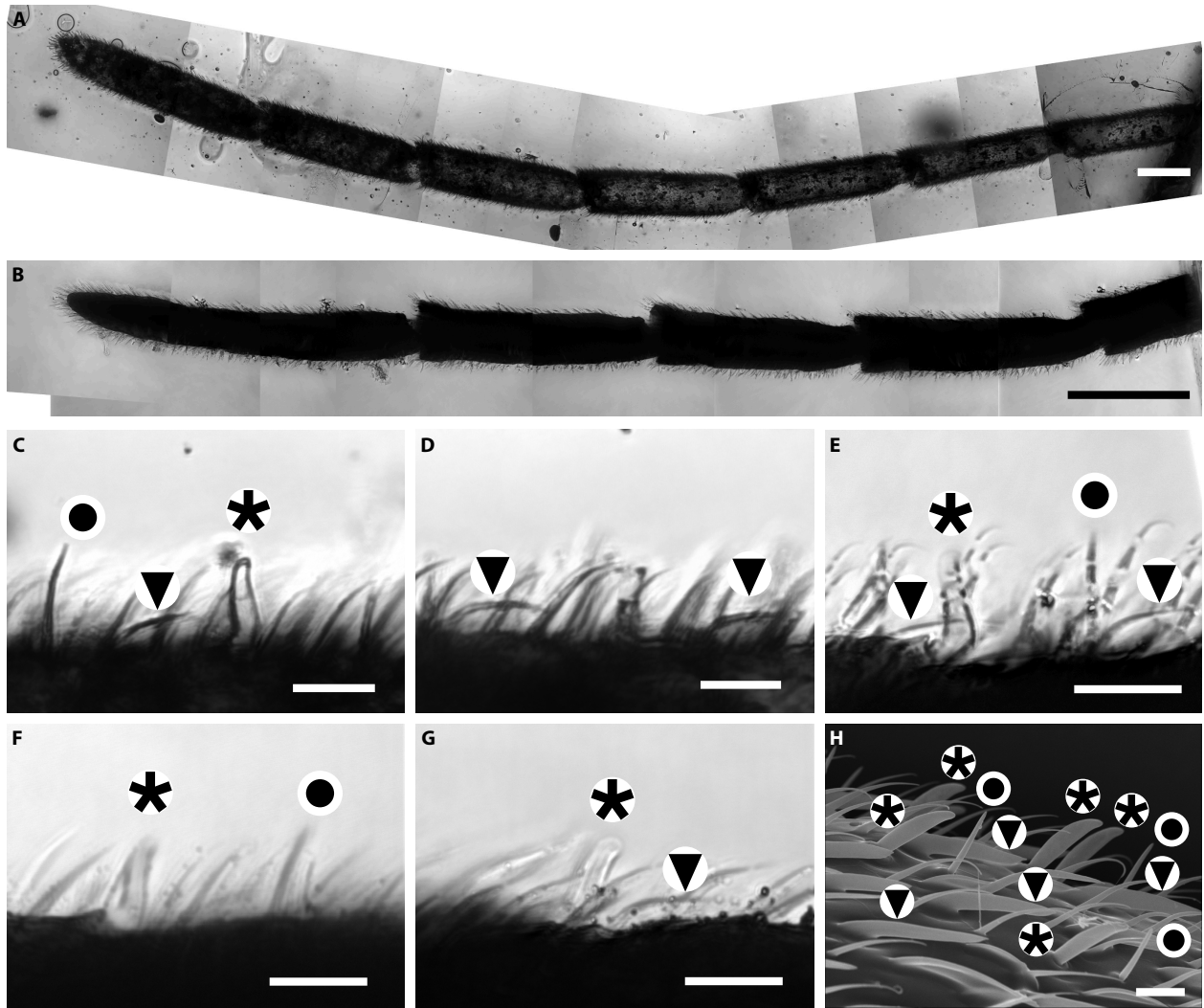


Fig. 2.4 (A) Left antenna of AMNH JZC Bu109. (B) Right antenna of specimen A in AMNH Bu-KL B1-21. (C, D) Antennal sensilla of AMNH JZC Bu109. (E) Antennal sensilla of specimen A in AMNH Bu-KL B1-21. (F, G) Antennal sensilla of specimen B in AMNH Bu-KL B1-21. (H) Antennal sensilla of *F. sanguinea*. Sensilla basiconica are marked by asterisks, trichodea curvata by triangles, trichodea by circles, and other hairlike sensilla without any marks are chatica or trichoid-II in (C) to (H). Scale bars: 100 μm (A, B); 10 μm (C–H)

visualize the orthogonal surface (Fig. 2.3). This two-time CLSM analysis of the specimen allowed observation throughout the dorsal, ventral, and inner and outer surfaces of the fossilized antenna. It revealed a concentrated pattern in the distribution of sensilla basiconica on the dorsal and inner surfaces (Fig. 2.5A, B). The same dorsal-inner distribution of sensilla basiconica was also observed in all six extant species via scanning electron microscopy (SEM) (Fig. 2.5C, D and Fig. 2.6).

2.4. Discussion

On extant ant antennae, there are eight types of sensilla based on their structure (28). These include five types of elongated protrusions (sensilla basiconica, trichodea, trichodea curvata, chaetica, and trichoid-II) and three types of peg-in-pit sensilla (sensilla ampullacea, coeloconica, and coelocapitular). The present study revealed at least four morphotypes of protruding sensilla on the Cretaceous *Gerontoformica* ants, all found in extant ants: sensilla basiconica, trichodea, trichodea curvata, and chaetica/trichoid-II (Fig. 2.4C–H).

Sensilla trichodea curvata are found uniquely in ants and occur in all ant taxa. Previous studies found that this sensillum type occurs in more than 60 species in 10 subfamilies of extant Formicidae (25, 28, 32–36). Sensilla trichodea curvata are considered homologous to plate-like sensilla placodea of some other hymenopterans (34). The transformation from flat plates embedded on the antennal surface (sensilla placodea) to protruding pegs (sensilla trichodea curvata) allows ants to detect molecules more effectively on a larger surface area. In extant ants, electrophysiological analysis has shown that sensilla trichodea curvata detect alarm pheromones (24, 27, 28). In detecting alarm pheromones, ants display aggressive or escape behavior in response to intruders (37, 38). †*Gerontoformica* already acquired this unique ant sensillum by 100 Ma ago (Fig. 2.4C–E, G) and correspondingly is likely to have used chemical alarm signaling. Acquiring alarm communication must have allowed stem ants to coordinate and mobilize nestmates to great advantage in inter- and intraspecific competition, attempted predation, and the invasion of territorial space, just like extant social insects (2, 39).

Sensilla basiconica are contact chemoreceptors for substrate molecules, and their distribution patterns on hymenopteran antennae are correlated with ecology. For instance, sensilla basiconica concentrated on the ventral surface in some parasitic wasps optimizes them for detecting molecular traces of their hosts (40). In eusocial hymenopterans, such as ants, hornets (Vespidae), and honey bees (*Apis*), the sensilla basiconica are usually distributed from the dorsal

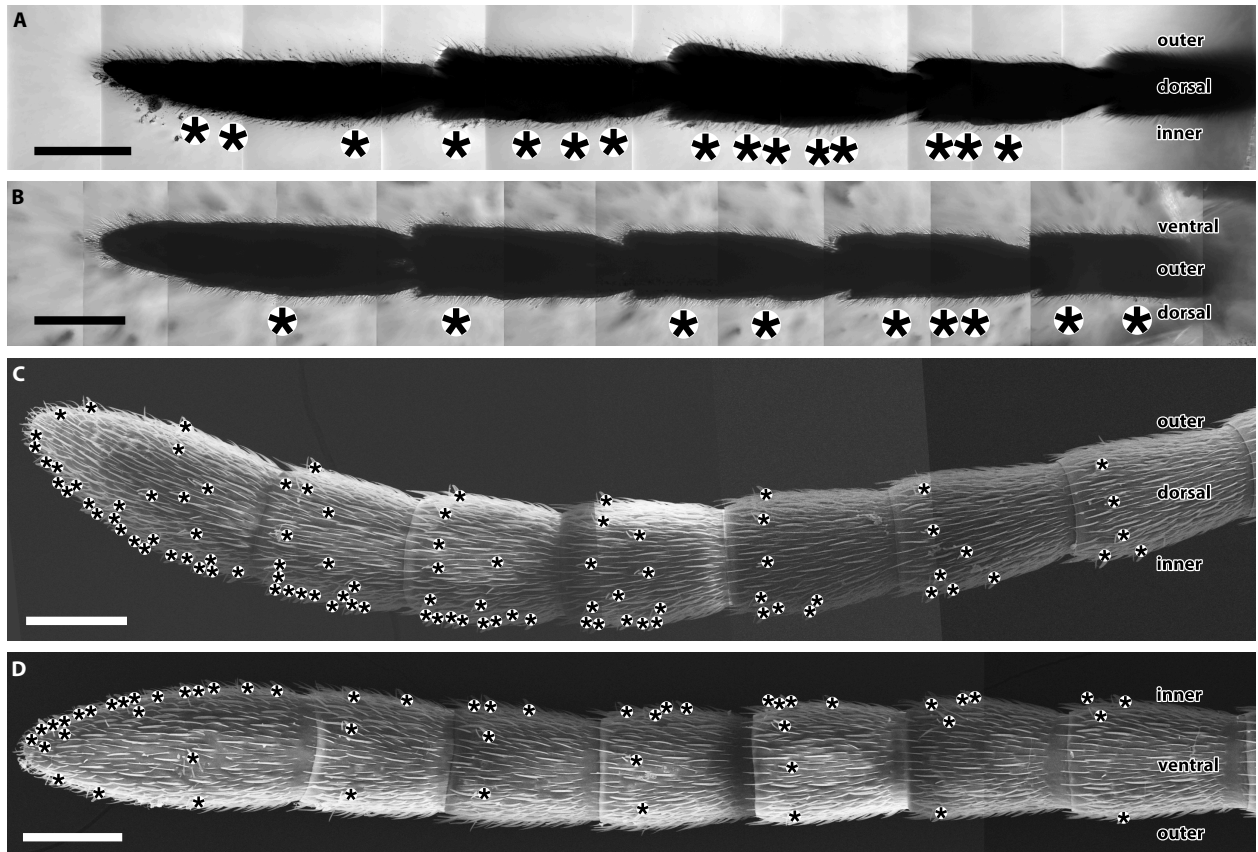


Fig. 2.5 (A, B) *G. gracilis*. (C, D) *C. quadrinotatus*. Sensilla basiconica marked by asterisks are biased on the dorsal and inner surfaces on the antennae. Scale bars: 100 μm (A–D)

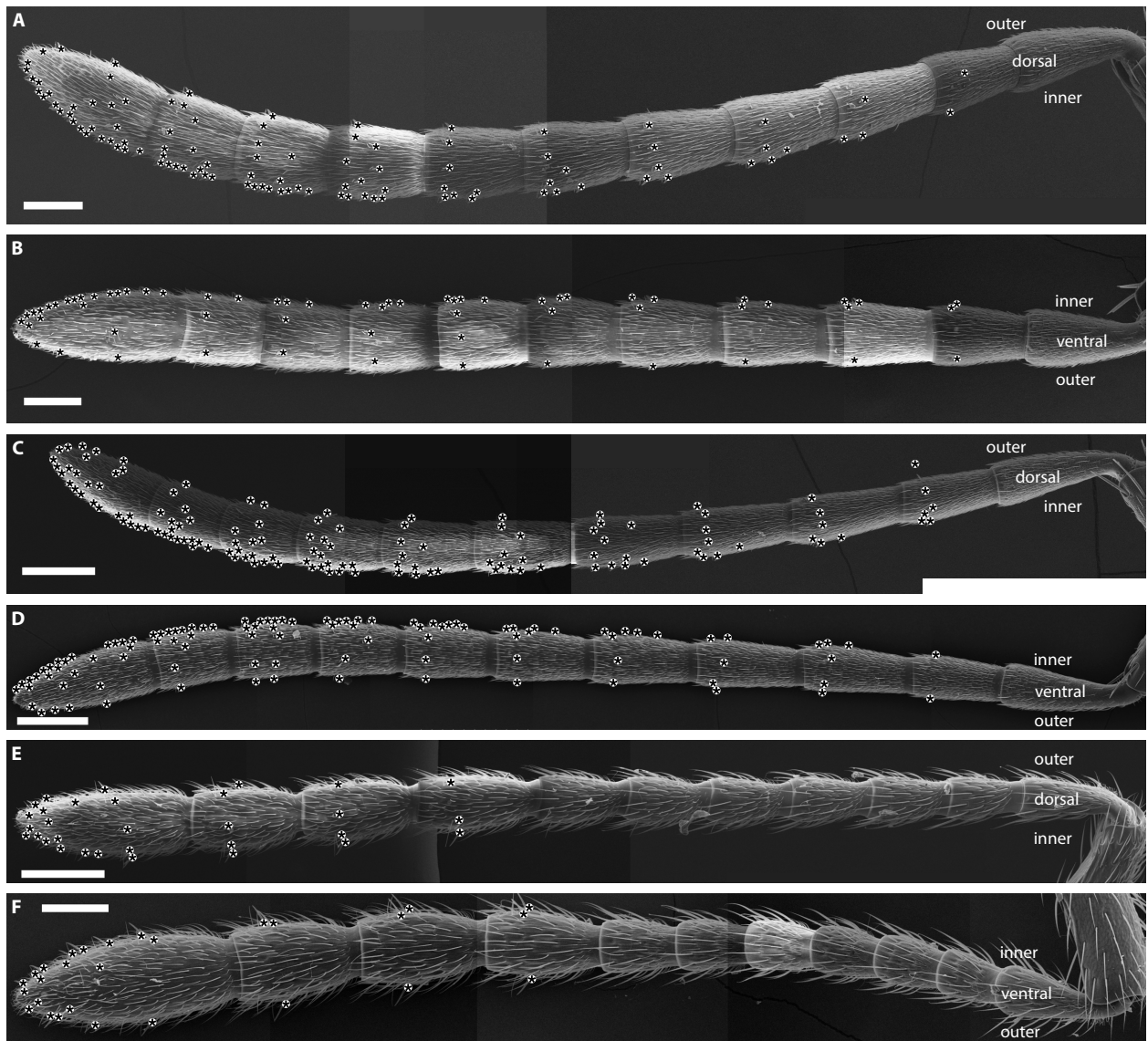




Fig. 2.6 (A, B) *Camponotus quadrinotatus*. (C, D) *Formica sanguinea*. (E, F) *Aphaenogaster famelica*. (G, H) *Tetramorium tsushimae*. (I, J) *Dolichoderus sibiricus*. (K, L) *Pachycondyla chinensis*. Sensilla basiconica marked by asterisks are biased on the dorsal and inner surfaces on the antennae. Scale bars: 100 μm (A, B, F–L); 200 μm (C–E)

to the inner surface (Fig. 2.5C, D and Fig. 2.6) (27, 28, 41, 42). The sensilla basiconica of eusocial species assist in distinguishing nestmates from intruders based on colony-specific cuticular hydrocarbon blends on the body surface (9, 43). The concentrations of these antennal sensilla on the dorsal and inner surfaces optimize nestmate recognition during antennation with other ants (27, 33). The same concentration of sensilla basiconica was observed in *Gerontoformica* as in modern ants (Fig. 2.5A, B). They likely identified their nestmates through chemosensory contact using their antennae like extant ants.

The presence on the antennae of *Gerontoformica* of abundant sensilla trichodea curvata and the concentration of sensilla basiconica on dorsal and inner surfaces indicate that these early, stem-group ants were adapted for social chemical communication via pheromones, as in modern ants. As such, this provides compelling, independent support for hypotheses that ants from the mid-Cretaceous were eusocial.

This raises the question posed by Grimaldi and Agosti (44): If early ants were eusocial, and eusociality confers substantial ecological advantages, why were ants so rare for the entire first half of their known existence? Throughout the Cretaceous, ants comprised 1% or less of individual insects in fossil deposits (the primary source of ant fossils is amber, and they are scarce in sedimentary rocks) (44, 45). It was not until the late Paleocene and Early Eocene that their abundance climbed to 10%, and not until the Neogene that it exceeded 20%. It could be argued that ants in Myanmar amber were not rare because hundreds of specimens and dozens of species are known (17). However, these have been found in huge, commercial-scale excavations of hundreds of tons of amber; all other Cretaceous deposits are a small fraction of this. In addition, noncommercial, unbiased samples of Myanmar amber show that ants comprise 1% of the inclusions (46).

The only explanation for this seems to be the colony size. Early ants probably lived in small colonies of several dozens, similar to modern amblyoponines and other basal ants today, and it was not until the origins of ant groups that formed immense colonies of many thousands in the Cenozoic that they became an overwhelming ecological force. Sophisticated social communication seems to have well preceded the ecological rise of ants.

References

1. C. Darwin, *On the Origin of Species by Means of Natural Selection* (John Murray, London, 1859).
2. E. O. Wilson, *Sociobiology: The New Synthesis* (Harvard University Press, Cambridge, 1975).
3. T. Clutton-Brock, Social evolution in mammals. *Science* **373**, eabc9699 (2021).
4. J. Tuma, P. Eggleton, T. M. Fayle, Ant-termite interactions: an important but under-explored ecological linkage. *Biol. Rev.* **95**, 555–572 (2020).
5. P. Schultheiss, S. S. Nooten, R. Wang, M. K. L. Wong, F. Brassard, B. Guénard, The abundance, biomass, and distribution of ants on Earth. *Proc. Natl. Acad. Sci.* **119**, e2201550119 (2022).
6. T. R. Schultz, In search of ant ancestors. *Proc. Natl. Acad. Sci.* **97**, 14028–14029 (2000).
7. S. N. Beshers, J. H. Fewell, Models of division of labor in social insects. *Annu. Rev. Entomol.* **46**, 413–440 (2001).
8. B. Hölldobler, E. O. Wilson, *The Ants* (Harvard University Press, Cambridge, 1990).
9. M. Ozaki, A. Wada-Katsumata, K. Fujikawa, M. Iwasaki, F. Yokohari, Y. Satoji, T. Nisimura, R. Yamaoka, Ant nestmate and non-nestmate discrimination by a chemosensory sensillum. *Science* **309**, 311–314 (2005).
10. M. A. Nowak, C. E. Tarnita, E. O. Wilson, The evolution of eusociality. *Nature* **466**, 1057–1062 (2010).
11. D. F. Simola, R. J. Graham, C. M. Brady, B. L. Enzmann, C. Desplan, A. Ray, L. J. Zwiebel, R. Bonasio, D. Reinberg, J. Liebig, S. L. Berger, Epigenetic (re)programming of caste-specific behavior in the ant *Camponotus floridanus*. *Science* **351**, aac6633 (2016).
12. J. Romiguier, M. L. Borowiec, A. Weyna, Q. Helleu, E. Loire, C. La Mendola, C. Rabeling, B. L. Fisher, P. S. Ward, L. Keller, Ant phylogenomics reveals a natural selection hotspot preceding the origin of complex eusociality. *Curr. Biol.* **32**, 2942-2947.e4 (2022).
13. S. G. Brady, T. R. Schultz, B. L. Fisher, P. S. Ward, Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proc. Natl. Acad. Sci.* **103**, 18172–18177 (2006).
14. E. O. Wilson, One giant leap: how insects achieved altruism and colonial life. *BioScience* **58**, 17–25 (2008).

15. R. S. Peters, L. Krogmann, C. Mayer, A. Donath, S. Gunkel, K. Meusemann, A. Kozlov, L. Podsiadlowski, M. Petersen, R. Lanfear, P. A. Diez, J. Heraty, K. M. Kjer, S. Klopstein, R. Meier, C. Polidori, T. Schmitt, S. Liu, X. Zhou, T. Wappler, J. Rust, B. Misof, O. Niehuis, Evolutionary history of the Hymenoptera. *Curr. Biol.* **27**, 1013–1018 (2017).
16. A. Nel, G. Perrault, V. Perrichot, D. Néraudeau, The oldest ant in the Lower Cretaceous amber of Charente-Maritime (SW France) (Insecta: Hymenoptera: Formicidae). *Geol. Acta* **2**, 23–29 (2004).
17. P. Barden, Fossil ants (Hymenoptera: Formicidae): ancient diversity and the rise of modern lineages. *Myrmecol. News* **24**, 1–30 (2017).
18. P. Barden, D. A. Grimaldi, Adaptive radiation in socially advanced stem-group ants from the Cretaceous. *Curr. Biol.* **26**, 515–521 (2016).
19. B. E. Boudinot, A. Richter, J. Katzke, J. C. M. Chaul, R. A. Keller, E. P. Economo, R. G. Beutel, S. Yamamoto, Evidence for the evolution of eusociality in stem ants and a systematic revision of †*Gerontoformica* (Hymenoptera: Formicidae). *Zool. J. Linn. Soc.*, zlab097 (2022).
20. R. Vander Meer K., “Pheromone directed behavior in ants” in *Pheromone Communication in Social Insects* (Westview Press, Oxford, 1998), pp. 159–192.
21. L. Van Oudenhove, E. Billoir, R. Boulay, C. Bernstein, X. Cerdá, Temperature limits trail following behaviour through pheromone decay in ants. *Naturwissenschaften* **98**, 1009–1017 (2011).
22. V. E. McCoy, C. Soriano, S. E. Gabbott, A review of preservational variation of fossil inclusions in amber of different chemical groups. *Earth Environ. Sci. Trans. R. Soc. Edinb.* **107**, 203–211 (2016).
23. A. Richter, B. Boudinot, S. Yamamoto, J. Katzke, R. G. Beutel, The first reconstruction of the head anatomy of a Cretaceous insect, †*Gerontoformica gracilis* (Hymenoptera: Formicidae), and the early evolution of ants. *Insect Syst. Divers.* **6**, 4 (2022).
24. K. Dumpert, Alarmstoffrezeptoren auf der Antenne von *Lasius fuliginosus* (Latr.) (Hymenoptera, Formicidae). *Z. Für Vgl. Physiol.* **76**, 403–425 (1972).
25. S. K. McKenzie, I. Fetter-Pruneda, V. Ruta, D. J. C. Kronauer, Transcriptomics and neuroanatomy of the clonal raider ant implicate an expanded clade of odorant receptors in chemical communication. *Proc. Natl. Acad. Sci.* **113**, 14091–14096 (2016).

26. H. Watanabe, S. Ogata, N. Nodomi, K. Tateishi, H. Nishino, R. Matsubara, M. Ozaki, F. Yokohari, Cuticular hydrocarbon reception by sensory neurons in basiconic sensilla of the Japanese carpenter ant. *Front. Cell. Neurosci.* **17**, 1084803 (2023).
27. A. Nakanishi, H. Nishino, H. Watanabe, F. Yokohari, M. Nishikawa, Sex-specific antennal sensory system in the ant *Camponotus japonicus*: structure and distribution of sensilla on the flagellum. *Cell Tissue Res.* **338**, 79–97 (2009).
28. F. Ramirez-Esquivel, J. Zeil, A. Narendra, The antennal sensory array of the nocturnal bull ant *Myrmecia pyriformis*. *Arthropod Struct. Dev.* **43**, 543–558 (2014).
29. P. Barden, D. Grimaldi, A Diverse Ant Fauna from the Mid-Cretaceous of Myanmar (Hymenoptera: Formicidae). *PLoS ONE* **9**, e93627 (2014).
30. B. E. Boudinot, V. Perrichot, J. C. M. Chaul, †*Camelosphecia* gen. nov., lost ant-wasp intermediates from the mid-Cretaceous (Hymenoptera, Formicoidea). *ZooKeys* **1005**, 21–55 (2020).
31. R. Taniguchi, H. Nishino, H. Watanabe, S. Yamamoto, Y. Iba, Reconstructing the ecology of a Cretaceous cockroach: destructive and high-resolution imaging of its micro sensory organs. *Sci. Nat.* **108**, 45 (2021).
32. K. Dumpert, Bau und Verteilung der Sensillen auf der Antennengeißel von *Lasius fuliginosus* (Latr.) (Hymenoptera, Formicidae). *Z. Für Morphol. Tiere* **73**, 95–116 (1972).
33. R. Renthal, D. Velasquez, D. Olmos, J. Hampton, W. P. Wergin, Structure and distribution of antennal sensilla of the red imported fire ant. *Micron* **34**, 405–413 (2003).
34. Y. Hashimoto, Unique features of sensilla on the antennae of Formicidae (Hymenoptera). *Appl. Entomol. Zool.* **25**, 491–501 (1990).
35. S. Hosoishi, K. Ogata, The relationship between the antennal club and the antennal sensilla in *Crematogaster* ants (Hymenoptera: Formicidae). *Sociobiology* **53** (2009).
36. F. Ramirez-Esquivel, N. E. Leitner, J. Zeil, A. Narendra, The sensory arrays of the ant, *Temnothorax rugatulus*. *Arthropod Struct. Dev.* **46**, 552–563 (2017).
37. E. O. Wilson, F. E. Regnier, The evolution of the alarm-defense system in the formicine ants. *Am. Nat.* **105**, 279–289 (1971).
38. H. Mizutani, K. Tagai, S. Habe, Y. Takaku, T. Uebi, T. Kimura, T. Hariyama, M. Ozaki, Antenna cleaning is essential for precise behavioral response to alarm pheromone and

- nestmate–non-nestmate discrimination in Japanese carpenter ants (*Camponotus japonicus*). *Insects* **12**, 773 (2021).
39. F. J. Verheggen, E. Haubruge, M. C. Mescher, “Alarm pheromones—chemical signaling in response to danger” in *Vitamins & Hormones* (Elsevier, 2010), vol. 83, pp. 215–239.
 40. N. Isidoro, R. Romani, F. Bin, Antennal multiporous sensilla: their gustatory features for host recognition in female parasitic wasps (Insecta, Hymenoptera: Platygastroidea). *Microsc. Res. Tech.* **55**, 350–358 (2001).
 41. H. Nishino, M. Nishikawa, M. Mizunami, F. Yokohari, Functional and topographic segregation of glomeruli revealed by local staining of antennal sensory neurons in the honeybee *Apis mellifera*. *J. Comp. Neurol.* **515**, 161–180 (2009).
 42. A. Couto, A. Mitra, D. Thiéry, F. Marion-Poll, J.-C. Sandoz, Hornets have it: a conserved olfactory subsystem for social recognition in Hymenoptera? *Front. Neuroanat.* **11**, 48 (2017).
 43. M. Ghaninia, K. Haight, S. L. Berger, D. Reinberg, L. J. Zwiebel, A. Ray, J. Liebig, Chemosensory sensitivity reflects reproductive status in the ant *Harpegnathos saltator*. *Sci. Rep.* **7**, 3732 (2017).
 44. D. Grimaldi, D. Agosti, A formicine in New Jersey Cretaceous amber (Hymenoptera: Formicidae) and early evolution of the ants. *Proc. Natl. Acad. Sci.* **97**, 13678–13683 (2000).
 45. J. S. LaPolla, G. M. Dlussky, V. Perrichot, Ants and the fossil record. *Annu. Rev. Entomol.* **58**, 609–630 (2013).
 46. D. A. Grimaldi, M. S. Engel, P. C. Nascimbene, Fossiliferous Cretaceous amber from Myanmar (Burma): its rediscovery, biotic diversity, and paleontological significance. *Am. Mus. Novit.* **3361**, 1–71 (2002).

Chapter 3.

Binocular stereopsis and predatory adaptation in a Cretaceous mantis

Abstract

Visual systems have been crucial for animals to detect light signals. Binocular stereopsis has affected prey-predator relationships throughout animal evolution by providing depth perception, among others. However, it has been difficult to reconstruct extinct binocular functions due to a lack of suitable fossil material. Here, we show, based on morphological analysis of well-preserved eyes, that an extinct mantis (*Ambermantis wozniaki* Grimaldi, 2003) in the Cretaceous New Jersey amber developed an advanced visual system as a predator. We found that *A. wozniaki* possesses large compound eyes with numerous, ca. 12000 ommatidia. The interocular distance is narrower than the eyes, and the estimated binocular visual field is broader than in the typical extant basal and derived taxa. The large number of ommatidia indicates that the compound eyes of *A. wozniaki* achieved high spatial resolution to capture objects visually. The broad binocular field supports that *A. wozniaki* increased the stereoscopic area and developed an advanced prey-recognition system. These findings suggest that the Cretaceous basal mantises were highly adaptive visual predators, implying the ecological domination of mantises as visual specialists for 90 million years.

3.1. Introduction

The eyes of animals detect light stimulation, and they are essential primary sensory organs responsible for simply perceiving environmental brightness to communicating complex social information (1–3). Although even one eye can achieve a broad visual field (e.g., nearly 180° in some vertebrates and insects) (4–6), most animals have two eyes and see objects ‘binocularly.’ Stereopsis is a binocular visual function that extracts depth information based on different images recognized by the right and left eyes (binocular disparity) (7, 8). Depth perception by binocular stereopsis is crucial, especially for predatory animals, since it is advantageous for successful visual hunting, such as judging the distance to prey and breaking camouflage (9, 10). Therefore, the evolution of stereopsis can be regarded as a critical adaptation that has strongly affected prey-predator relationships in the deep-time evolution of animals. Although some previous studies reconstructed the binocular visual function of extinct vertebrates, they lack robust bases such as soft tissue: the eyes themselves (4, 11, 12). Extinct binocular systems have remained hard to access using direct fossil evidence despite their evolutionary significance.

Praying mantises (order Mantodea) include about 2500 extant species, all predatory with spiny raptorial forelegs (13). Mantises were the first invertebrates proven to possess stereopsis based on neurobiological analysis, and they have been traditional models of binocular visual studies (14–16). When prey-capturing, mantises need to recognize an appropriate distance to prey and judge whether to strike based on their stereoscopic ability with a large binocular visual field and complex information process. Their visual ability has been investigated by combining behavioral experiments and neurophysiological and morphological analyses (17–19). Since compound eyes of arthropods have chitinous exoskeletons, their morphological characteristics, such as micron-scale ommatidia, are potentially preserved as fossils (20, 21). In some cases of insect eyes fossilized in amber, their three-dimensional structures are exceptionally preserved with almost their original morphology (22–24), although some taphonomic biases need to be noted (25). Fossilized mantises in amber can be therefore examined using the same morphological analysis as their extant counterparts. Fossil records of mantodeans are relatively rare: 38 species have been described, 25 from the Mesozoic and 13 from the Cenozoic (26–30). All Mesozoic taxa, mostly from the Cretaceous and a few from the Late Jurassic, show plesiomorphic characters represented by short prothoraxes and forelegs (29, 31–33), and thus, the binocular vision of such basal taxa potentially can reveal the early evolution and ecology of the visual system of mantises.

Here, we analyzed the exceptionally well-preserved compound eyes of a fossilized mantis (*Ambermantis wozniaki* Grimaldi, 2003) in mid-Cretaceous amber and compared the morphological property to extant relatives. Based on the comparison, we reconstructed the binocular visual system and prey-recognition/capture ability of the primitive mantis.

3.2. Material and methods

The fossilized adult male mantis derived from the Raritan Formation (Turonian: 90–94 Ma) in central New Jersey (34, 35) and was described as *Ambermantis wozniaki* (32), deposited in the American Museum of Natural History (AMNH NJ1085). The specimen is three-dimensionally well preserved and shows nearly complete body remains except for some parts of the antennae and legs (Fig. 1).

The fossil macrophotos were taken using a Canon EOS 5DS R (8688×5792 pixels) with a Canon MP-E 65 mm macro lens (F2.8, 1–5×) and a ZEISS LED Cold Light Source Dual Pipe Light System. We also used a Canon Extender EF 2× III for higher-magnified images. The amber was immersed in clove oil (Wako Pure Chemical Industries) to improve optical resolution by covering the surface cracks. The focus-stacked images of the specimen were obtained via Helicon Focus 8.2.3 (Helicon Soft). Facet diameters on the compound eyes were measured from these images, and the area of each facet was calculated by being regarded as a regular hexagon to estimate the ommatidium number explained below. The head was scanned with a microfocus X-ray CT system (60 kV, 3.5 $\mu\text{m}^3/\text{voxel}$; Bruker SKYSCAN 2214) at Tomakomai Industrial Technology Center (Hokkaido, Japan). The CT data were visualized as a 3D model, and its surface data were created with Amira 3D 2023.2 (Thermo Fisher Scientific) after being converted to 8-bit images. The surface data were imported into Blender 4.3.2 (Blender Foundation), and the surface area of the compound eyes was digitally measured on the software. The number of ommatidia was estimated by dividing this surface area by the facet area.

We measured the binocular visual field angle to estimate the stereoscopic and prey-recognition ability of *A. wozniaki* from the 3D model, following the methodology for extant mantis species (36) (Fig. 2). Frontal, lateral, and rear parts of the compound eye were determined in the dorsal view by drawing a line from the frontal-medial-most to rear-most edge and dividing the straight angle into three 60-degree parts. The curvature of the frontal part was represented by the osculating circle passing the edges, and the angle between the two lines from the circle centers to

the medial edges of each eye was regarded as the maximum binocular visual field angle. The drawing and measurement were performed on Serif Affinity Designer 2.5.5.

3.3. Results

Many facets are visible on the compound eyes (Fig. 1D). The diameter of each facet is $\sim 30\ \mu\text{m}$, and its area was calculated as $5.85 \times 10^{-4}\ \text{mm}^2$. The total surface area of both compound eyes was measured at $7.26\ \text{mm}^2$ from the 3D model. The compound eyes were estimated to comprise 12410 ommatidia from these data.

The compound eyes of *A. wozniaki* are well-developed and slightly protrude anteriorly (Figs. 1C, 2A–C). They are distributed with an anterior tilt from the dorsal view and have an enlarged frontal area of the eyes. (Fig. 2A, C). The head width and interocular distance were 2.87 mm and 0.96 mm, respectively. The ratio of interocular distance to head width of *A. wozniaki* is 0.34; i.e., from the frontal view, the eyes of *A. wozniaki* occupy about two-thirds of the head in their width. By geometrical drawing of osculating circles on the frontal parts, the maximum binocular visual field angle of *A. wozniaki* is estimated at 90.5° (Fig. 2D).

3.4. Discussion

Insects recognize objects as images subdivided via ommatidia, and many ommatidia achieve a high spatial resolution (37). The spatial resolution is also restricted by body size, limiting the number of ommatidia (38). The number of ommatidia in the specimen of *A. wozniaki* was estimated at ca. 12000. The extant mantises generally used as a model for visual function, whose body lengths are 8–10 mm in early nymphs and 50–80 mm in adults, increase their ommatidia ontogenetically from 8000 to 18000 ommatidia (e.g., *Tenodora aridifolia*, *Mantis religiosa*, and *Polyspilota* sp.) (39, 40). Considering the small adult body length (13 mm), *A. wozniaki* was equipped with a large number of ommatidia relative to the body size, indicating high visual resolution.

We calculated the ratio of interocular distance and head width of *A. wozniaki* at 0.34. This value is significantly smaller than for extant mantises; basal and small species: 0.44 in *Chaeteessa valida*, 0.38 in *Metallyticus splendidus*, 0.45 in *Mantoida maya*, 0.44 in *Amorphoscelis hamata*; derived and large species: 0.35 in *T. aridifolia*, 0.41 in *M. religiosa*, 0.52 in *Clinia humeralis* (33, 36, 41) and for other Cretaceous mantises recorded an adult with the complete and clearly

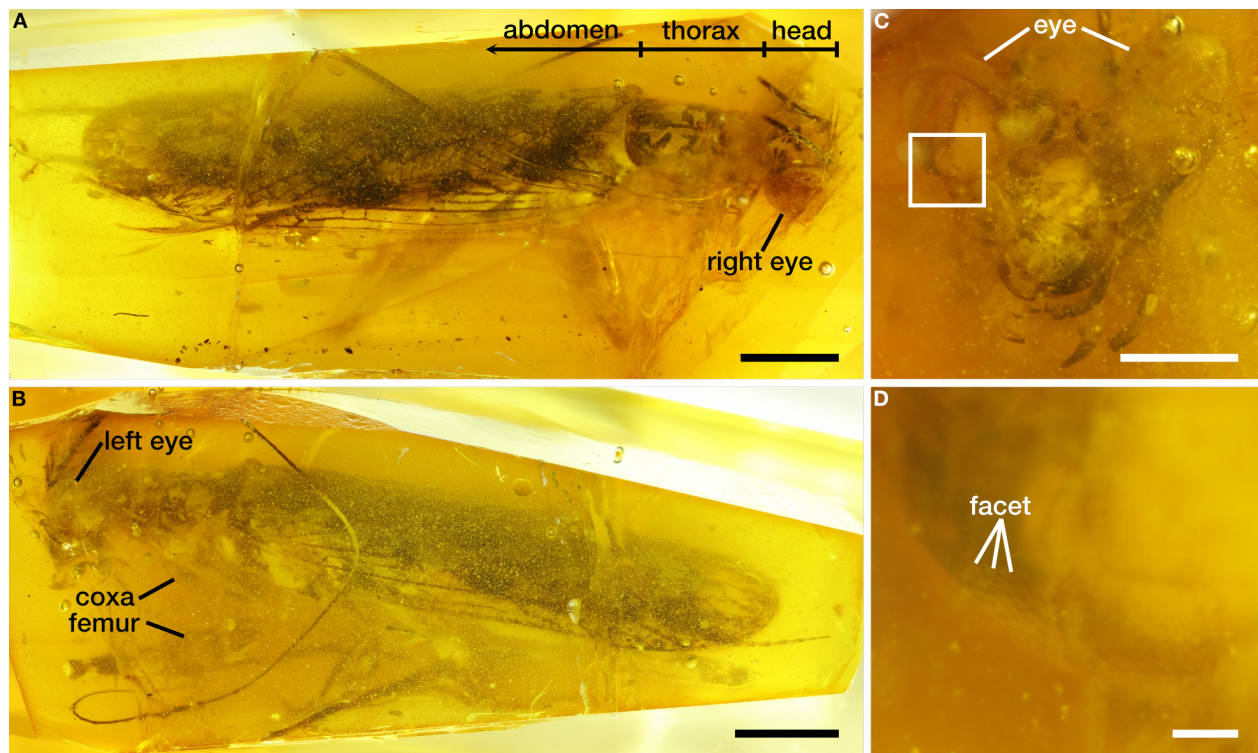


Fig. 3.1 Photographs of the Cretaceous mantis, *Ambermantis woźniaki* (AMNH NJ1085) in New Jersey amber. (A) dorsal view. The thorax is short, $1.8\times$ the head in length. (B) left lateral view, showing the spiny raptorial foreleg. (C) head with large compound eyes. (D) enlarged image of the framed area in (C) showing many facets on the eye. Scale bars: 2 mm (A, B); 1 mm (C); 0.1 mm (D)

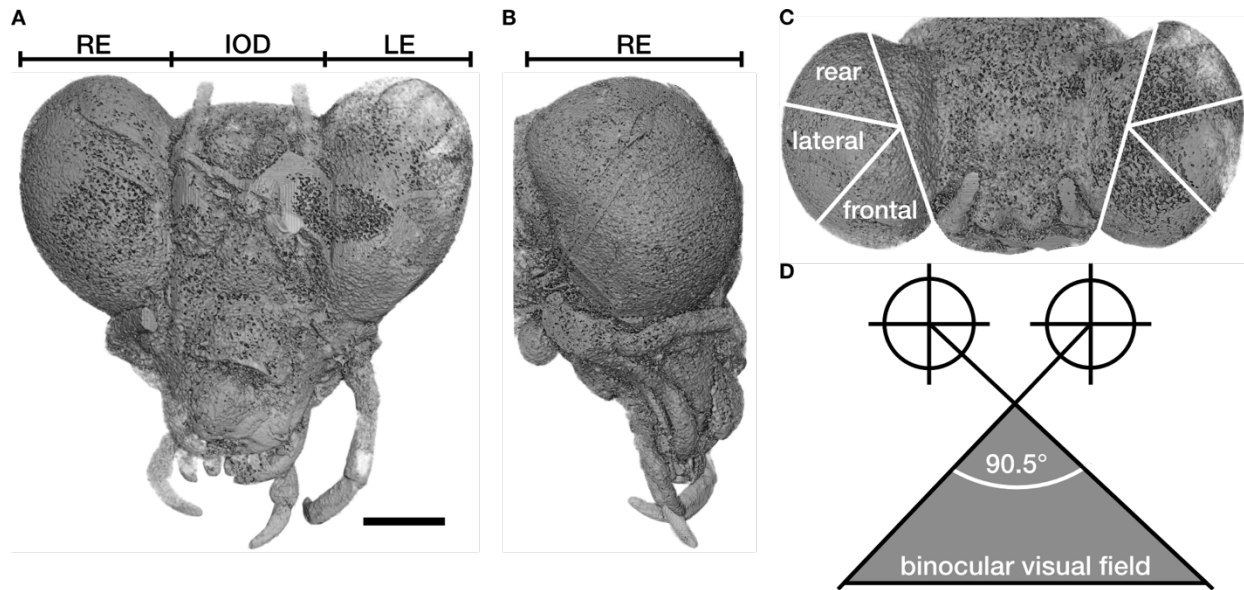


Fig. 3.2 Three-dimensional models of the head from CT data (A–C) and estimated binocular visual field angle (D) of *Ambermantis*. (A) frontal view. (B) right lateral view. (C) dorsal view, showing three divided parts on the eyes. *Abbreviations*: IOD, interocular distance; LE, left eye; RE, right eye. Scale bar: 0.5 mm (A–C)

observable heads/eyes; 0.41 in *Burmantis hexispinea*, 0.42 in *Santanmantis axelrodi* (27, 42). The short interocular distance to head width indicates that compound eyes occupy a large proportion of the head. *A. wozniaki* possessed relatively large eyes, even compared to modern eumantodeans. The interocular distance to head value 0.34 of *A. wozniaki* is also smaller than for extinct Cretaceous cockroaches, closely related to mantodeans in phylogeny and ecology: 0.53 in *Stavba babkaeva* (Liberiblattindiae), 0.56 in *Pseudomantina occisor* (Blattulidae), 0.48 in *Manipulatoides obscura* (Manipulatoridae), 0.52 in *Caputoraptor elegans* (Alienopteridae) (29, 43–48). These comparisons imply that *A. wozniaki* evolved the broad frontal visual field after splitting from the cockroach-like ancestral stage.

Mantises utilize their stereoscopic ability to perceive the distance to prey and strike them at a critical distance (Nityananda et al. 2016b; Read 2023). They detect the kinetic disparity of images between right and left compound eyes for the stereopsis (8, 51); thus, large binocular visual field angles enlarge the range of depth perception. The potential binocular visual field angle of *Ambermantis* is 90.5°, considerably broader than extant derived species (ca. 70° in *T. australasiae*, *T. aridifolia*, *M. religiosa* and 44.0° in *C. humeralis*) (14, 36) (Fig. 2D). This suggests that *A. wozniaki* increased the area where distance can be measured through binocular stereopsis and search their prey in a broad range. Behavioral experiments with extant species have shown that mantises cannot define the target as prey if it is too large to capture enough within the binocular visual field (36, 52, 53). In other words, the broader the binocular field, the greater the opportunity to recognize prey. *A. wozniaki* likely developed a broad binocular visual field and effectively captured their prey.

The extant derived mantises (most species are larger than 50 mm) are top predators among terrestrial invertebrates, and their predators are often also vertebrates (54). In contrast, *A. wozniaki* is very small (13 mm in body length), meaning the genus occupied middle-rank predatory status. They were likely preyed on by various higher predators, including insects or other arthropods. The large and high-resolution eyes of *A. wozniaki* may have also helped detect predators and escape from them, considering that extant species rely on visual stimulation in their defensive behavior (54, 55). Our lines of evidence suggest that *A. wozniaki* was a highly adaptive visual animal, enhancing the ability to search and recognize prey by equipping numerous ommatidia into the compound eyes and broadening its binocular stereoscopic visual field. The ancient tiny mantis from the Cretaceous period may have occupied the ecological niche as an adaptive predator

comparable to modern derived relatives. The advanced visual ability of praying mantises seems to have supported their role as principal predators for 90 million years.

References

1. M. F. Land, R. D. Fernald, The evolution of eyes. *Annu. Rev. Neurosci.* **15**, 1–29 (1992).
2. N. J. Emery, The eyes have it: the neuroethology, function and evolution of social gaze. *Neurosci. Biobehav. Rev.* **24**, 581–604 (2000).
3. T. E. White, J. Zeil, D. J. Kemp, Signal design and courtship presentation coincide for highly biased delivery of an iridescent butterfly mating signal. *Evolution* **69**, 14–25 (2015).
4. K. A. Stevens, Binocular vision in theropod dinosaurs. *J. Vertebr. Paleontol.* **26**, 321–330 (2006).
5. A. Borst, Drosophila's view on insect vision. *Curr. Biol.* **19**, R36–R47 (2009).
6. M. Bergman, J. Smolka, D.-E. Nilsson, A. Kelber, Seeing the world through the eyes of a butterfly: visual ecology of the territorial males of *Pararge aegeria* (Lepidoptera: Nymphalidae). *J. Comp. Physiol. A* **207**, 701–713 (2021).
7. A. J. Parker, Binocular depth perception and the cerebral cortex. *Nat. Rev. Neurosci.* **8**, 379–391 (2007).
8. J. C. A. Read, Binocular vision and stereopsis across the animal kingdom. *Annu. Rev. Vis. Sci.* **7**, 389–415 (2021).
9. V. Nityananda, J. C. A. Read, Stereopsis in animals: evolution, function and mechanisms. *J. Exp. Biol.* **220**, 2502–2512 (2017).
10. W. J. Adams, E. W. Graf, M. Anderson, Disruptive coloration and binocular disparity: breaking camouflage. *Proc. R. Soc. B Biol. Sci.* **286**, 20182045 (2019).
11. T. D. S. Marinho, F. V. Iori, I. D. S. Carvalho, F. M. De Vasconcellos, *Gondwanasuchus scabrosus* gen. et sp. nov., a new terrestrial predatory crocodyliform (Mesoeucrocodylia: Baurusuchidae) from the Late Cretaceous Bauru Basin of Brazil. *Cretac. Res.* **44**, 104–111 (2013).
12. T. Konishi, M. W. Caldwell, T. Nishimura, K. Sakurai, K. Tanoue, A new halisaurine mosasaur (Squamata: Halisaurinae) from Japan: the first record in the western Pacific realm

- and the first documented insights into binocular vision in mosasaurs. *J. Syst. Palaeontol.* **14**, 809–839 (2016).
13. A. Izquierdo-López, C. Kiesmüller, C. Gröhn, J. T. Haug, C. Haug, M. K. Hörnig, Patterns of morphological evolution in the raptorial appendages of praying mantises. *Insect Sci.*, 1744-7917.13423 (2024).
 14. S. Rossel, Binocular stereopsis in an insect. *Nature* **302**, 821–822 (1983).
 15. K. Kral, M. Poteser, Relationship between body size and spatial vision in the praying mantis - an ontogenetic study. *J. Orthoptera Res.* **18**, 153–158 (2009).
 16. R. Rosner, G. Tarawneh, V. Lukyanova, J. C. A. Read, Binocular responsiveness of projection neurons of the praying mantis optic lobe in the frontal visual field. *J. Comp. Physiol. A* **206**, 165–181 (2020).
 17. S. Rossel, Regional differences in photoreceptor performance in the eye of the praying mantis. *J. Comp. Physiol. A* **131**, 95–112 (1979).
 18. Y. Yamawaki, Y. Toh, Response properties of visual interneurons to motion stimuli in the praying mantis, *Tenodera aridifolia*. *Zoolog. Sci.* **20**, 819–832 (2003).
 19. R. Rosner, J. Von Hadeln, G. Tarawneh, J. C. A. Read, A neuronal correlate of insect stereopsis. *Nat. Commun.* **10**, 2845 (2019).
 20. M. S. Y. Lee, J. B. Jago, D. C. García-Bellido, G. D. Edgecombe, J. G. Gehling, J. R. Paterson, Modern optics in exceptionally preserved eyes of Early Cambrian arthropods from Australia. *Nature* **474**, 631–634 (2011).
 21. J. Lindgren, D.-E. Nilsson, P. Sjövall, M. Jarenmark, S. Ito, K. Wakamatsu, B. P. Kear, B. P. Schultz, R. L. Sylvestersen, H. Madsen, J. R. LaFountain, C. Alwmark, M. E. Eriksson, S. A. Hall, P. Lindgren, I. Rodríguez-Meizoso, P. Ahlberg, Fossil insect eyes shed light on trilobite optics and the arthropod pigment screen. *Nature* **573**, 122–125 (2019).
 22. X. Lin, C. C. Labandeira, C. Shih, C. L. Hotton, D. Ren, Life habits and evolutionary biology of new two-winged long-proboscid scorpionflies from mid-Cretaceous Myanmar amber. *Nat. Commun.* **10**, 1235 (2019).
 23. R. Kundrata, A. Bukejs, A. S. Prosvirov, J. Hoffmannova, X-ray micro-computed tomography reveals a unique morphology in a new click-beetle (Coleoptera, Elateridae) from the Eocene Baltic amber. *Sci. Rep.* **10**, 20158 (2020).

24. P. Vršanský, H. Sendi, J. Hinkelman, M. Hain, *Alienopterix* Mlynský et al., 2018 complex in North Myanmar amber supports Umenocoleoidea/ae status. *Biologia (Bratisl.)* **76**, 2207–2224 (2021).
25. I. Koubová, T. Mlynský, Two new mid-Cretaceous dictyopterans (Umenocoleidae: Vitisminae) from northern Myanmar exemplify taphonomic bias. *AMBA Proj.* **10**, 1–16 (2020).
26. X. Delclòs, E. Peñalver, A. Arillo, M. S. Engel, A. Nel, D. Azar, A. Ross, New mantises (Insecta: Mantodea) in Cretaceous ambers from Lebanon, Spain, and Myanmar. *Cretac. Res.* **60**, 91–108 (2016).
27. X.-R. Li, D. Huang, A new praying mantis from middle Cretaceous Burmese amber exhibits bilateral asymmetry of forefemoral spination (Insecta: Dictyoptera). *Cretac. Res.* **91**, 269–273 (2018).
28. J. Terríquez-Beltrán, F. Riquelme, F. Varela-Hernández, A new species of mantis (Insecta: Mantodea: Amelidae) from the Miocene Amber-Lagerstätte in Mexico. *Hist. Biol.* **35**, 2127–2134 (2023).
29. P. Vršanský, Late Mesozoic cockroaches s.l. from the Karabastau Formation in Kazakhstan. *AMBA Proj.* **14**, 1–700 (2024).
30. P. Vršanský, Z. Kováčová, D. V. Vasilenko, H. Pálková, Š. Nagy, D. Kosnáč, L. Vidlička, S. K. Martin, Systematics of Mesozoic ‘Arctic’ polar cockroaches. *Biologia* **80**, 51–77 (2025).
31. P. Vršanský, Origin and the early evolution of mantises. *AMBA Proj.* **6**, 1–16 (2002).
32. D. Grimaldi, A revision of Cretaceous mantises and their relationships, including new taxa (Insecta: Dictyoptera: Mantodea). *Am. Mus. Novit.* **3412**, 1–47 (2003).
33. F. Wieland, The phylogenetic system of Mantodea (Insecta: Dictyoptera). *Species Phylogeny Evol.* **3**, 3–222 (2013).
34. M. A. Gandolfo, K. C. Nixon, W. L. Crepet, D. A. Grimaldi, A late Cretaceous fagalean inflorescence preserved in amber from New Jersey. *Am. J. Bot.* **105**, 1424–1435 (2018).
35. X. Delclòs, E. Peñalver, E. Barrón, D. Peris, D. A. Grimaldi, M. Holz, C. C. Labandeira, E. E. Saupe, C. R. Scotese, M. M. Solórzano-Kraemer, S. Álvarez-Parra, A. Arillo, D. Azar, E. A. Cadena, J. Dal Corso, J. Kvaček, A. Monleón-Getino, A. Nel, D. Peyrot, C. A. Bueno-Cebollada, A. Gallardo, B. González-Fernández, M. Goula, C. Jaramillo, I. Kania-Kłosok, R. López-Del Valle, R. P. Lozano, N. Meléndez, C. Menor-Salván, C. Peña-Kairath, V.

- Perrichot, A. Rodrigo, A. Sánchez-García, M. Santer, V. Sarto I Monteys, D. Uhl, J. L. Viejo, R. Pérez-de La Fuente, Amber and the Cretaceous resinous interval. *Earth-Sci. Rev.* **243**, 104486 (2023).
36. F. R. Prete, J. L. Komito, S. Dominguez, G. Svenson, L. Y. López, A. Guillen, N. Bogdanivich, Visual stimuli that elicit appetitive behaviors in three morphologically distinct species of praying mantis. *J. Comp. Physiol. A* **197**, 877–894 (2011).
 37. E. K. Buschbeck, M. Friedrich, Evolution of insect eyes: tales of ancient heritage, deconstruction, reconstruction, remodeling, and recycling. *Evol. Educ. Outreach* **1**, 448–462 (2008).
 38. M. Streinzer, W. Huber, J. Spaethe, Body size limits dim-light foraging activity in stingless bees (Apidae: Meliponini). *J. Comp. Physiol. A* **202**, 643–655 (2016).
 39. K. Kral, The functional significance of mantis peering behaviour. *Eur. J. Entomol.* **109**, 295–301 (2012).
 40. K. Kral, Orientation behavior with and without visual cues in newly hatched and adult praying mantis. *J. Insect Behav.* **27**, 192–205 (2014).
 41. R. Roy, M. B. D. Stiewe, Contribution to the knowledge of Eastern African *Amorphoscelis* Stål, 1871, with description of two new species (Dictyoptera, Mantodea, Amorphoscelidae). *Bull. Société Entomol. Fr.* **114**, 195–209 (2009).
 42. M. K. Hörnig, J. T. Haug, C. Haug, An exceptionally preserved 110 million years old praying mantis provides new insights into the predatory behaviour of early mantodeans. *PeerJ* **5**, e3605 (2017).
 43. P. Vršanský, L. Vršanská, M. Beňo, T. Bao, X. Lei, X. Ren, H. Wu, L. Šmídová, G. Bechly, L. Jun, M. Yeo, E. Jarzembowski, Pathogenic DWV infection symptoms in a Cretaceous cockroach. *Palaeontogr. Abt. A* **314**, 1–10 (2019).
 44. P. Vršanský, J. Hinkelman, I. Koubová, H. Sendi, T. Kúdelová, M. Kúdela, M. Barclay, A single common ancestor for praying mantids, termites, cave roaches and umenocoleoids. *AMBA Proj.* **11**, 1–16 (2021).
 45. B. Wipfler, P. Kočárek, A. Richter, B. Boudinot, M. Bai, R. G. Beutel, Structural features and life habits of †Alienoptera (Polyneoptera, Dictyoptera, Insecta). *Palaeoentomology* **2**, 465–473 (2019).

46. X. Li, D. Huang, Predators or herbivores: cockroaches of Manipulatoridae revisited with a new genus from Cretaceous Myanmar amber (Dictyoptera: Blattaria: Corydioidea). *Insects* **13**, 732 (2022).
47. C. Luo, R. G. Beutel, M. S. Engel, K. Liang, L. Li, J. Li, C. Xu, P. Vršanský, E. A. Jarzembowski, B. Wang, Life history and evolution of the enigmatic Cretaceous–Eocene Alienopteridae: A critical review. *Earth-Sci. Rev.* **225**, 103914 (2022).
48. H. Sendi, P. Vršanský, D. Azar, Jordanian–Lebanese–Syrian cockroaches s.s. from Lower Cretaceous amber – Monograph. *Biologia (Bratisl.)* **78**, 1447–1541 (2023).
49. V. Nityananda, G. Bissianna, G. Tarawneh, J. Read, Small or far away? Size and distance perception in the praying mantis. *Philos. Trans. R. Soc. B Biol. Sci.* **371**, 20150262 (2016).
50. J. C. A. Read, Stereopsis without correspondence. *Philos. Trans. R. Soc. B Biol. Sci.* **378**, 20210449 (2023).
51. V. Nityananda, G. Tarawneh, S. Henriksen, D. Umeton, A. Simmons, J. C. A. Read, A novel form of stereo vision in the praying mantis. *Curr. Biol.* **28**, 588-593.e4 (2018).
52. F. R. Prete, T. McLean, Responses to moving small-field stimuli by the praying mantis, *Sphodromantis lineola* (Burmeister). *Brain. Behav. Evol.* **47**, 42–54 (1996).
53. F. R. Prete, R. Theis, J. L. Komito, J. Dominguez, S. Dominguez, G. Svenson, F. Wieland, Visual stimuli that elicit visual tracking, approaching and striking behavior from an unusual praying mantis, *Euchomenella macrops* (Insecta: Mantodea). *J. Insect Physiol.* **58**, 648–659 (2012).
54. Y. Yamawaki, Decision-making and motor control in predatory insects: a review of the praying mantis. *Ecol. Entomol.* **42**, 39–50 (2017).
55. Y. Yamawaki, Defence behaviours of the praying mantis *Tenodera aridifolia* in response to looming objects. *J. Insect Physiol.* **57**, 1510–1517 (2011).

General discussion and conclusions

This study analyzed the anatomical features of insect micro-sensory organs preserved in amber, which have been overlooked due to limited perspectives and techniques. These sensors composed of chitinous exoskeleton show exceptional preservation even to sub-micron scale structures in 3D, such as surface grooves and thin cuticular walls of antennal sensilla (Figs. 1.2, 1.3, 2.4, and 2.5). The fossil preservation potential of amber is much better than previously known.

In Chapters 1 and 2, I reconstructed two types of intra-specific communication (sexual and social) mediated by insect antennae. This involved the first discovery of fossilized sensilla specialized to detect pheromones, chemical signals affecting the behavior of other individuals, by developing new visualization techniques (Figs. 1.2, 1.3, 2.4, and 2.5). Pheromone communication is widespread in insects (e.g., Lepidoptera, Hymenoptera, Coleoptera, Blattodea, Mantodea) (1–4); cf. sound communication by files on wings, previously known in extinct insects, is restricted in ensiferan orthopterans (crickets) (5, 6). The imaging of amber inclusions developed here potentially reveals the extinct communication in various insect taxa and their evolution. Insect communication patterns often differ among the sexes or developmental stages (4, 7, 8), easily determined in amber based on reproductive organs, wings, and body sizes. Unfortunately, this study examined only adults or one sex because of restrictions on accessible specimens. Further investigation using more fossils arranged in both sexes and sequential developmental stages can extend breakthroughs in reconstructing the complicated communication of various extinct insects.

Chapters 1 and 3 focused on the vision of cockroaches and mantises. The light signal is the fastest sensory stimulation detected by the visual system; thus, the visual function is critical for animals to respond rapidly to their circumstances. Eyes and the remains are the primary visual organs and keys to revealing the light condition in the owners' habitats and behaviors. However, we must be cautious in interpreting eye morphology and light environments. For instance, in the same dark environments, some animals lose their eyes in caves while others have large eyes to condense light in night environments, and some diurnal animals who highly rely on vision also evolve large eyes (9–11). In this study, I reconstructed the well-developed visual system and behavior depending on the light information of a fossilized cockroach and mantis, considered with other sensory (diurnal antennae) and motor organs (raptorial forelegs), which are insect-unique fossilizable parts. The fossilized compound eyes can preserve ommatidia, visual pixel sensors themselves, since they have chitinous corneal lenses (Figs. 1.1 and 3.1) (11, 12). Estimation of

ommatidium number, therefore, enables to reveal the visual resolution of extant and extinct arthropods, including insects. Insect eye fossils show much exoskeletal morphology and combinable information, and become suitable materials for visual evolution.

I demonstrated the reconstruction of evolutionally notable ecology (sociality and stereopsis) in Chapters 2 and 3, based on sensory evidence made accessible by the new approaches. Although these behaviors are closely involved with sensory organs, previous studies cannot have accessed such organs, and they relied on indirect basis such as statistical “group” fossils and casts of the nervous system without soft-tissued neural organs (13–16). This study revealed sensors directly mediating sensory signals for such advanced behaviors. The results suggest that sociality has allowed ants to dominate competitively solitary species, and stereopsis has provided mantises of the niches as adaptive visual predators, both for 90–100 million years.

Neurobiology has worked on functional, physiological, and anatomical analyses of the nervous system, developing microscopic imaging and staining techniques for over a century (17–21). This field has traditionally focused on the peripheral and central nervous systems, which play roles in inputting and processing neural information, respectively, while recent researchers are additionally trying to reveal neural mechanisms that are outputting behavior (22, 23). Such comprehensive neurobiology, which connects neural input, processing, and output, is one of the most significant scientific fields, as it aims to discover the principles of complex animal behavior and nervous systems.

In contrast to modern neurobiology, paleo-neurobiology focusing on fossilized animals has expanded the strongly limited discussion for extinct nervous systems with poor neuroanatomical evidence. Although discoveries of the Cambrian nervous system are highly featured and praised, they are just described in the macro-scale morphology and used as indicators for the emergence periods of the particular, easily visualizable neural features in deep time without functional reconstruction (24–27). The transmitted electron microscopy of the fossilized fly eye in Baltic amber revealed that the fly-unique distribution pattern of rhabdoms in the ommatidia had already evolved in the Eocene period (28). This study is notable as the discovery of fossilized internal neural microstructures. The authors, however, did not discuss their function and are confined to anchoring the morphological evolution timing on the geological timeline, like the Cambrian fossils.

The principal materials in previous studies for functional and ecological reconstruction of the ancient nervous system have been vertebrates, focusing on the casts of neural tissues such as

brain cases (29–31). These studies estimate the sensory function of extinct vertebrates based on the brain outline shape; e.g., large olfactory bulbs imply well-developed olfaction, and large optic lobes (midbrains) imply well-developed visual ability. This approach cannot estimate the connection between concrete sensory input and behavioral output since it cannot deal with the fossil evidence of peripheral sensory systems. Recently, some studies analyzed the facial neurovascular canals of *Tyrannosaurus rex* (32, 33). The authors argued that the tactile neurons in the *Tyrannosaurus* jaw show highly complex branching and suggest their high sensitivity comparable to that of extant crocodiles and tactile-foraging birds. However, the sensitive tactile systems of these aquatic animals are adapted to search for food resources in/under the water and are unsuitable for analogous animals for the ecological reconstruction of *Tyrannosaurus*, which likely lived in the terrestrial environment. The authors only speculated the *Tyrannosaurus* ecology that they might have utilized their tactile sensors for predation, nest construction, paternal care, or communication due to a lack of direct modern analogs (33). Such indirect evidence of neural morphology and mismatches with accessible analogs obscure the specific sensory information inputs, resulting in the current situation where the behavioral ecology of fossilized animals can only be reconstructed in a highly ambiguous manner.

In this study, I focused on the peripheral sensory systems of insects, functioning specific sensory input and behavioral output. Insect sensory organs are not only fossilized directly but functionally specialized (e.g., sensilla for odors/physical contacts, ommatidia for light). The comparative studies show that their function and morphology are likely conservative among many insect orders (2, 34–37). These features of insect sensors enable us to reconstruct the sensory function and behavior based on their original morphology. This study proposes insects as novel models for paleo-neurobiology, whose peripheral sensory system can be applied to functional and ecological reconstruction in great detail. The miniaturized but sophisticated peripheral nervous systems of insects will significantly contribute to revealing extinct environmental information processing, biological interactions, advanced social systems, and their deep-time evolution, all of which are on the basis of the primary sensory systems.

References

1. H. Sass, Production, release and effectiveness of two female sex pheromone components of *Periplaneta americana*. *J. Comp. Physiol. A* **152**, 309–317 (1983).
2. T. Carle, Y. Toh, Y. Yamawaki, H. Watanabe, F. Yokohari, The antennal sensilla of the praying mantis *Tenodera aridifolia*: a new flagellar partition based on the antennal macro-, micro- and ultrastructures. *Arthropod Struct. Dev.* **43**, 103–116 (2014).
3. K. Fukuda, A. Yanagawa, M. Tuda, G. Sakurai, S. Kamitani, N. Furuya, Sexual difference in antennal sensilla abundance, density and size in *Callosobruchus rhodesianus* (Coleoptera: Chrysomelidae: Bruchinae). *Appl. Entomol. Zool.* **51**, 641–651 (2016).
4. H. Watanabe, Y. Koike, K. Tateishi, M. Domae, H. Nishino, F. Yokohari, Two types of sensory proliferation patterns underlie the formation of spatially tuned olfactory receptive fields in the cockroach *Periplaneta americana*. *J. Comp. Neurol.* **526**, 2683–2705 (2018).
5. O. Béthoux, A. Nel, J. Lapeyrie, G. Gand, The Permostridulidae fam. n. (Panorthoptera), a new enigmatic insect family from the Upper Permian of France. *Eur. J. Entomol.* **100**, 581–585 (2003).
6. J.-J. Gu, F. Montealegre-Z, D. Robert, M. S. Engel, G.-X. Qiao, D. Ren, Wing stridulation in a Jurassic katydid (Insecta, Orthoptera) produced low-pitched musical calls to attract females. *Proc. Natl. Acad. Sci.* **109**, 3868–3873 (2012).
7. H. Nishino, M. Iwasaki, M. Mizunami, Pheromone detection by a pheromone emitter: a small sex pheromone-specific processing system in the female american cockroach. *Chem. Senses* **36**, 261–270 (2011).
8. J. K. Yuvaraj, M. N. Andersson, O. Anderbrant, C. Löfstedt, Diversity of olfactory structures: a comparative study of antennal sensilla in Trichoptera and Lepidoptera. *Micron* **111**, 9–18 (2018).
9. R. G. Bland, D. P. Slaney, P. Weinstein, Antennal sensilla on cave species of Australian *Paratemnopteryx* cockroaches (Blattaria : Blattellidae). *Int. J. Insect Morphol. Embryol.* **27**, 83–93 (1998).
10. K. Kral, M. Poteser, Relationship between body size and spatial vision in the praying mantis - an ontogenetic study. *J. Orthoptera Res.* **18**, 153–158 (2009).
11. M. Streinzer, W. Huber, J. Spaethe, Body size limits dim-light foraging activity in stingless bees (Apidae: Meliponini). *J. Comp. Physiol. A* **202**, 643–655 (2016).

12. E. K. Buschbeck, M. Friedrich, Evolution of insect eyes: tales of ancient heritage, deconstruction, reconstruction, remodeling, and recycling. *Evol. Educ. Outreach* **1**, 448–462 (2008).
13. P. Barden, D. A. Grimaldi, Adaptive radiation in socially advanced stem-group ants from the Cretaceous. *Curr. Biol.* **26**, 515–521 (2016).
14. T. Konishi, M. W. Caldwell, T. Nishimura, K. Sakurai, K. Tanoue, A new halisaurine mosasaur (Squamata: Halisaurinae) from Japan: the first record in the western Pacific realm and the first documented insights into binocular vision in mosasaurs. *J. Syst. Palaeontol.* **14**, 809–839 (2016).
15. L. N. Weaver, D. J. Varricchio, E. J. Sargis, M. Chen, W. J. Freimuth, G. P. Wilson Mantilla, Early mammalian social behaviour revealed by multituberculates from a dinosaur nesting site. *Nat. Ecol. Evol.* **5**, 32–37 (2020).
16. B. E. Boudinot, A. Richter, J. Katzke, J. C. M. Chaul, R. A. Keller, E. P. Economo, R. G. Beutel, S. Yamamoto, Evidence for the evolution of eusociality in stem ants and a systematic revision of †*Gerontoformica* (Hymenoptera: Formicidae). *Zool. J. Linn. Soc.*, zlab097 (2022).
17. A. L. Hodgkin, A. F. Huxley, B. Katz, Measurement of current-voltage relations in the membrane of the giant axon of *Loligo*. *J. Physiol.* **116**, 424–448 (1952).
18. R. Butler, The anatomy of the compound eye of *Periplaneta americana* L. - 2. Fine Structure. *J. Comp. Physiol.* **83**, 239–262 (1973).
19. E. D. Jarvis, O. Güntürkün, L. Bruce, A. Csillag, H. Karten, W. Kuenzel, L. Medina, G. Paxinos, D. J. Perkel, T. Shimizu, G. Striedter, J. M. Wild, G. F. Ball, J. Dugas-Ford, S. E. Durand, G. E. Hough, S. Husband, L. Kubikova, D. W. Lee, C. V. Mello, A. Powers, C. Siang, T. V. Smulders, K. Wada, S. A. White, K. Yamamoto, J. Yu, A. Reiner, A. B. Butler, Avian brains and a new understanding of vertebrate brain evolution. *Nat. Rev. Neurosci.* **6**, 151–159 (2005).
20. H. Nishino, M. Nishikawa, F. Yokohari, M. Mizunami, Dual, multilayered somatosensory maps formed by antennal tactile and contact chemosensory afferents in an insect brain. *J. Comp. Neurol.* **493**, 291–308 (2005).
21. C. A. H. Allard, G. Kang, J. J. Kim, W. A. Valencia-Montoya, R. E. Hibbs, N. W. Bellono, Structural basis of sensory receptor evolution in octopus. *Nature* **616**, 373–377 (2023).

22. M. Kössl, J. Hechavarría, Smart bats click twice. *eLife* **7**, e36561 (2018).
23. H. Nishino, M. Sakai, “Tonic immobility in a cricket: behavioral characteristics, neural substrate, and functional significance” in *Death-Feigning in Insects*, M. Sakai, Ed. (Springer Singapore, 2021), *Entomology Monographs*, pp. 93–108.
24. G. Tanaka, X. Hou, X. Ma, G. D. Edgecombe, N. J. Strausfeld, Chelicerate neural ground pattern in a Cambrian great appendage arthropod. *Nature* **502**, 364–367 (2013).
25. J. Yang, J. Ortega-Hernández, N. J. Butterfield, Y. Liu, G. S. Boyan, J. Hou, T. Lan, X. Zhang, Fuxianhuiid ventral nerve cord and early nervous system evolution in Panarthropoda. *Proc. Natl. Acad. Sci.* **113**, 2988–2993 (2016).
26. J. Ortega-Hernández, R. Lerosey-Aubril, S. Pates, Proclivity of nervous system preservation in Cambrian Burgess Shale-type deposits. *Proc. R. Soc. B Biol. Sci.* **286**, 20192370 (2019).
27. J. Ortega-Hernández, R. Lerosey-Aubril, S. R. Losso, J. C. Weaver, Neuroanatomy in a middle Cambrian mollisoniid and the ancestral nervous system organization of chelicerates. *Nat. Commun.* **13**, 410 (2022).
28. G. Tanaka, A. R. Parker, D. J. Siveter, H. Maeda, M. Furutani, An exceptionally well-preserved Eocene dolichopodid fly eye: function and evolutionary significance. *Proc. R. Soc. B Biol. Sci.* **276**, 1015–1019 (2009).
29. D. C. Evans, R. Ridgely, L. M. Witmer, Endocranial anatomy of lambeosaurine hadrosaurids (Dinosauria: Ornithischia): a sensorineural perspective on cranial crest function. *Anat. Rec.* **292**, 1315–1337 (2009).
30. C. R. Torres, J. A. Clarke, Nocturnal giants: evolution of the sensory ecology in elephant birds and other palaeognaths inferred from digital brain reconstructions. *Proc. R. Soc. B Biol. Sci.* **285**, 20181540 (2018).
31. L. M. Chiappe, G. Navalón, A. G. Martinelli, I. D. S. Carvalho, R. Miloni Santucci, Y.-H. Wu, D. J. Field, Cretaceous bird from Brazil informs the evolution of the avian skull and brain. *Nature* **635**, 376–381 (2024).
32. F. Bouabdellah, E. Lessner, J. Benoit, The rostral neurovascular system of *Tyrannosaurus rex*. *Palaeontol. Electron.*, doi: 10.26879/1178 (2022).
33. S. Kawabe, S. Hattori, Complex neurovascular system in the dentary of *Tyrannosaurus*. *Hist. Biol.* **34**, 1137–1145 (2022).

34. T. A. Keil, Functional morphology of insect mechanoreceptors. *Microsc. Res. Tech.* **39**, 506–531 (1997).
35. J. R. Riesgo-Escovar, W. B. Piekos, J. R. Carlson, The *Drosophila* antenna: ultrastructural and physiological studies in wild-type and lozenge mutants. *J. Comp. Physiol. A* **180**, 151–160 (1997).
36. A. Nakanishi, H. Nishino, H. Watanabe, F. Yokohari, M. Nishikawa, Sex-specific antennal sensory system in the ant *Camponotus japonicus*: structure and distribution of sensilla on the flagellum. *Cell Tissue Res.* **338**, 79–97 (2009).
37. C. Missbach, H. K. Dweck, H. Vogel, A. Vilcinskas, M. C. Stensmyr, B. S. Hansson, E. Grosse-Wilde, Evolution of insect olfactory receptors. *eLife* **3**, e02115 (2014).

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