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Asian citrus psyllid stylet morphology and applicability to the model for inter-instar stylet replacement in the potato psyllid

Joseph M. Cicero [a, *], Javier Alba-Tercedor [b], Wayne B. Hunter [c], Liliana M. Cano [d], S. Saha [e], Lukas A. Mueller [e], Susan J. Brown [f]

[a] Entomology and Nematology Dept., 1881 Natural Area Dr., Steinmetz Hall, University of Florida, Gainesville, 32611, USA
[b] Dept. de Zoologia, Facultad de Ciencias, Universidad de Granada, 18071, Spain
[c] USDA-ARS, U.S. Horticultural Research Lab, 2001 S. Rock Rd., Fort Pierce, FL, 34945, USA
[d] IRREC, University of Florida, 2199 S. Rock Rd., Ft. Pierce, FL, 34945, USA
[e] Boyce Thompson Institute, 533 Tower Rd., Ithaca, NY, 14853, USA
[f] Dept. Biology, Ackert Hall, Kansas State University, Manhattan, KS, 66506, USA

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ABSTRACT

In Hemiptera, presumptive stylets for each consecutive postembryonic instar are manufactured prior to ecdysis to replace the ecdysial stylets discarded with the exuviae. With the discovery that the bacterium "*Candidatus*" Liberibacter solanacearum accesses the tissues involved in the stylet replacement process of the potato psyllid, a hypothesis was formed that the bacterium could adhere to the stylets of freshly emerged instars and hence gain access to the host plant when feeding is resumed. Although unproven, it was imperative that a model for stylet replacement be built. Stylet morphology and the stylet replacement process of the Asian citrus psyllid (ACP), vector of "C." L. asiaticus, causal pathogen of citrus greening disease, are comparable to the potato psyllid model system. Morphology consists of a basal terminus with its tab-shaped auricle, a base, shaft, and an apical terminus. Each of the four auricles act as a platform for the replacement apparatus, which is compacted into a tight aggregate of cells, the 'end-cap'. As modeled, on apolysis of larval instar hypodermis, the aggregate 'deconstructs' and expands into a snail shell-shaped tube, the 'atrium', that houses the presumptive stylet as it is synthesized. Completed stylets then despool from the atrium and are fitted into their functional positions as the next instar emerges from its exuviae.

1. Introduction

The discovery that biofilms of the bacterium "*Candidatus*" Liberibacter solanacearum occur on the stylet replacement apparatus of the adults of the potato psyllid, *Bactericera cockerelli* (Sulc, Triozidae) (PoP) led to the hypothesis that they may also be on the same tissues in earlier instars of that species, and of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Liviidae) (ACP). If so, then it is possible that transmission of the Liberibacters may occur when consecutively new instars resume feeding. This hypothesis prompted intensive study of the internal structure of the PoP oral region (Cicero et al., 2015), followed by a model of the processes by which a new (presumptive) stylet is formed in association with the old (ecdysial) stylet prior to rupture of the dorsal exoskeletal cuticle, and its fitting into the intrastadial (functional) position as the teneral adult lifts away from its exuviae (Cicero, 2017).

It has been known since antiquity that hemipteran stylets are lost with the exuviae of each consecutive molt, and that replacement stylets are coiled inside the head on either side of the midline for deployment on ecdysis. Davidson (1913) was the first to publish an English text and sketches describing these coils in *Schizoneura lanigera* (Aphididae), calling them 'retort-shaped organs', so named by 19th Century authors. In addition to the PoP model, two others were published on the coils for Sternorrhyncha (Hemiptera: aphids, psyllids, whiteflies, scales) since then, Weber (1929) for *Cacopsylla* (=*Psylla*) *mali* (Schmidberger, Psyllidae) and Pesson (1951) for the scale *Icerya purchasi* Maskell (Margarodidae).

The two earlier authors correctly based their models on observation of the lifting out of cells from the hollow core of the larval stylet and organizing into a coiled housing for manufacture of

* Corresponding author.
E-mail address: jcicero@ufl.edu (J.M. Cicero).

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presumptive stylets from the cells that were adherent to the core walls. Greater clarity to the general process, molting, came from the collective works that followed later in the 20th Century wherein technical terms such as 'apolysis', 'molting space', 'molting fluid', 'pharate' and several others were coined (Jenkin and Hinton, 1966; Wigglesworth, 1973), rendering the general physiology of the molting process well known (Chapman, 1998) and applicable to many modern studies.

Cicero (2017) used transmission electron microscopy (TEM) to study the hemispherical mass of cells that Pesson (1951:1398) discovered at the basal terminus of each stylet and that were continuous with the core cells and referred to as a 'cell bulb' or 'cell matrix' ('*bulbe cellulaire*' … '*La matrice cellulaire de chacun des stylets se trouve réduite à un bourgeon, que coiffe sa racine creuse et dilatée*'). For PoP, Cicero called them 'end-caps' and characterized a mass of tightly folded, extremely flattened cells, referred to as the 'matrix', in its interior. The core cells were modeled to lift out of the PoP larval stylets in preparation for a molt also, but to dissociate, or 'deconstruct', allowing the matrix and the end-cap cells peripheral to the matrix to expand into a snail-shell shaped tube to house the presumptive stylet as it is secreted. The basis behind this model came from electron micrographs of pharate adults, adults and the exuviae the adults leave behind, the latter retaining the stylets and other cuticles that were associated with them in the last instar larva.

In PoP, this snail-shell shaped housing, termed the 'atrium' for *Rhodnius* (Pinet, 1968), consists of a 'hub' and a 'rim'. The hub is a discoid helix of extremely flat cells representing the expanded matrix, and the rim is a tubular helix of cells that forms the hub's circumference. The rim has the hollow that houses the growing stylet. As expected from the fact that the functional stylet protrudes to the outside, this rim hollow is lined with a cuticular intima which, like the stomodeal and proctodeal intimas, is continuous with all other cuticles of the endo- and exoskeleton, and, correspondingly, an airspace occurs between it and the stylet it houses. The atrium is therefore an impressively deep, coiled invagination of the exoskeleton. As modeled, on completion of secretion of the presumptive stylet within the pharate adult, and during emergence of the teneral adult through the exuvial rupture, the stylet 'despools' from the atrium, allowing the atrium to collapse down into an end-cap of the next instar. An understanding of the actual morphology of the proximal end of the stylet is of critical

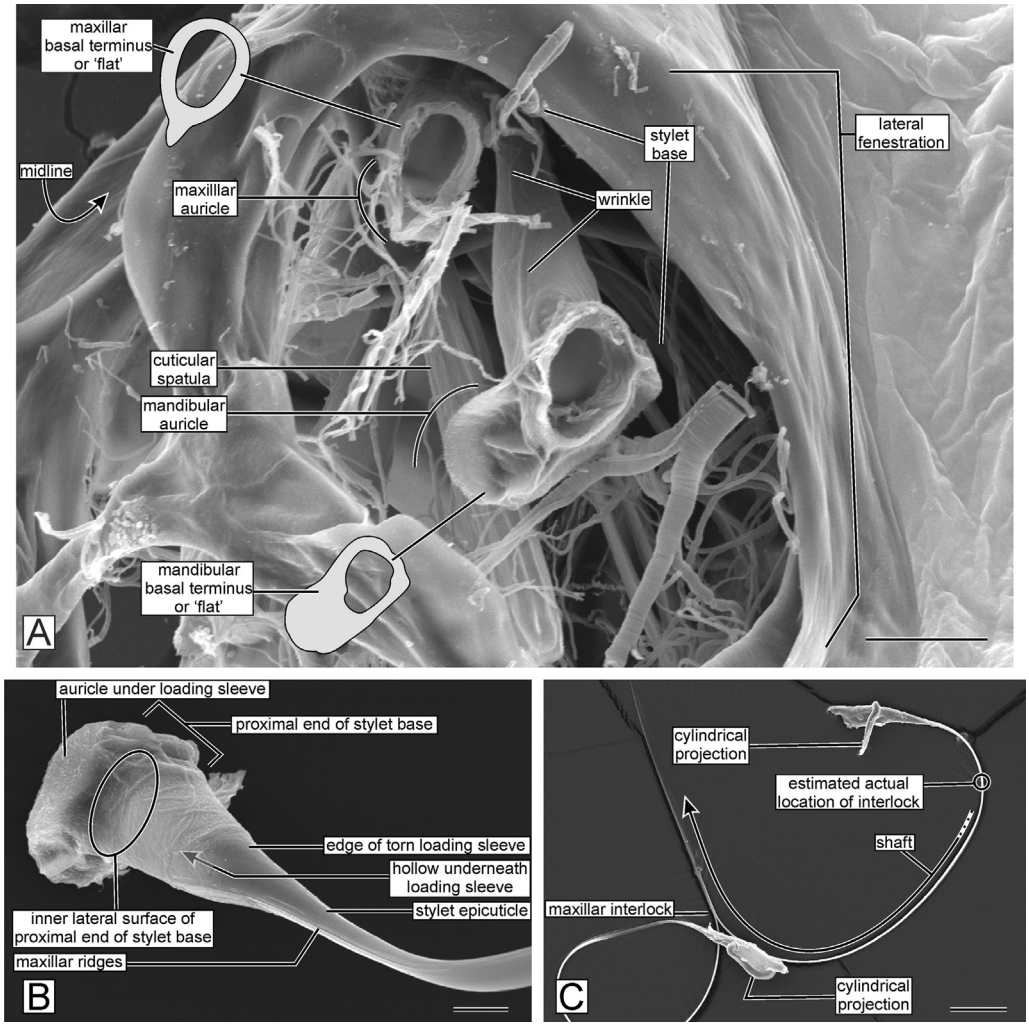

Fig. 1. Asian citrus psyllid, *Diaphorina citri*, stylets. **(A)** Head digested with proteinase K. View by SEM through the lateral fenestration of the tentorium. Wrinkle indicates that the loading sleeve is present and intact on the mandibular. The cuticular spatula is interpreted to serve as an attachment anchor for muscles. Line = 20μ. **(B)**–**(C)** Free dissections. **(B)** Inner lateral surface of the maxillar's proximal base is underneath the loading sleeve. The white ellipse indicates the space created by the protruding auricle. The stylet epicuticle is smooth in contrast to the wrinkled loading sleeve overlying it. The tracking of maxillar ridges appears to curve longitudinally. See Discussion. Line = 10μ. **(C)** Maxillar Interlock was retained at a mesal location of the two shafts. A cylindrical projection is retained at the basal end in some dissected specimens but not in others (cf. Fig. 4C). Line = 50μ.

significance because, based on micrograph interpretations (Cicero, 2017), it is modeled to have pores or slits allowing core cells to reach the epicuticular surface and assist in gathering the intima into a bolus called the 'cushion' during the despooling event.

The dynamic behavior of cells during stylet replacement is so complex as to render the traditional, monographic style of exposition woefully inadequate. In resolution, this work builds upon the use of animation graphics (Cicero et al., 2018) for interpreting sample micrographs of ACP in order to determine possible similarities and differences in the stylet replacement apparatus between the two species.

2. Methods and materials

Psyllids were collected from the citrus orchard of the Horticultural Sciences Department, University of Florida, Gainesville.

Free, open dissections of male and female ACP (n = 16) were performed with ultrasharp tweezers (Electron Microscopy Supplies, Hatfield, PA) to isolate stylets. Other Methods are detailed elsewhere (Cicero et al., 2018). Briefly, heads of live ACP adults and alcoholed PoP (n = 10) were decapitated and their tissues digested with 75.0 μg/ml proteinase K in 1.0% Triton® X100 at 45 °C overnight. They, and the isolated stylets, were then critical point dried, sputter coated, and examined with a Hitachi SU5000 Schottky Field Emission scanning electron microscope (SEM). For transmission electron microscopy (TEM), heads of ACP adults and pharate last instar larvae were fixed in 4% formaldehyde, 0.5% glutaraldehyde in Na^+ K^+ phosphate buffered saline (Sambrook et al., 1989), pH 7.8, then rinsed, dehydrated, infiltrated with LR White embedment medium (EMS), and polymerized. Ultrathin sections were stained with UranyLess EM stain (EMS) and lead citrate and viewed with a Hitachi H-7000 TEM. Intervening semithin sections were stained with Toluidine Blue 0 (Sigma) for light microscopy.

3. Results

The basic configuration of the ACP stylets, as imaged by SEM (e.g. Fig. 1) and TEM (e.g. Fig. 2), compare well with those presented for PoP herein, and in prior publications.

3.1. Scanning electron microscopy

Terminology, developed to address the complexity of PoP stylet anatomy, can be applied to ACP (Fig. 1A). The 'base' of the ACP stylet was the length inside the head. The 'basal terminus' was the 'flat', or basal-most aspect of the stylet, perpendicular to its long axis. It was wrinkled but otherwise more or less planar. The 'auricle' was a tab-shaped protuberance of the basal terminus. In both species, the mandibular auricle was larger and truncate in contrast to the shorter, acute maxillar auricle. The inner lateral stylet surface immediately distad to the auricle was functionally very significant and could be addressed by identifying the 'proximal end of the base' (Fig. 1B). The base of each stylet was housed in a fluting that consisted of a loading sleeve and a holster (Figs. 1B, 2 and 3A). A clear demarcation was observed between holster and loading sleeve in one specimen of ACP (Fig. 4A). The 'shaft' of the stylet was the section outside the head (Fig. 1C). The maxillar interlock and tightly, laterally appressed mandibulars of the four shafts formed the 'stylet bundle'. In Fig. 4A, the intima of the 'atrium' (the tube of the prior pharate instar that housed the presumptive stylet of the adult) unraveled itself from its repose as a bolus (the 'cushion') inside the loading sleeve apex and survived processing for SEM.

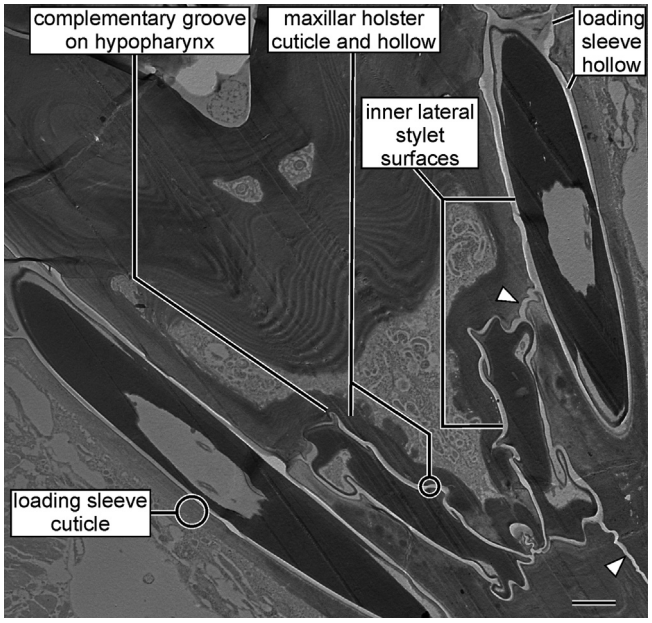

Fig. 2. TEM cross-section of bases of Asian citrus psyllid maxillars and mandibulars, that is, of their lengths inside the head. Cross-section is anterior to the maxillar interlock. The loading sleeve hollow surrounding the mandibular is the interior of an invagination-- arrowheads locate continuation of the invagination posteriorly into and out of the maxillar holster hollows. Maxillars have loading sleeves of their own, but their holsters transition into them more anteriorly (cf. Fig. 4A). The hollow eventually reaches the open air outside the body. Ridges of the maxillars are fitted into grooves of the opposing wall of the hypopharynx. No discrepancies are indicated between this configuration and that of PoP (Cicero et al., 2015). Line = 2μ.

The loading sleeve was retained at full length on non-extirpated stylets (e.g. Fig. 1A), and retained at the proximal end only on extirpated stylets-- the rest of the loading sleeve length was torn away (e.g. Figs. 1B and 3A). As exemplified by these latter figures, the exposed epicuticle could be distinguished from the retentive loading sleeve cuticle by its smooth surface. Minute tuberculate structures, apparently sensilla, were detected. In one specimen, the retentive loading sleeve was ripped where it joined the stylet basal terminus, exposing epicuticle of the proximal base (Fig. 3B and C). The epicuticle had a smooth surface dotted with numerous circular, pore-like structures. Without more examples, the proximal basal epicuticle could not be inspected for structures that might allow cells to pass from the core to the epicuticular surface (see Discussion). Anomalous, unexplained holes were found in the viewable surface of certain specimens (Fig. 5).

Stylets of ACP and PoP, mounted for SEM in such a way as to afford an anterior view, possessed a smooth-walled core that was recessed from the basal terminus, exposing a second cuticular layer basad of it, which, with the epicuticle coating the stylet exterior, indicated that three cuticular layers were present. In PoP, this basally exposed layer was rough and fibrous in contrast to a more uniform texture in ACP (Figs. 4B and 6A).

A cylindrical structure projected from the stylet bases in certain dissected ACP specimens but not in others (Figs. 1C and 4C). A similar cylindrical structure was observed in an undissected specimen of PoP in the same location (Fig. 6D).

In both species, the loading sleeve cuticle consisted of fibrous bundles that ran longitudinally and cross-connected to each other producing a carinate, alveolate pattern (Figs. 7 and 8). The PoP specimen in Fig. 7A had a much tighter mesh. In both species, the loading sleeve cuticle could also be distinguished by the presence of wrinkles (Figs. 1A,B, 3A, B, 4A, B and, 5A). In Fig. 4B, the loading

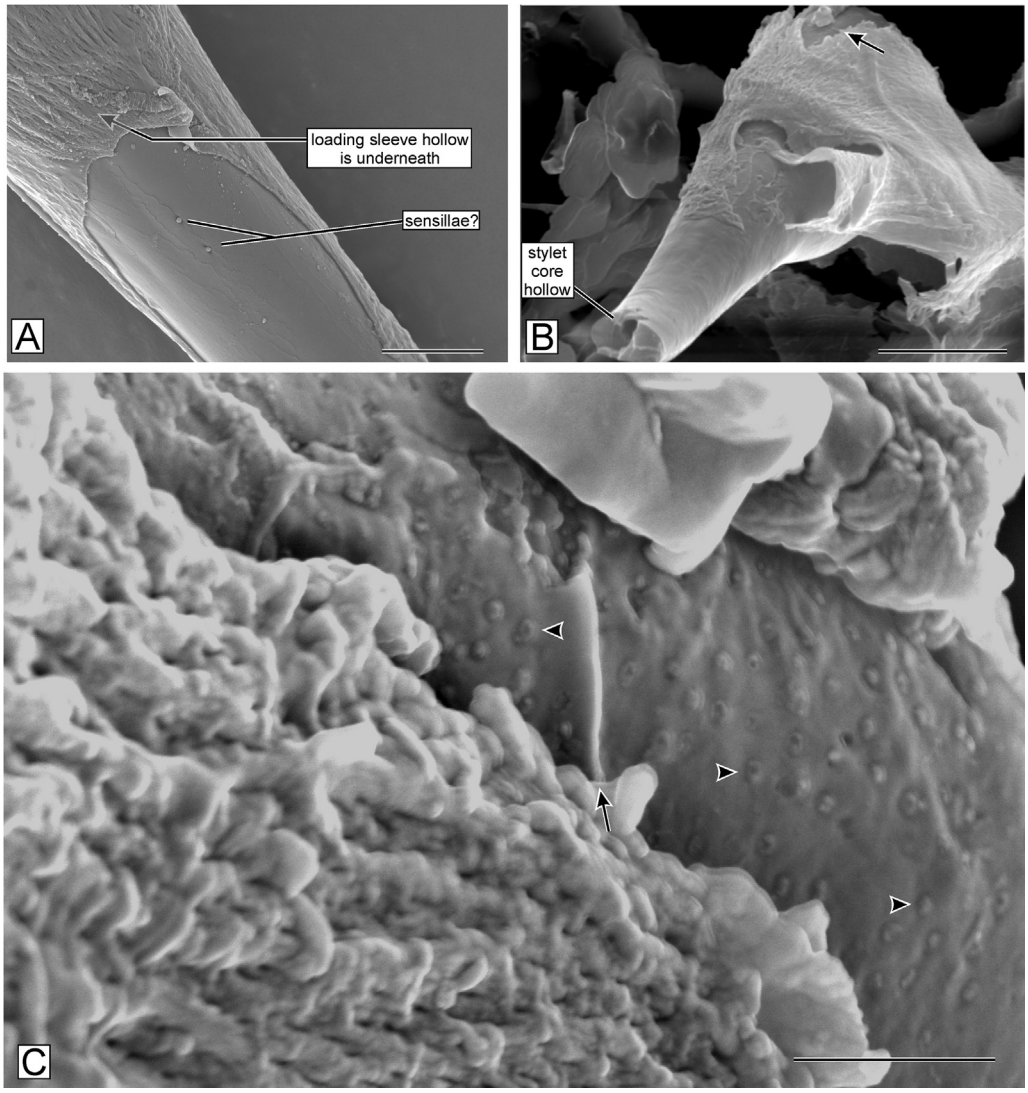

Fig. 3. Free dissections of Asian citrus psyllid stylets. **(A)** Stylet with loading sleeve mostly torn away, exposing apparent sensillae on its basal surface. Line = 5μ. **(B)** The loading sleeve was torn away from its attachment to the stylet basal terminus, exposing the previously unknown ultrastructure of the stylet's proximal base. Arrow identifies position of arrow in **(C)**. Line = 10μ. **(C)** Magnification of tear in **(B)**. Location of arrow is indicated by arrow in **(B)**. The stylet proximal base is smooth with numerous pore-like structures not known anywhere else in the stylet's full length. Arrowheads point to three of the many pores. Line = 1μ.

sleeve was interpreted to be uplifted proximally and dried down in a wrinkled condition distally.

3.2. Transmission electron microscopy

As with PoP, the end-cap of intrastadial ACP had a matrix of folds embedded within it, and a bolus of cuticle called the 'cushion', representing the atrial intima, collected in a cuticular pocket at a specific location on one side (Fig. 9A). Which side could not be determined by TEM without extensive Z-sectioning.

The atrial rim of ACP in Fig. 9B was directly comparable to several key features known for PoP. The maxillar was positioned inside the rim hollow so that its ridges were directed outward, away from the hub center. A cytosol, interpreted as the stylet-secreting cytosol, filled the maxillar hollow. A homogeneous, dark-fringed bed and a granular material occurred between the ridges and outer aspect of the rim just as it did in PoP. Also, the cells surrounding the rim tapered as they transitioned into the hub. The apparent dissociation of the intima was interpreted as a processing artifact-- the intima is substantive in Fig. 4A.

4. Discussion

Prior studies of homopteran stylets were largely focused on their design, as it facilitates host plant penetration, feeding, and pathogen trafficking. In contrast, this paper focuses on the design of Asian citrus psyllid (ACP) stylets, as it compares to the model for the processes involved in stylet replacement, built from intensive study of the molt from last instar larva to adult of the potato psyllid (PoP) (Cicero, 2017). This focus is in response to the observation that "*Ca.*" L. solanacearum establishes biofilms on the stylet replacement apparatus of PoP (Cicero et al., 2015). Readers are referred to these two papers and the animation (Cicero et al., 2018) for detailed explanation of the summaries below.

Stylet replacement involves biogenesis, despooling, and fitting of presumptive stylets into their functional positions. "Biogenesis" refers to the secretion of the presumptive stylet cuticle. It is believed that the shape of the stylet is fashioned by the secretion process itself. "Despooling" refers to the evacuation of the presumptive stylet from its snail-shaped atrium. For despooling to initiate, the insect must commence its lift away from the exuviae

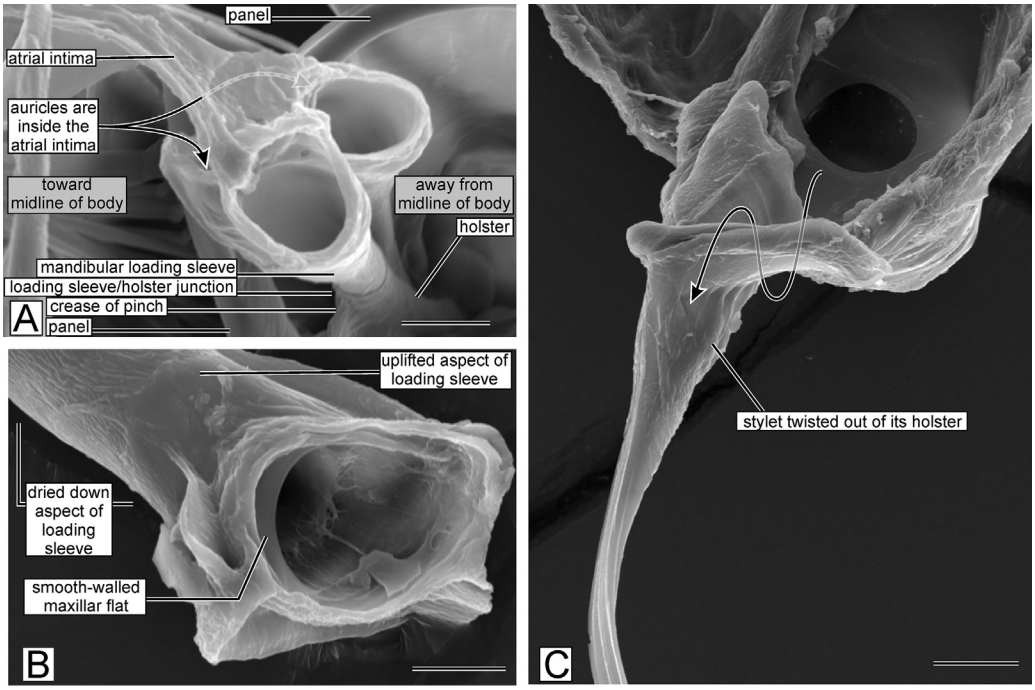

Fig. 4. Asian citrus psyllid stylets. **(A)** Head digested with proteinase K. Thin cuticles are retained that would otherwise be lost with KOH digestion. The atrial intima unraveled from its repose as cushions underneath the auricles. Auricles are directed interiorly. Holsters pinch away from the panel that backs them, and the pinch ends in a crease while the tubular cuticles continue anteriorly as loading sleeves free from the panel (Cicero et al., 2015). Lines = 10μ. **(B)** Free dissection. Maxillar hollow and proximal base. Analysis of the abrupt change in cuticular surface texture pattern indicates that during processing, the more distal aspect of the loading sleeve dried down to a wrinkled pattern, while the proximal aspect uplifted. Line = 10μ. **(C)** Free dissection. Identification of cylindrical projections in Fig. 1C. The stylet twisted out of its holster during dissection, and the twisting was about the holster's cylindrical, belt-shaped rim (cf. Fig. 6D). Line = 20μ.

through the dorsal rupture, because the apex of the presumptive stylet is within the proximal base of the ecdysial stylet core, and clearance from it must be provided. According to Cicero et al. (2015:746, f. 2B), the despooling of PoP stylets progresses to a considerable extent by the time the anterior half of the teneral has passed through the rupture. “Fitting” refers to the passage of the presumptive stylet through the presumptive loading sleeves, thence through the presumptive holsters, until its full length has

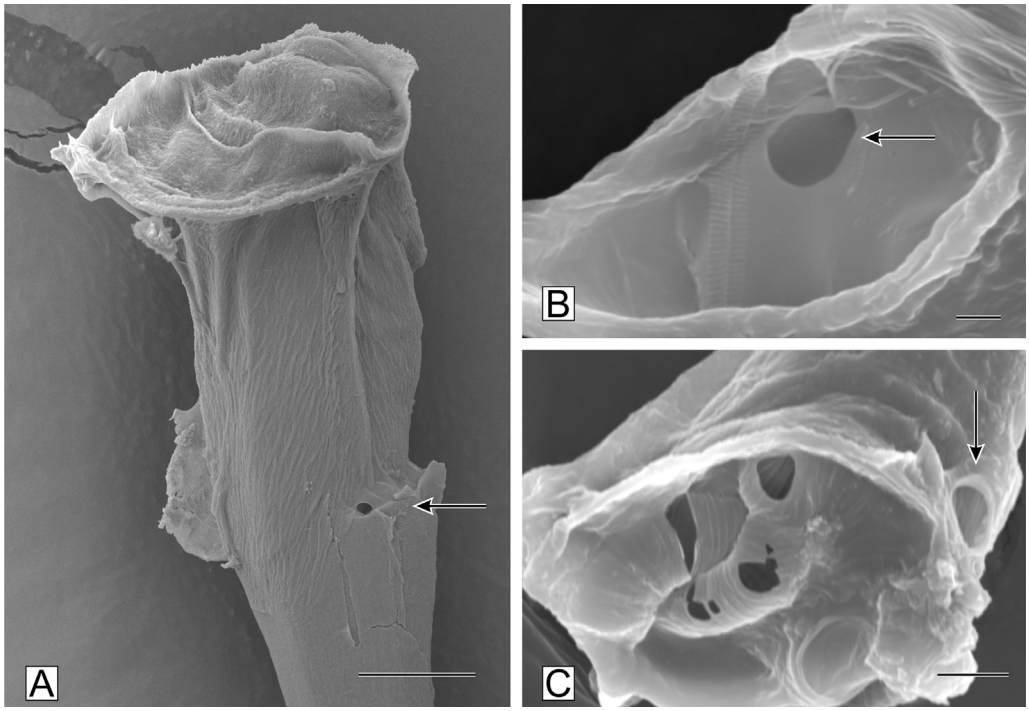

Fig. 5. Free dissections of Asian citrus psyllid stylets. Anomalous holes in cuticles (arrows). **(A)** A small hole in the loading sleeve. Line = 10μ. **(B)** A large hole in the surface of the stylet core. Line = 2μ. **(C)** A large hole in the loading sleeve. Tracheal perforations are an artifact. Line = 5μ.

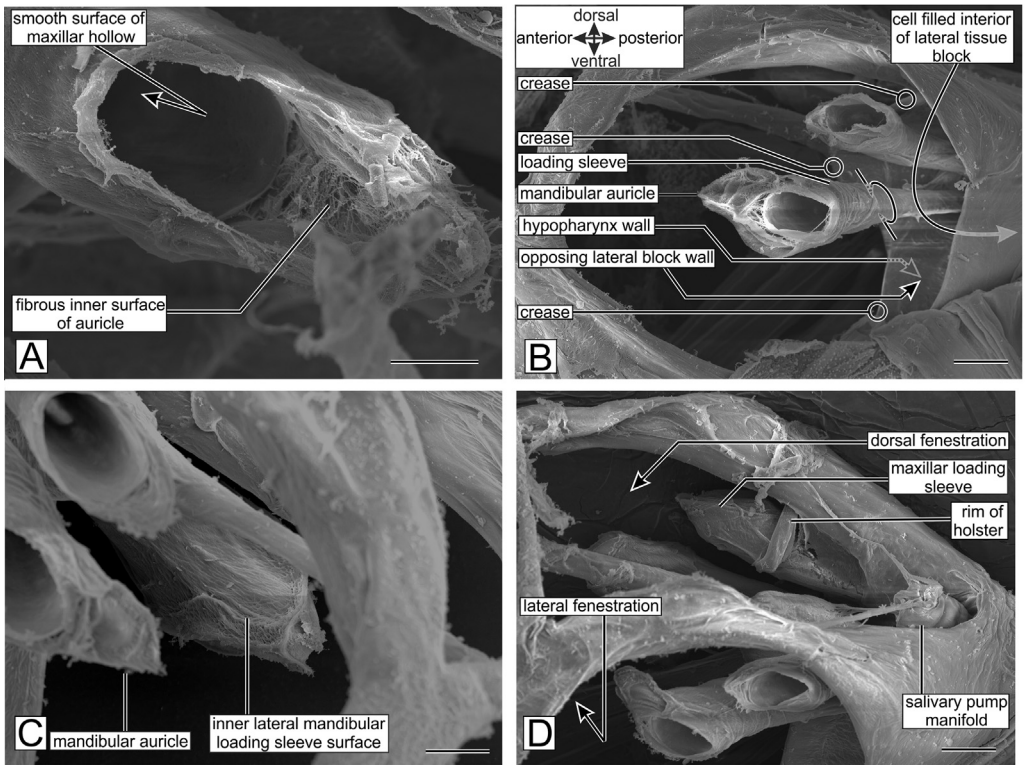

Fig. 6. Anterior and lateral views of PoP stylets through tentorial fenestrations. (A) Hollow of a maxillar. Line = 10μ. (B) The crease is the end of an invagination of the exoskeleton. The invagination is panel-shaped, and its two walls respectively define the hypopharynx and the lateral blocks, both of which are, in life, filled with cells. Stylets pass inside the fluting ('holsters') of the lateral block wall (Cicero et al., 2015:749, f. 5A, HW, LH). Each fluting continues anteriorly as a loading sleeve (note identifying wrinkles). Horseshoe-shaped inset indicates that the flute of the lateral block wall wraps over the stylet to form its holster. Line = 20μ. (C) Mandibular loading sleeve. Line = 10μ. (D) Fenestrations are labeled. The maxillar loading sleeve is retained (cf. Fig. 7A). The holster rim is cylindrical and belt-shaped (cf. Figs. 1C and 4C). Line = 20μ.

left the atrium and its basal terminus is seated upon the loading sleeve apex.

All three are dynamic events and, although time lapse for them has not been measured, the first may be brief, and the other two are well known to be very brief by direct observation of the time it takes for the species under study to emerge from the exuviae.

Comparison of ACP stylet design to aspects of PoP stylet design known to be critical in the replacement process yielded positive correlations, and it can be concluded *a priori* that the general features of replacement in PoP can be applied to ACP. In brief summary, ACP stylets are invaginated into the head, and, according to the tenet of 'cuticular continuity' (Cicero et al., 2015), the cuticle of each invagination, namely the holster/loading sleeve complex, is continuous with the cuticle of the stylet's basal terminus and thence with all other cuticular aspects of the stylet (Fig. 10C). On apolysis, these cuticles become (terminologically) ecdysial as the end-cap cell extensions lift out of the stylet hollow (Fig. 10B). The end-cap deconstructs to release the matrix (Fig. 9A), allowing it to expand into the rim and hub of the atrium, and allowing initiation of presumptive stylet secretion.

Atrial cells generate an intima which is continuous with the presumptive loading sleeve/holster complex basally, and the basal terminus of the presumptive stylet apically. The design of the presumptive stylet, with the tab-shaped, protruding auricle on its inner lateral surface, facilitates gathering of the intima after it apolyses from the atrial cells that secreted it. At onset of despooling the intima is tubular, but on completion of despooling, the only evidence of it is the bolus of crumbled cuticle, the 'cushion' (Fig. 9A). The bolus is therefore interpreted to be the intima that was forced into the only available space directly underneath the auricle (Fig. 1B, inner lateral surface of proximal end of stylet base). The cushion is detected by TEM underneath the loading sleeve (e.g. Fig. 9A), but is not detected by SEM as a bulge at this location in Fig. 1B. It may have flattened during dehydration, or it may have pulled out with the loading sleeve, to which it is connected, during dissection. Support for these interpretations comes from Fig. 4A, wherein the bolus unraveled itself out of this location.

The pores exposed at the proximal base, underneath the loading sleeve (Fig. 3B and C), are diametrically opposite the location of the cushion, but their overall distribution around this circumferential area is unknown. They are not observed in more distal areas that were exposed when the loading sleeves were torn away (e.g. Figs. 1B, 3B and 4C). Since this area is where stylet manipulation muscles are attached (Cicero et al., 2015), the loading sleeve hollow directly underneath (Fig. 3A) would be subject to friction during probing and drilling, suggesting the function of the pores may possibly be to secrete lubrication fluid.

In life, the bolus is in a position where it can function to cushion flexations of stylet manipulation muscles after the latter attach to the loading sleeve apex. The apparent end-cap/cushion ligation seen in Fig. 9A and others seen in Cicero (2017:646) are not understood, and when studied further may eventually require revising this model.

The gradual curve of the tracking of maxillar ridges from the inner lateral aspect of the specimen in Figs. 1B and 4C may be significant during the fitting process. If a relaxed state of the cuticle was assumed when the stylet was dissected out of the locked position inside its holster, then the curving of the ridges might be an aspect of design rather than a mechanical twist. If so, torsion is implicated in the installation of stylets into their functional positions. In other words, the stylets may be 'screwed' into their functional positions during despooling. The complementary ridges and

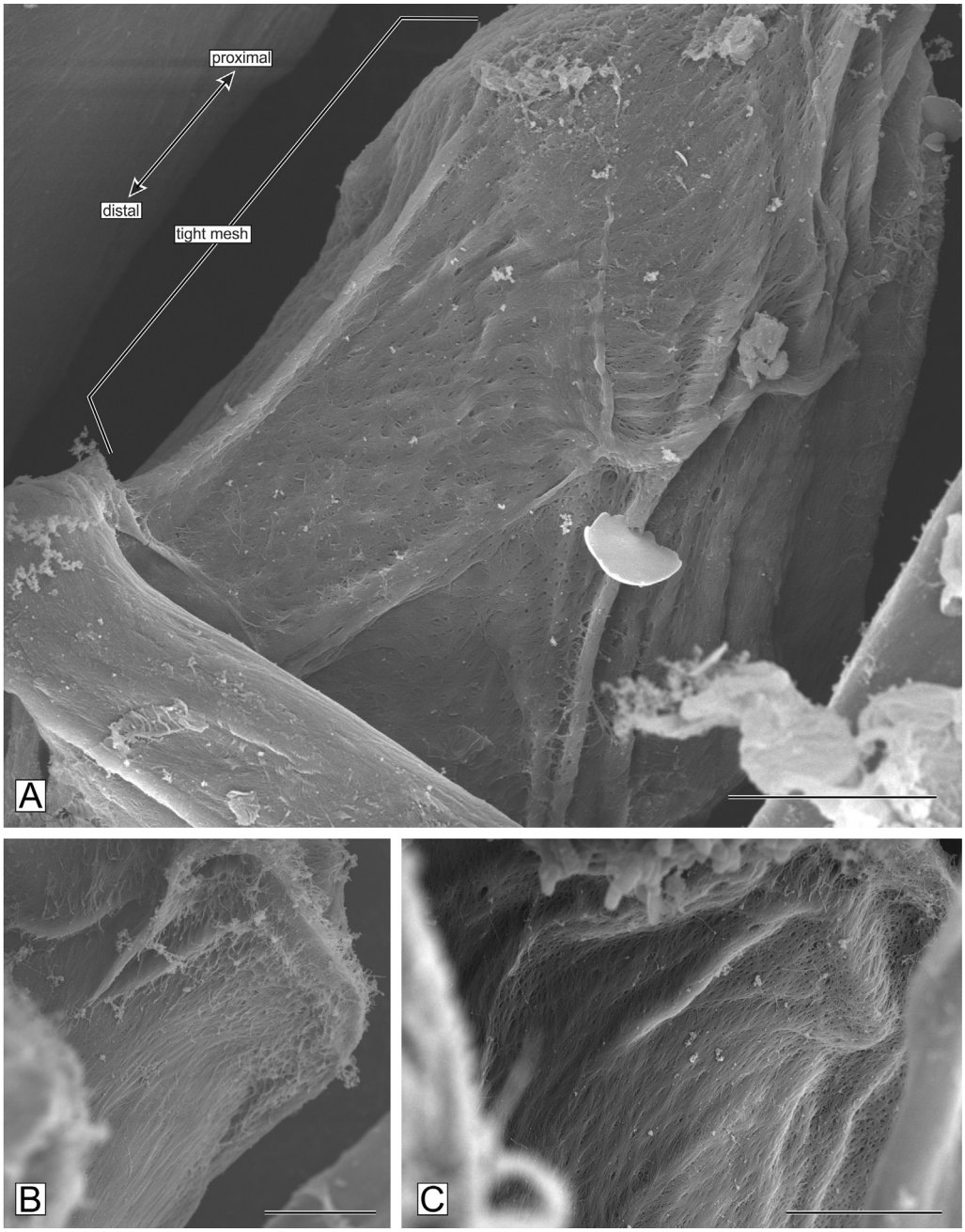

Fig. 7. Inner lateral stylet surfaces from three different potato psyllid specimens. **(A)** Maxillar. Enlargement of Fig. 6D. Loading sleeve is retained. Loading sleeve is a tight fibrous mesh. **(B)**–**(C)** Mandibulars. Loading sleeves are retained. They are a wrinkled, fibrous mesh also, but the meshes are carinate and more loosely woven than in (A). Lines = 5μ.

grooves on the opposing wall of the hypopharynx (Fig. 2) lend some support to this idea (Cicero et al., 2015:755, f.10D).

The maxillar ridges appear to attenuate proximally in Fig. 1B, but the residual loading sleeve hides their point of origin. However, cross-sectioning of the PoP proximal base (Cicero, 2017: 648, f. 3A) shows their absence at that locus. Functionally, the maxillars and mandibulars need to be spaced apart at their proximal base so that muscles can attach to their loading sleeves, and the ridges are not needed until interlock. This correlation is supported empirically in Fig. 2, where it is seen that the ridges are very short in gradual transition to a height and shape needed to conjoin.

The cylindrical projection adhering to the proximal end of stylet bases (Figs. 1C and 4C) matches that of the so-called maxillar and mandibular 'levers' of classical authors (Parsons, 1964), but in this case they are an aspect of the holsters that was twisted out of place during extirpation. The 'levers' of classical authors have not yet been given modern characterization for psyllids and remain tentative with regard to identity, derivation, and function.

The last instar larval stasis configuration has not been detailed, but it is assumed to be comparable to the adult end-cap, since its stylets were formed during the prior (penultimate) pharate instar and despooled also. However, the configuration of the adult end-cap is not definitive in this regard, since the adult does not molt further. It is possible that the larval end-cap is shaped differently and/or deconstructs precociously relative to apolysis of the rest of the body, which would mean that stylet biogenesis may be ongoing intrastadially.

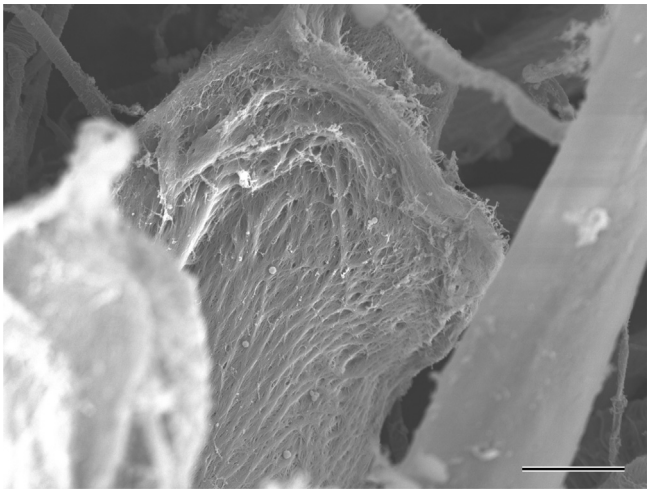

Fig. 8. Potato psyllid maxillar. Different specimen from those in Fig. 7. Inner lateral view of proximal base. Note loose, carinate mesh of loading sleeve fibers. Line = 5μ.

The two earlier authors (Weber, 1929; Pesson, 1951) variously mention observing the stylet replacement apparatus in various ways-- by dissecting larvae, by observing it through the transparent integument of undissected larvae, as well as with the use of coagulant fixatives, sectioning, and staining, without indicating which observations resulted from which preparations. As above, Cicero (2017) based his observations on TEM micrographs of adults, pharate adults and last instar larval exuviae. These differences in technological approach and subject insect led to considerable agreement and disagreement in interpretation and modeling.

All three model builders are in agreement with regard to certain basic aspects of the stylet replacement apparatus. The cells adhering to the ecdysial stylet core walls are continuous with those of the end-cap and those of the tube that surrounds the ecdysial stylet exterior (Fig. 10B) (the 'sheath or muff for stylet implantation'-- '*gaine ou manchon d'implantation des stylets*' of Pesson; the 'loading sleeves' of Cicero). This continuity defines a length of cells that can be referred to as the "core cell-loading sleeve transition" (Fig. 10A). The authors' interpretation of their respective microscopical observations relied critically on identification of this transition because it is the location of the source of the atrium. On apolysis, the core cells, the transitional cells, and the loading sleeve cells disengage from their respective cuticles and all cells in the core lift out, leaving a deep molting space behind. The atrial cells can then be seen as an extension of the loading sleeve cells (Fig. 10B).

The Weber model depicts the cells adherent to the stylet core walls to be small relative to the volume of the hollow, and nucleate. The Pesson and Cicero models recognize that the core is too narrow for the nuclei, and as a consequence, the cytosolic component of these cells remains in its adherent position, but the nuclei are crowded into the end-cap (Fig. 9A). This adherent cytosol is interpreted to be hypodermis and therefore stylet-secreting in function (Fig. 9B), but other cell types may occur in the core too. The end-cap is also interpreted as having other cell types, some of which may be associated with the matrix.

The illustrations of Weber and Pesson indicate that, after apolysis, the core cells that were adherent to the functional stylet core wall, secrete the new stylet, apical terminus first, and gradually elaborate its full length until the basal terminus is completed.

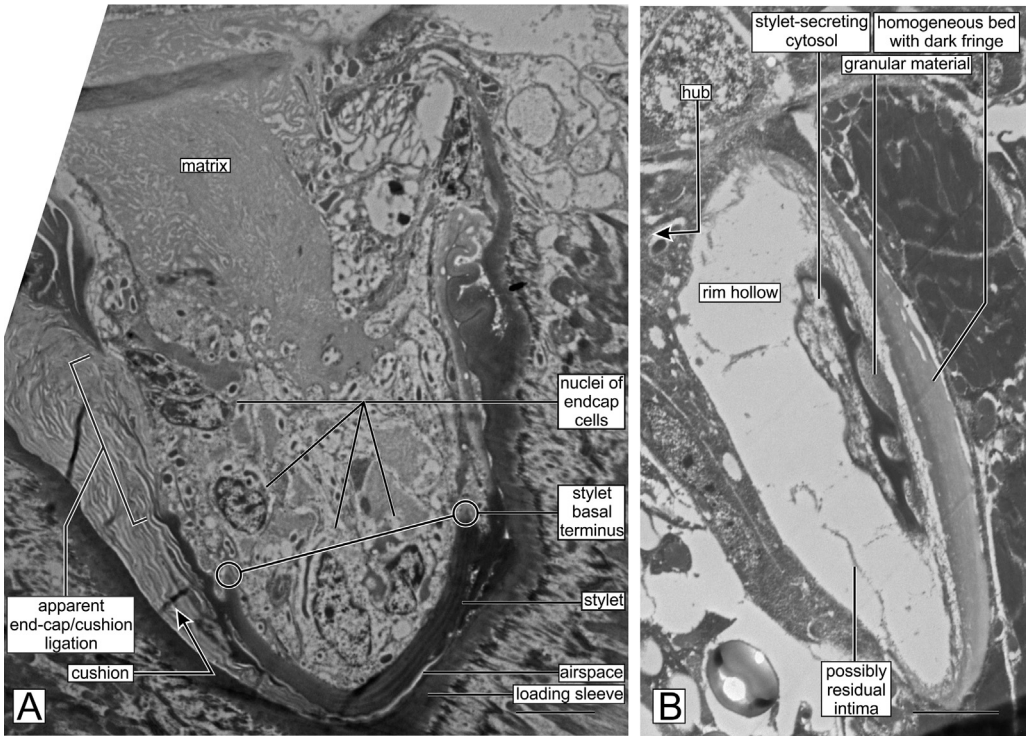

Fig. 9. Asian citrus psyllid, *Diaphorina citri*, stylets. **(A)** Intrastadial specimen. Diagonal section through the basal terminus. The basal terminus is therefore represented by two loci at either end of the stylet cuticle's crescent shape. The matrix is inside the end-cap and the end-cap is seated on the stylet basal terminus or 'flat' (cf. Fig. 1A). Some nuclei occur at this proximal location in association with those that occur in the end-cap, but none are present distally (cf. Fig. 10B, cytosolic extensions of the end-cap cells). The cushion is interpreted to be the intima of the atrium of the prior pharate stage that was collected into a bolus when the stylet despooled to its current position. The apparent ligation between end-cap and cushion is not understood. Line = 3μ. **(B)** Pharate adult. Maxillar atrial rim. The cytoplasm inside the maxillar is a cytosolic extension of one or more of the stylet secreting cells. The rim hollow is a molting space. The rim intima appears to have dissociated during processing, possibly due to poor fixation. Line = 2μ.

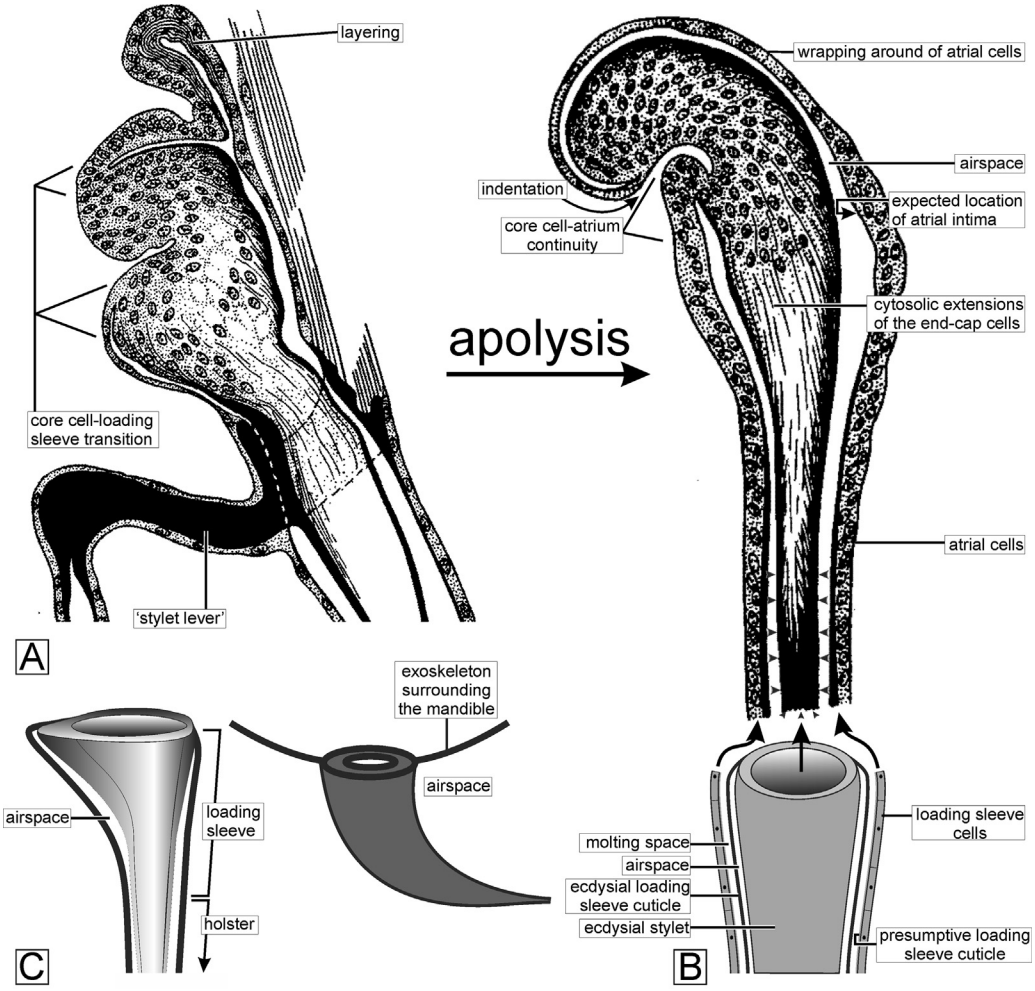

Fig. 10. Diagrams instructive of the dynamic process of end-cap apolysis. **(A)**–**(B)** Modified from (Pesson, 1951:1396, f. 1224). See text for detailed interpretations. **(A)** End-cap of functional stylet. Layering may represent Pesson's visualization of the 'matrix' of extremely flattened cells that expand into the atrial hub. The so-called 'levers' ('*la base du stylet est en rapport avec un levier articulaire*') of Pesson and many other classical authors are not yet clarified. Indications are that they correspond to the cylindrical, belt-shaped rim of the holsters (cf. Fig. 6D). The loading sleeve cell tube is continuous with the end-cap. **(B)** Apolysis of the end-cap of a pharate stage. The loading sleeve cell tube is continuous with the atrial cell tube. Pesson gave no indication of deconstruction of the end-cap in his model. Arrowheads indicate the impending 'front of elaboration'. **(C)** Correlation of invaginated stylets to the primitive configuration of a mandible, intended to illustrate the tenet of 'cuticular continuity'. The walls of the invagination are actually a complex of continuous cuticles, (loading sleeve + holster), but in this simplified rendering they can arguably be considered analogous to the exoskeleton surrounding the mandible.

Cicero did not specifically observe this by tracking the fate of the singular apical membrane surface after it detaches from the core wall to see if it is the exclusive cellular aspect that engages in secretion, but agrees that it can be so modeled. Since the entire length of the presumptive stylet descends into the atrium to await ecdysis, it is suggested that exocytotic activity along the singular apical membrane surface secretes the stylet in a manner somewhat analogous to industrial production of extruded metal shapes. Hence, the singular membrane surface was coined the 'front of elaboration' (Cicero, 2017: 659) (Fig. 10B, arrowheads).

Even with these commonalities, major differences occur between the three models. Inferentially by their text and drawings, both early authors reported the onset of stylet secretion to occur at the base of the atrium, and Pesson (1951) indicated that the secreting cells are actually involuted inside the atrium for the entire secretion process. In contrast, the contemporary model shows the onset of stylet secretion to occur at the apex (Cicero, 2017: 660, f. 14A). This apical position is so modeled because the hub of the atrium is crumbled into a matrix inside the end-cap of adult ACP (Fig. 9A) and PoP, and is preconfigured as a discoid helix that expands outward. Neither the Weber nor the Pesson model identify a matrix, although Pesson did show a series of parallel lines alongside the core cell-loading sleeve transition that may correspond to a matrix in his margarodid (Pesson, 1951) (Fig. 10A). Expansion of these aggregates is herein suggested to be accomplished by aquaporin control of influx of water.

The most perplexing observation along these lines comes from comparison of Pesson's uplifting core cell mass (Fig. 10B) to the TEM micrograph of a 'hub aggregate' with an apparently nearly completed mandibular within its rim (Cicero, 2017: 655, f. 11A). Both are very similar in their ovoid, indented shape and cell type, with nuclei at one end and cytosolic extensions at the other. Moreover, Pesson shows two cross-sectional aspects of a tube of cells originating from the indentation and extending outward into continuity with the atrium which, in turn, is in continuity with the loading sleeves. This extension is clearly the "core cell-loading sleeve transition" mentioned above, and at least one such cross-sectional aspect is present in the TEM micrograph of the hub aggregate. However, according to this micrograph, the hub cells converge into the indentation of the hub aggregate, and there is no indication that the basal end of the mandibular is sourced from it.

Two other aggregates, the rim and companion aggregates, were not indicated in the early authors' works. Further, the early

authors showed that on onset of apolysis, the cells associate with the stylet core somehow, by cell biological means unknown to them, produce a tube, the atrium, that extends outward and actively winds into a coil in the hemocoel, where tracheae and other obstructions would interfere. Pesson showed the full diameter of the atrium to be preoccupied by coils. Neither model recognized a hub that affords the coils (e.g. the rims) a wide, helical circumference that would facilitate a freshly secreted stylet with a smoothly curving path rather than a tight one. Neither recognized that the apical cell membranes facing the molting space inside the atrium (the "*cavité de la gaine interne*" of Pesson) are lined with an intima.

Weber's (1929: 98, abb. 15) placement of onset of stylet secretion inside the ecdysial stylet core and his putative observation that the presumptive stylet apical terminus is attached to the ecdysial stylet core wall are somewhat supportive of each other. That is, the stylet apex might be "sticky" and able to adhere to the ecdysial core wall, when freshly secreted at that locus, whereas, if secreted at the apex of the fully expanded atrium, the stylet apical terminus would have to descend through its full length before it reaches the ecdysial stylet to adhere to its core. Weber interpreted this attachment as the mechanism by which the presumptive stylet is pulled out of the atrium as the insect lifts away from the exuviae (1929:97): "*Da die neue Borste mit der Spitze an der Basis der alten haftet, wird sie von der letzteren bei der Häutung selbsttätig in die Lage der alten gebracht, erst wenn das vollzogen ist und die neue Borste völlig gestreckt in die richtigen Bahnen gebracht ist, wird die alte Borste vollends von ihr losgerissen*." Pesson (1951: 1399, Fig. 1226) drew this attachment in one figure, but not in another (p. 1397, f. 1225), and does not mention it in his text. The only known features of the homopteran stylet apex are grooves on the outer lateral mandibular surface, and an acrostyle on the inner lateral maxillar surface (Uzest et al., 2010). Garzo et al. (2012) counted 10 grooves for ACP and indicated that there is no acrostyle. There is no report of a cicatrix or scar in the literature that might suggest a detachment site (but see Fig. 5B, C).

Continued investigation of ACP stylet replacement needs to target the stasis configuration in the larval stylet end-cap, its deconstruction into the atrium, presumptive stylet secretion *in vivo* using contrast features of modern light microscopy, and most importantly, whether "*Ca*." L. asiaticus is able to infiltrate this system in such a way as to use it as a portal of exit in a previously unrealized transmission pathway.

Author Contributions

JMC performed the microscopy and composed the manuscript. JA-T founded one of the themes of the study using microCT technology. WBH reviewed the manuscript in its formative versions. SS and LAM contributed with database support and presentation of the animation. WBH, LMC, SS, LAM and SJB were the Principal Investigators administrating the research directive.

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References

Chapman, R.F., 1998. The Insects: Structure and Function, fourth ed. Cambridge Univ. Press, NY.

Cicero, J.M., 2017. Stylet biogenesis in *Bactericera cockerelli* (Hemiptera: Triozidae). Arthropod Struct. Dev. 46, 644–661. https://doi.org/10.1016/j.asd.2016.12.007.

Cicero, J.M., Stansly, P.A., Brown, J.K., 2015. Functional anatomy of the oral region of the potato psyllid (Hemiptera: Psylloidea: Triozidae). Ann. Entomol. Soc. Am. 108 (5), 743–761. https://doi.org/10.1093/aesa/sav059.

Cicero, J.M., Alba-Tercedor, J., Hunter, W.B., Cano, L.M., Saha, S., Brown, S.J., 2018. An Animated Correspondence of Asian Citrus Psyllid Stylets to the Model for Biogenesis of Potato Psyllid Stylets. https://citrusgreening.org/microtomography/cicero_stylet_2018. Last viewed, June 27, 2018.

Davidson, J., 1913. The structure and biology of *Schizoneura lanigera*, Huasmann or woolly aphis of the apple tree. Q. J. Microsc. Sci. 58, 653–701.

Garzo, E., Bonani, J.P., Lopes, J.R.S., Fereres, A., 2012. Morphological description of the mouthparts of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Arthropod Struct. Dev. 41, 79–86. https://doi.org/10.1016/j.asd.2011.07.005.

Jenkin, P.M., Hinton, H.E., 1966. Apolysis in arthropod molting cycles. Nature 211 (5051), 871. https://doi.org/10.1038/211871a0.

Parsons, M.C., 1964. The origin and development of the hemipteran cranium. Can. J. Zool. 42, 409–432. https://doi.org/10.1139/z64-036.

Pesson, P., 1951. Ordre des Homopteres (Homoptera Leach, 1815). In: Grasse, P.P. (Ed.), Traite de Zoologie: Anatomie, Systematique, Biologie, vol. 10, II. Masson, Paris, France. https://doi.org/10.1126/science.115.2990.432.

Pinet, J.M., 1968. Structure et formation des coaptations des stylets maxillaires de *Rhodnius prolixus* [Heter. Reduviidae]. Ann Soc Entomol France (NS) 4 (2), 455–475.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: a Laboratory Manual, second ed. Cold Spring Harbor Laboratory Press, New York, NY.

Uzest, M., Gargani, D., Dombrovsky, A., Cazevieille, C., Cot, D., 2010. The "acrostyle": a newly described anatomical structure in aphid stylets. Arthropod Struct. Dev. 39, 221–229. https://doi.org/10.1016/j.asd.2010.02.005.

Weber, H., 1929. Kopf und Thorax von *Psylla mali* Schmidb. (Hemiptera-Homoptera). Eine morphogenetische Studie. Z Morphol Ökol Tiere 14, 59–165. https://doi.org/10.1007/bf00419345.

Wigglesworth, V.B., 1973. The significance of "apolysis" in the moulting of insects. J. Entomol. 47 (2), 141–149.