An animated correspondence of Asian citrus psyllid stylets to the model for biogenesis of potato psyllid stylets

Joseph M. Cicero,[1] Javier Alba-Tercedor,[2] Wayne B. Hunter,[3] Liliana M. Cano,[4] S. Saha,[5] Lukas Mueller,[5] Susan J. Brown[6]

Hemiptera shed their stylets with the exuviae during each molt to the next instar. New stylets are manufactured inside consecutive pharate instars to replace them, and, as each new instar lifts away from the exuviae, these are fitted into their functional positions so that feeding can be resumed. The discovery that biofilms of the bacterium "*Candidatus*" Liberibacter solanacearum occur on the adult stylet replacement apparatus of the potato psyllid, *Bactericera cockerelli* (Sulc, Triozidae, PoP) led to hypothesizing that the same may be true for 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 new instars resume feeding. This hypothesis prompted intensive study of PoP mouthparts and development of a model for stylet replacement. The following presentation demonstrates positive correlation of ACP mouthparts to key features of the model.

The manufacture ('biogenesis') and fitting ('despooling') of new (presumptive) stylets into their functional (intrastadial) positions is an extremely complex, dynamic process, and traditional publication platforms are inadequate for elucidating it. Therefore, this animation was crafted to simplify exposition of the potato psyllid model so that it can be understood quickly and easily. Only a basic comprehension of textbook molting events is needed, i.e. apolysis, molting space, secretion of new cuticle, and ecdysis. This approach will allow future researchers to identify anatomical features of the ACP oral region in their TEM cross-sections.

This work was funded by USDA-NIFA grant award 2015-70016-23028:
Developing an infrastructure and product test pipeline to deliver novel therapies for citrus greening disease.
Lead, Susan J. Brown

[1] Entomology and Nematology Dept., 1881 Natural Area Dr., University of Florida, Gainesville 32611
[2] Dept. de Zoologia, Facultad de Ciencias, Universidad de Granada 18071, Spain
[3] USDA-ARS, Horticultural Research Lab, 2001 S. Rock Road, Fort Pierce, FL 34945
[4] IRREC, University of Florida, 2199 S. Rock Rd., Ft. Pierce, FL 34945
[5] Boyce Thompson Institute, 533 Tower Road, Ithaca, NY 14853
[6] Dept. Biology, Ackert Hall, Kansas State University, Manhattan, KS 66506

Methods

Two hundred eighty-six sequential animation frames representing the model proposed for the stylet replacement process in the potato psyllid (Cicero 2017) were drawn using CorelDRAW® X8 2016 (Corel Corporation, Ottawa, Ontario, Canada). The frames were exported as jpegs, and imported into animation sequences on an Adobe Animate CC HTML5 canvas (Adobe Systems Inc. San Jose,CA).

Heads of ACP were decapitated and their tissues digested with 75.0µg/ml proteinase K in 1.0% Triton X100, 45ºC overnight, and critical point dried and sputter coated for scanning electron microscopy.

Heads of ACP adults and pharate last instar larvae were fixed overnight in 1M Na^+ K^+ phosphate buffered saline (pH 7.8), 4% formaldehyde, 1.5% glutaraldehyde, then rinsed, dehydrated in a graded ethanol series, infiltrated with LR White embedment medium (25%, 75%, 100%) (Electron Microscopy Supplies, Hatfield, PA), and polymerized at 60º C. Semi-thin sections were stained with Toluidine Blue 0 (Sigma). Ultrathin sections were stained with UranyLess EM stain (EMS) and lead citrate. Light, SEM and TEM of ACP stylets corresponding to key features of the PoP model were added to the video.

Cicero, J.M. 2017. Stylet biogenesis in *Bactericera cockerelli*. Arthropod Structure and Development. 46: 644-661. https://doi.org/10.1016/j.asd.2016.12.007

Select frames

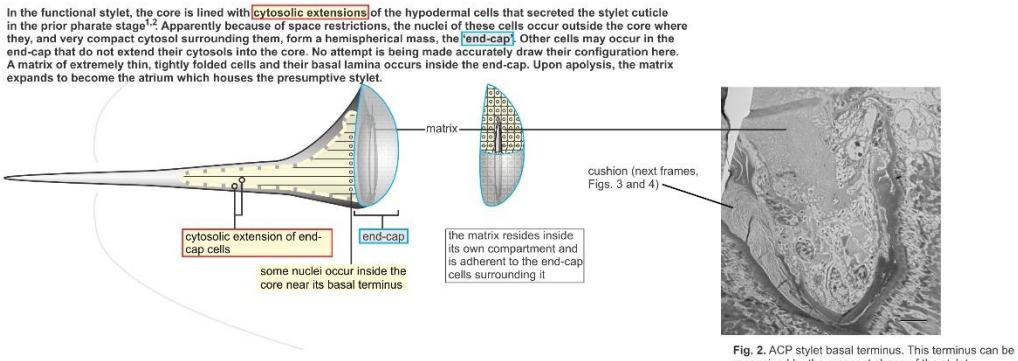


Fig. 2. ACP stylet basal terminus. This terminus can be recognized by the crescent shape of the stylet in diagonal section. The matrix is mass of tightly folded cells that served as the atrium when the stylet was being secreted. Line = 3μ.

[1] 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

[2] Cicero, J.M. 2017. Stylet biogenesis in *Bactericera cockerelli*. Arthropod Structure and Development. 46: 644-661. https://doi.org/10.1016/j.asd.2016.12.007

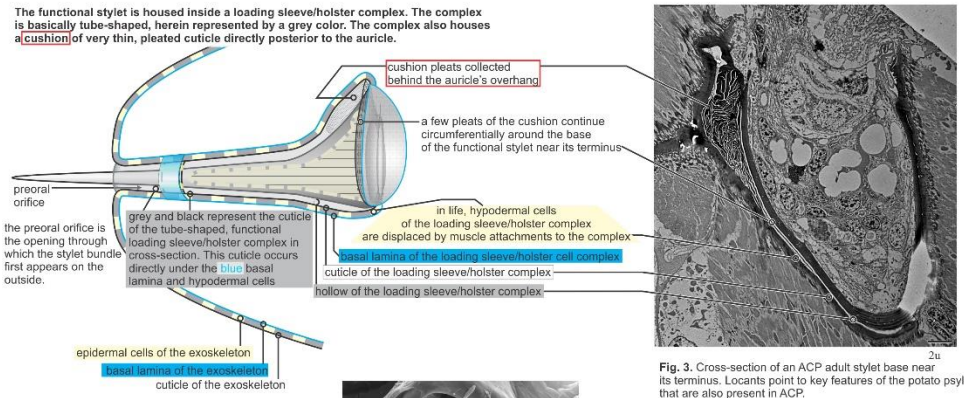


Fig. 3. Cross-section of an ACP adult stylet base near its terminus. Locants point to key features of the potato psyllid that are also present in ACP.

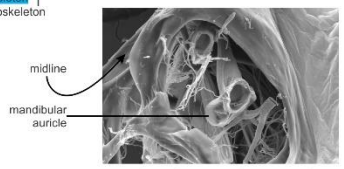


Fig. 4. SEM of the lateral fenestration of an ACP tentorium showing the interiorly directed stylet auricle.

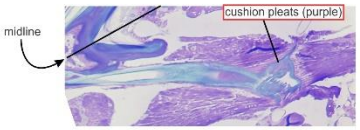
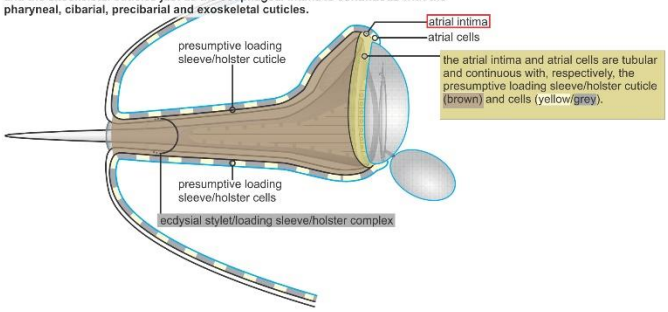
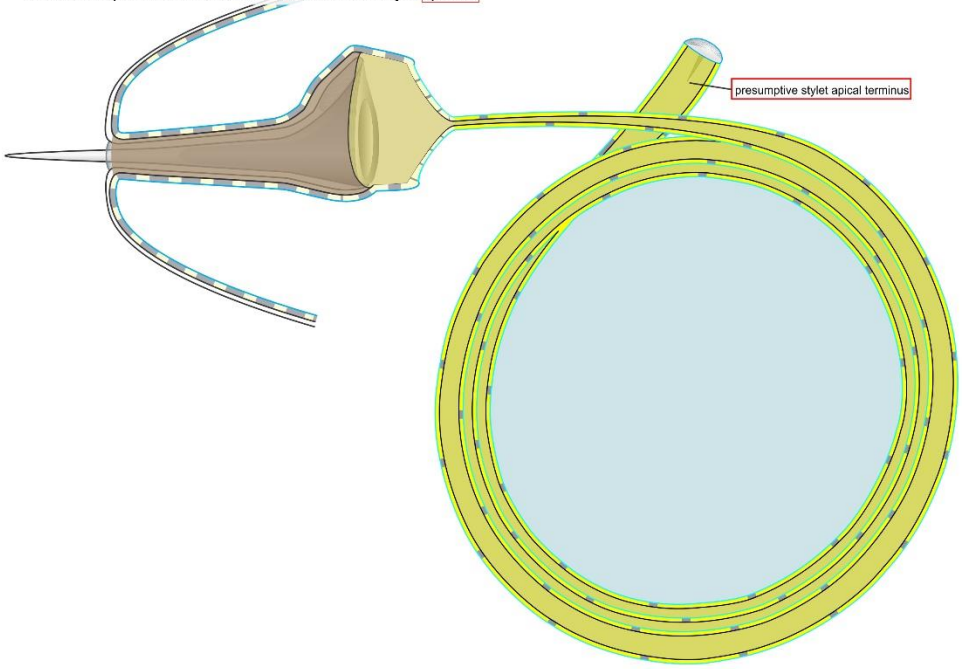


Fig. 5. Light micrograph of a longitudinal section of an adult ACP stylet confirming that the cushion is directly posterior to the auricle, which in turn is directed interiorly relative to the midline.

The cells of the atrium secrete an intima at some unknown point in the expansion of the matrix. The intima is continuous with the presumptive loading sleeve/holster cuticles and the exoskeletal cuticles just as the esophageal intima is continuous with the pharyneal, cibarial, precibarial and exoskeletal cuticles.



The cells at the apex of the atrium are modeled to secrete the new stylets apex first



Despooling of the presumptive stylet follows its synthesis. It is animated in Cicero (2017) but not in this presentation.

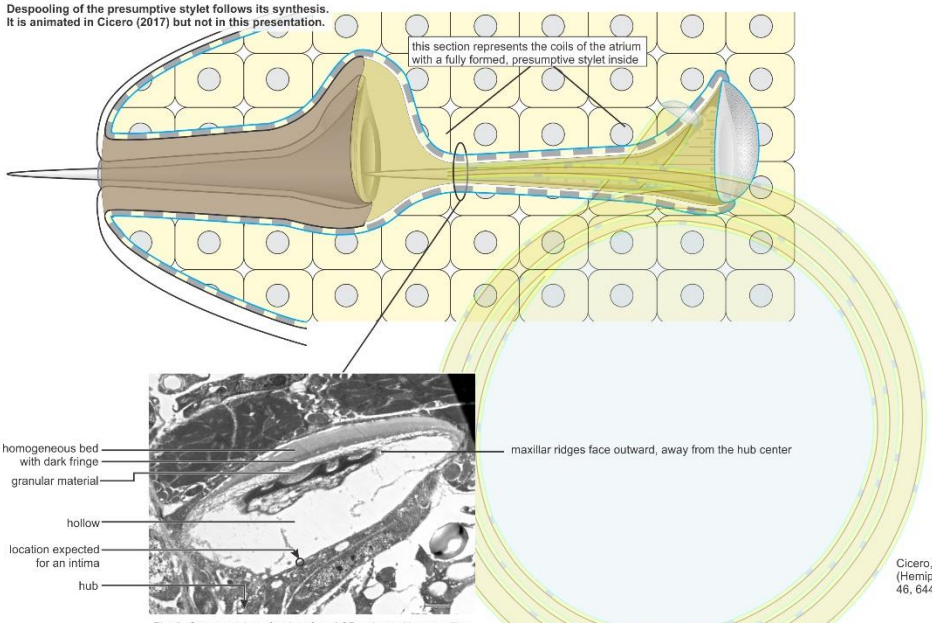
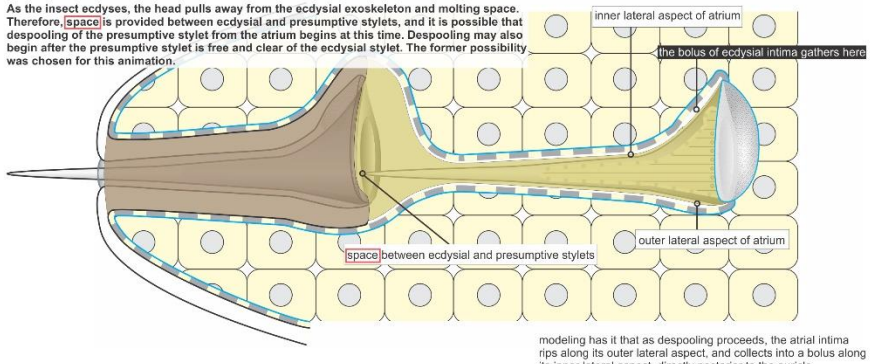


Fig. 9. Cross-section of a rim of an ACP atrium with a maxillar inside. Key features of the PoP model are present as indicated. An intima did not resolve with processing but Figure 10 shows its presence in a specimen of a head digested with proteinase K. Line = 2µ.

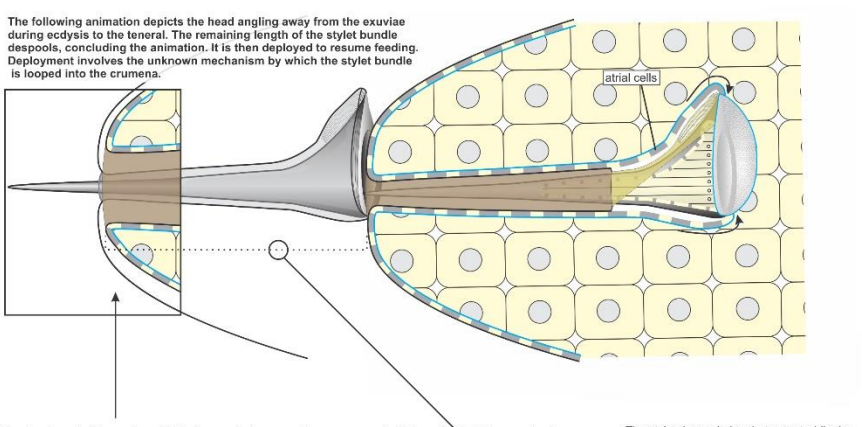
Cicero, JM. (2017) Stylet biogenesis in *Bactericera cockerelli* (Hemiptera: Triozidae) Arthropod Structure and Development 46, 644-661. https://doi.org/10.1016/j.asd.2016.12.007

As the insect ecdyses, the head pulls away from the ecdysial exoskeleton and molting space. Therefore, space is provided between ecdysial and presumptive stylets, and it is possible that despooling of the presumptive stylet from the atrium begins at this time. Despooling may also begin after the presumptive stylet is free and clear of the ecdysial stylet. The former possibility was chosen for this animation.



modeling has it that as despooling proceeds, the atrial intima rips along its outer lateral aspect, and collects into a bolus along its inner lateral aspect, directly posterior to the auricle.

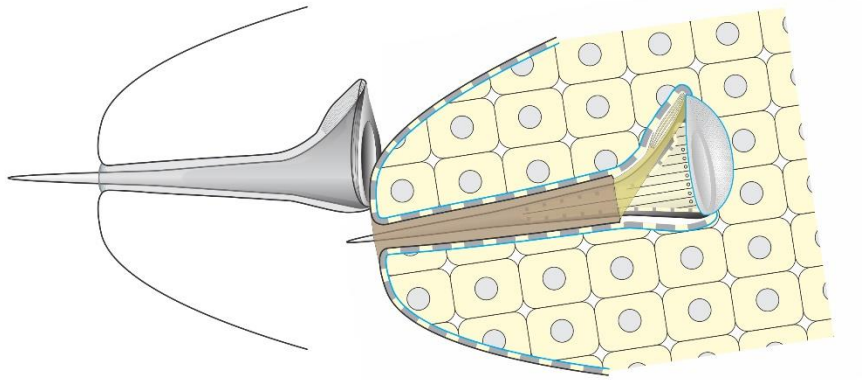
The following animation depicts the head angling away from the exuviae during ecdysis to the teneral. The remaining length of the stylet bundle despools, concluding the animation. It is then deployed to resume feeding. Deployment involves the unknown mechanism by which the stylet bundle is looped into the crumena.



The head was in this position at left when apolysis occurred.....

.....and withdrew for this distance, clearing the exuviae so that it could angle away from it.....

.....The stylet despooled to that extent while the atrial cells passed over it (arrows) to reconstruct the end-cap. In keeping with the model, as the head angles and lifts away from the exuviae, the stylet finishes despooling.



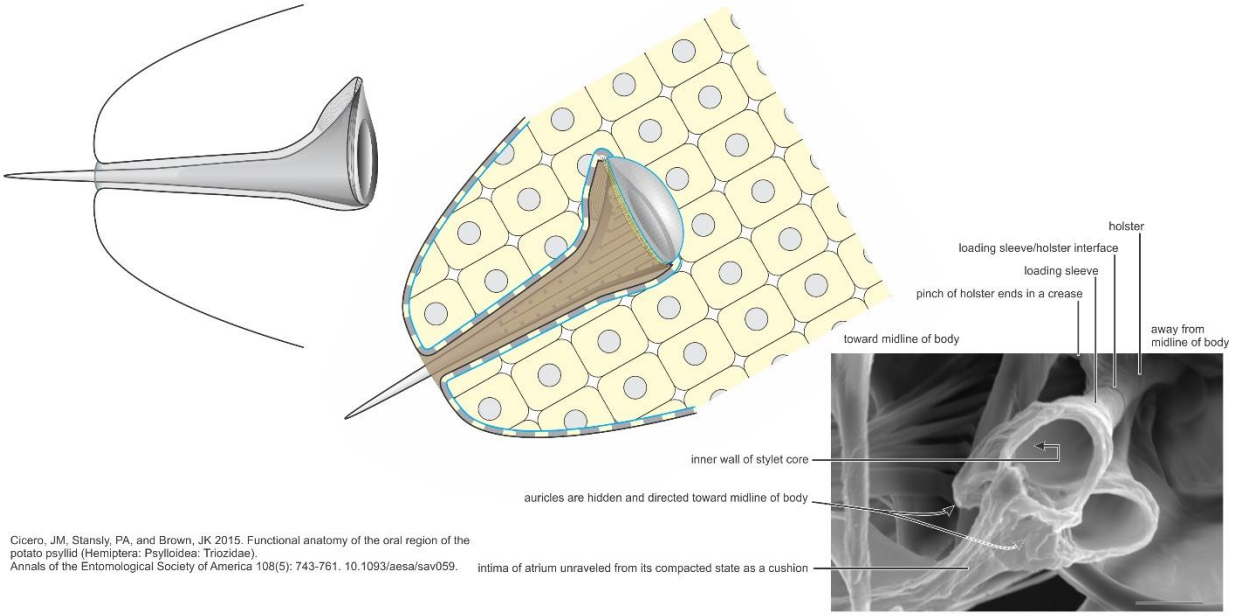


Figure 10. Anterior view of the stylet bases of an adult ACP cleared with proteinase K. The undigested structures are cuticles of the tentorium. The holster pinches away from the side panels and the pinch ends in a crease, but the tubular shape of the holster that the stylet resides in continues anteriorly as a loading sleeve (Cicero et al. 2015:750, f. 6Cq; 752, f. 7Be,f). Line = 10µ.

Cicero, JM, Stansly, PA, and Brown, JK 2015. Functional anatomy of the oral region of the potato psyllid (Hemiptera: Psylloidea: Triozidae).
Annals of the Entomological Society of America 108(5): 743-761. 10.1093/aesa/sav059.