EPI-Cuticle, an interdisciplinary team-project on the biomimetics of insect Cuticle: the case study of the hemipteran stylet - a chitin-protein polymeric assemblage and an articulated biosyringe & drilling micro-engine.

Yvan Rahbé, Guillaume Sudre, Stéphane Trombotto, Pierre Alcouffe, Andrea Bareggi, Mathias M. Choquer, Guy Condemine, Matthieu Fumagalli, Erwan Gueguen, Aline Bel-Brunon, et al.

EPI-CUTICLE, AN INTERDISCIPLINARY TEAM-PROJECT ON THE BIOMIMETICS OF INSECT CUTICLE: THE CASE STUDY OF THE HEMIPTERAN STYLET — A CHITIN-PROTEIN POLYMERIC ASSEMBLAGE AND AN ARTICULATED BIOSYRINGE & DRILLING MICRO-ENGINE.

IN BIOMIM’2020, F. GUITTARD, ED. (NICE: GDR 2088)

Yvan Rahbé

RAHBÉ, Y., SUDRE, G., TROMBOTTO, S., ALCOUFFE, P., BAREGGI, A., CHOQUER, M., CONDÉMINE, G., FUMAGALLI, M., GUÉGUEN, E., BEL-BRUNON, A., ALBERTINI, D., UZEST, M., DAVID, L. (2020)

THE HEMIPTERAN STYLET: A MULTIFUNCTIONAL DRILLING MACHINE

EPIQTICLE

PROJECT

WHAT IS AN HEMIPTERA ?

1: AN ORDER OF INSECTS COMPRISING GROUPS SUCH AS THE CICADAS, APHIDS, PLANTHOPPERS, LEAFHOPPERS, BED BUGS AND SHIELD BUGS. THEY RANGE IN SIZE FROM 1 MM TO AROUND 15 CM AND SHARE A COMMON ARRANGEMENT OF SUCKING MOUTHPARTS. MOST HEMIPTERANS FEED ON PLANTS, USING THEIR SUCKING AND PIERCING MOUTHPARTS TO EXTRACT PLANT SAP. SOME ARE HEMATOPHAGOUS.

DATE

2020 OCT 14

MEETING

NICE BIOMIM’2020

PROJECT

WHAT IS AN HEMIPTERA ?

2: THE PEA APHID (ACYRTHOSIPHON PISUM): A MODEL INSECT FOR BIOAGRNONOMY RESEARCH

vector

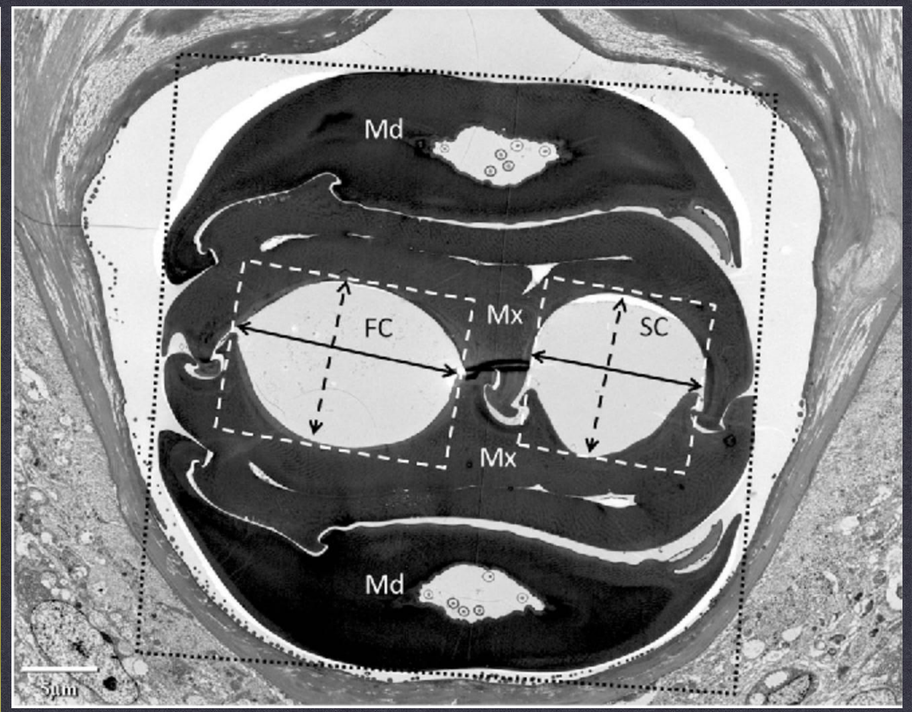
DATE 2020 OCT 14

MEETING NICE BIOMIM'2020

cyclic sexuality
parthenogenetic

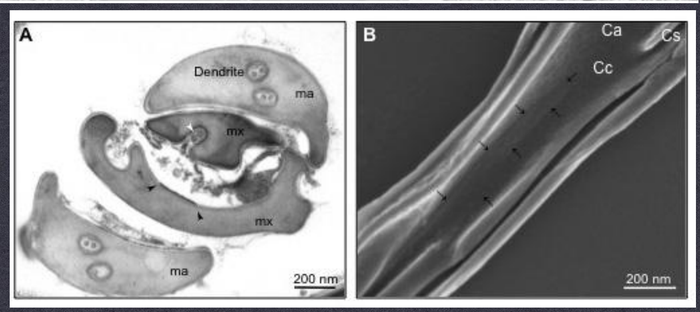
polyphenic

symbiotic

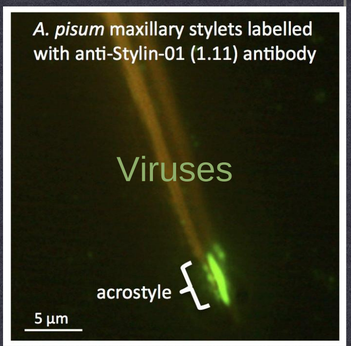


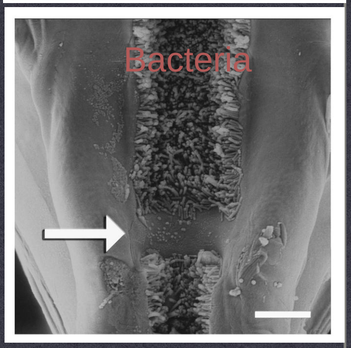
Bug Stylet

Aphid Stylet



Stylets and microbes





PROJECT

WHAT IS A STYLET ?

1

DATE

2020 OCT 14

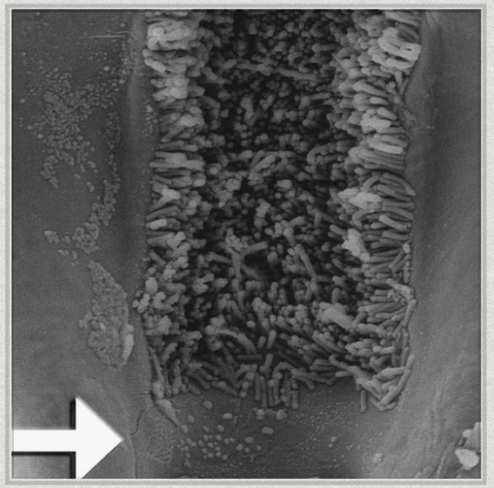
MEETING

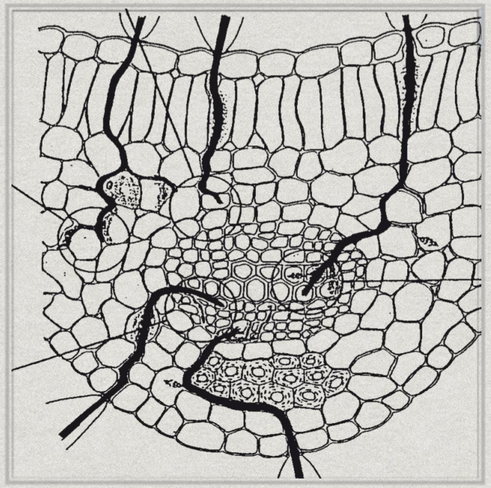
NICE BIOMIM’2020

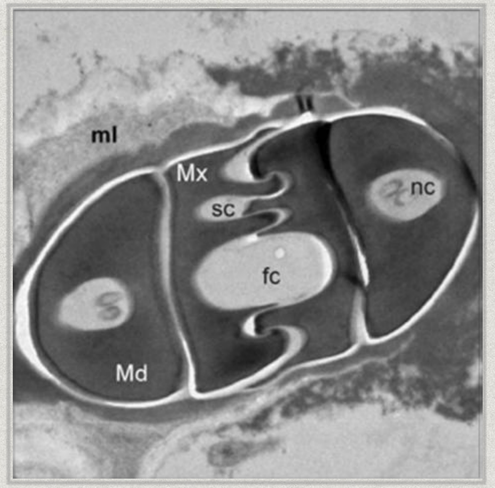
The questions (4 avenues)

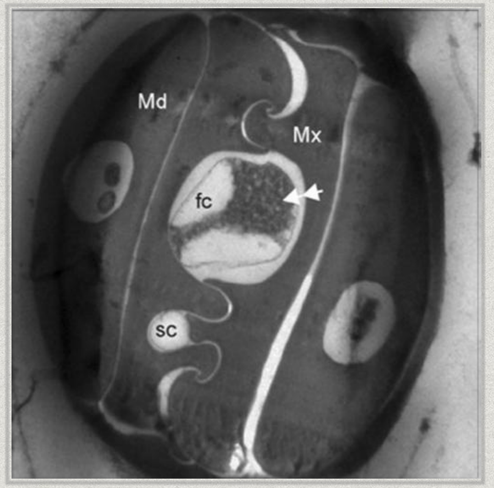
- **Polymer** sciences (4 slides)
  Cuticular (µ)territories as microbial platforms for (plant) diseases transmission
- **Mechanics** (4 slides)
  Aphid stylets as an exceptional precision drilling machine (plant parasitism as en evolutionary driving force for mechanical optimisation)
- **Sensor**(y) physiology (to come, 1 slide)
  Mandibular stylets as proprioceptor devices (?)
- **µfluidics** and fluid mechanics (to come, 1s.)
  Phloem sap and saliva: optimisation of sugar- and protein-loaded fluids transport through cuticular surface physical and chemical adaptation (?)

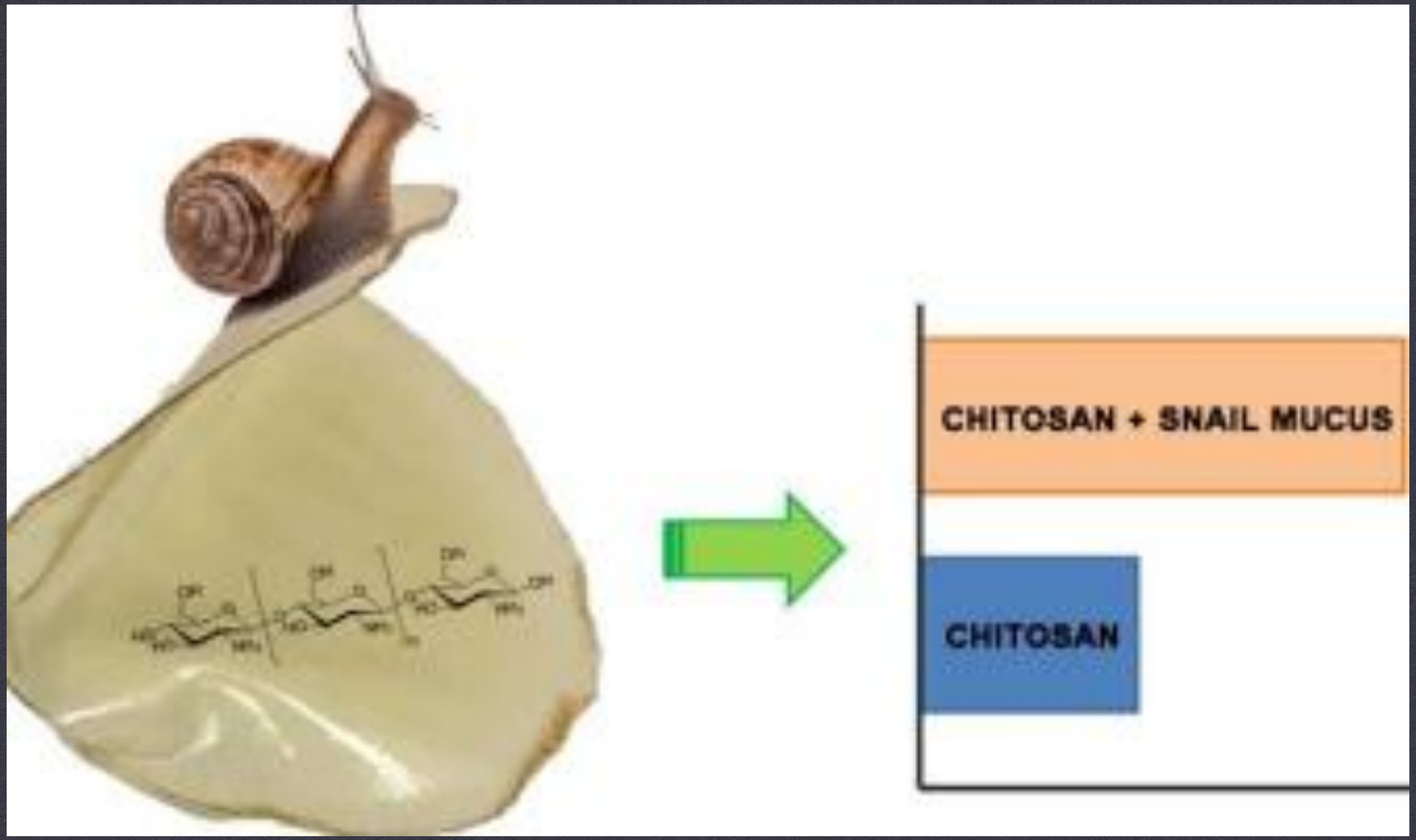
Xylella fastidiosa in Sharpshooter











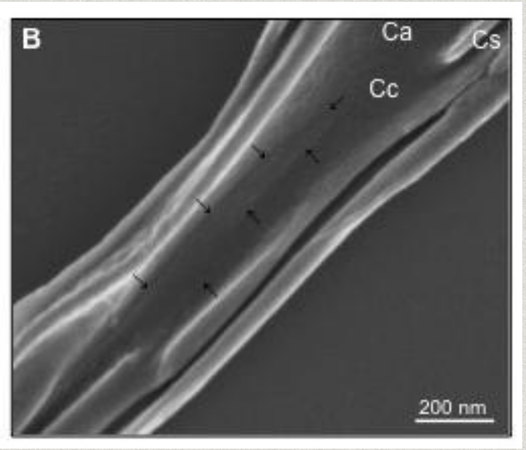
POLYMER SCIENCES

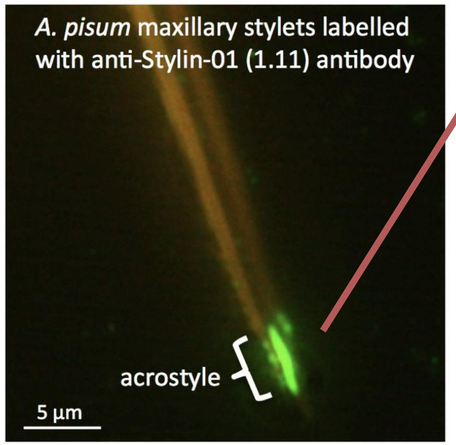
GLYCOSAMINOGLYCAN POLYMERS : CHITIN AND CHITOSAN (IMP MPISV GROUP —MATÉRIAUX BIO-INSPIRÉS POUR LES MILIEUX BIOLOGIQUES—, LAURENT DAVID & AL.)

A: Polymers

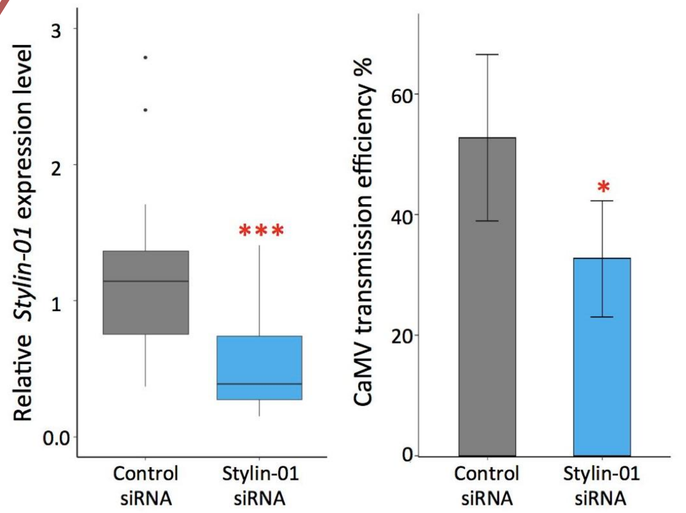
Biology of the acrostyle and aphid virus transmission

- a specific organ is responsible for the (specific) binding of non-persistent plant viruses: the acrostyle.
- proteins are responsible for the virus binding activity of the acrostyle, not chitin.
- *Stylins* (1 & 2) are the RR-family cuticular proteins that are responsible of viral retention (= putative receptors)





Stylin-01, the first cuticular protein identified in insect mouthparts, displays a motif directly accessible at the surface of the acrostyle, an organ on which non-circulative plant viruses can bind.



Reducing Stylin-01 transcripts is correlated to a decrease in *Cauliflower mosaic virus* (CaMV) transmission by aphids.

Stylhook Project

AGENCE NATIONALE DE LA RECHERCHE ANR

Webster, C., Thillier, M., Pirolles, E., Cayrol, B., Blanc, S., and Uzest, M. (2017). Proteomic composition of the acrostyle: novel approaches to identify cuticular proteins involved in plant- and virus-insect interactions. Insect Science, 1-47.

A: Polymers

Comprehensive stylet proteo-transcriptomic

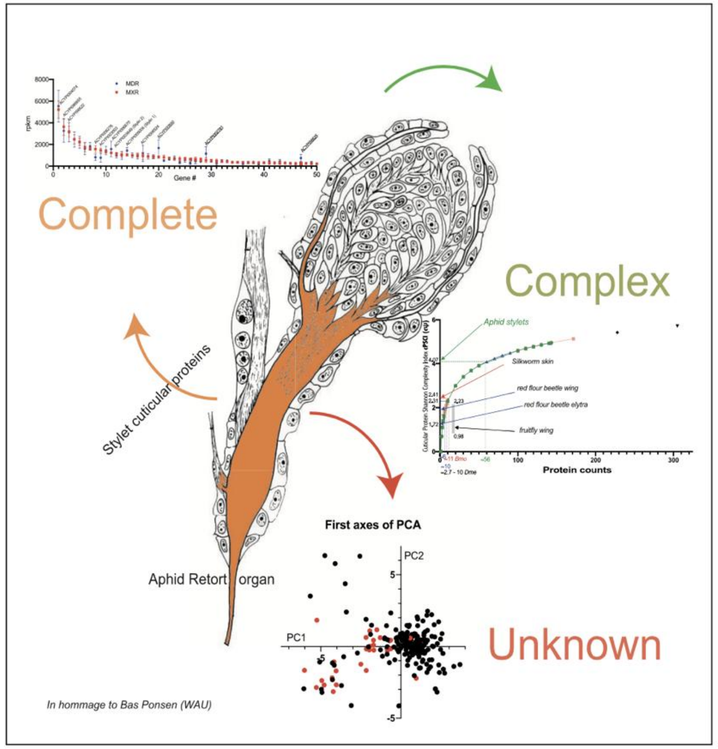
- Deep stylet transcriptomics (retort organ) revealed feasible
- No differenciation between MX and MD stylet
- Stylets are (far) more complex than other cuticles analysed so far (Drosophila or Mosquito, Coleopteran elytra, Bombyx epidermis)
- A major extension of cuticular protein repertoire is revealed (low complexity, non chitin-mining)

iScience

CellPress

Article

Insect Mouthpart Transcriptome Unveils Extension of Cuticular Protein Repertoire and Complex Organization



Natalia Guschinskaya, Denis Ressnikoff, Karim Arafah, Sébastien Voisin, Philippe Bulet, Marilyne Uzest, Yvan Rahbé

yvan.rahbe@inra.fr

HIGHLIGHTS

First transcriptome of aphid retort glands and stylet cuticular protein composition

A pyrokinin transcript is mandibular gland specific at the onset of adult moult

Stylet cuticle is of higher protein complexity than other insect cuticles

A new class of low-complexity cuticular proteins is predicted

Guschinskaya et al., iScience *23*, 100828
February 21, 2020 © 2020 The Author(s).
https://doi.org/10.1016/j.isci.2020.100828

Natalia Guschinskaya

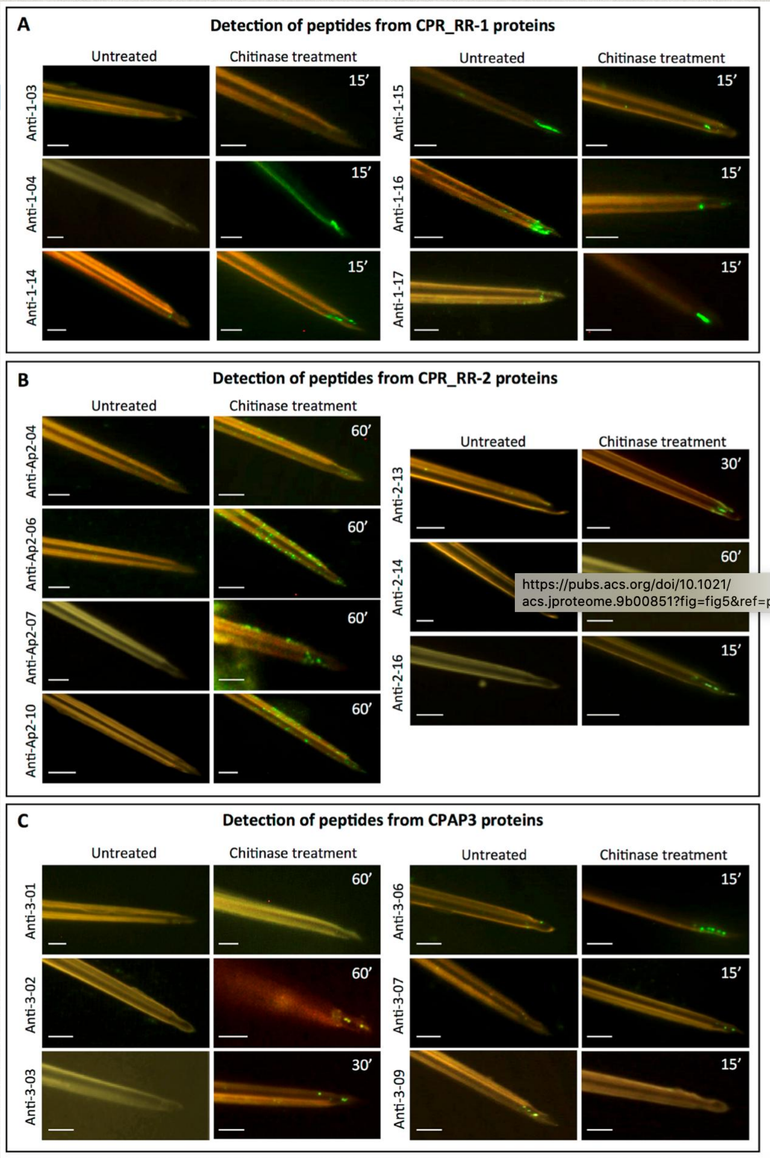
Stylhook Project ANR AGENCE NATIONALE DE LA RECHERCHE

Guschinskaya, N., Ressnikoff, D., Arafah, K., Voisin, S., Bulet, P., Uzest, M., and Rahbé, Y. (2020). Insect mouthpart transcriptome unveils extension of cuticular protein repertoire and complex organization. iScience *23*, 100828.

A: Polymers

Stylet (*per*
proteomic

- Comprehensive stylin identification



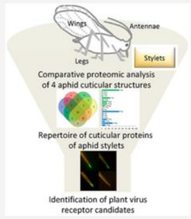
Journal of proteome research
pubs.acs.org/jpr
Article

Cuticular Structure Proteomics in the Pea Aphid *Acyrthosiphon pisum* Reveals New Plant Virus Receptor Candidates at the Tip of Maxillary Stylets

Maëlle Deshoux,[‡] Victor Masson,[‡] Karim Arafah, Sébastien Voisin, Natalia Guschinskaya, Manuella van Munster, Bastien Cayrol, Craig G. Webster, Yvan Rahbé, Stéphane Blanc, Philippe Bulet,*[,‡] and Marilyne Uzest*[,‡]

Cite This: *J. Proteome Res.* 2020, 19, 1319−1337
Read Online
ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Aphids are phloem-feeding insects known as major pests in agriculture that are able to transmit hundreds of plant viruses. The majority of these viruses, classified as noncirculative, are retained and transported on the inner surface of the cuticle of the needle-like mouthparts while the aphids move from plant to plant. Identification of receptors of viruses within insect vectors is a key challenge because they are promising targets for alternative control strategies. The acrostyle, an organ discovered earlier within the common food/salivary canal at the tip of aphid maxillary stylets, displays proteins at the cuticle−fluid interface, some of which are receptors of noncirculative viruses. To assess the presence of stylet- and acrostyle-specific proteins and identify putative receptors, we have developed a comprehensive comparative analysis of the proteomes of four cuticular anatomical structures of the pea aphid, stylets, antennae, legs, and wings. In addition, we performed systematic immunolabeling detection of the cuticular proteins identified by mass spectrometry in dissected stylets. We thereby establish the first proteome of stylets of an insect and determine the minimal repertoire of the cuticular proteins composing the acrostyle. Most importantly, we propose a short list of plant virus receptor candidates, among which RR-1 proteins are remarkably predominant. The data are available via ProteomeXchange (PXD016517).



KEYWORDS: *cuticle, cuticular proteins, stylets, acrostyle, virus receptor, proteomics, aphid*

INTRODUCTION

Aphids are phloem-feeding insects, well-known as major pests in agriculture. More than 5,000 aphid species have been described. They colonize countless plant species and have been reported on 300 plant families ranging from gymnosperms to angiosperms.[1,2] Aphids have a complex life cycle alternating between sexual and asexual reproduction and seasonal host changes. They are and have long been extensively studied, not only because of interesting life traits such as reproductive and wing polyphenisms but also because they transmit numerous plant diseases.[3] With more than 300 species transmitted, aphids are one of the most efficient and important vectors of plant viruses and are the best studied-model to characterize the mechanisms of vector-mediated virus transmission (recently reviewed by Whitfield and colleagues).[4−7] Most aphid-transmitted viruses bind reversibly to retention sites on the inner cuticle of insect mouthparts to which they remain attached during transport to a new host plant.[4] This so-called noncirculative virus transmission is particularly difficult to limit and control in field conditions, as the viruses can be acquired and inoculated by nonresident aphids mostly within a single puncture lasting only a few seconds.[8] During this process, an intimate association occurs between viruses and their vectors. This interaction is highly specific and involves the capsid proteins or viral-encoded proteins called helper components, together with poorly characterized molecular compounds in the insect cuticle acting as receptors.[9−12] These vector molecules are promising targets for alternative viral control strategies, and their identification would help characterizing further the molecular mechanisms of virus−vector interaction.

Aphids have piercing-sucking mouthparts, composed of the short triangular labrum covering the base of the stylet bundle, and the labium, a segmented organ which contracts to facilitate stylets penetration into plant tissues. The stylet bundle arises in the head from its secreting glands and extends outside the head in a dorsal groove of the labium.[13,14] A pair of external mandibular stylets innervated by two dendrites, surround two (noninnervated) inner maxillary stylets and together form the stylet bundle. Specific anatomical features are visible on both types of stylets. Barb-like ridges are present at the tip of

Received: December 20, 2019
Published: January 28, 2020

Maëlle Deshoux & Victor Masson
Natalia Guschinskaya

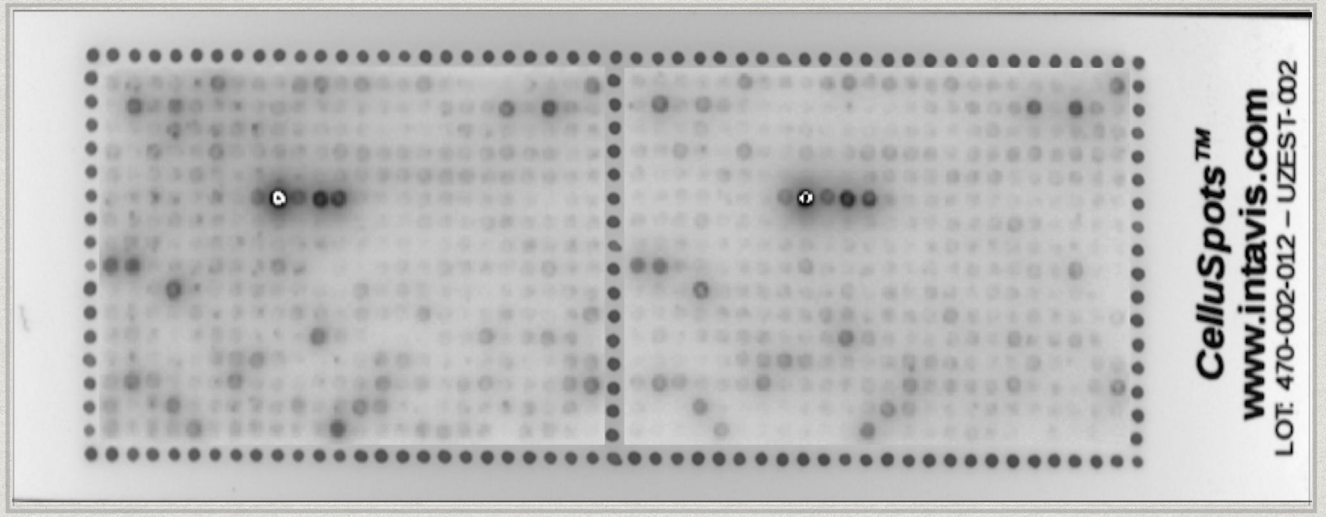
Stylhook Project

AGENCE NATIONALE DE LA RECHERCHE ANR

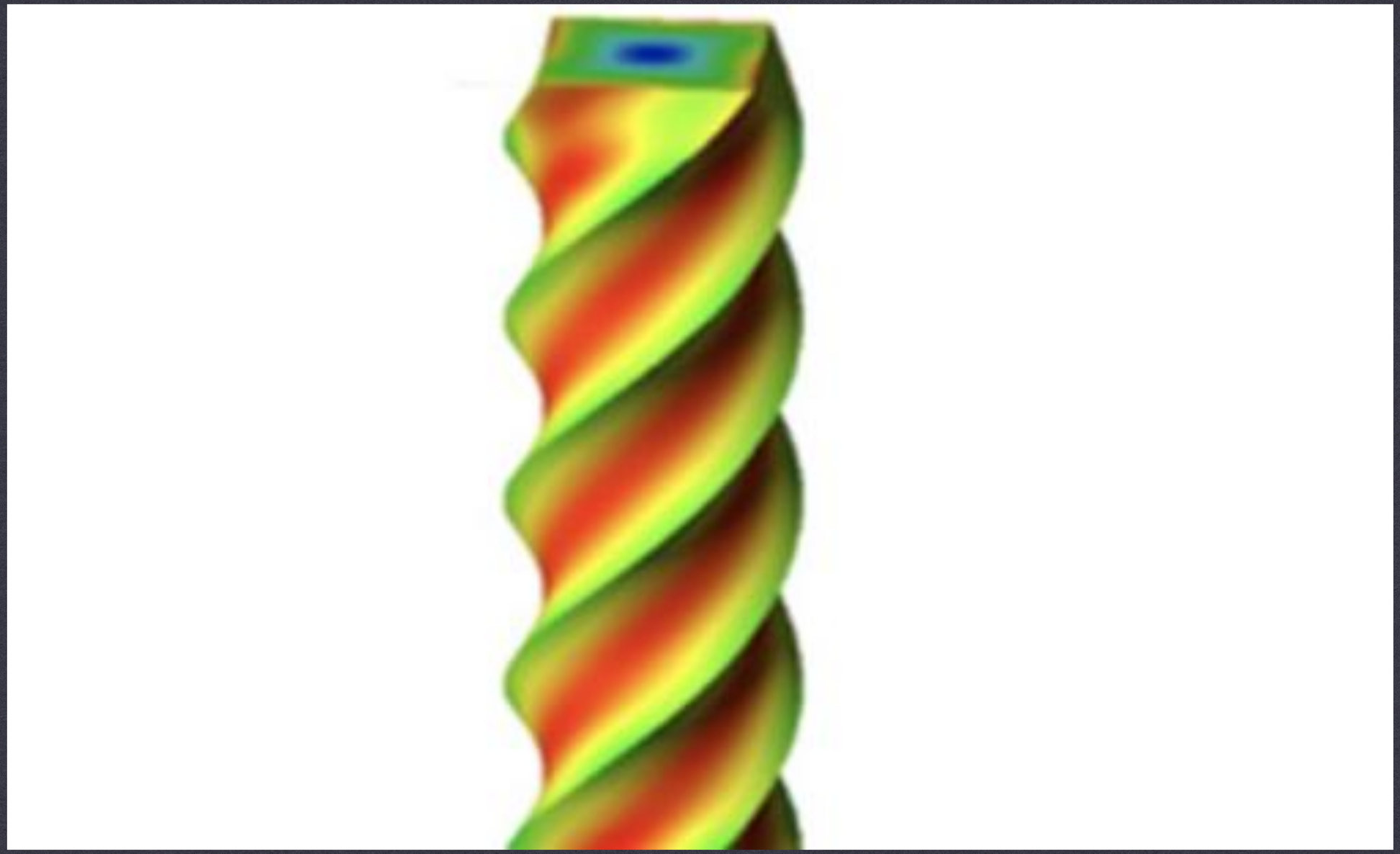
Deshoux, M., Masson, V., Arafah, K., Voisin, S., Guschinskaya, N., van Munster, M., Cayrol, B., Webster, C.G., Rahbé, Y., Blanc, S., et al. (2020). Cuticular structure proteomics in the pea aphid *Acyrthosiphon pisum* reveals new plant virus receptor candidates at the tip of maxillary stylets. Journal of Proteome Research *19*, 1319-1337.

Applications

- A platform for the interaction biology of cuticle (planar chitin films with protein spots = « cuticular micro-arrays »)
- Specific uses of chitosan films for biomedical diagnostics
- Fine tuning and understanding the microbial properties of GAG films
- Mechanical functionalization of implantable chitosan devices



Faivre, J., **Sudre, G.**, Montembault, A., Benayoun, S., Banquy, X., Delair, T., and **David, L.** (2018). Bioinspired microstructures of chitosan hydrogel provide enhanced wear protection. Soft Matter.
Treguier, J., Bugnicourt, L., Gay, G., Diallo, M., Islam, S.T., Toro, A., **David, L.,** Theodoly, O., **Sudre, G.**, and Mignot, T. (2019). Chitosan Films for Microfluidic Studies of Single Bacteria and Perspectives for Antibiotic Susceptibility Testing. mBio *10*.
Yan, X., **Alcouffe, P.**, Bernard, J., and Ganachaud, F. (2020). Functional Hybrid Glyconanocapsules by a One-Pot Nanoprecipitation Process. Biomacromolecules.
Goncalves, I.R., Brouillet, S., Soulie, M.C., Gribaldo, S., Sirven, C., Charron, N., Boccara, M., and **Choquer, M.** (2016). Genome-wide analyses of chitin synthases identify horizontal gene transfers towards bacteria and allow a robust and unifying classification into fungi. BMC Evol Biol *16*, 252.
Fumagalli, M., Berriot, J., de Gaudemaris, B., Veyland, A., Putaux, J.L., Molina-Boisseau, S., and Heux, L. (2018). Rubber materials from elastomers and nanocellulose powders: filler dispersion and mechanical reinforcement. Soft Matter *14*, 2638-2648.



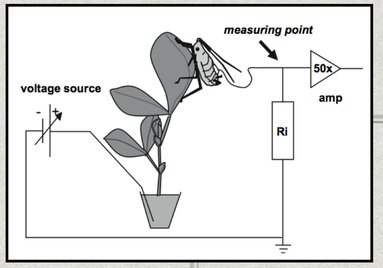
MECHANICS

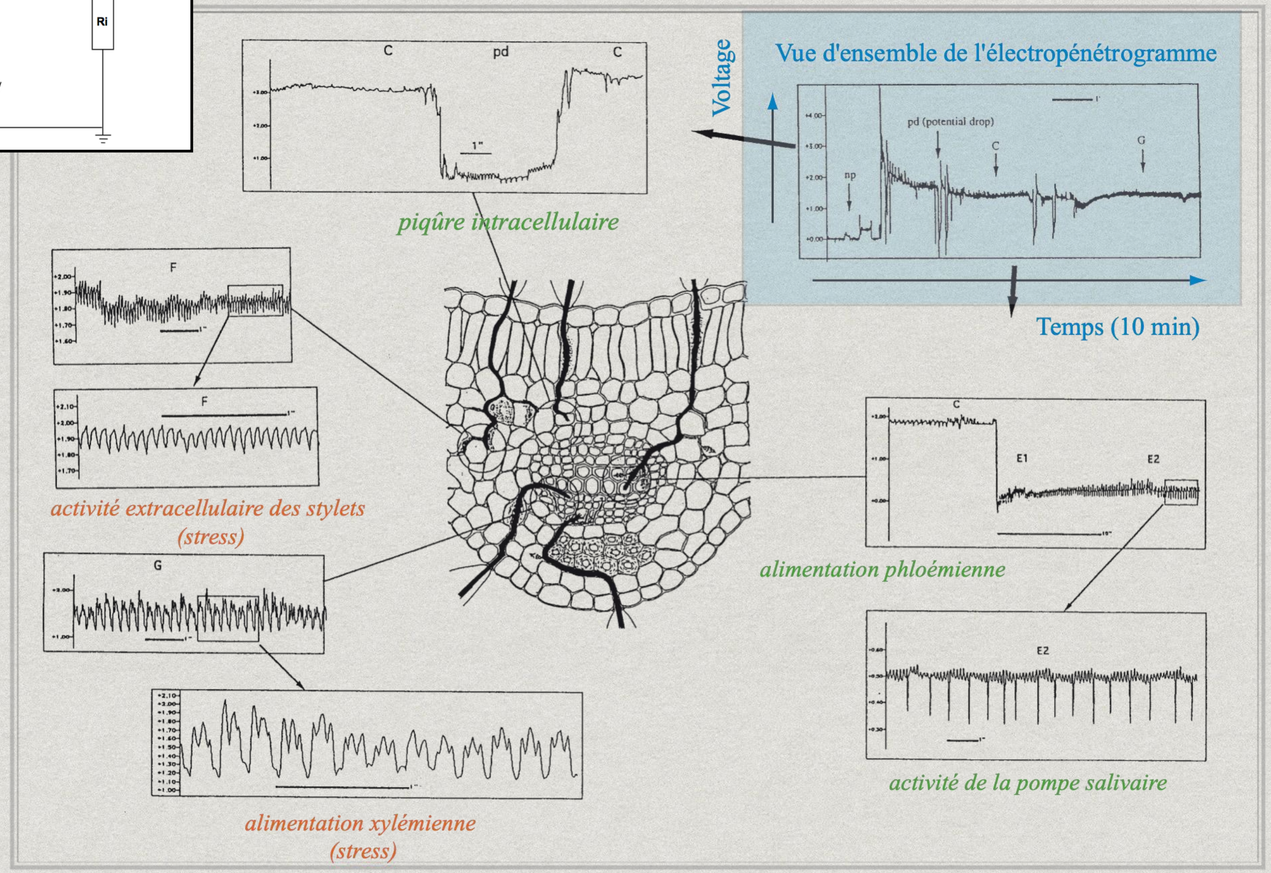
STRUCTURE MECHANICS AND SIMULATION (LAMCOS MIMESIS GROUP —MULTISCALE MECHANICS FOR SOLIDS—, ALINE BEL-BRUNON & AL.)

B: Mechanics

precision drilling: what does this mean ?

- no or very low cell injury
- many types of salivary secretions
- mechanically adapted stylets
- occurred many times in *Sternorrhyncha*
- is a parasitic (*vs* predatory) evolutionary adaptation





B: Mechanics evolution of mouthparts and cuticular proteins

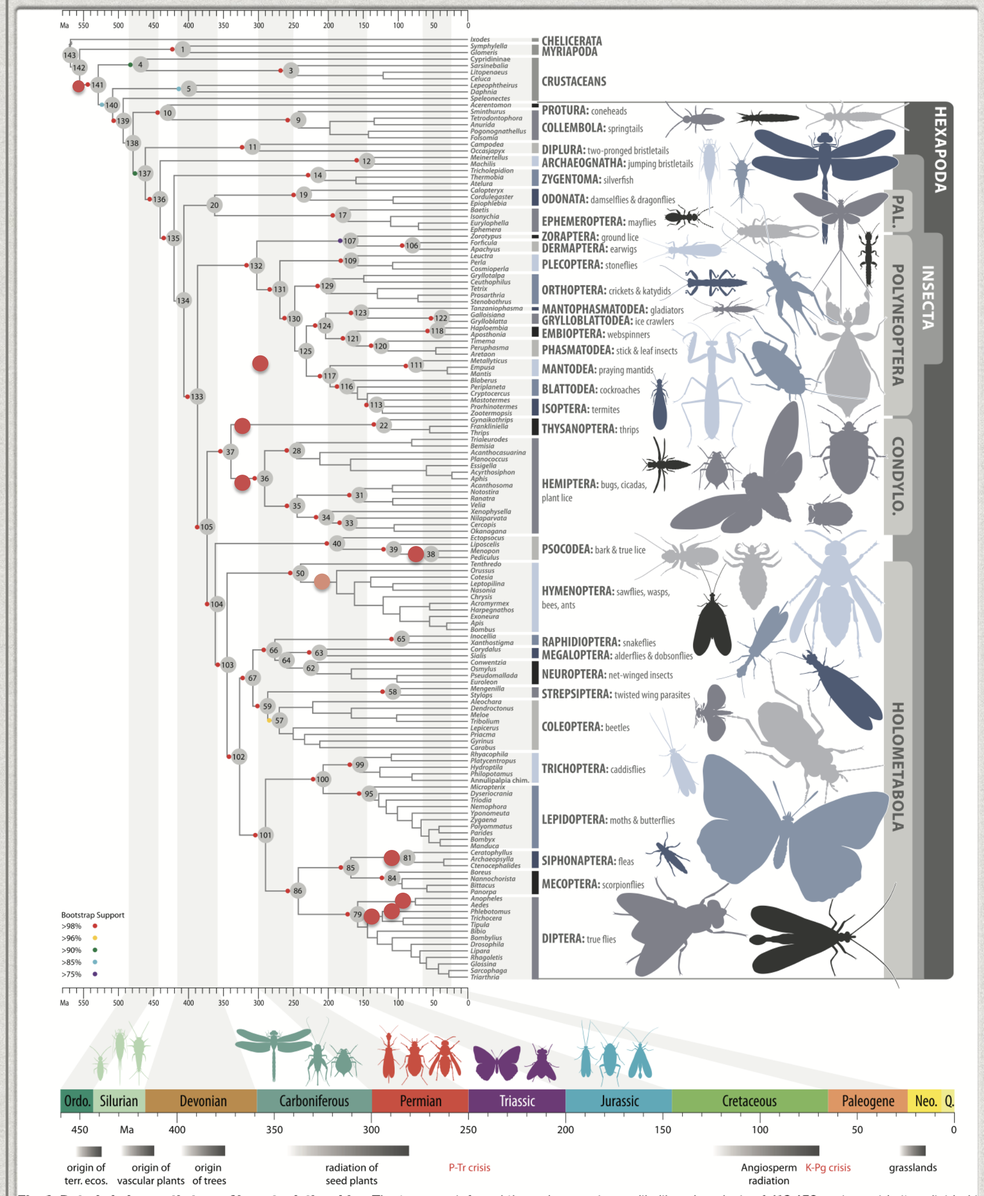
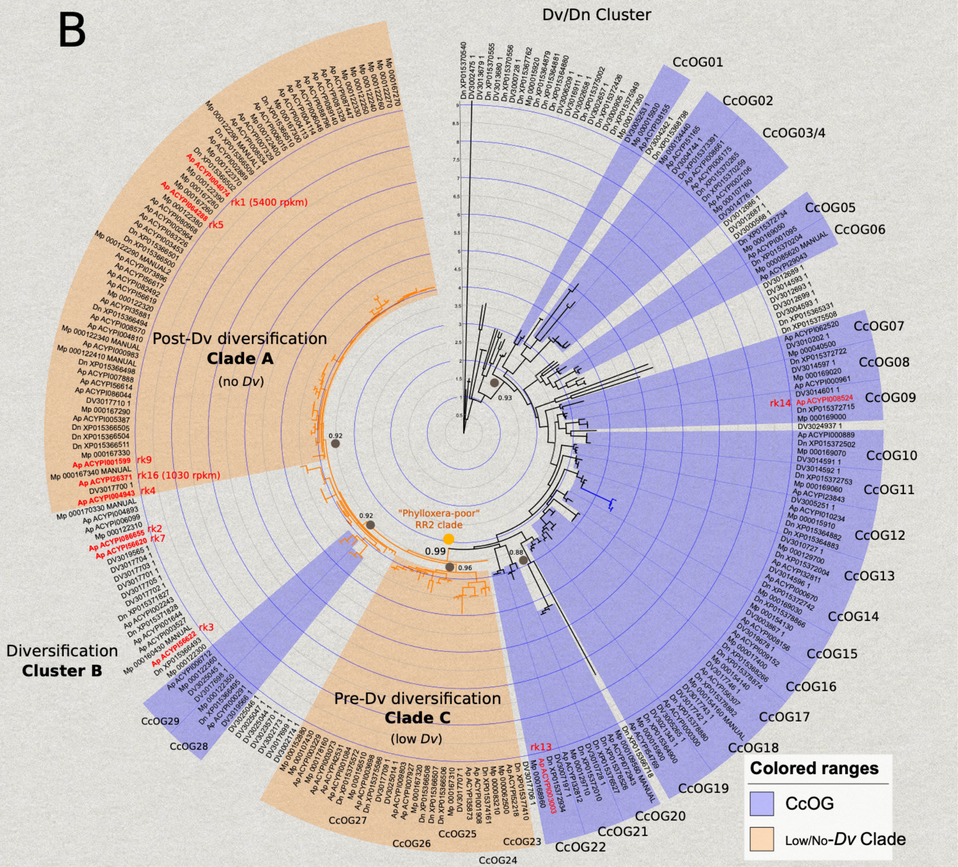
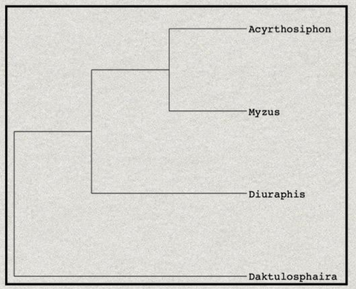
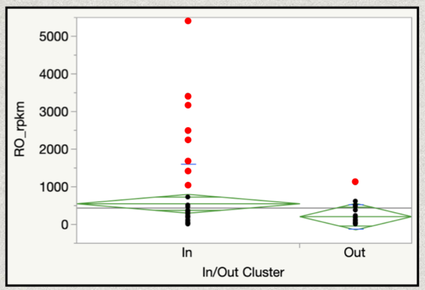


Fig. 1. Dated phylogenetic tree of insect relationships. The tree was inferred through a maximum-likelihood analysis of 413,459 amino acid sites divided i

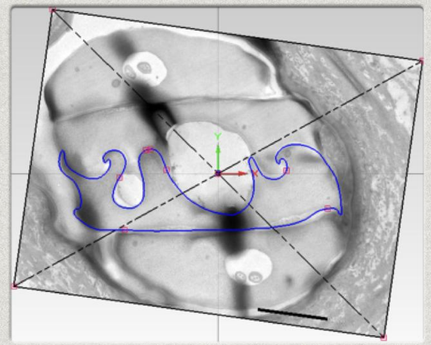




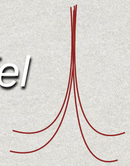


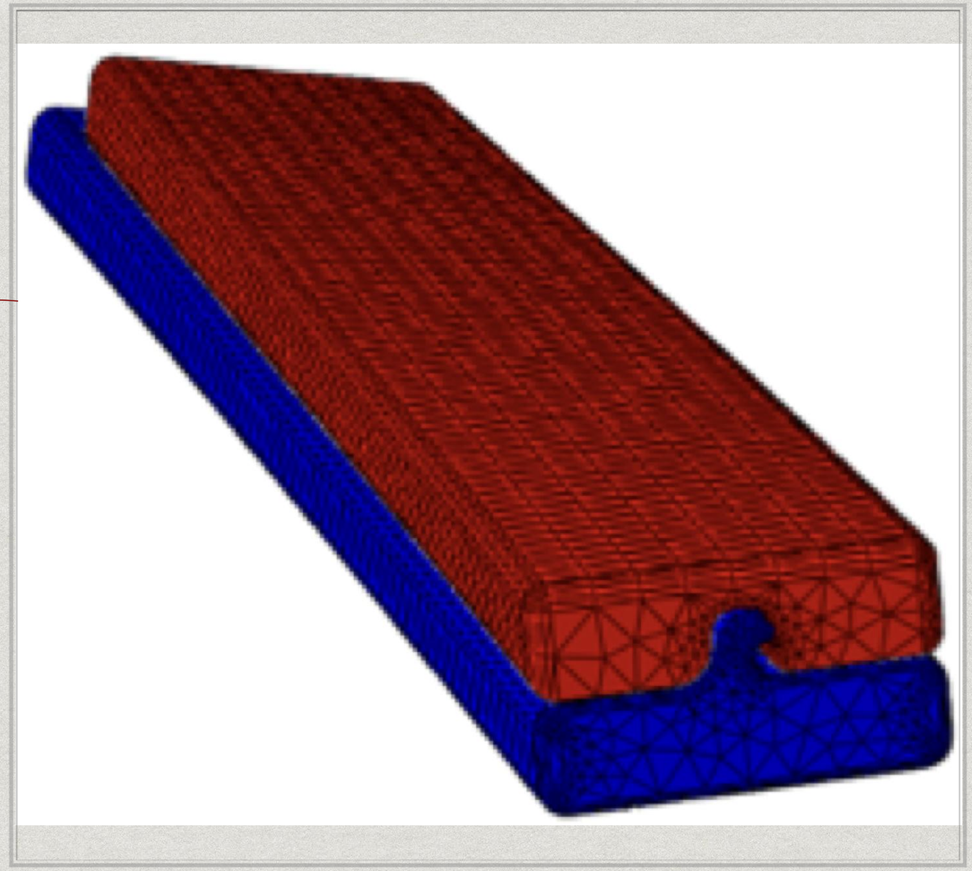
Rispe, C., Legeai, F., Nabity, P., Fernández, R., Arora, A.K., Baa-Puyoulet, P., Banfill, C.R., Bao, L., Barberà, M., Bouallègue, M., et al. (2020). Genome sequence of the grape phylloxera: insights into the genome evolution and invasion routes of an iconic pest. BMC Biology *18*, 90.

stylet bundle mechanics



- finite element modelling
- influence of internal tension (clipping) in the stability of the structure
- mechanisms of apical steering (governing of pd-behaviour)
- exclusive role of mandibular stylets in penetration properties (z-relative movements and external shapes)



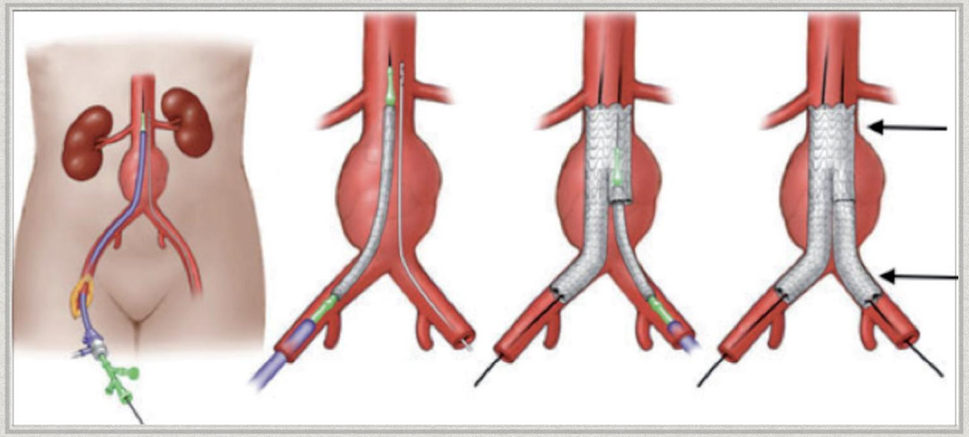


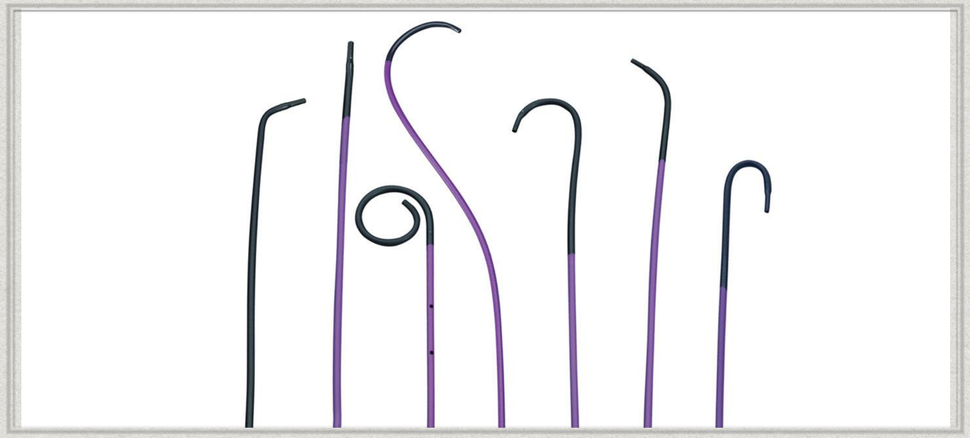
Quentin Le May

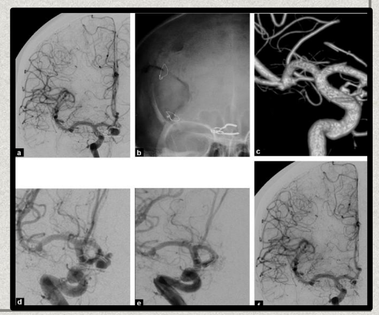
Le May, Q. (2018). Biomimétisme : modélisation des stylets d'un puceron. In Mechanical engineering (GM), Volume Ingénieur INSA-Lyon. (Lyon (Fra): INSA-Lyon), p. 47.

Applications

- Conception de sondes et cathéters de chirurgie vasculaire (rénale, cardiaque, cérébrale…)



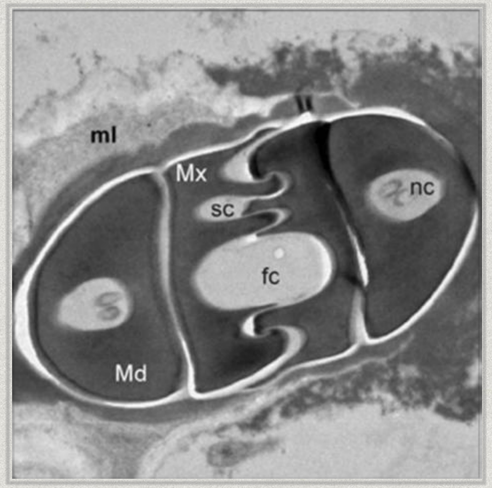




Gindre, J., **Bel-Brunon, A.**, Kaladji, A., Dumenil, A., Rochette, M., Lucas, A., Haigron, P., and Combescure, A. (2015). Finite element simulation of the insertion of guidewires during an EVAR procedure: example of a complex patient case, a first step toward patient-specific parameterized models. Int J Numer Method Biomed Eng *31*, e02716.
Puyoo, E., and **Albertini, D.** (2020). Conductive Filament Localization Within Crossbar Resistive Memories by Scanning Joule Expansion Microscopy. IEEE Electron Device Letters *41*, 848-851.

C: Mechanosensors

Looking for collaboration



- Confirmation or not of homology with insect chordotonal organ ?
- Cellular mechanisms and modelling of mecanotransduction
- Testing the hypothesis of *nanocrack* mechanoresistivity sensing ?
  analogy with spider leg sensors ?

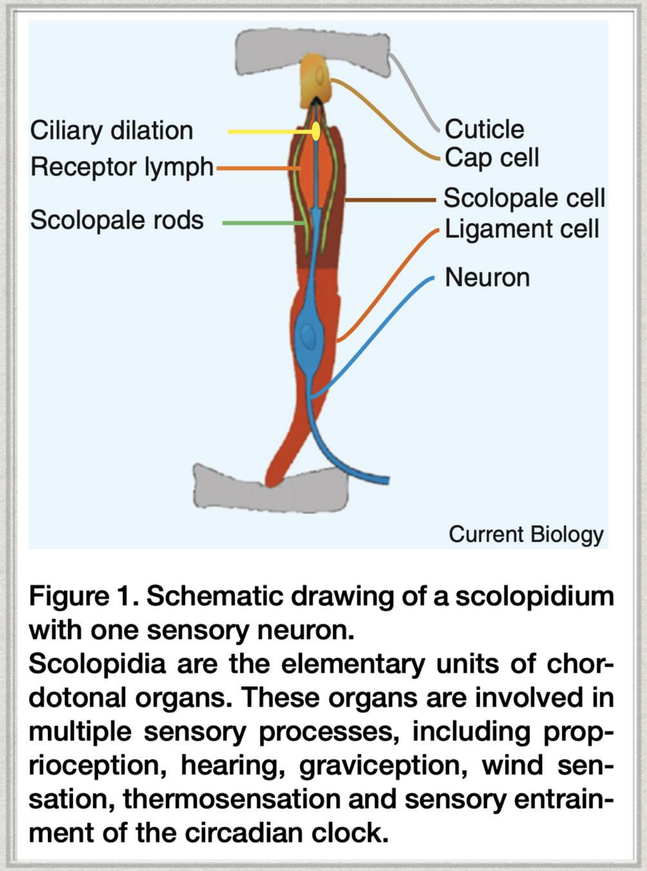


Figure 1. Schematic drawing of a scolopidium with one sensory neuron.
Scolopidia are the elementary units of chordotonal organs. These organs are involved in multiple sensory processes, including proprioception, hearing, graviception, wind sensation, thermosensation and sensory entrainment of the circadian clock.

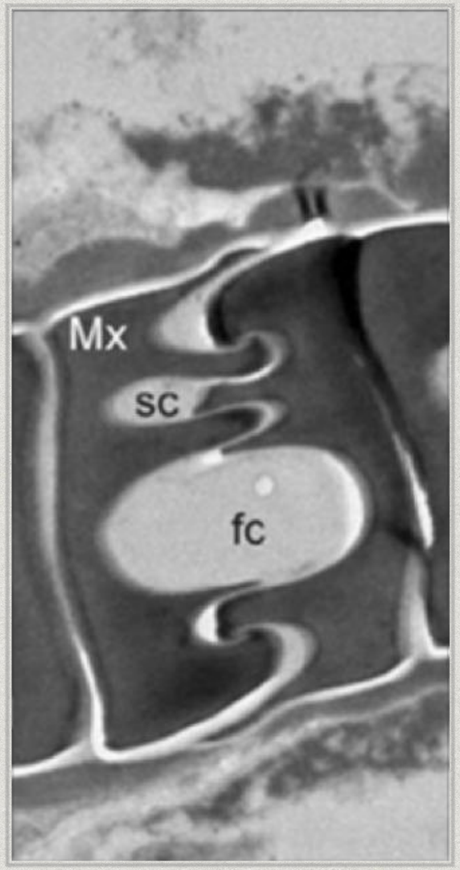
D: Microfluidics

Collaboration still in progress

1. Analytics of *fc* and *sc* tubing wettability measures feasibility ?

2. Devicing microfluidics for food canal experimentation with live (bacterial) particulate phases.
Includes fluid dynamics modelling.

3. Mimicking insect mouth / food canals for artificial selection « module » for bacterial genetics



THE TEAM:

IMP (LAURENT DAVID ET AL.)

LAMCOS (ALINE BEL-BRUNON ET AL.)

INL (DAVID ALBERTINI ET AL.)

MAP (YVAN RAHBÉ ET AL.)

BGPI / PHIM (MARILYNE UZEST ET AL.) (MONTPELLIER)

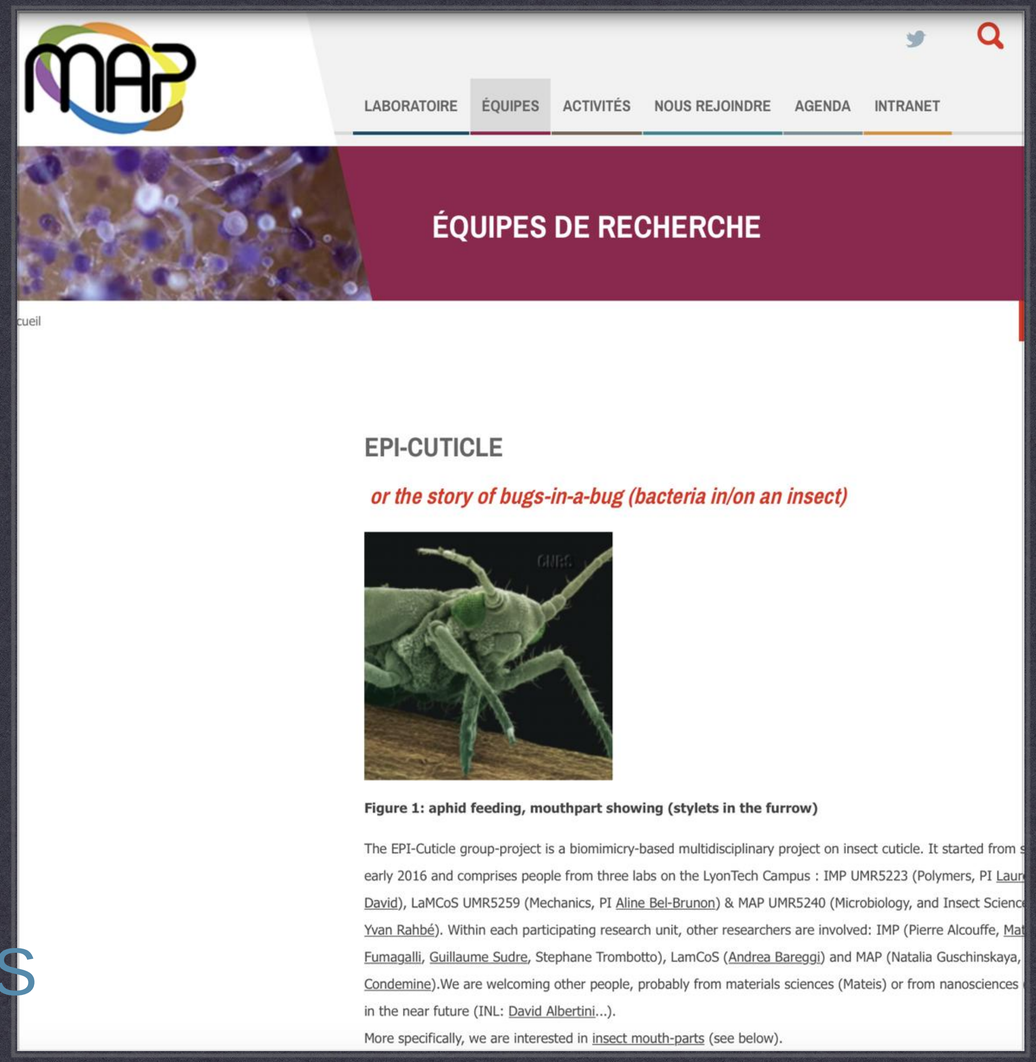
HTTPS://MAP.INSA-LYON.FR/FR/CONTENT/EPI-CUTICLE

OR

HTTP://TINYURL.COM/EPIQTICLE

StylHook ANR Project (MU)

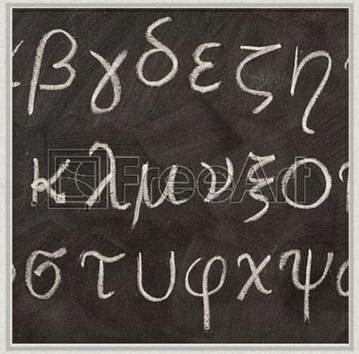
« green chitin » pre-maturation project CNRS



RAHBÉ, Y., SUDRE, G., TROMBOTTO, S., ALCOUFFE, P., BAREGGI, A., CHOQUER, M., CONDEMIN

Take home 4message

- How to (nano) communicate on a surface with a chitin blackboard and a protein alphabet ?



- How to perform precision drilling with a (simple ?) 4piece structure ?

- How to propriosense a solid appendix when you are a (soft, living) organism ?

- How to (µ)inject / (µ)ingest when you are a (µ)parasite ?

mostly questions of surfaces and scales in biological systems

You may find this in HAL

- **Poster**: Rahbé, Y., Sudre, G., Trombotto, S., Alcouffe, P., Bareggi, A., Choquer, M., Condemine, G., Fumagalli, M., Guéguen, E., Bel-Brunon, A., et al. (2020). EPI-Cuticle, an interdisciplinary team-project in biomimetic research (of insect Cuticle). In Biomim’2020. (Nice: GDR 2088).
- **Talk** (this slide-show): Rahbé, Y., Sudre, G., Trombotto, S., Alcouffe, P., Bareggi, A., Choquer, M., Condemine, G., Fumagalli, M., Guéguen, E., Bel-Brunon, A., et al. (2020). EPI-Cuticle, an interdisciplinary team-project on the biomimetics of insect Cuticle: the case study of the hemipteran stylet — a chitin-protein polymeric assemblage and an articulated biosyringe & drilling micro-engine. In Biomim’2020, F. Guittard, ed. (Nice: GDR 2088).