RETRACTED STYLETS IN NYMPHS OF THE ASIAN CITRUS PSYLLID ARE HELD EXTERNALLY AGAINST THE CLYPEUS BY A SPECIAL PAIRED ORGAN NOT FOUND IN THE ADULTS

El-Desouky Ammar* and David G. Hall
USDA-ARS, US Horticultural Research Laboratory, Fort Pierce, FL 34945, USA

*Corresponding author; E-mail: eldammar@hotmail.com

Supplementary material for this article in Florida Entomologist 96(1) (2013) is online at http://purl.fcla.edu/fcla/entomologist/browse

The Asian citrus psyllid *Diaphorina citri* Kuwayama (ACP, Hemiptera, Psyllidae) is the main vector of the bacterium '*Candidatus* Liberibacter asiaticus' associated with huanglongbing (HLB), one of the most devastating citrus diseases worldwide. The piercing sucking mouthparts play an important role in acquisition and transmission of this bacterium, and late instar nymphs of ACP are more efficient transmitters than adults (Inoue et al. 2009). Ultrastructure of the mouthparts in ACP adults has been described by Garzo et al. (2012), but that of nymphs has not yet been described. In the present work, a scanning electron microscopy (SEM) study was undertaken to compare the mouthparts of nymphs and adults of ACP as well as those of the melaleuca psyllid (*Boreioglycaspis melaleucae* Moore; Hemiptera: Psyllidae).

Healthy (non-HLB infected) nymphs and adults of ACP were taken from a laboratory colony reared on healthy citrus plants (*Citrus macrophylla* Wester; Sapindales: Rutaceae). Leaves and young shoots of melaleuca trees (*Melaleuca quinquenervia* (Cav.) S. T. Blake; Myrtales: Myrtaceae) infested with nymphs and adults of the melaleuca psyllid were provided by Matthew Hentz (USDA-ARS, Fort Pierce, Florida). Psyllid nymphs or adults were fed in groups (5-10/group) on small pieces of young shoots or excised leaves of their respective hosts in Petri-dishes or 50-mL polypropylene tubes as described by Ammar & Hall (2012).

Most exuviae of nymphal instars in psyllids and other hemipterans have fully extended stylets that usually remain attached to their host plants (Ammar & Hall 2012). Thus, to study the mouthparts of nymphs or adults in their extended state exuviae or live insects were used. Live nymphs and adults (immobilized during feeding with chloroform vapor) were pulled out gently from the plant parts they were feeding on using fine forceps. However, to study the mouthparts during non-feeding periods, psyllid nymphs or adults that were kept in glass tubes for 1-3 h away from any plant material were used. For SEM, exuviae or insects were prepared, coated and examined as described by Ammar & Hall (2012). To study partially extended stylets of feeding nymphs, live 5th instar ACP nymphs were caged in small plastic Petri-dishes covered with transparent membrane (Morgan et al. 2012), and while probing through this membrane, they were photographed by a stereomicroscope (Leica M60) fitted with a video camera (Leica DFC290 HD) (Leica, Switzerland). Supplementary material for this article in Florida Entomologist 96(1) (2013) is online at http://purl.fcla.edu/fcla/entomologist/browse; in this link a color version of a feeding 5th instar nymph is shown.

During feeding by ACP nymphs and adults on plant hosts, the stylets were fully or partially extended while the base of the compact stylet bundle was housed in the labial groove (Figs. 1A, 1C, 2A & 2B). In the labium of ACP nymphs, only 2 segments were observed (Figs. 1B & 1C) compared to 3 segments found in the adult labium (Garzo et al. 2012). In nymphs, 2 large clamps on each side of the labial groove of the first (basal) labial segment were observed to hold the base of the stylet bundle firmly in place during feeding (Fig. 1C). On each side of the second (terminal) labial segment in ACP nymphs at least 5 sensilla at the tip and 4 sensilla on the anterior face were found (Figs. 1C & 1D). Only 4 sensilla on each side of the labial tip have been reported in the adult ACP (Garzo et al. 2012). However, the 2 long setae on each side of the terminal (third) labial segment in adults (Fig. 2B) have not been found on the labium of ACP nymphs (Figs. 1B & 1C). Also, the labrum reported earlier in psyllid adults (Weber 1928, Ullman & McLean 1986), was not observed in ACP nymphs (Figs. 1B and 3A). The terminal part of the mandibular stylets in ACP nymphs is serrated (Fig. 1E) as is the case with ACP adults (Garzo et al. 2012). The salivary flange (top of the salivary sheath formed during feeding) was frequently observed around the stylet bundle in both nymphs (Fig. 1E) and adults (Figs. 2A and 2B).

In ACP nymphs, a paired organ that we will call 'stylet-holding organ' was found very close to the base of the labium between the 2 front coxae (Figs. 1A, 1B, 3A and 3B, Suppl. 3B). In ventral view, this organ appeared as 2 convex banana shaped projections, slightly curved inward, pointed at the tip, and measured 77-138 µm in length and 20-50 µm in width in various instars. Howev-

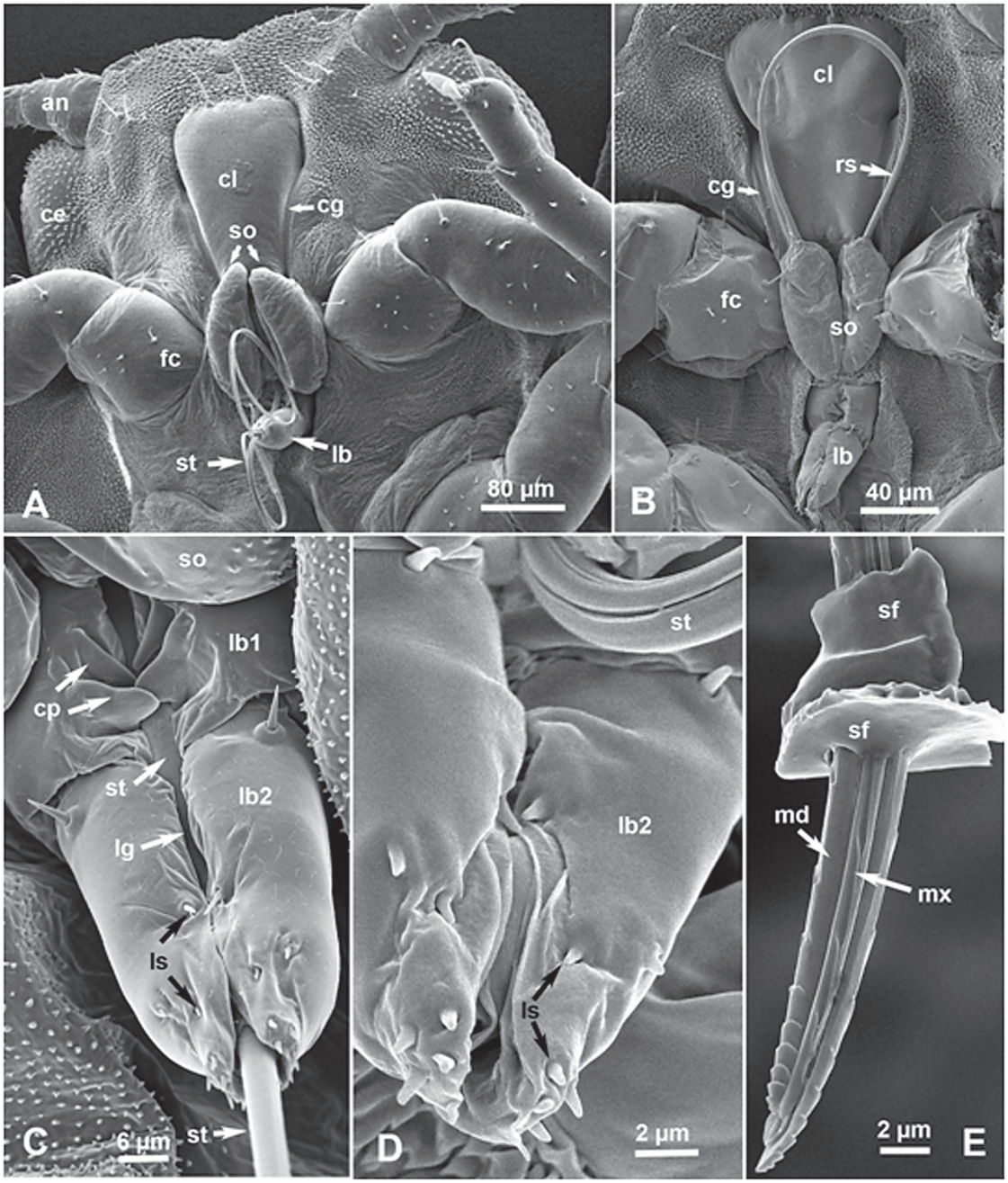

Fig. 1. SEM of the head and mouthparts of *Diaphorina citri* nymphs, either with extended stylets (A, C, D & E) or with retracted/looped stylets (B); ventral views except in E side view. Abbreviations an, antenna; ce, compound eye; cg, clypeal groove, cl, clypeus; cp, clamp; lb, labium; lb 1 & lb 2, labial segments 1 & 2; lg, labial groove; ls, labial sensilla; md, mandibular stylets; mx, maxillary stylets; rs, retracted/looped stylets; sf, salivary flange; so, stylet-holding organ; st, stylets.

er, in lateral (or ventro-lateral) view these projections appeared as flat, curved, flaps/plates (Fig. 3A). The role of these projections, which were not found in ACP adults, is apparently to hold the retracted/looped stylets behind them externally against the clypeus during the non-feeding periods (Fig. 1B) as well as during partial probing/feeding when the stylets are not fully extended

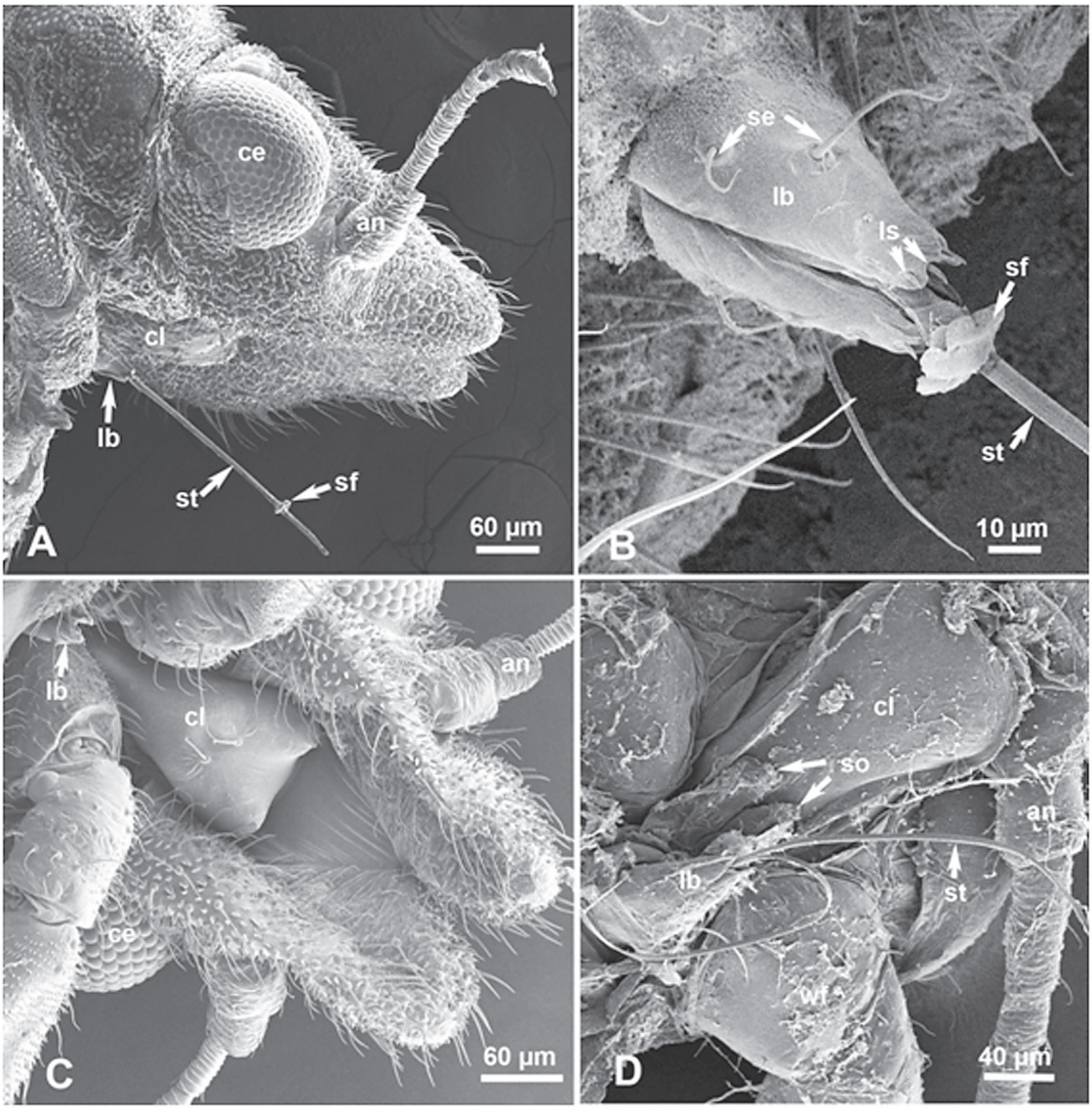

Fig. 2. SEM of the head and mouth parts of *Diaphorina citri* adults (A-C) and melaleuca psyllid nymph, *Boreioglycaspis melaleucae* (D); ventro-lateral views except in D ventral view. Abbreviations: an, antenna; ce, compound eye; cl, clypeus; lb, labium; ls, labial sensilla; se, setae; sf, salivary flange; so, stylet-holding organ; st, stylets, wf, waxy filaments (secreted by the melaleuca psyllid).

(Fig. 3B; Suppl. Fig. 3B). A groove around the clypeus, 'the clypeal groove', appeared to house the retracted or looped stylets, especially the part of the loop closer to the labium (Figs. 1B & 3A). It is possible that this groove may facilitate the retraction and extension movements of the stylets during feeding and/or probing. The retracted/looped stylets are not visible externally in adults (Fig. 2C). They are housed instead in the crumena, a membranous bag inside the prothorax or head of ACP, other psyllids, and some other hemipterans (Garzo et al. 2012).

The 'stylet-holding organ' described above was also found in nymphs, but not in adults, of the melaleuca psyllid (Fig. 2D) and is likely to be found in nymphs of other psyllids. To our knowledge, this organ has not been described earlier in *D. citri* nymphs (Husain & Nath 1927), but docking of the retracted/looped stylets outside the heads of nymphs has been illustrated in other psyllid species (Brocher 1925; Weber 1928; Snodgrass 1935). Since the labrum, reported earlier in psyllid adults (Weber 1928; Ullman & McLean 1986) has not been observed in ACP nymphs, it is pos-

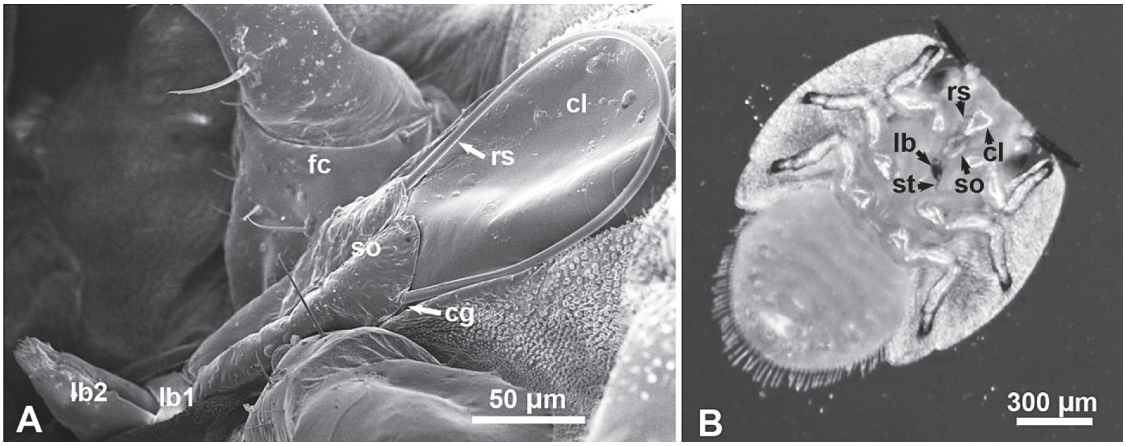

Fig. 3. A. SEM (ventro-lateral view) of the head of *Diaphorina citri* showing the flat aspect of the stylet-holding organ (so). B. A micrograph taken from a video of a feeding 5th instar *D. citri* nymph, showing the extended tip of the stylets (st) and the partially retracted/looped part of the stylets (rs): cg, clypeal groove, cl, clypeus; lb, labium; lb1 & lb2, labial segments 1 & 2; rs, retracted/looped stylets; so, stylet-holding organ. NOTE: A video and color image of a feeding 5th instar nymph (Suppl. Fig. 3B) are available online at http://purl.fcla.edu/fcla/entomologist/browse .

sible that the stylet-holding organ described here might be a 'modified labrum', but this requires further investigation. Also, the fact that fully or partially retracted stylets in psyllid nymphs are visible externally, unlike the case in adults, makes larger nymphs ideal for studying the feeding mechanism and stylet movement of ACP and other psyllids in future research on this economically important group of hemipteran insects.

We thank the Florida Citrus Research Foundation for funding this research. We are also grateful to the 3 anonymous reviewers and to Dr. Joseph M. Cicero (School of Plant Sciences, University of Arizona) for their comments and suggestions that helped improve an earlier version of our manuscript.

Summary

Differences in ultrastructure of the mouthparts in nymphs and adults of the Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae), vector of the bacterium associated with citrus huanglongbing disease, were studied using scanning electron microscopy. The number of sensilla on the labial tip in nymphs was greater than that in adults. Furthermore, during the resting (non-feeding) periods, the retracted/looped stylets in the adults are hidden inside a bag called the crumena, whereas the fully or partially retracted stylets in nymphs are externally visible between the clypeus and 2 large projections, not found in the adults, at the base of the labium. These projections, that we called 'stylet-holding organ', were found also in nymphs of the melaleuca psyllid, *Boreioglycaspis melaleuca*, retracted/looped stylets.

Key Words: *Boreioglycaspis melaleuca*, stylet-holding organ, stylets, pysillids, Hemiptera

References Cited

Ammar, E.-D., and Hall, D. G. 2012. New and simple methods for studying hemipteran stylets, bacteriomes, and salivary sheaths in host plants. Ann. Entomol. Soc. Am. 105: 731-739.

Brocher, F. 1925. L'appareil buccal des larves de *Psylla pyrisuga* Fstr. Etude anatomique et physiologique. Ann. Soc. entom. Fr. 94: 55-66.

Garzo, E., Bonani, J. P., Lopes, J. R. S., and Fereres, A. 2012. Morphological description of the mouthparts of the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). Arthrop. Struc. Develop. 41: 79-86.

Husain, M. A., and Nath, D. 1927. The citrus psylla (*Diaphorina citri*, Kuw.) [Psyllidae: Homoptera]. Mem. Dept. Agr. India, Entomol. Series 10: 27 pp.

Inoue, H., Ohnishi, J., Ito, T., Tomimura, K., Miyata, S., Iwanami, T., and Ashihara, W. 2009. Enhanced proliferation and efficient transmission of *Candidatus* Liberibacter asiaticus by adult *Diaphorina citri* after acquisition feeding in the nymphal stage. Ann. Appl. Biol. 155: 29-36.

Morgan, J. K., Shatters, R. G., Egan, E. A., Luzio, G. A., Ammar, E.-D., Hunter, W. B., and Hall, D. G. 2012. Plant or diet interactions are not required for stylet sheath formation in phytophagous hemipterans from six species (Suborders: Auchenorrhyncha and Sternorrhyncha). Florida Entomological Society Meeting, July 2012, Jupiter, FL. (Abstract).

Snodgrass, R. E. 1935. Principles of Insect Morphology, McGraw-Hill, N.Y. 667pp.

Ullman, D. E., and McLean, D. L. 1986. Anterior alimentary canal of the pear psylla, *Psylla pyricola* Forester (Homoptera, Psyllidae). J. Morphol. 189: 89-98.

Weber, H. 1928. Zur vergleichenden Physiologie der Saugorgane der Hemipteran. Zeitschr. vergl. Physiol. 8: 145-186.