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# Analysis of the retort organs, producing mouthpart cuticle, in (the pea) aphid

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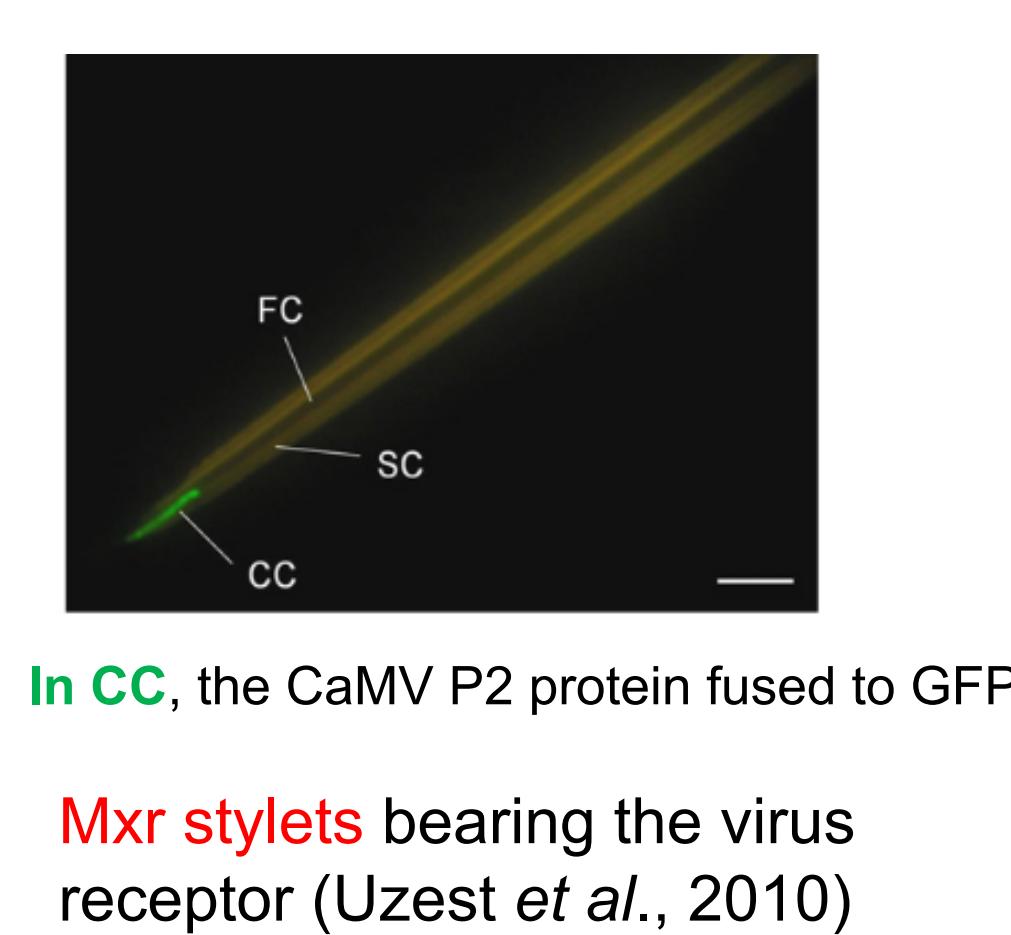
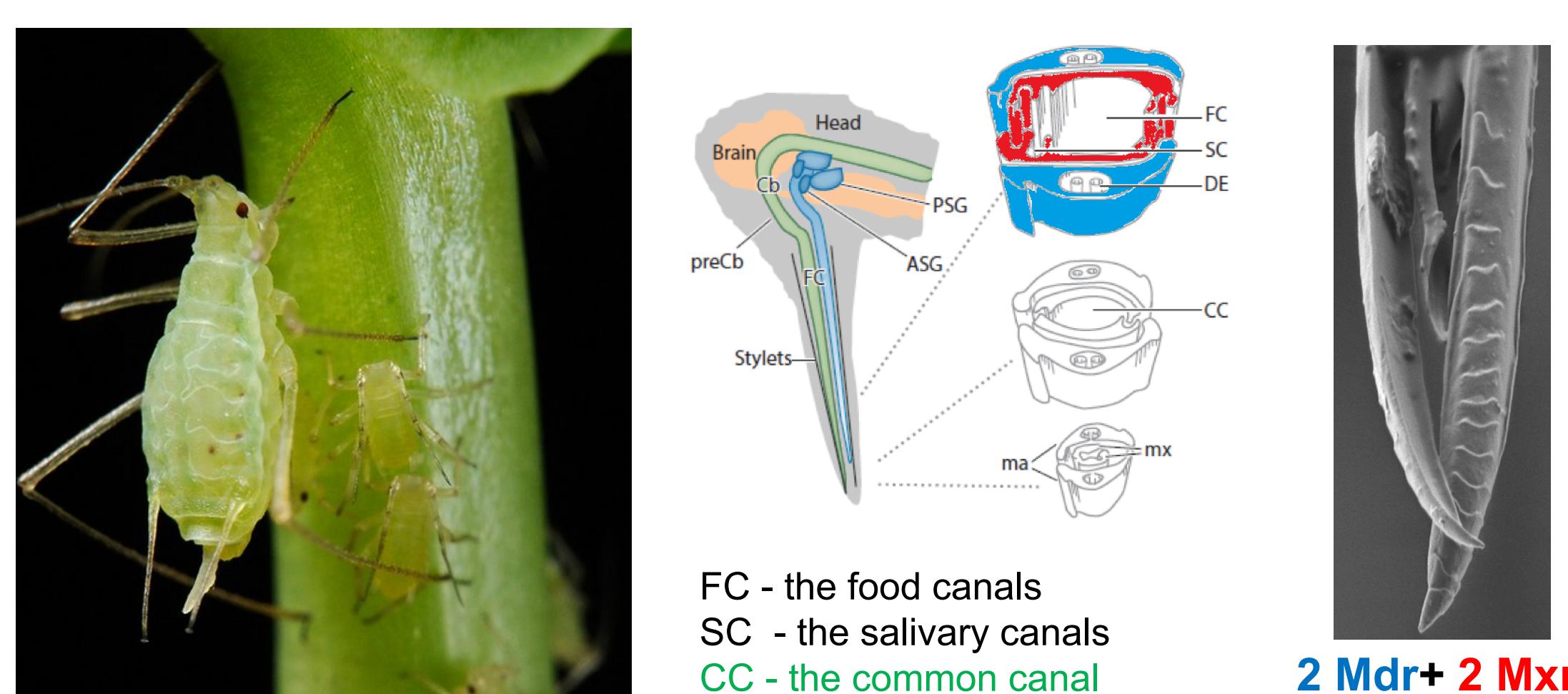
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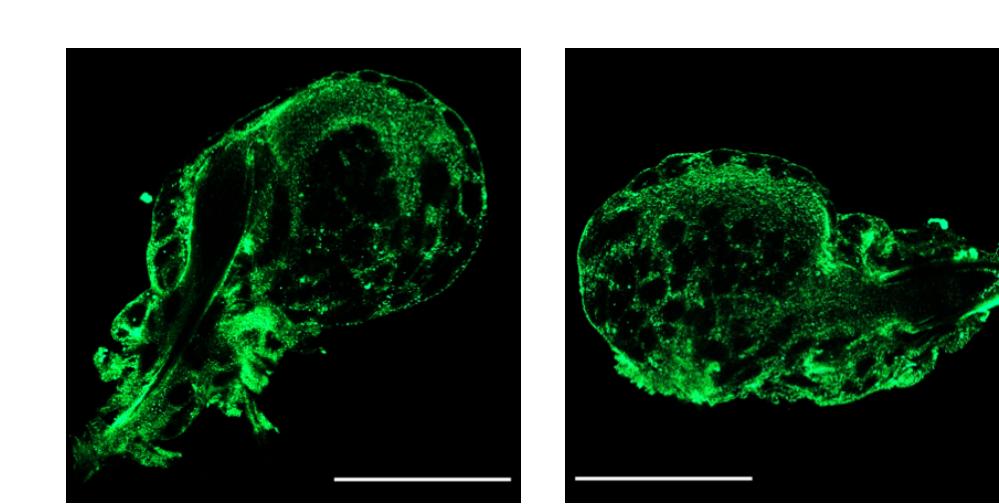
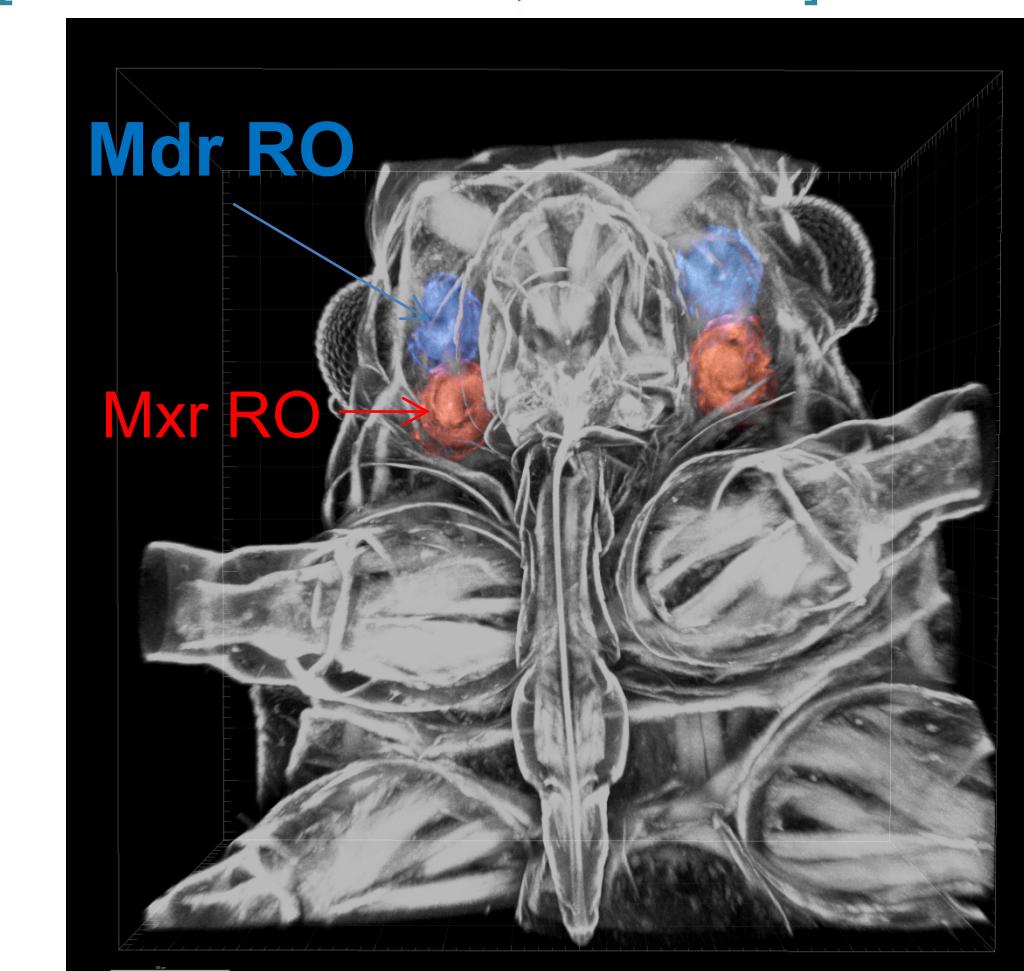
## Introduction

Insects display specialized cuticle territories with very specific molecular and architectural structures and functions, among which micro-structures governing their interactions with transmitted microbes (viruses, bacteria). Very often, these territories are displayed by mouthparts, a very specialized subset of insect cuticular structures, and yet poorly studied. In aphids, the main transmitters of noncirculative phytopathogens, a piercing-sucking mouthpart composed of two external mandibular stylets that surround two inner maxillary stylets bearing the virus receptor. We provide the first analysis of any insect's mouthpart cuticle, through aphid "retortiforms organs" (ROs) specialized in producing these (stylet, mandibular and maxillary) mouthparts.



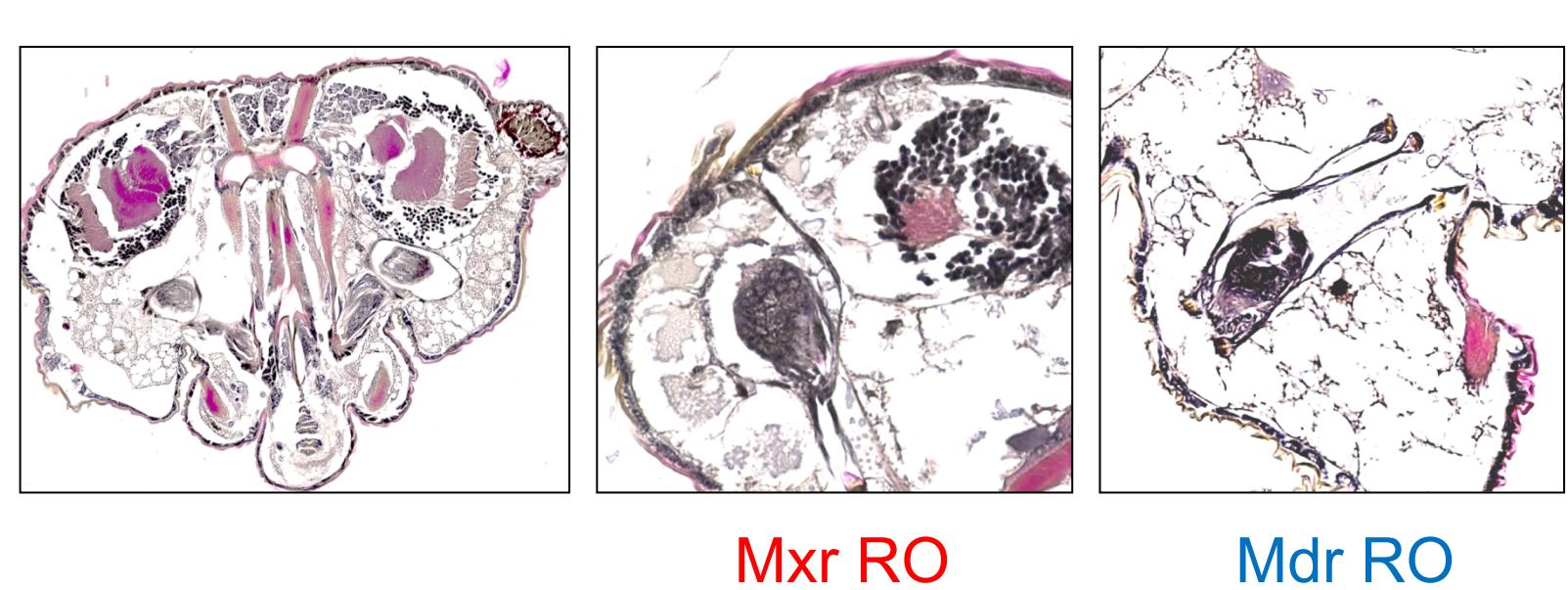
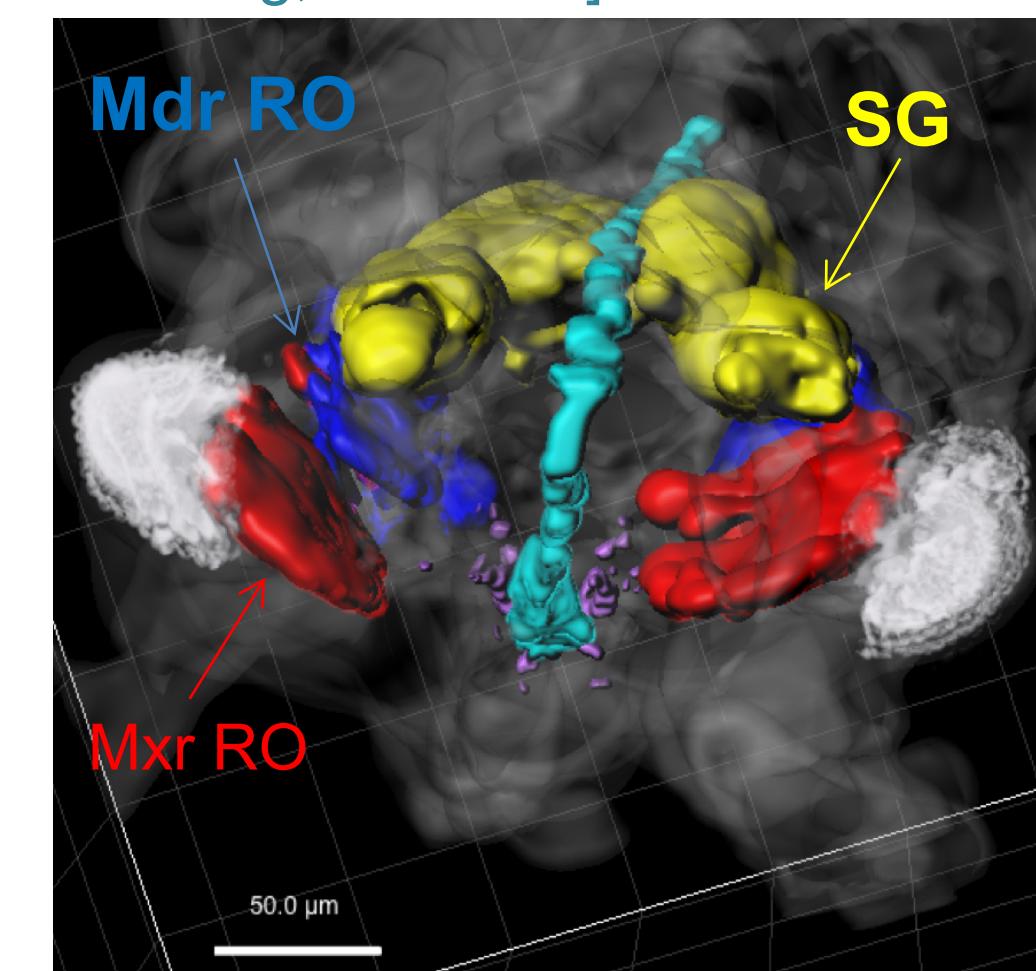
## Imaging of Retort Organs

❖ *in situ* laser-scanning Microscopy [Confocal autofluo; Imaris3D]



Mxr RO Mdr RO

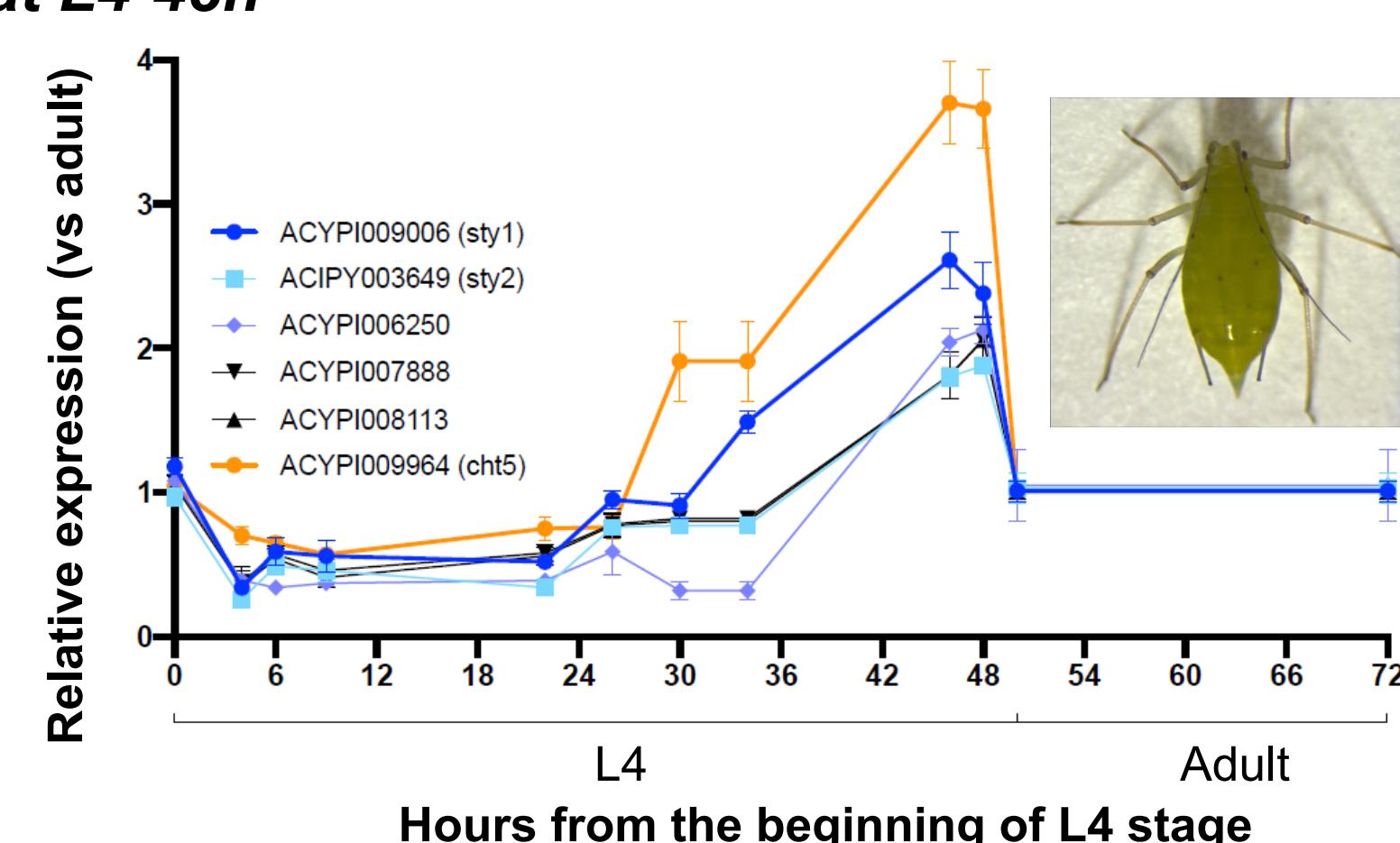
❖ Cutting sections of paraffin-embedded tissues and 3D-reconstruction [optical, \$\$\$ staining; Imaris3D]



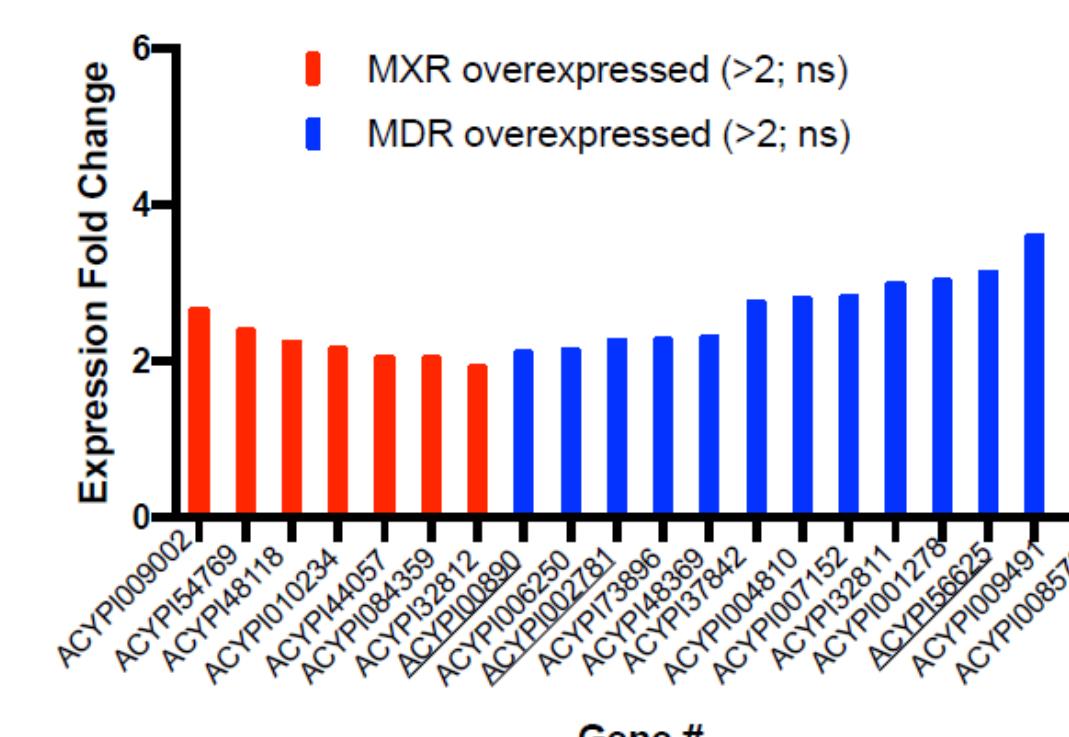
Mxr RO Mdr RO

## Transcriptomics of Retort Organs

Aphid genes coding for cuticular protein have expression peaks at L4-48h

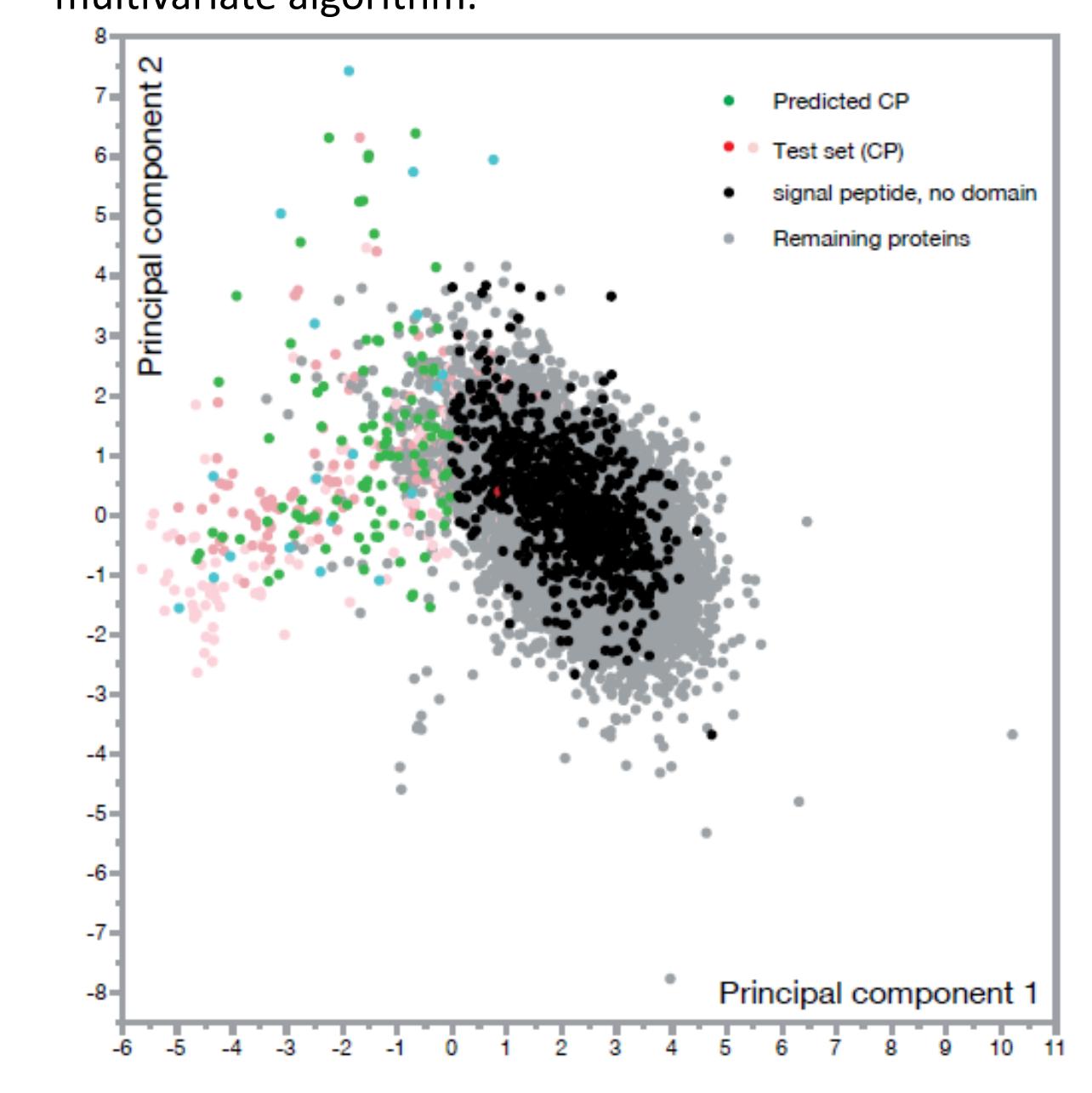


Mxr and Mdr stylets share a same global cuticular protein composition



RO expression analysis defines a potential new class of cuticular proteins

We identified, within the 200 RO-most expressed genes, a series of unannotated secreted proteins that show a striking imbalance in amino-acid composition, similar to that of cognate CuPs. From this, we identified 121 new cuticular proteins (in the whole genome) with such an multivariate algorithm.



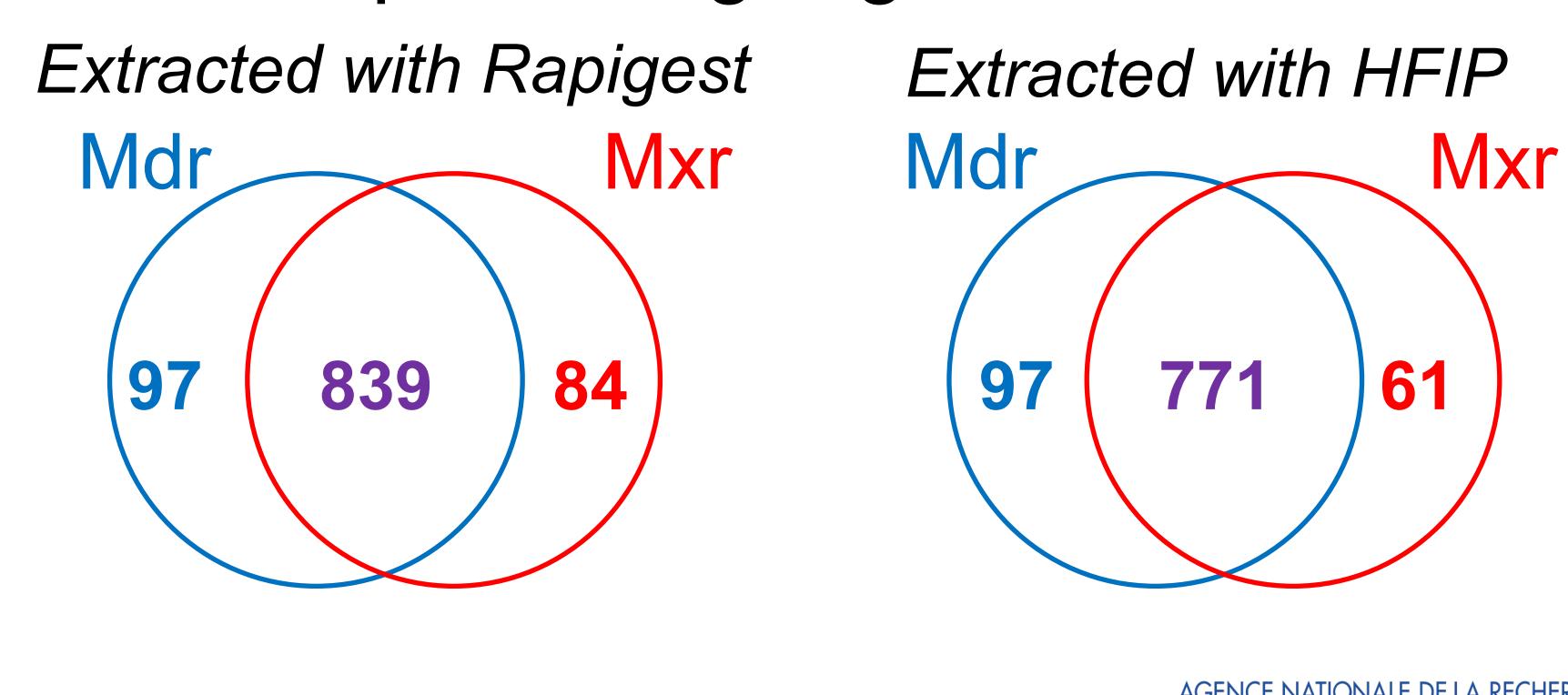
## Proteomics of Retort Organs

### Methods:

- ❖ Sample preparation by direct dissection
- Mdr RO**
- Mxr RO**
- ❖ Extraction with 0,1% Rapigest and HFIP
- ❖ nano-LC/MS-MS
- ❖ Identification with Proteome Discoverer 2.0
- ❖ Label Free Quantification

### Results:

- a) First proteome of retort glands as cuticle producing organs



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### b) Detection of cuticular proteins:

Known CP families:  
53 of 143 (37%)

Mdr Mxr

ACYPI003003 RR-2  
ACYPI00983 RR-2  
ACYPI005387 RR-2

3 50 3

New CP family:  
31 of 121 (31%)

Mdr Mxr

ACYPI001932 CDA  
ACYPI002005 RR-2  
ACYPI45536 CPAP1

7 23 1

Core "cuticulome" of RO:

1 CPAP1 34 CPR\_RR-2  
6 CPAP3 1 Tweedle  
7 CPR\_RR-1 1 CP Unchar

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## Proteomics of Stylets

- ❖ First package of WP1 (Stylet analytics from ANR StylHook)
- ❖ Separate work by Deshoux, Masson et al. (in prep, see refs)
- ❖ First proteomic analysis of stylet (mouthpart) cuticle of any insect

## Publications from ANR Stylhook WP1

- Guschinskaya, N., Ressnikoff, D., Arafah, K., Voisin, S., Bulet, P., Uzest, M., and Rahbé, Y. Insect mouthpart transcriptome unveils extension of cuticular protein repertoire and complex organization. *iScience* (2019) in revision
- Deshoux, M., Masson, V., Guschinskaya, N., van Munster, M., Cayrol, B., Webster, C. G., Arafah, K., Voisin, S., Blanc, S., Rahbé, Y., Uzest, M., and Bulet, P. Cuticular structure proteomics in the pea aphid *Acyrtosiphon pisum* reveals new plant virus receptor candidates at the surface of the acrostyle. *Mol. Cell. Proteomics* (2018) (2019) in prep
- Rahbé, Y. Pathogen transmission by insects: Impact of vector evolutionary histories, & comparative vector biology between plant and animal pathogens (2019). In *Microbes 2019*, invited (SFM ed., SFM, Cités Sciences et de l'Industrie, Paris (Fr))
- Guschinskaya, N., Arafah, K., Voisin, S., Uzest, M., Rahbé, Y., and Bulet, P. Differential proteomic analysis of a couple of cuticular insect glands, the aphid stylet (retort) organ. *Mol. Cell. Proteomics* (in prep 2019)
- Guschinskaya, N., Yvon, M., Bulet, P., Rahbé, Y., and Uzest, M. Apomictic (pea) aphids display altered salivary behaviour and proteome, but are not impeded in their transmission behaviour for non-circulative viruses. (in prep 2019)
- Guschinskaya, N., Deshoux, M., Ressnikoff, D., Uzest, M., and Rahbé, Y. Morphology and immunological/ transcriptomic characterization of the Retort Glands of pea aphid *Acyrtosiphon pisum*. *Arthropod Structure and Development* (prev 2020)
- Guschinskaya, N., Ribot, C., and Rahbé, Y. Insect interactions with cellular pathogens at the cuticular interface. (prev 2020)
- Rahbé, Y., Guschinskaya, N., and Uzest, M. Comparative genomics of cuticular proteins in the pea aphid and other insect models. (prev 2020)