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Histological Observation of Helmet Development in the Treehopper *Poppea capricornis* (Insecta: Hemiptera: Membracidae)

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The treehoppers (Hemiptera, Membracidae) are known for possessing a large three-dimensional structure called a helmet. Although some ecological functions of the helmet have already been elucidated, the developmental mechanisms underlying the complex and diverse morphology of the helmet are still largely unknown. The process of helmet formation was first described in *Antianthe expansa*, which possesses a simple roof-shaped helmet. However, the developmental process in species with more complex helmet morphologies remains largely unexplored. Hence, in this study, we used *Poppea capricornis*, which possesses a more complex helmet structure than *A. expansa*, to investigate the helmet development using paraffin sections, micro-CT, and scanning electronic microscopy. Our focus was on the overall helmet developmental process common to both species and formation of structures unique to *Poppea* and its comparison to *Antianthe*. As a result, we discovered that miniature structures were also formed in *Poppea*, similar to *Antianthe*, during the helmet formation. Common structures that were shared between the two species were discernible at this stage. Additionally, we observed that suprahumeral horns and posterior horns, two morphological traits specific to the *Poppea* helmet that are apparently similar anatomically, are formed through two distinctly different developmental mechanisms. The suprahumeral horns appeared to be formed by utilizing the nymphal suprahumeral bud as a mold, while we could not detect any nymphal structures potentially used for a mold in the posterior horns formation. Our findings suggest that the helmet formation mechanisms of *Antianthe* and *Poppea* employ a common mechanism but form species-specific structures by multiple mechanisms.

Key words: 3D structure, treehopper, micro-CT, development, Hemiptera, pronotum, Costa Rica

INTRODUCTION

Most treehopper species (Membracidae, Hemiptera) possess a complex three-dimensional structure on their dor-

sal thorax (Buckton and Poulton, 1903; Stegmann, 1998). This structure, commonly referred to as a “helmet”, is a highly modified pronotum and exhibits remarkable diversity in terms of shape and color across the family. The developing adult helmet primordium is densely folded inside the nymphal helmet sheaths during the final nymphal instar. At eclosion, the folded primordium undergoes an unfolding process similar to

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that of wings (Stegmann, 1998; Prud'homme et al., 2011; Fisher et al., 2020; Adachi et al., 2020; Kudla et al., 2022).

The adult helmet is a large structure composed of two thin cuticular layers. In contrast, the horn sheath observed in the final instar nymph is relatively small, consisting of a single cuticular layer (Stegmann, 1998; Mikó et al., 2012; Yoshizawa, 2012; Adachi et al., 2020). Each cuticular layer is underlain by an epithelial cell layer in both nymph and adult. During the development from final instar nymph to adult, the helmet undergoes two significant changes. One is the change in size from a small helmet sheath to a large helmet, and the other is the structural transformation from single-layered to double-layered (Stegmann, 1998; Adachi et al., 2020). Although the details of these changes have not been investigated, the developmental process has recently been described using *Antianthe expansa*, which possesses a roof-shaped helmet (Godoy et al., 2006; Maruyama, 2011; Adachi et al., 2020).

However, the helmet morphology of treehoppers exhibits striking diversity, with many species having structures that are absent in *Antianthe* helmets. Two typical examples are the suprahumeral horns, seen as a pair of large projections on the anterior portion of the helmet, and the posterior horns, which are a branching structure of the posterior process (Godoy et al., 2006; Maruyama, 2011). Understanding when and how these structures form during helmet formation is crucial for comprehending the diverse types of helmet morphogenesis in treehoppers. In this study, we observed and described the developmental process of the entire helmet and each landmark in *Poppea capricornis* Fowler, 1895, a species which is placed in the same subfamily as *Antianthe*, Smiliinae (Godoy et al., 2006), but possessing both the suprahumeral horns and the posterior horns.

MATERIALS AND METHODS

Insect collecting

All treehopper samples (*Poppea capricornis*), both nymphs and adults, used in the study were collected at La Selva Biological Station at Puerto Viejo de Sarapiquí, Costa Rica in November of 2016, 2017, and 2019. All collection activities were conducted in accordance with the permission of MINAE-SINAC, Costa Rica, resolution numbers SINAC-SE-GASP-PI-R-122-2016, 056-2017-ACC-PI, M-P-SINAC-PNI-ACAT-047-2017, and SINAC-ACC-PI-R-094-2019. Sampled insects were immediately fixed in formalin or 95% EtOH. Identification of the subfamily and genus was performed following Godoy et al. (2006). As we could not bring live treehoppers back to the laboratory due to permit restrictions, the chronological order (early, mid-early, middle, and late) was reconstructed based on the observations from a previous study (Adachi et al., 2020) and the development of adult wing tissues inside the nymphal wing bud.

Imaging of adult and nymphal morphology

Photographs of adult and nymphal animals and their helmets were captured with a digital camera (KISS EOSX7, Canon, Japan) attached to a binocular microscope (SZ61, Olympus, Tokyo, Japan). To observe the adult helmets from the ventral side we removed the head, T2, T3, and abdomen under a binocular microscope.

Micro-CT

Micro-CT scans were performed to observe the adult helmet structure by using a micro-CT-scanner SkyScan 1172 (SkyScan NV, Belgium) following the manufacturer's instructions. Details of sample preparation methods, scanning, and 3D image construction methods were described in the previous study (Adachi et al., 2020).

Observations were conducted using two individuals in mid-early stage and late stage.

Paraffin sections

Paraffin sections were prepared to observe the fine structures of adult helmets. Observations were conducted using one individual at each stage. Fixations and sectioning procedures were performed as previously described (Adachi et al., 2020). The thickness of each section was set at 6 µm. Sections were stained with hematoxylin and eosin and captured with a digital camera (KISS EOSX7, Canon, Tokyo, Japan) attached to a microscope (CX43, Olympus, Tokyo, Japan).

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) (JSM-6510LV, JEOL Co., Tokyo, Japan) was used to observe the dense folded structure of helmet primordia inside the helmet sheath of the final instar nymph. The nymphal cuticle was carefully removed via forceps under a binocular microscope (SZ61, Olympus, Tokyo, Japan). Details of sample preparation for SEM observation were described in the previous study (Adachi et al., 2020). Observations were conducted using two individuals in late stage.

RESULTS AND DISCUSSION

External morphology of adult helmet and nymphal helmet sheath

First, we describe the external morphology of *Poppea capricornis* in both adult and nymphal stages. The morphological terms of each helmet part and tissues are based on previous studies of closely related species within the subfamily Smiliinae. Specifically, Stegmann (1998) (describing adult and nymphal morphology of *Stictocephala bisonia*) and Sakakibara and Evangelista (2008) (describing several species within the tribe Ceresini including *Poppea*) were used for morphological terminology of nymphal and adult helmet. We also referred to Adachi et al. (2020), a previous study on the developmental process of *Antianthe expansa*, which belongs to the same subfamily.

The adult has a large colorful helmet (Fig. 1A, B) which is covered with black hairs and has a knobbed shape. In this study the knobs were divided into three parts using the narrow points of the helmet as the boundaries. The parts are called the anterior bulb (ab), median bulb (mb), and posterior bulb (pb), in order from anterior to posterior (Fig. 1A). The suprahumeral horns (sh) and humeral angles (ha) are formed on the anterior bulb. The suprahumeral horn is a horn-like structure that extends outwards on both sides from the anterior bulb and the tip bends backward. On the ventrally facing portion on each side of the anterior bulb, a pair of humeral angles can be recognized, which are small triangular spine-like structures.

A septum (sp), a membranous double-layered structure that is widely seen across treehoppers, can also be easily recognized in the ventral view of the helmet in this species (Fig. 1B). The posterior bulb has a three-branched structure consisting of a posterior process (pp) and a pair of posterior horns (ph), forming the characteristic trifurcation of this genus (Fig. 1A). The posterior process extends like a spike from the middle of the posterior bulb. The posterior horns extend from the lateral sides of the posterior bulb. The base of the horn is thick but becomes thinner where it bends backward.

The helmet sheath of the final instar nymph is smaller

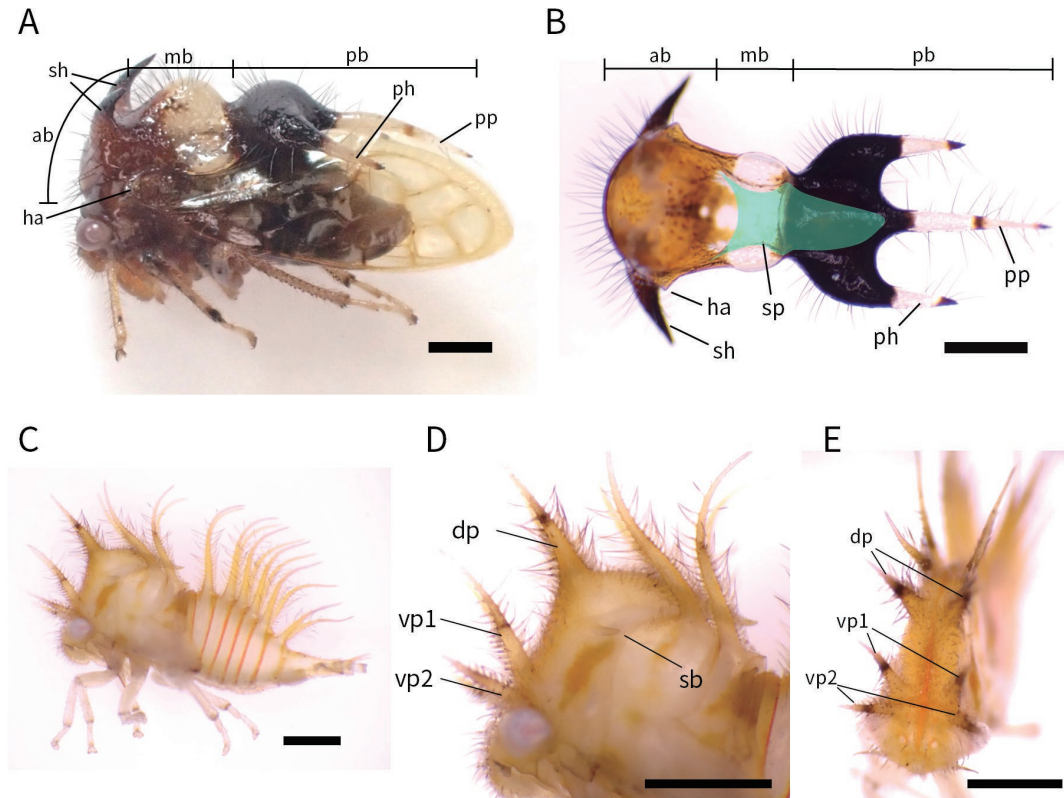


Fig. 1. External morphology of *Poppea capricornis*. **(A)** Lateral view of adult *P. capricornis*. The adult helmet is well developed and has a knob-like structure. From anterior to posterior, the anterior bulb (ab), median bulb (mb), and posterior bulb (pb) are arranged. A pair of supra-humeral horns (sh) and humeral angles (ha) are present on the anterior bulb and a pair of posterior horns (ph) as well as a posterior process (pp) are present on the posterior bulb. **(B)** Ventral view of the adult helmet (head, T2, T3, and abdominal segments were removed) with the septum (sp) highlighted in green. The humeral angle (ha), a small protruding structure to the left and right, can also be seen. **(C, D)** Lateral view of the final instar nymph of *P. capricornis*. **(E)** Dorsal view of the final instar nymph. The final instar nymph has a helmet sheath with three pairs of large spine-like projections which are, from anterior to posterior: ventral processes 2 (vp2), ventral processes 1 (vp1), and dorsal processes (dp). A pair of small suprahumeral buds (sb) on the lateral side can also be recognized. Scale bars: 1 mm.

than the adult helmet. The hairs on the surface are thicker and shorter than those of adults. There are no bulb-like structures, only three paired spine-like structures can be seen (Fig. 1C–E). The spine-like structures consist of three types: dorsal processes (dp) and ventral processes 1 and 2 (vp1, vp2) on the dorsal side of the helmet sheath, from posterior to anterior. A pair of small suprahumeral buds (sb) on the lateral sides can also be recognized (Fig. 1D). The dorsal processes are a pair of spike-like structures that extend diagonally from the anterior-dorsal side. Among these three types of projections, the dorsal processes and ventral processes disappear in adult helmets, while only the suprahumeral bud retains a horn-like structure in the adult stage, since it serves as a mold for the formation of the adult suprahumeral horn (described in later section).

Developmental process of adult helmet primordia inside nymphal helmet sheath

Next, we describe the developmental process of the adult helmet within the nymphal helmet sheath of *Poppea*. Previous studies using *Antianthe* (Adachi et al., 2020) have demonstrated that in the final instar of *Antianthe*, adhering dorsal (upper) and ventral (lower) cell layers of the nymphal helmet sheath are eventually transformed into a double-layered adult helmet. The double-layered structure of the

nymph undergoes contraction and deformation to form a helmet miniature, which grows and is folded in the late final instar; during eclosion body fluid flows into the miniature thereby expanding the helmet (Adachi et al., 2020). Similar processes were observed in *Poppea* (Fig. 2A, B). That is, at the early stage the dorsal and ventral layers do not fully adhere to one another (Fig. 2A[i]: the area enclosed by a dashed line is the interior of the helmet sheath, which is filled with hemolymph). At the mid-early stage, adherence of the dorsal and ventral layers is almost complete (Fig. 2A[ii]). At the middle stage (miniature stage), the septum, lateral carina, and median carina are clearly recognizable (Fig. 2A[iii], D).

The median carina is a tubular structure running through the center of the helmet from the anterior to posterior end. The lateral carinae are also tubular, vein-like structures on both sides of the helmet. In this species, at least two lateral carinae (we named them lateral carina 1 and 2) can be recognized. Lateral carina1 (lc1) runs through the ventral edge of the helmet while lateral carina2 (lc2) runs through the edge of the septum. Both of the lateral carinae run from anterior to posterior, but the exact starting point is not well defined.

The dorsal layer was thicker than the ventral layer and these two layers were connected by bridging cells (green arrowhead in Fig. 2C). At this stage, the dorsal layer seems to have started secreting the adult cuticle and some hairs

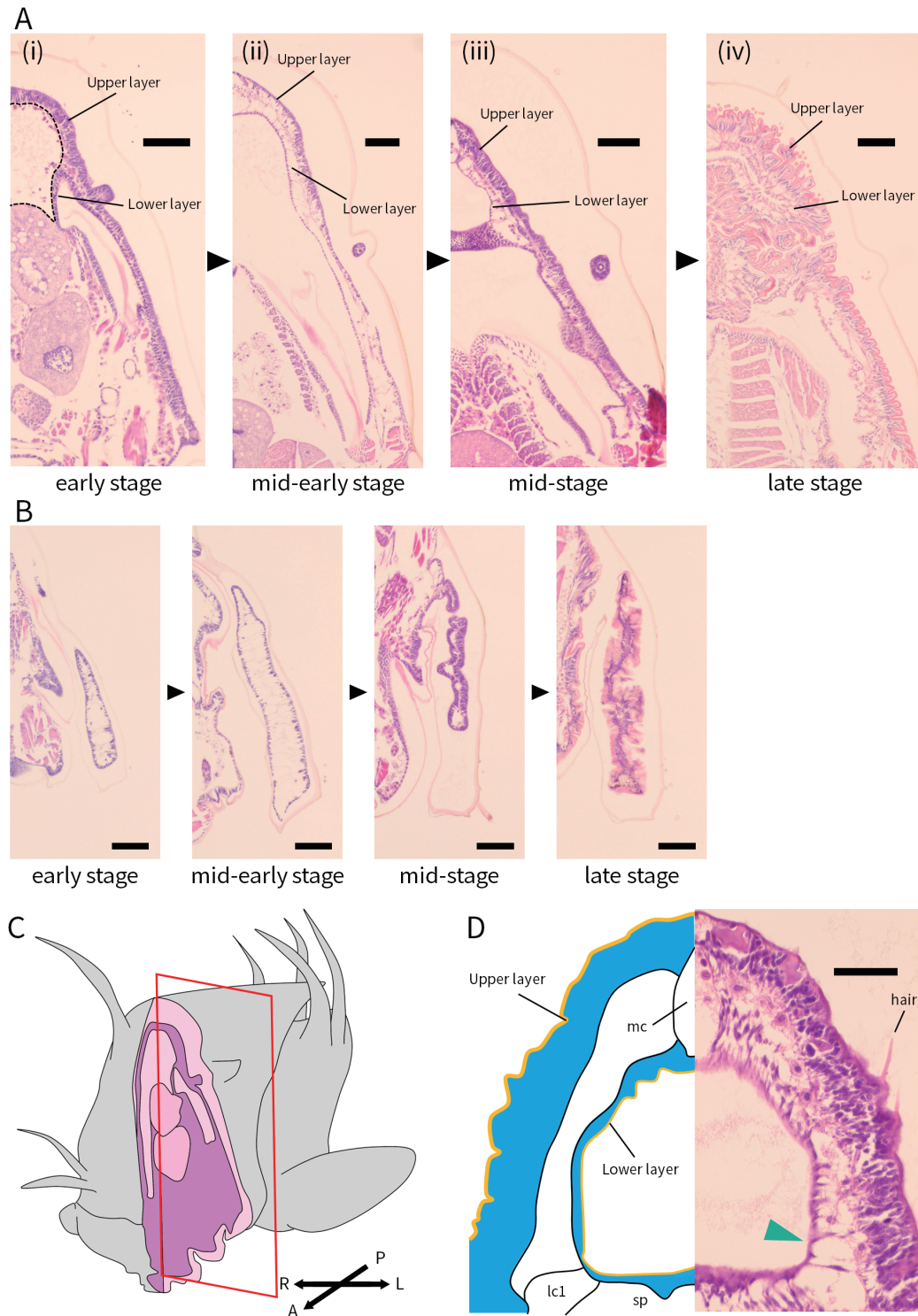


Fig. 2. Developmental process of helmet primordia observed using paraffin section. **(A)** Developmental process of helmet primordium inside the nymphal helmet sheath in last instar. (i) early stage, (ii) mid-early stage, (iii) mid-stage, (iv) late stage. Dorsal layer and ventral layers adhered to one another during early to mid-stage (i–iii) and finally became a densely folded structure at late stage (iv). The area enclosed in black dashed line is hemolymph (between cell layers). Scale bar: 100 μm . **(B)** Developing wing disc inside the last instar wing buds, serving as cues for the chronological order of helmet development. The developing wing had thin epithelium at early stages; then the epithelium became thicker (mid-stage), and eventually double-layered and folded (late stage). **(C)** Schematic view of sectioning position of nymphal helmet sheath and developing helmet primordium shown in (A[i]). This schematic diagram is drawn with reference to histological observations at early stage (A[i]). The darker pink areas indicate tissues and cells, while the lighter pink areas indicate hemolymph. **(D)** Magnified view of developing helmet at mid-stage, focusing on median carina (mc), septum (sp) and lateral carina 1 (lc1). Epithelial layers are indicated in light blue and cuticles in orange. Clearly, the dorsal layer has a thicker epithelium than the ventral layer. The two layers are bridged by elongated cells (green arrowhead). Scale bar: 50 μm .

were also observed on the cuticle (Fig. 2C). At the late stage, densely folded structures were developed (Fig. 2A[iv], 3), as in *Antianthe* (Adachi et al., 2020). Many long hairs were observed, especially on the anterior-dorsal side of the helmet (Fig. 3A, enclosed by dashed line) inserted in the dorsal process. The dorsal and ventral processes (dp: dorsal process, vp1: ventral process 1, vp2: ventral process 2) disappeared in adult helmets. A large groove running laterally across helmet is also seen (Fig. 3A, white arrowhead; 3D[i]). The dorsal surface of the helmet was covered with very dense folds (microfolds) (Fig. 3B). Among these folds, two

types were recognized: parallel folds and zigzag folds (Fig. 3B, highlighted by green and purple, respectively). The parallel area is located along the edge of the helmet and median carina, allowing directional expansion, whereas the zig-zag area is located in other regions, enabling nondirectional expansion. Such a pattern of folding (parallel area on edge and median carina, zig-zag area in other regions) on the developing helmet at the late stage was also reported in the previous study (Adachi et al., 2020). In addition to microfolds, on the ventrally facing portion of the helmet (Fig. 3C[i]) many finer folds were found on the surface of microfolds, (Fig. 3C[ii]) many finer folds were found on the surface of microfolds,

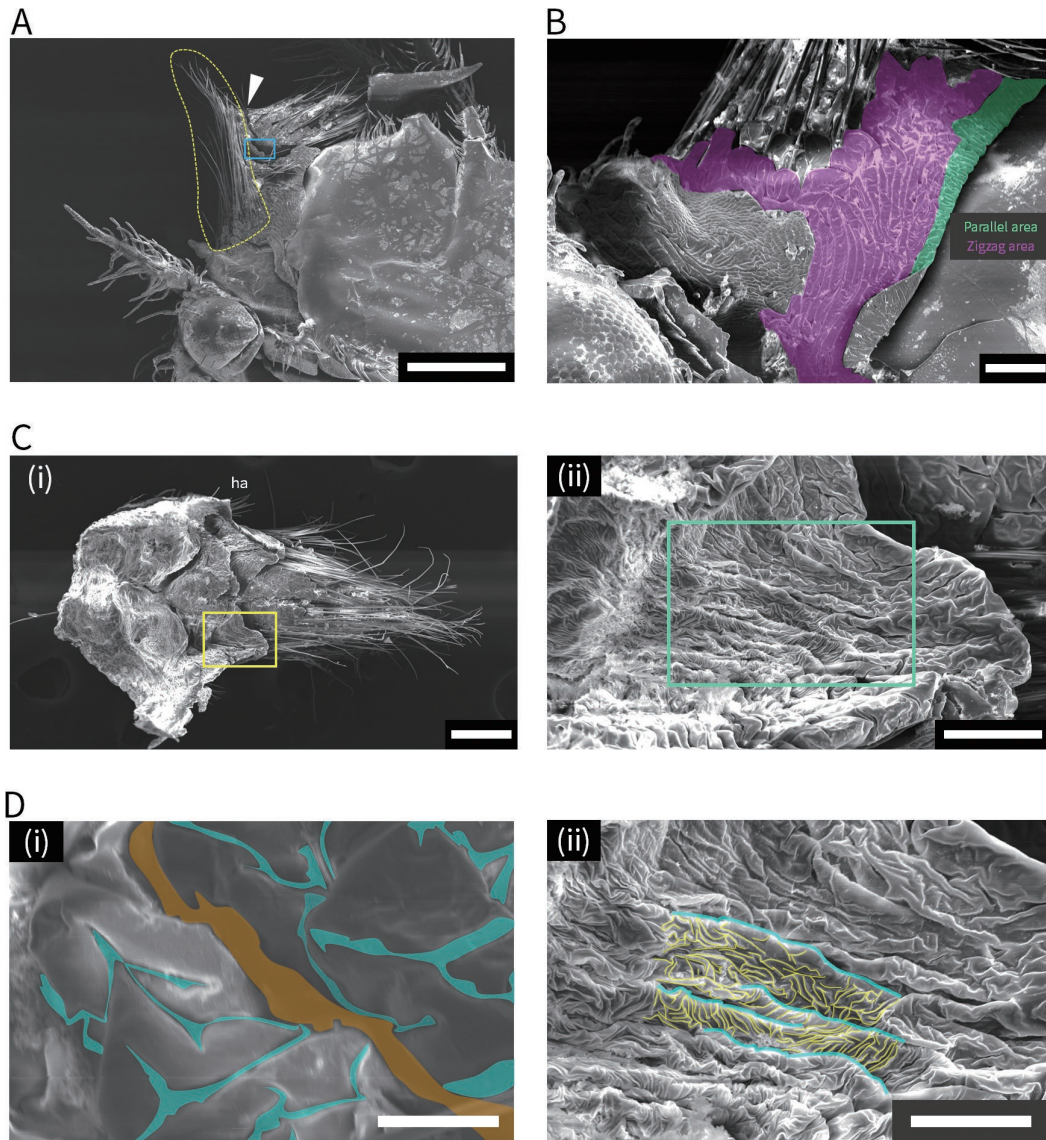


Fig. 3. Surface of fully developed helmet primordium at late stage observed by SEM. **(A)** Lateral view of fully developed helmet primordium at late stage. Nymphal cuticle of the helmet sheath was removed to observe inner helmet primordium. Most of the helmet surface was covered with dense folded structures termed microfolds. Also, a large groove can be seen (white arrowhead). The anterior region (enclosed in yellow dashed line) was covered with long dense hairs inserted in the nymphal cuticle of the dorsal process. Scale bar indicates 500 μm . **(B)** Magnified view of fully developed helmet primordium at late stage. Two types of microfolds are highlighted in purple and green. Scale bar indicates 100 μm . **(C[i])** Ventral view of removed helmet primordium at late stage. Developed humeral angle (ha) can be recognized as small lateral projection. Scale bar indicates 200 μm . **(C[ii])** Magnified view of ventral side of helmet corresponding to yellow window in **(C[i])**. Many finer folds (super microfolds) were found on the surface of microfolds. Scale bar indicates 50 μm . **(D)** Comparison of the macro folds, the microfolds, and the super microfolds in same scale. (i) Magnified view of the area indicated by blue box in **(A)**. (ii) Magnified view of the area indicated by green box in **(C[iii])**. Furrow(s) of macrofolds, microfolds and super microfolds are highlighted in orange, cyan, and yellow, respectively. Scale bars: 50 μm .

which we term super microfolds (Fig. 3C[iii], D[iii]). Super microfolds were only observed on the ventral layer, probably because it is much thinner and more deformable than the dorsal layer (Fig. 2A[iii, iv], C), allowing for finer folds.

Morphogenesis of miniature helmet inside the nymphal helmet sheath

The process of adult helmet formation during the final nymphal instar was elucidated by Adachi et al. (2020) using *Antianthe expansa*. During the early stage of the final instar,

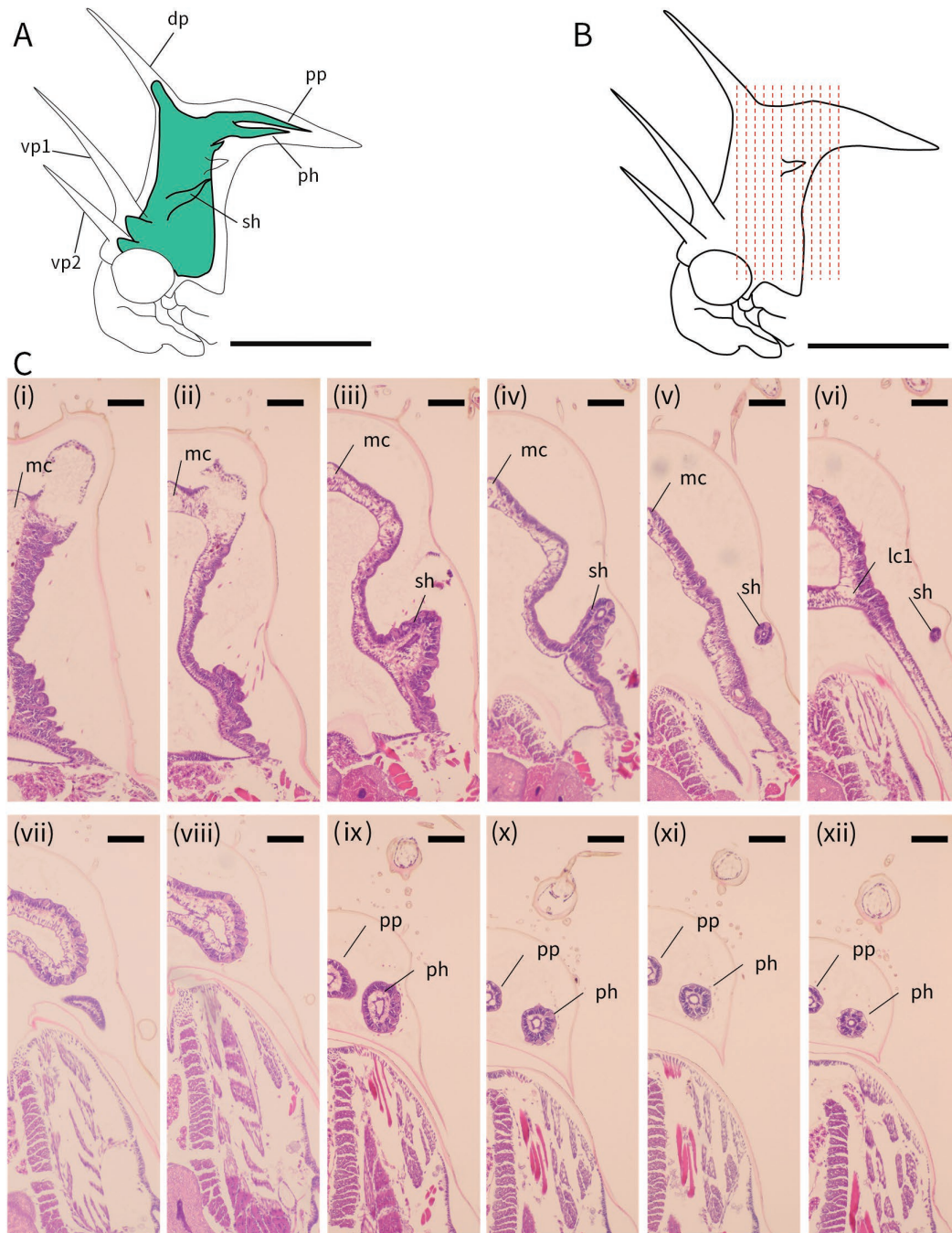


Fig. 4. Miniature helmet at mid-stage nymph. **(A)** Schematic diagram of miniature helmet inside nymphal helmet sheath colored in light green. Scale bar: 1 mm. At this stage, suprahumeral horns and posterior horns can be clearly recognized, while three pairs of nymphal projections (vp1, vp2 and dp) were degenerated in developing adult helmet. **(B)** Sectioning points are indicated in red dashed lines. Each vertical line corresponds to a section image from **(C[i]–C[xii])**, from anterior to posterior. Scale bar indicates 1 mm. **(C)** Paraffin section images of developing helmet. Left-half of coronal sections (as indicated in Fig. 2B) are shown serially at approximately 60- μ m intervals. There was a loss of one section between vi and vii (thus, the interval of vi and vii might be 120–140 μ m). All adult helmet structures (mc, sh, pp, and ph) were clearly recognized at this stage. Scale bars: 100 μ m. vp1, ventral processes 1; vp2, ventral processes 2; dp, dorsal processes; mc, median carina; lc1, lateral carina1; sh, suprahumeral horn; pp, posterior process; ph, posterior horn.

the epithelial layer supporting the horn sheath detaches and separates from the cuticle layer. Nearly simultaneously, the epithelial layer fuses together to form a bilayer structure. The two-layered structure undergoes deformation and contraction, resulting in the formation of a miniature structure resembling a scaled-down version of the adult helmet in the mid-stage of the final instar. This process of miniature formation is thought to play a crucial role in determining the overall

shape and structure of the adult helmet (Adachi et al., 2020).

We hypothesized that miniature structures would also be formed in *Poppea* during the final instar nymph. By observing serial paraffin sections of the middle stage nymph, we found that structures such as the median carina, lateral carina, septum, posterior process, posterior horn, and suprahumeral horn, which are characteristic of the adult helmet morphology of *Poppea*, were already present at this stage (Fig. 4). Since

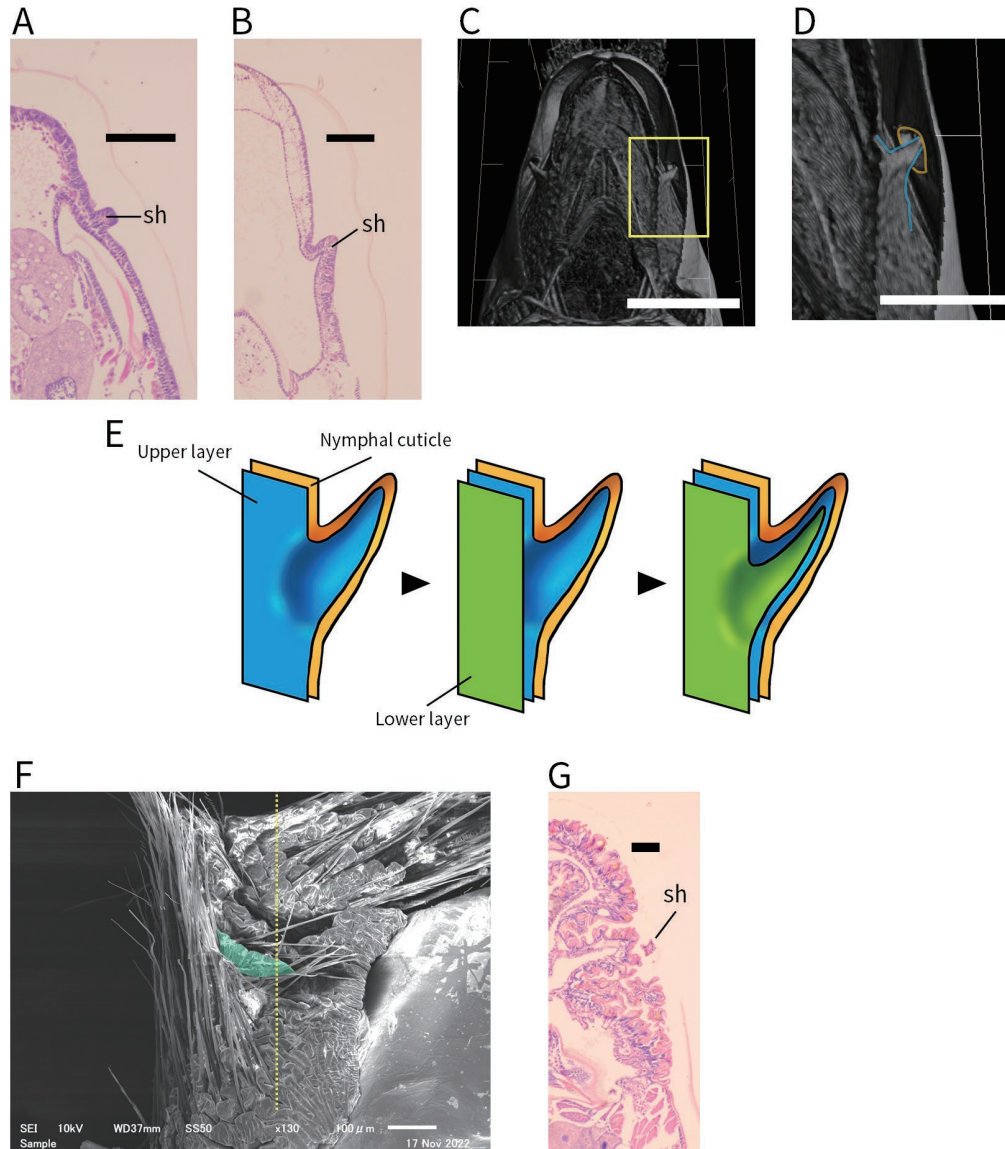
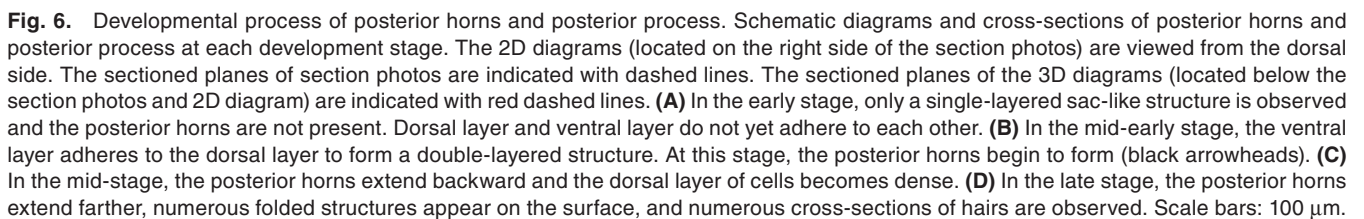


Fig. 5. Developmental process of suprahumeral horn. **(A)** Paraffin section of developing suprahumeral horn at the early stage. The future suprahumeral horn (sh, the small projection) was made by only the dorsal layer, with the ventral layer remaining flat. Scale bar: 100 μm. **(B)** Paraffin section of developing suprahumeral horn at the mid-early stage. The small projection became double-layered. Note that the ventral layer adhered to the dorsal layer. Scale bar: 100 μm. **(C)** Micro-CT image of developing suprahumeral horn at the mid-early stage. Scale bar: 250 μm. **(D)** Magnified view of developing suprahumeral horn, corresponding to yellow window in **(C)**. Developing suprahumeral horn (light blue) likely to be detached from nymphal suprahumeral bud (orange) used as mold. Scale bar: 125 μm. **(E)** Schematic diagram of development of suprahumeral horn according to the observation of paraffin sections **(A, B)** and micro-CT **(C, D)**. The rough shape of the suprahumeral horn is determined by the dorsal cell layer (light blue) that underlies the cuticle of the nymphal suprahumeral bud (orange) (left). Then, the flat ventral layer is attached like a lid to the invaginating dorsal layer (center, corresponding to **[A]**), and sinks in to match the shape of the dorsal layer, forming the double-layered suprahumeral horn (right, corresponding to **[B]**). **(F)** Fully developed suprahumeral horn at the late stage, observed by SEM. The suprahumeral horn is highlighted in green. The base of the suprahumeral horn cannot be seen due to the dense covering of hairs. Scale bar: 120 μm. **(G)** Paraffin section of the fully developed suprahumeral horn, corresponding to the yellow dashed line in **(F)**. Scale bar: 100 μm.



this developing helmet primordium already exhibited the morphology of the adult helmet, we considered this developing primordium to be a miniature helmet of this species. The miniature of *Poppea* was larger relative to the volume of the helmet sheath compared to *Antianthe*.

Morphogenesis of *Poppea*-specific structures (suprahumeral horns and posterior horns)

As seen in the previous section, the helmet morphology of adult *Poppea* had already formed via the formation of miniatures inside the nymphal helmet sheath at the middle stage. Next, we will describe the formation of *Poppea*-specific structures (suprahumeral horns and posterior horns) which are already clearly formed in the miniature.

Suprahumeral horns

The angular structure located on the side of the developing helmet primordium is considered to correspond to the suprahumeral horn of the adult (Fig. 4). It was also confirmed to be the suprahumeral horn based on the suprahumeral bud on the side of the helmet sheath (Fig. 5A–D). By examining the shape of the developing suprahumeral horn it was confirmed that in the early stage, the flat ventral layer adheres to the convex dorsal layer of the suprahumeral horn (Fig. 5A). In other words, at this point the suprahumeral horn is not yet a double-layered structure. In the mid-early stage, the ventral layer invaginates and adheres to the dorsal layer of the suprahumeral horn, to form a double-layered conical shaped structure (Fig. 5B). Therefore, the suprahumeral horn likely changed from a single-layered structure to a double-layered one (Fig. 5E). During this change, the nymphal suprahumeral bud acts as a mold (Fig. 5C, D), and the shape of the dorsal layer does not change, while the ventral layer invaginates and adheres to it (Fig. 5E). Since the formation of suprahumeral horns can be observed before the middle stage when the miniature is formed, the formation of suprahumeral horns is not caused by the contraction that occurs

during miniature formation. In the late stage, the suprahumeral horns can also be observed (Fig. 5F, G).

Posterior horns

The posterior horns, along with the posterior process, form the characteristic trifurcated helmet shape of *Poppea* (Fig. 1A, B). At the early stage, the posterior horns had not yet formed (Fig. 6A) and the posterior region of the helmet sheath was still a single-layered structure (Fig. 6A). By the mid-early stage, the posterior horns were present (Fig. 6B). At this stage, the posterior horns were short and small, but had already transformed into a double-layered structure (Fig. 6B). According to the other mid-early-stage sample observed via micro-CT, the formation of the posterior horns likely begins before the transformation from a single-layered to a double-layered structure (Fig. 7). At the middle stage, the branching point becomes deeper, causing the posterior horns to elongate (Fig. 6C). Additionally, the outer cell layer becomes thicker compared to that in the mid-early stage (Fig. 6B, C). In the late stage, the posterior process and posterior horns were densely packed within the nymphal helmet sheath (Fig. 6D) and many folded structures could be recognized. The posterior process is positioned dorsally and the posterior horns ventrally. Adult helmet cuticle and surface hairs are also clearly recognized at this stage (Fig. 6D).

CONCLUSION AND PERSPECTIVE

In this study we examined the developmental process of adult helmet formation in *Poppea capricornis*, which has a more complex helmet shape compared to *A. expansa* used in the previous study (Adachi et al., 2020). We investigated whether miniature formation, which is thought to be an important developmental event for transforming the helmet morphology from nymph to adult in treehoppers, occurs in *Poppea* as well as *Antianthe*. We also observed and described the development of specific helmet structures that are not seen in *Antianthe*, namely, the suprahumeral horns

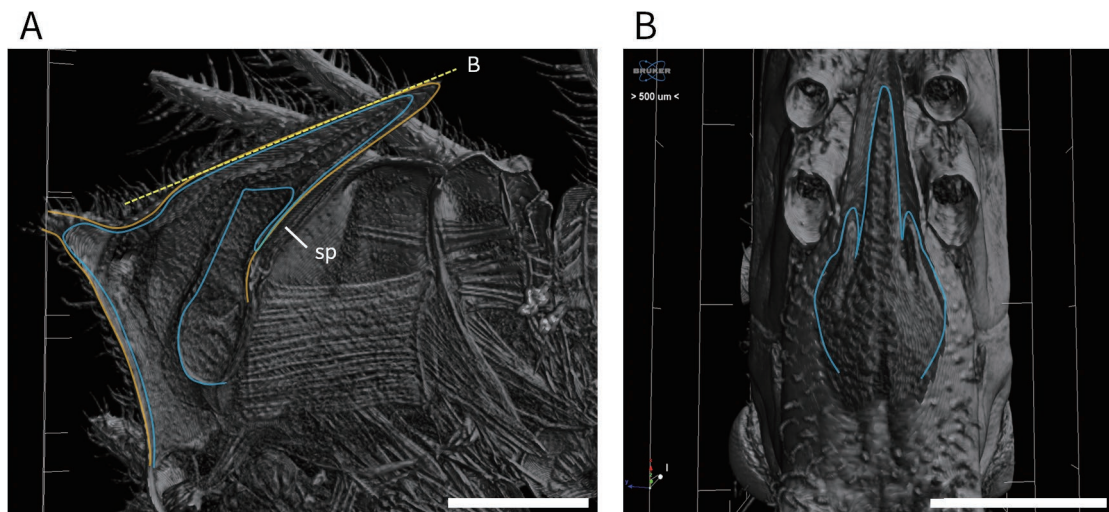


Fig. 7. Micro-CT images of mid-early stage. Micro-CT images of a mid-early-stage sample. **(A)** In this micro-CT image in sagittal section, the posterior region of the developing adult helmet is still a single-layered structure. Epithelial layers of developing adult helmet are indicated in blue, while nymphal helmet sheath cuticle is indicated in orange; sp indicates developing septum. **(B)** Dorsal view of developing helmet in transverse section indicated by yellow dashed line in **(A)**. A pair of short posterior horns have already formed at this stage. Scale bars: 500 μm .

and posterior horns. We found that miniature formation also occurred in *Poppea* as in *Antianthe*. The helmet miniature already possessed common helmet structures such as the septum and median carina. Characteristic structures of *Poppea* (the suprahumeral horns and the posterior horns) were also recognized at this stage. Although the suprahumeral horns and posterior horns have similar structures as “projections growing from the helmet that are only seen in specific species”, it has become clear that their formation mechanisms are likely to be different. The suprahumeral horns seem to be formed by using the nymphal suprahumeral buds as a mold, while the posterior horns are made without any nymphal mold structure. Recent studies suggest that a combination of epithelial cell sheet contraction and adhesion of this sheet to old cuticle plays an important role in the development of adult structures (Matsuda et al., 2023; Tsuboi et al., 2023). We hypothesized that the posterior horns are also generated by a combination of contraction during miniature formation and adhesion between the nymphal helmet sheath cuticle and dorsal layer of the developing adult helmet.

These results suggest that the helmet formation of *Antianthe* and *Poppea* use a common mechanism (miniature formation) but form species-specific structures (suprahumeral horn and posterior horns) by different mechanisms (using a nymphal bud as mold and generating it during miniature formation, respectively). In the future, we will use other treehopper species with more complex helmet shapes to investigate whether the commonality and diversity of developmental processes exists throughout the treehoppers as in the case of *Antianthe* and *Poppea*.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Study design: HG. Sample preparation and observation for micro-CT, SEM and paraffin section: KS, TT, HA, KM and HG. Contribution to field sampling: HA, HG, KN, SK and PH. Figure preparation: KS, TT and HG. Manuscript preparation: KS and HG. Other authors reviewed, made corrections, and approved the manuscript.

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