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The development of the piercing mouth during the last molt of the diseases-transmitting aphids and mosquitoes as revealed by synchrotron X-ray microtomography

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With 5 figures and 1 table

Abstract: Piercing-sucking insects are extremely efficient vectors of animal and plant pathogens. This group is polyphyletic with the piercing organ highly heterogeneous in its structure and morphogenesis, adapted to a specific host and feeding mechanism. For instance, hemimetabolous aphids feed on plant sap from nymphs to adults and they renew their stylets at each molt, thanks to specialized secreting glands. Distinctly, holometabolous mosquitoes have two feeding modes: larvae feed in water by filtering and scraping surfaces with their mouths, while female adults can acquire blood from vertebrate hosts. The pupal metamorphosis allows switching from one feeding habit to another. Here, we present a deep characterization of the biogenesis of the adult mouthparts in parthenogenetic females of the pea aphid *Acyrtosiphon pisum* (Harris 1776) (Hemiptera: Aphididae) and in females of the mosquito *Aedes albopictus* (Skuse 1895) (Diptera: Culicidae), investigated using non-invasive X-ray synchrotron-based microtomography. Comparing datasets collected from aphid juvenile and adult stages and from preimaginal and adult stages of the mosquito, we were able to track the morphological changes of secreting glands and the synthesis of the adult stylet in aphid heads and to follow the *de novo* formation of mosquito mouthparts in pupae. Our study provides a baseline for investigating the evolution and the development of piercing-sucking mouthparts and to better understand how morphogenesis works in insects.

Keywords: Tiger mosquito; *Aedes albopictus*; pea aphid; *Acyrtosiphon pisum*; mouthparts

1 Introduction

Piercing-sucking insects, characterized by their fluid-feeding behavior, possess specialized mouthparts adapted for extracting blood or plant sap. They move from host to host in search of food, behaving as extremely efficient disease vectors since they pick up pathogens from infected organisms and transport them to others.

The diverse mouth appendices of these insects, crucial for fluid-feeding, have evolved along various paths (Fig. 1A) (Labandeira 2019) and the morphogenetic developmental plans governing the formation or renewal of these structures exhibit considerable variation among species (Snodgrass 1943; Matsuda 1965; Zumpt 1973; Grimaldi & Engel 2006;

Krenn & Aspöck 2012; Burkett-Cadena 2019). For instance, aphids, like *Acyrtosiphon pisum* (Harris 1776), showcase structural similarity between juvenile and adult feeding apparatuses, with new mouthparts developing at each molt. In contrast, mosquitoes, such as *Aedes albopictus* (Skuse 1895), employ a distinct feeding strategy between larval and adult stages.

Acyrtosiphon pisum, a model aphid thriving on legume crops, poses a threat due to its role in spreading over 30 circulative and non-circulative plant viruses (Blackman & Eastop 2007; Brault et al. 2010). The species exhibits reproductive polyphenism, alternating between cyclical parthenogenesis and sexual reproduction (Le Trionnaire et al. 2008), with both nymphs and adults sharing the same dietary habits (Fig. 1B).

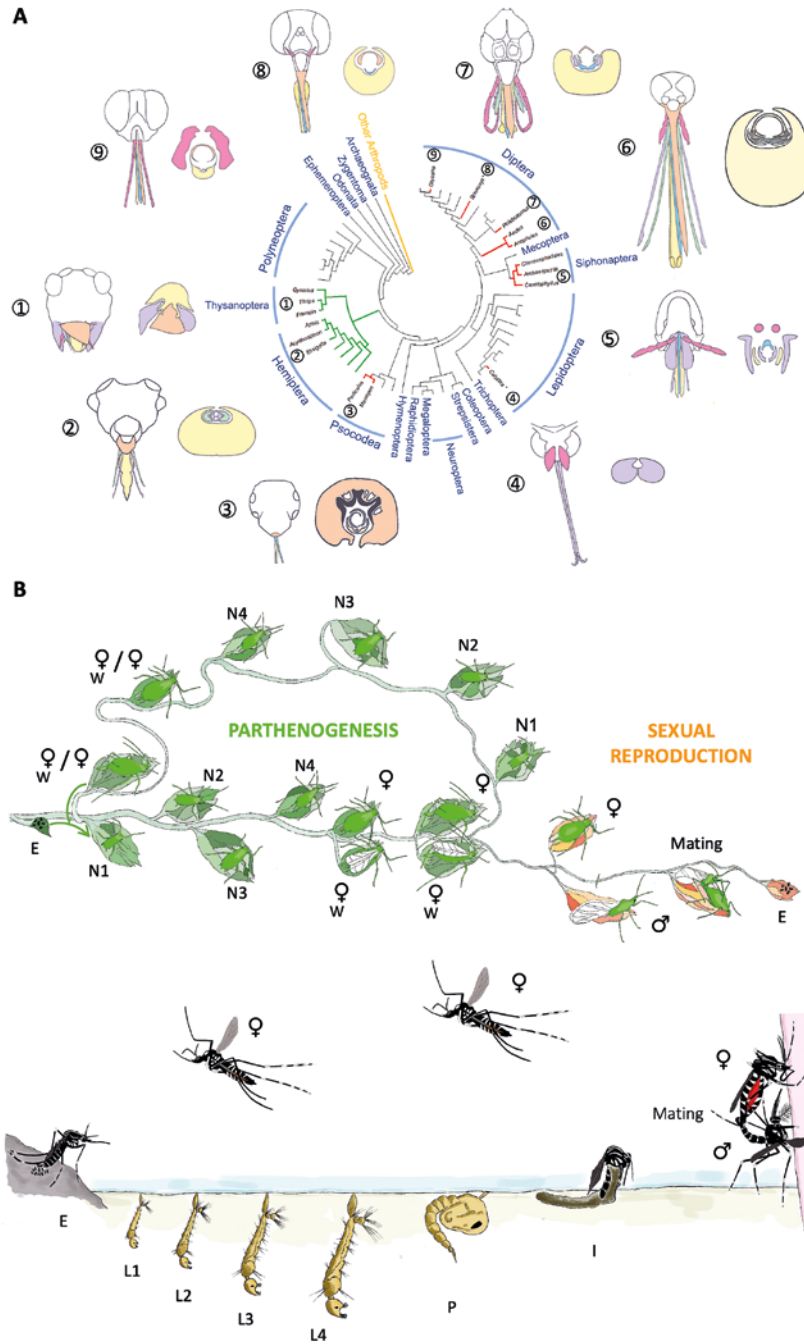


Fig. 1. Evolution of insect mouthparts and aphid and mosquito life cycles. In (A), a phylogenetic tree showing the relations between different insect orders of blood feeders (red branches) and plant pests (green branches) is reported (data from Misof 2014). Representative mouthparts of Thysanoptera (*Heliothrips* spp., 1), Hemiptera (Aphidoidea, 2), Psococoea (*Pediculus* spp., 3), Lepidoptera (*Calyptra* spp., 4), Siphonaptera (*Ctenocephalides* spp., 5) and Diptera (Culicidae, 6; *Phlebotomus* spp., 7; *Stomoxys* spp., 8; *Glossina* spp., 9) are shown. The frontal view of the mouthparts and their transversal section were realized *ex novo* or re-drawn from Snodgrass 1943; Matsuda 1965; Zumpt 1973; Grimaldi & Engel 2006; Krenn & Aspöck 2012; Burkett-Cadena 2019. For each organism, the stylets usually gathered in the fascicle are represented as separated. The labium is colored in yellow, the labrum in orange, the maxillae in violet, the mandibles in green, the hypopharynx in light blue and the maxillary palps in pink. In (B), the life cycle of the pea aphid *Acyrtosiphon pisum* (up) and of the mosquito *Aedes albopictus* (down) are shown. For *Acyrtosiphon pisum*, at the beginning of the spring season, overwintering fertilized eggs (E) hatch into a wingless (♀) asexual female, the foundatrix, which gives birth to nymphs by viviparity. Aphids then alternate several parthenogenetic reproduction cycles in spring and summer (represented in green leaves in the image), and one sexual reproduction in autumn (represented in orange leaves in the image). Aphids develop through four nymphal instars (N1–N4) and finally molt into adult winged (♀W) or unwinged (♀) females. During the autumn season, both male (♂) and female (♀) sexual morphs are produced. After mating, females will lay fertilized eggs (oviparity) that will hatch in the following spring. *Aedes albopictus* eggs (E) hatch in water and develop throughout four larval instars (L1–L4). Larvae metamorphosize into pupae (P) from which, after approximately 48 hours, the imago originates (I). Mature male (♂) and female (♀) mosquitoes mate. *Aedes* females take a blood meal from a vertebrate host to complete egg development; subsequently, they seek for a feasible breeding site to lay eggs, preferring to oviposit on wet surfaces in proximity of water pools.

The tiger mosquito *Aedes albopictus*, native to South-Eastern Asia, is globally known for being a competent vector of human and animal viruses (Ogunlade et al. 2021), such as chikungunya and dengue (Battaglia et al. 2022). It undergoes holometabolous development, with larvae mostly eating particles, microorganisms, algae, and protozoa from suspension, submerged substrates or from the surface microbial biofilms, while adults feeding on blood (females) or nectar sources (males and females) (Fig. 1B).

Both species as adults exhibit piercing-sucking mouth apparatus adaptations from the orthopteroid chewing mouth (Grimaldi & Engel 2006; Misof et al. 2014). Aphid mouthparts consist of the labrum, labium, and stylet bundle (Fig. S1A) (Davidson 1913). The labium, a telescopic tubular segmented organ, facilitates the insertion of the stylet bundle into plant tissues (Pollard 1973). The stylet bundle, formed by mandibular and maxillary stylets, is synthesized by specialized cuticular glands during molts, the retort organs (ROs) (Heriot 1934). The maxillary stylets are tightly interlocked each other on their inner face, forming two ducts, a large food canal and a smaller salivary canal. The two canals merge into a single common duct at the distal tip (Forbes 1966).

In female adult mosquitoes, the stylet bundle comprises the labrum, mandibles, maxillae, and hypopharynx, surrounded by the labium (Fig. S1B). The food canal is constituted by the labrum, which forms a tube, closed at the ventral side by the hypopharynx, which carries the salivary duct. The paired mandibles and maxillae lie on the ventral portion of the labrum-hypopharynx canal: they are long and flat cuticular structures. The maxillae carry also fine teeth at the apex, which are used to anchor the stylet to the skin surface and to favour its penetration. Unlike aphids, the labium forms an external sheath, not inserted during feeding, distally carrying labella, having a sensory function (Lee 1974).

Although the aphid and mosquito stylets differ in their anatomic components, they share a common feature: both are formed by long, cuticle-rich parts, which lacks cuticle-secreting cells. This condition poses an interesting developmental constrain: these appendices cannot be renewed from “inside”, i.e. from a cuticle-secreting epithelial layer beneath the “old” cuticle, during metamorphosis.

Our study, utilizing high-resolution synchrotron-based X-ray tomography and 3D reconstruction, explores and compares the developmental dynamics of mouth apparatus in these two insect species during the last nymphal or pre-imaginal stages. The last molt, indeed, foresees the development of adults. The aphids evolved a system to substitute these cuticular structures at each molt, while mosquitoes produce them *de novo* during the last molting stage, i.e. during pupal development. By supplying a detailed reconstruction of temporal and anatomical changes preceding the last molt in both species, our data provide novel insights into the formation of mouth structures. This research establishes a foundation for

understanding the evolution and development of piercing-sucking mouthparts in insects.

2 Materials and methods

2.1 Insect rearing

Acyrtosiphon pisum established parthenogenetic clone (LL01) was maintained on faba bean plants (*Vicia faba*, L. cv. Sutton) at 18 °C, with a 16:8 h light/dark photoperiod at the Plant Health Institute of Montpellier, France.

An *Aedes albopictus* colony derived from the “Rimini” strain, established in 2004 from mosquitoes collected in Rimini (Italy), was reared in the Insectary of the Department of Biosciences (University of Milan, Italy) at 27 ± 1 °C, 65–80% relative humidity, 12:12 h light/dark photoperiod. Larvae were fed with carp fish pellets (Tetramin, Tetra) until pupation. Pupae were collected in a small plastic cup of water and moved to the mosquito cage. A 10% sucrose solution was supplied to adults *ad libitum* and sexually mature and mated females were blood-fed *in vitro* on animal blood using the Hemotek system.

2.2 Collection of insects

The focus of this study is the last molt event in both the species analyzed. This event leads to the formation of adult mouthparts in both the species, with the death of the cuticle-secreting cells.

The aphid colony was synchronized to allow sampling at precise times during development. To that end, *Acyrtosiphon pisum* adults were allowed to produce progeny for four hours on faba bean plants and were then eliminated. The synchronized aphids were collected at four different time points, three during N4 instar (2 h, 24 h and 46 h from the beginning of the N4 instar corresponding to early-, mid- and late-stage, respectively, with the N4 stage lasting around 50 hours under our insectary conditions). Aphids were also collected at adult stage (24 h post imaginal molt).

Collected aphids were fixed in a 1:1 ethanol:Bouin solution (75% picric acid, 20% formaldehyde, 5% acetic acid) overnight at room temperature, and dehydrated in graded ethanol series (50, 70, 100 %).

Pupae of *Aedes albopictus* were separated from L4 larvae soon after pupation. Sex was determined for each pupa using the sexual dimorphism of the paddles of the last abdominal segment. Female pupae were kept in a dedicated tray and maintained in the insectary till the desired age (0, 1, 2, 3, 4, 5, 6, 7, 8, 20, 30, 40 and 47 hours after pupation), when they were removed and readily transferred to pure ethanol. The pupal stage of *Aedes albopictus* lasts for two days under laboratory conditions (Marini et al. 2020), therefore our time points cover up the entire pupal development. Pupal aging can be also tracked by the color change of the cuticle from white or pale yellow to brown or black (Qiao et al. 2016).

All samples were stored at 4 °C until the analysis.

2.3 Synchrotron micro-computed tomographic scans, segmentation and 3d reconstructions

Tomographic scans were performed at the TOMographic Microscopy and Coherent rAdiology experiments (TOMCAT) beamline of the Swiss Light Synchrotron facility (SLS) of the Paul Scherrer Institute (PSI, Villigen, Switzerland) using an indirect detector system composed of a scintillating screen, a Pioneering in Cameras and Optoelectronics PCO.Edge 5.5 camera and an Optique Peter microscope with a 10× magnification, resulting in an effective pixel size of 0.65 μm. Each sample was run at 17 keV using 100 milliseconds of exposure, 2000 projections and a rotation around the Y axis of 180°. The data were analyzed in both the absorption based and edge-enhanced radiography using Gridrec and Paganini reconstruction pipelines at the SLS facility. For aphid datasets, volume rendering, image segmentation, surface reconstruction and movies, calculation of the volumes of the retort organs, were done with the software Avizo 2019.1 and Fiji ImageJ operated on a workstation of Montpellier Ressources Imagerie Platform (MRI). In the case of mosquito datasets, the data were first reduced to 8 bits using Fiji ImageJ and then analyzed using Slicer 3D software.

2.4 Transmission electron microscopy of aphid stylets

Heads of aphids collected at N4 late stage were severed and fixed in 0.1M sodium cacodylate (Sigma-Aldrich, MO, USA) buffer pH 7.2 (FB) containing 4% glutaraldehyde (Sigma-Aldrich, MO, USA) for 4 h at 4°C, and stored at 4°C in FB containing 0.5% glutaraldehyde until being processed. After rinses in FB followed by post-fixation in FB containing 1% osmic acid for 2 h at 4°C, samples were included in 3% low melting agarose and further dehydrated in a graded ethanol series (30–100%). Finally, samples were embedded in EmBed 812 using an automated microwave tissue processor for electron microscopy, Leica EM AMW. Sections 65 nm thick were observed in a JEOL JEM1400 microscope.

3 Results

3.1 Morphogenesis of adult stylets in the pea aphid *Acyrtosiphon pisum*

We reconstructed the external anatomy of the head and thorax of the pea aphid at the mid N4 stage, followed by segmentation and reconstruction of internal organs such as the nervous system, anterior alimentary tract, and principal and accessory salivary glands (Fig. 2A–E). The labium of *Acyrtosiphon pisum*, when at rest, bends backward and aligns with the ventral part of the thorax. The four functional N4 stylets originate from the retort organs (ROs), two maxillary and two mandibular positioned ventrally to the nervous system, with the mandibular located anteriorly to the

maxillary glands. These stylets converge at the pharynx's tip to form the stylet bundle, extending along the longitudinal labial groove. The alimentary tract, connected to the maxillary stylets' food canal, includes the pharyngeal duct, pharynx, esophagus, and stomach or upper midgut (Ponsen 2006) (Fig. 2D, E).

Our 3D reconstructions traced the development of adult stylets from early N4 nymphal instar, revealing notable remodeling of mandibular (green) and maxillary (violet) ROs (Fig. 3). At the commencement of the fourth instar (2 hours post ecdysis), the ROs exhibit a tubular bud shape, likely corresponding to the stylogenic tissue at rest (Pinet 1968). By mid N4 stage (24 hours post ecdysis), all four ROs elongate, bend, coil, and continue expanding until the stage's conclusion (46 hours post ecdysis). This morphological transformation coincides with a substantial increase in gland volumes (1.5 to 1.7 fold), indicating intensive tissue growth during the latter half of the stage (Pinet 1970) (Fig. S3).

In the second half of the N4 instar, retort organs secrete the cuticle of new stylets which will become those of adults. Their synthesis is complete at the end of the nymphal stage. These newly formed cuticular structures are distinguishable from the cellular material of secretory glands (gray) (Fig. 3, bottom panel). Before the imaginal molt, the apices of the newly formed maxillary and mandibular stylets lie within the basal funnel of the old N4 stylets (Fig. S2A–G and Movie 1). These apices appear surrounded by an amorphous substance of undetermined origin on electron micrographs (Fig. S2H–I).

As adult aphids no longer need to synthesize stylets, the now obsolete ROs regress. In our 3D reconstructions, these regressed ROs appear significantly smaller compared to N4 stage ROs, regardless of their developmental stage (Fig. 3).

3.2 *De novo* formation of the stylet during mosquito molt

In mosquitoes, the transition from juveniles to adults involves a distinctive pupal stage, when individuals are still motile and swim in water. However, most of the molting germinative buds, including those of legs and wings, are already visible during the fourth instar larva, in the prepupal stage. Germinative buds of mouth appendices remain involuted in the larval head capsule and extrude during pupation, as already noted by previous authors (Salem 1931). In our reconstructions, we could recognize both the labrum and the labium buds in the larva (Fig. 4A, Movie 2). Reconstructions reveal the development of labrum (orange) and labium (yellow) buds in larvae, with the labrum forming as a fold of the epidermis extending backward beneath the cuticle of the anterior dorsal wall of the head, while the labium bud emerges behind a triangular cuticle sclerite, named mental sclerite (grey; Fig. 4A, Movie 2). The germinal buds of the mandibles (green) and maxillae (purple) form directly from the epidermis retracted into the basis of the corresponding larval structures.

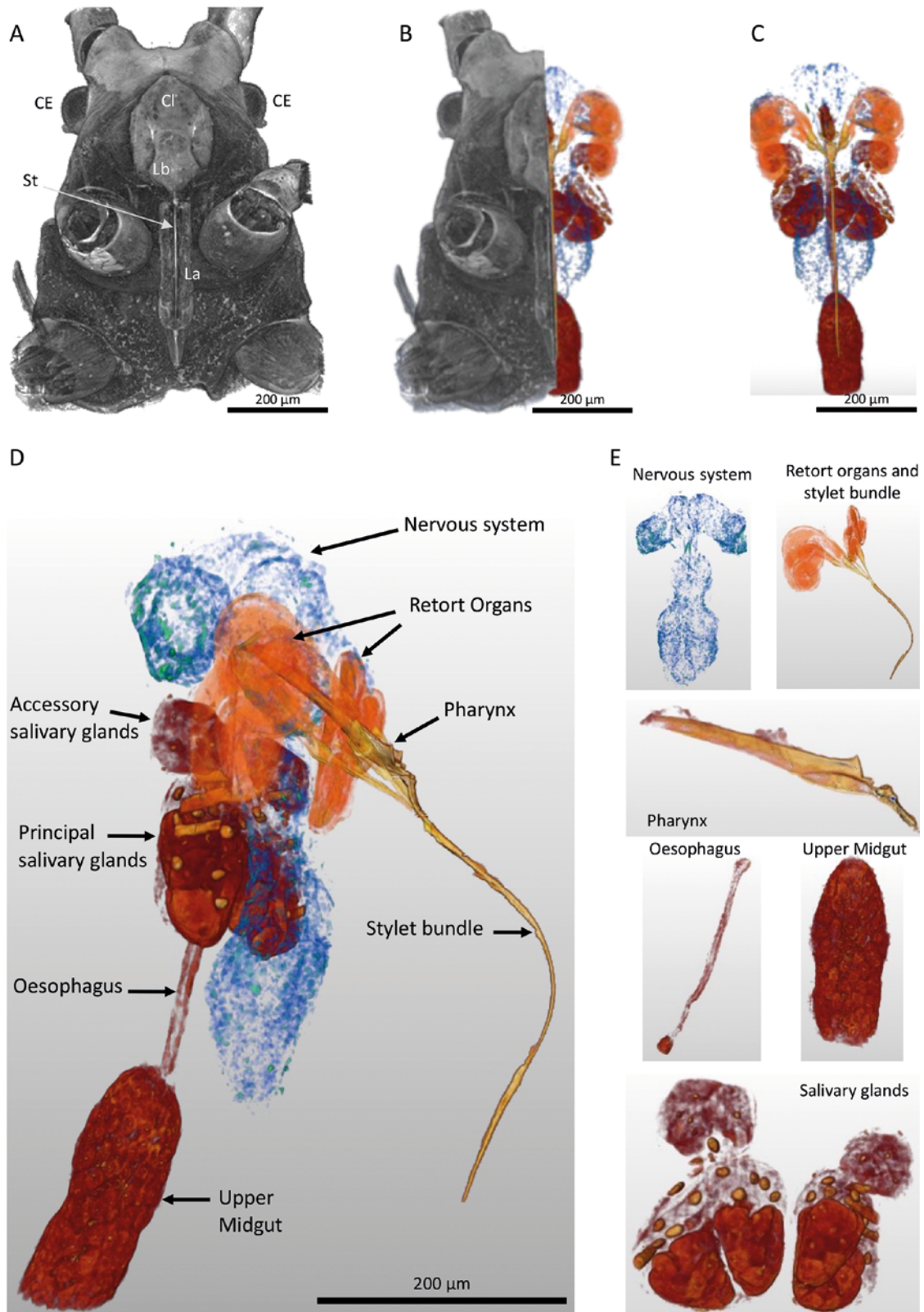


Fig. 2. 3D reconstruction of the head and internal organs of the pea aphid *Acyrthosiphon pisum* from X-ray microtomography datasets. (A–B) Ventral view of the head and the thorax of an aphid at N4 nymphal mid-stage. (A), External anatomy. (B), 3D display of the head optically sliced and with superficial section virtually removed using AVISO software, revealing the main internal organs including nervous and digestive systems, and salivary glands. (C), 3D reconstructions of aphid internal organs with the nervous system, the accessory and principal salivary glands, esophagus, upper midgut, pharynx, stylet bundle and retort organs, also visualized in lateral view (D), and separately (E). CE, compound eyes; Cl, clypeus; Lb, labrum; La, labium; St, stylet bundle.

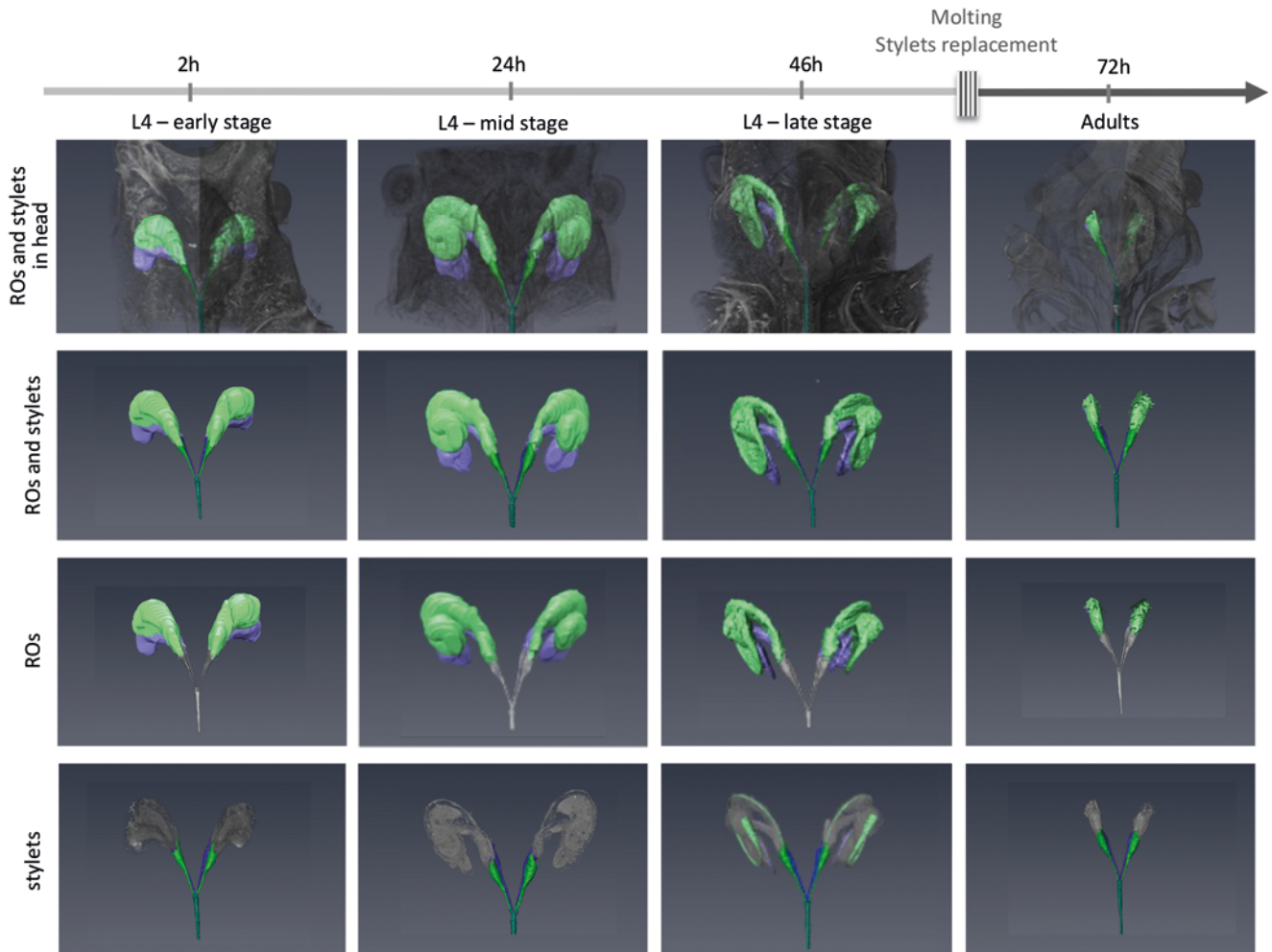


Fig. 3. Developmental anatomy of aphid retort organs during the synthesis of adult stylets of *Acyrtosiphum pisum* obtained by X-ray microtomography. 3D reconstructions of aphid retort organs (ROs) and stylets from different N4 and adult developmental stages as indicated on the timeline. For each stage, four 3D views are displayed. Upper panel, mandibular (green) and maxillary (violet) ROs and stylets within aphid heads to figure out their respective location and volumes; second and third panels, ROs (coloured) and functional stylets (only coloured in the 2nd panel). The 4th panel distinguishes cellular material (grey) from cuticular one (coloured). Note that the newly synthesized adult stylets (light colours) are only visible at N4-late stage where they appear tightly related to the base of the functional N4 nymphal stylets (dark colours).

Major tissue remodeling and imago formation events occur within the first 6 hours after pupal formation (Fig. 5). Larval eyes disappear shortly after pupation, and imago features become distinguishable from 7 hours onward until the acquisition of their final shape.

Most parts of the proboscis are synthesized in the early stages of pupal development, though the hypopharynx becomes distinguishable only after 20 hours after pupation (Fig. 5), confirming the earlier observation of its absence as a germinative bud in larvae and in early pupae (Snodgrass 1959). It most likely forms from the labium or the chitinous setting separating the labium and the labrum.

During the first four hours, pupal cuticle forms sacs serving as scaffolds for mouth and maxillary palp formation. Cell layers cover the sacs' interior side, resembling *Drosophila*

imaginal discs, likely composed of a sheet of monolayered epithelial cells (Kojima 2004). After 5 hours, the cell layer shrinks, forming tubular-like structures visible after 7–8 hours. The tomographic scans suggest that the cell layers persist till 30 hours in the labral, maxillary and mandible primordia, while in the labium and in the maxillary palp they persist till the adult stage. The pupal chitinous sacs persist until adult eclosion. The palps achieve their final morphology after 20 hours, with scales fully formed at 40 hours, just before adult hatching. Tubular-like structures of maxillae and mandibles collapse at 20 hours, forming flat chitinous structures. The labrum and labium maintain a tubular morphology until late developmental stages, acquiring their final shapes at 40 hours.

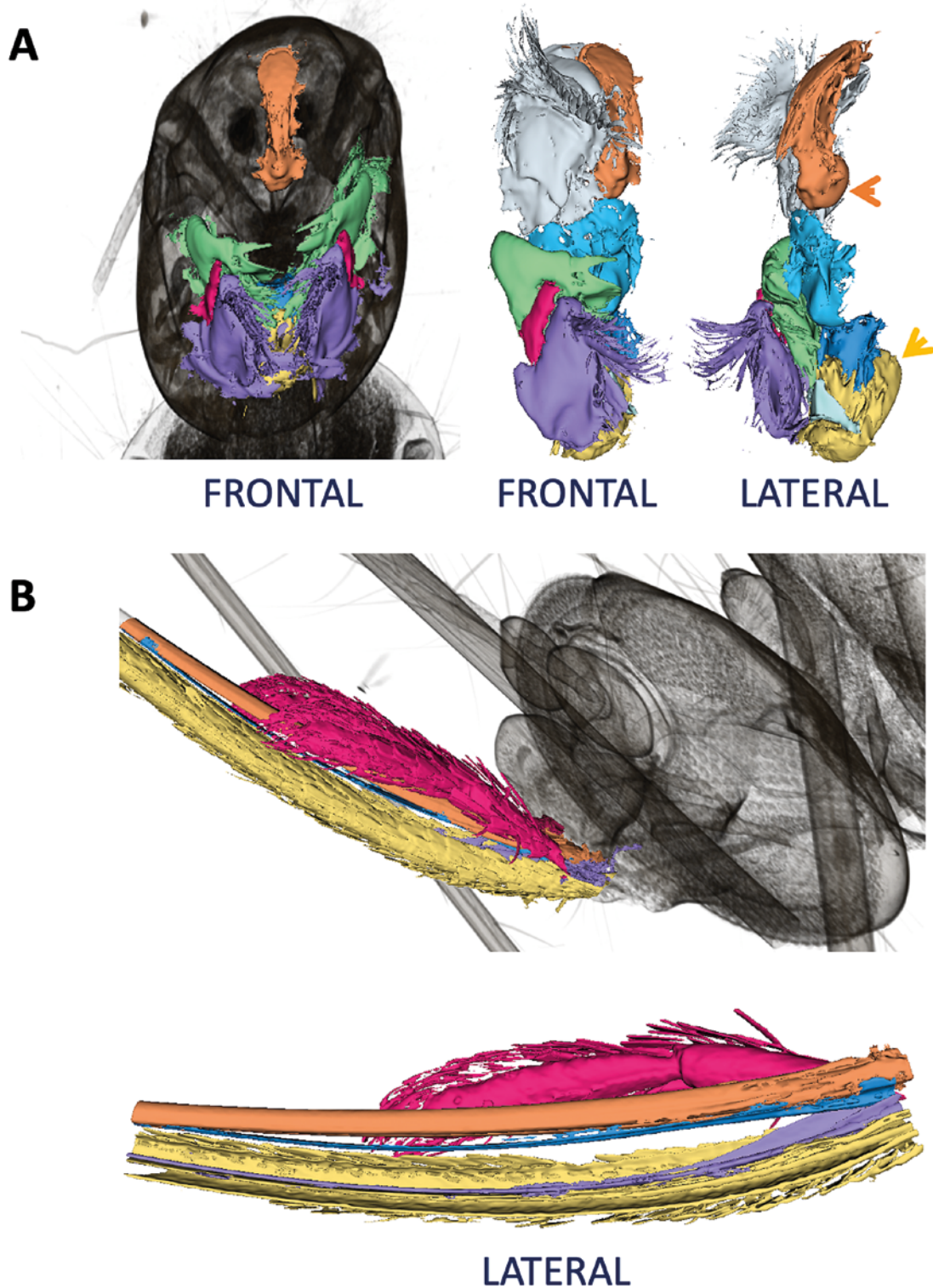


Fig. 4. Mouthparts of the Asian tiger mosquito *Aedes albopictus* as revealed by X-ray microtomography. 3D reconstructions of the mouthparts of the 4th instar larva (A) and of the female adult mosquito (B) of the species *Aedes albopictus*. The frontal view of the head of the larva (A, left panel) shows both the left and right sides of the mouthparts; the segmentations (frontal and lateral views, right panels) show only one side of the mouthparts, to highlight the germinal buds of the labrum and of the labium (indicated by orange and yellow arrows respectively). The lateral view of the segmented adult mouthparts is shown (B, bottom panel) to point out the reciprocal organization of the labium, the maxillae and the hypopharynx in the labial sheath. The labrum (or the epypharynx in the larva) is colored in orange, the mandibles in green, the maxillae in violet, the hypopharynx in light blue and the labium in yellow. Maxillary palps are colored in pink. The mental sclerite (grey) is a triangular sclerite surrounding the labial bulb which is visible in the larva.

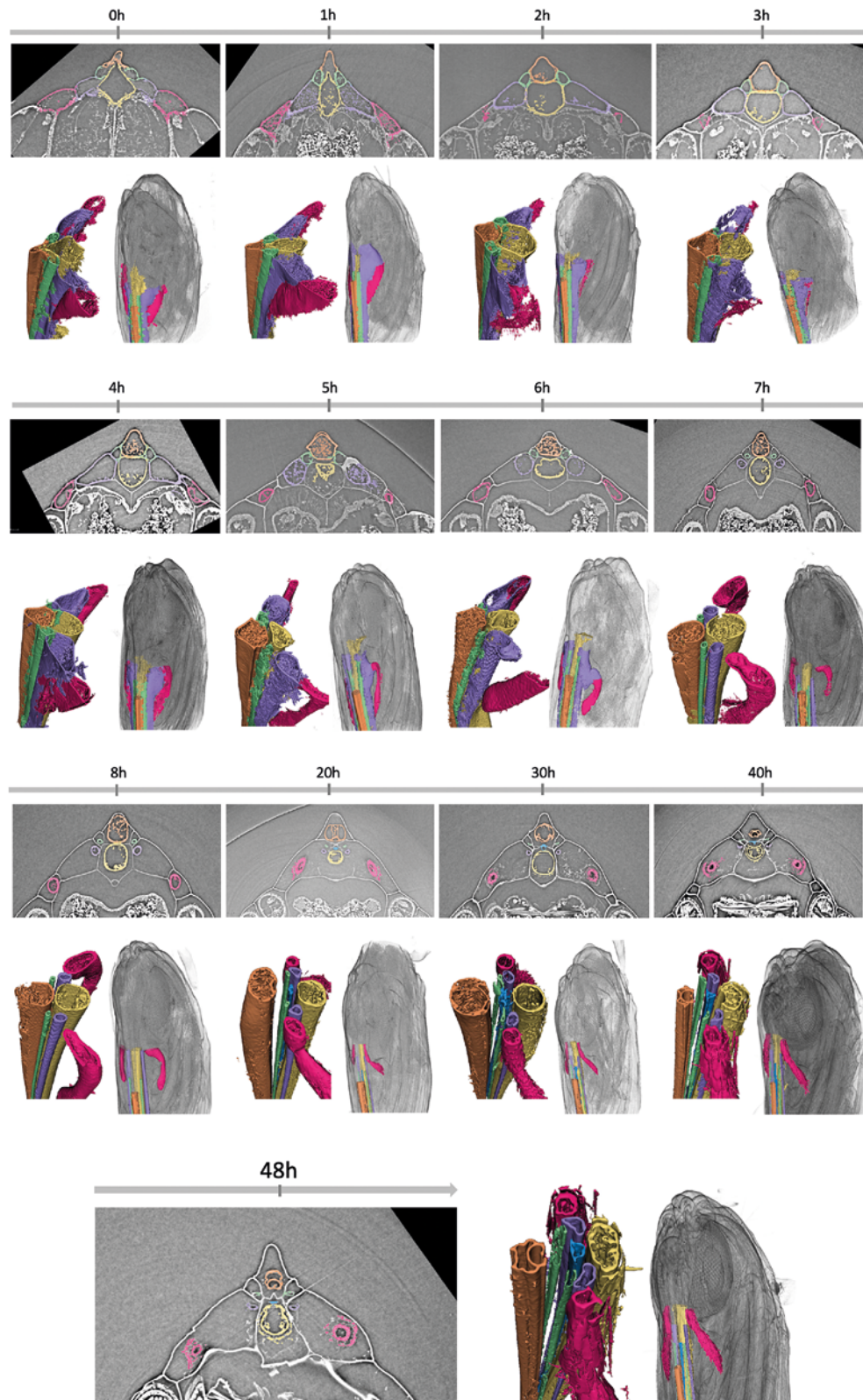


Fig. 5. Morphology of mouthparts during the pupal development of the Asian tiger mosquito *Aedes albopictus* imaged using X-ray microtomography. The segmentation of the mouthparts of *Ae. albopictus* during the pupal development is shown, both using transversal sections and 3D reconstructions. Pupae collected after 0, 1, 2, 3, 4, 5, 6, 7, 8, 20, 30, 40 and 48 hours from the pupation were analyzed. The labrum is colored in orange, the mandibles in green, the maxillae in violet, the hypopharynx in light blue and the labium in yellow. Maxillary palps are colored in pink.

Original tomogram data are available at Zenodo using the doi reported in the Table of content.

4 Discussion

The morphogenesis of adult stylets in the pea aphid *Acyrtosiphon pisum* and the formation of mouthparts during the molt of the tiger mosquito *Aedes albopictus* present intriguing parallels and contrasts that highlight the diversity of developmental processes in insects.

The stylet bundles of both adult mosquitoes and aphids are long chitin-rich structures forged to pierce and penetrate host tissues and to transport fluids. These structures do not present any cuticle-secreting epithelia in their adult form, and only few neuronal cells are present in the stylet of both the species. Therefore, insects adopted multiple strategies to form this type of mouth apparatus during development.

In hemimetabolous insects, such as the pea aphid *Acyrtosiphon pisum*, nymphs and adult mouthparts are closely similar, varying only in size. This anatomical similarity is reflected in the feeding habits of nymphs and adults, both of which feed on plant sap. Aphids renew their stylet at each molt cycle, changing the cuticle structures but maintaining the dendrites. During the styletogenesis process, retort organs undergo dynamic changes. Growth of the secreting organs begins very early during the last nymph to adult molt. They are visible in *A. pisum* head of early 4th instar nymphs (2 hours after molt) where they appear tubular at the base of the old nymph stylet. Along the N4 instar, the retort organs first expand and elongate. They then secrete the cuticle at late stage, leaving behind the cell-free nascent stylet only visible at this time point in our data sets covering the outer surface of RO coils. Hypertrophy of the retort organs suggests an intense remodeling and active secretion. This phenomenon seems to correlate with the expression profiles of genes encoding for cuticular proteins identified in aphid stylets, especially those that are abundant on the surface of the tip of maxillary stylets (Deshoux et al. 2020; Guschinskaya et al. 2022; Uzest et al. 2007). Expression of these genes generally peaks at 46–48 hour during the N4 stage, and drop off in adults, mirroring the ROs dynamics and stylet formation (Guschinskaya et al. 2020). The developmental process involves four rounds of stylet bundle synthesis from the first nymphal instar to the adult stage in *Acyrtosiphon pisum*. While retort organs degenerate after the production of adult stylets, the last ones synthesized by the insect, as suggested by the analysis of the ROs volume, gland epithelium should not atrophy but instead persist from one nymphal instar to the other. After dynamic restructuring of their epithelium, secreting glands should therefore return to an initial state similar to that observed at N4-early stage during the ecdysis of stages N1 to N2, N2 to N3, and N3 to N4. It would be interesting in the future to compare the process of styletogenesis during the different developmental stages in aphids.

By the end of the N4 stage, the new stylets are fully formed and the assembly of the new stylet in the bundle occurs likely as in most of the hemipterans by passive placement during ecdysis: the newly formed stylets are pulled out in their functional position thanks to the attachment to the exuvial cuticle (Cicero 2017).

Mosquitoes, instead, are holometabolous insects and the elongated mouthparts appear only in the adults. The transition between a chewing mouth apparatus, typical of the larval stages, to an elongated piercing-sucking apparatus with sex-specific anatomical differences occurs during the pupal metamorphosis, even if germinative buds form already during the fourth instar larva, in the prepupal stage (Lee 1974; Snodgrass 1943; Snodgrass 1959). The germinative buds of the mouth appendices are still involuted in the larval head capsule and will extrude in the act of the pupation (Salem 1931), with the exception of the hypopharynx, which does not form from a dedicated bud, but it is formed most likely by the labium later in the pupal development, between 8 and 20 hours post pupal formation. Most of the morphological changes of the mouthparts occur soon after pupation, with the formation of a cell layer secreting the stylet cuticle. The mouth development closely resembles that of wings, with cuticle-secreting epithelia that degenerate after the formation of the organ. Further studies are needed to deeply describe the cellular dynamics of this process. Interestingly, like as in aphids, the expression profile of a cuticular protein localized at the tip of the *Aedes albopictus* labrum and interacting with a protein of the mosquito saliva mirrors the timeline of the epithelia forming the labrum as described here (Arnoldi et al. 2022), reaching its maximum expression at around 24 hours of pupal development and drastically diminishing while approaching the adult eclosion. The stylet bundle remains separated in the pupal case till adult emergence, when the imago comes out from the pupa in the water and the stylet is assembled in its final form.

In conclusion, micro-computed comparative analyses help us understanding how the development of long, cell-depleted piercing-sucking mouth apparatus occurs in insects. Aphids and mosquitoes present anatomically distinct stylets, but they originate from germinal regions where cuticle-secreting cells are located in both the species, such as retort organs in aphids and germinal buds in mosquitoes. Therefore, the developmental morphogenetic potential in insects strictly depends on the organization of these germinal regions bearing cuticle-secreting cells. The organization of these cells seems to be highly flexible (retort organs in aphids, imaginal discs in *Drosophila*, germinative buds and sacs in mosquitoes), giving rise to potentially multiple forms as seen for the highly diversified mouth typologies observed in insects. The study of the arrangement of these epithelia during molting in insects can help us better understanding how does morphogenesis works in these organisms.

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Figure S1–S3

Table of contents stored in Zenodo database

Dataset	DOI
Female pupa 0h <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.8410276
Female pupa 1h <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10065931
Female pupa 2h <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10066873
Female pupa 3h <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10066658
Female pupa 4h <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10067133
Female pupa 5h <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10068416
Female pupa 6h <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10069098
Female pupa 7h <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10069487
Female pupa 8h <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10074830
Female pupa 20h <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10075902
Female pupa 30h <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10076269
Female pupa 40h <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10078283
Female pupa 47h <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10079345
Adult female <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10079819
Larva <i>Aedes albopictus</i>	doi.org/10.5281/zenodo.10079727
<i>Acyrtosiphon pisum</i> N4 nymph 2 hours post moulting	doi.org/10.5281/zenodo.10090599
<i>Acyrtosiphon pisum</i> N4 nymph 24 hours post moulting	doi.org/10.5281/zenodo.10081928
<i>Acyrtosiphon pisum</i> N4 nymph 46 hours post moulting	doi.org/10.5281/zenodo.10083486
<i>Acyrtosiphon pisum</i> adult 72 hours post moulting	doi.org/10.5281/zenodo.10091109
Presumptive adult stylets of <i>Acyrtosiphon pisum</i> with their apices hosted in the old N4 nymphal stylets prior to ecdysis (Movie 1)	doi.org/10.5281/zenodo.12706450
The mouth development during pupal stage in <i>Aedes albopictus</i> female (Movie 2)	doi.org/10.5281/zenodo.12706721