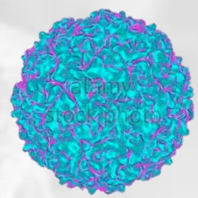
INRA SCIENCE & IMPACT


# Metagenomics: a tool to develop the health diagnosis of Insect mass rearing



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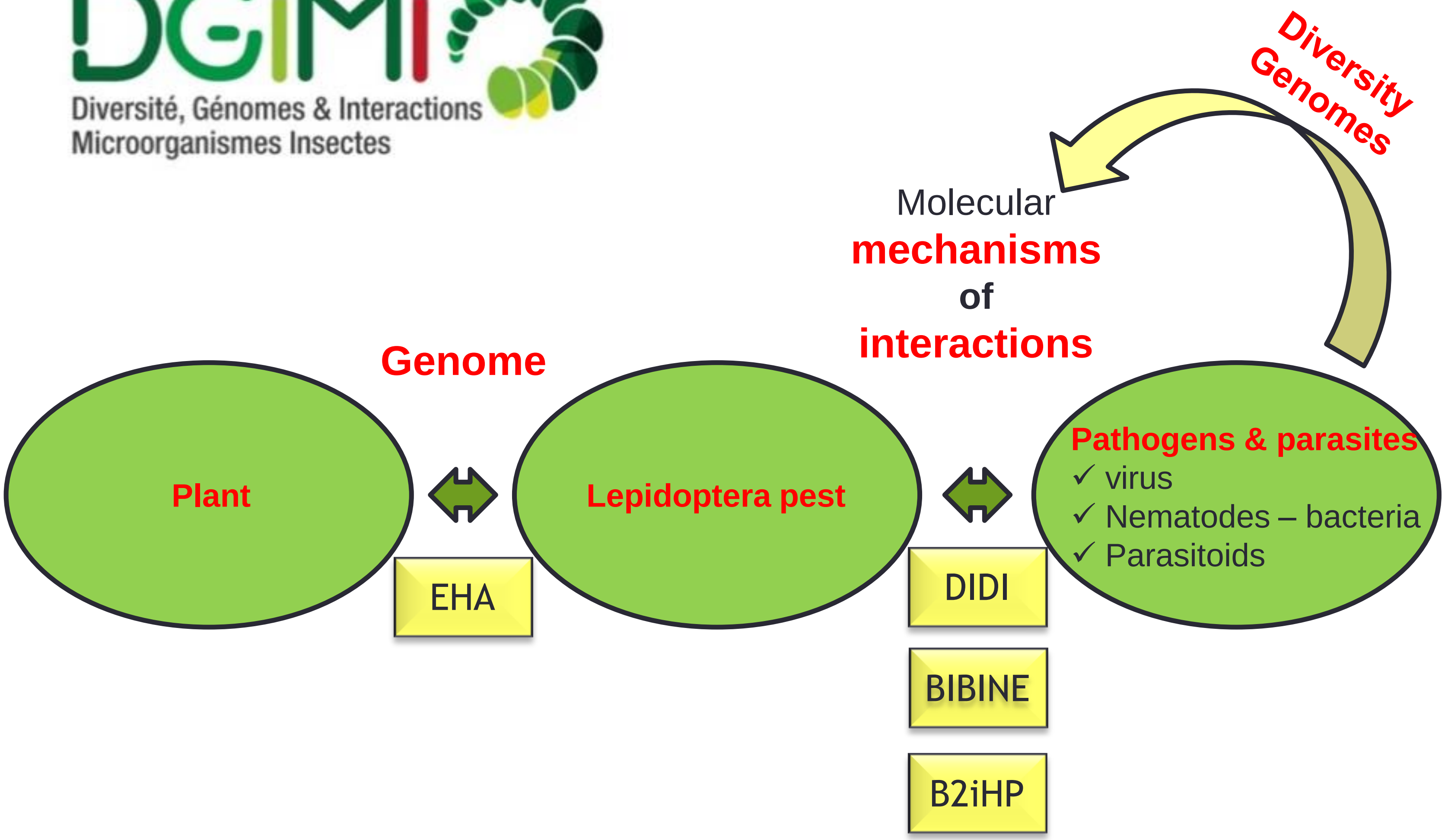
DGIMI Diversité, Génomes & Interactions Microorganismes Insectes

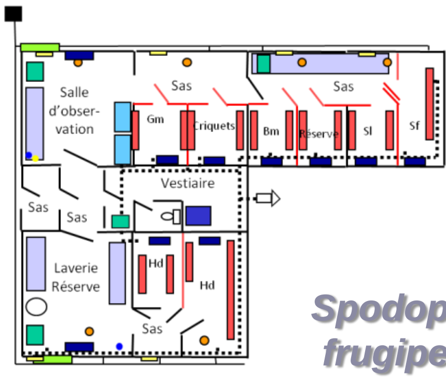
*Insectinov 2 – Adebiotech / AgroParisTech – Romainville - Octobre 2017*

DGIMI
Diversité, Génomes & Interactions Microorganismes Insectes




❖ With an integrative approach: molecules – organisms - ecosystems

# Facilities dedicated to the production and experimentation on insects under biological containment (NS2)

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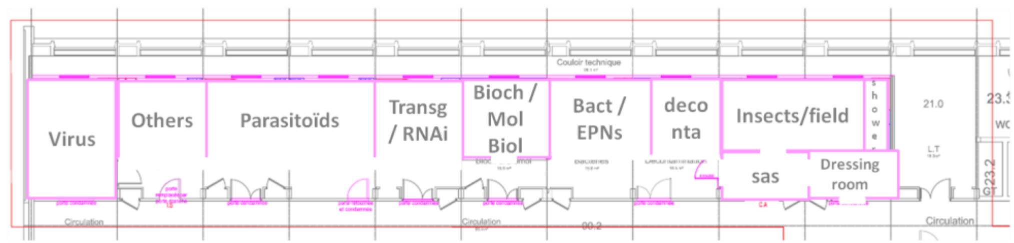



***Spodoptera frugiperda***

***Spodoptera littoralis***

~90 000 larvae/y

***Hyposoter didymator***

***Galleria mellonella***

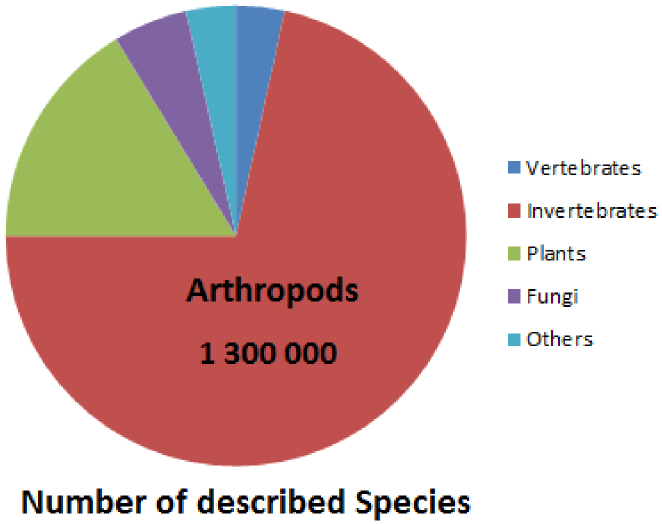
~1500 females/y

9000 larvae/y

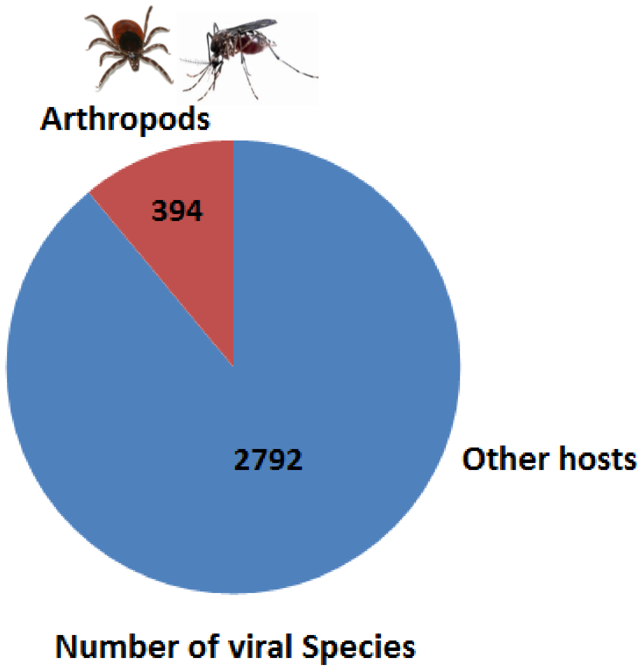
- **Surface 80 m² + 150 m²**
- **Budget (investments) > 300 k€**
- **Annual budget 17 k€**
- **Agreement 20120800-0001 2012-03-20 (validity: 5years)**

# Arthropods: reservoirs of viral diversity

- **Arthropods**: the largest animal phylum (80% animal species)




(Chapman, 2009)



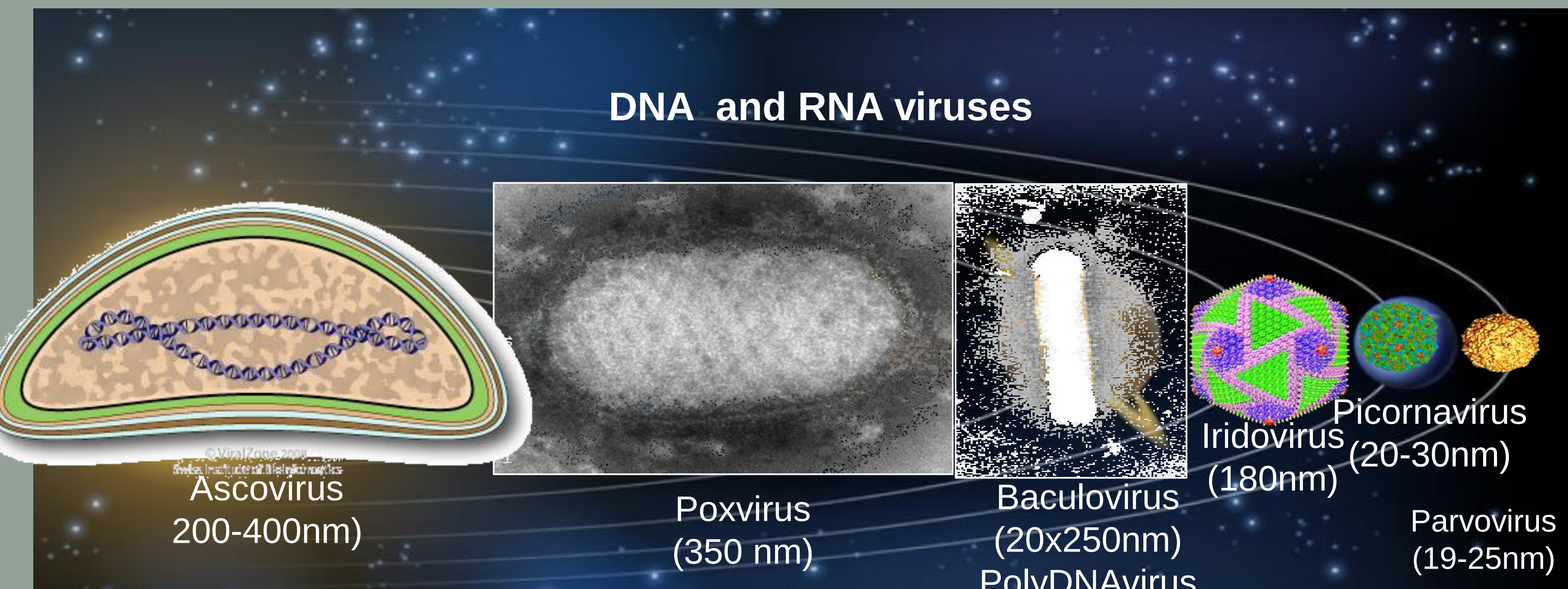

(ICTV, 2014)

**… but lack of knowledge about their viral diversity**

# The arthropod virosphere

<u>504 virus species associated with Arthropods (25 virus families)</u>
(3186 viruses, ICTV; 6544 complete viral genomes, NCBI )

- 176 pathogens for plants
- 40 pathogens for vertebrates
- 288 pathogens for arthropods



# Aims to « hunt » viruses

**Fundamental and applied issues**

- Optimise the use and the safety of viruses (in biocontrol)
- Find viral reservoirs
- “Control insect health and health safety”
- Control sanitation
- Control outbreaks
- …

⇨ **Prevent (Understand) virus emergence in insects**

# Viruses are over-represented among emerging pathogens

- Human : 25 % (Jones, 2008)
- Crops : 47% (Anderson, 2004)

**What can cause viral emergence ?**

# 1960s – Viral diseases in insect rearings

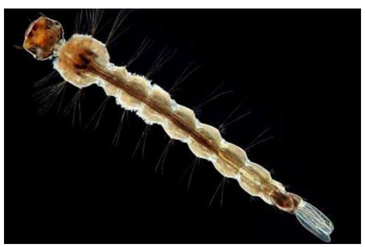
Wax moth caterpillars

Extrait de la *Revue de Zoologie Agricole et Appliquée*, **63**, 207-208 (1964).

**Virose d'un type inhabituel**
**chez le Lépidoptère « GALLERIA MELLONELLA » L.**

**Meynadier et al. 1964**

Mosquitos



*Acta virol.* 17 : 253—256, 1973

INVESTIGATION OF A VIRUS DISEASE
OF THE DENSONUCLEOSIS TYPE IN A LABORATORY CULTURE
OF *AEDES AEGYPTI*

O. P. LEBEDEVA, M. A. KUZNETSOVA, A. P. ZELENKO, A. P. GUDZ-GORBAN

The T. G. Shevchenko Kiev State University and Division of Molecular Biology and Genetics of the Academy of Sciences of the Ukrainian S.S.R., Kiev, U.S.S.R.

*Received August 7, 1972*

*Summary.* — A virus disease of the larvae in a laboratory colony of *Aedes aegypti* L. was studied. Data on the histological changes

genomeAnnouncements

genomeA Article | Journal Info. | Authors | Permissions | Journals.ASM.org

Genome Announc. 2013 Nov-Dec; 1(6): e00914-13. PMCID: PMC3820778
Published online 2013 Nov 7. doi: 10.1128/genomeA.00914-13

**A Novel Ambisense Densovirus, *Acheta domesticus* Mini Ambidensovirus, from Crickets**

Hanh T. Pham, Qian Yu, Max Bergoin, and Peter Tijssen

Author information ▶ Article notes ▶ Copyright and License information ▶

**cricket paralysis virus**

:ket Paralysis Virus (CrPV) was initially discovered in Australian field
:kets (Teleogryllus commodus and Teleogryllus oceanicus) by Carl
nganum and his colleagues at the Victorian Plant Research Institute
rnley, Melbourne, Australia). The paralytic disease spread rapidly
ough a breeding colony as well as through a laboratory population
ısing about 95% mortality. This was the first recorded isolate of the virus
l is generally referred to as CrPVvic to distinguish it from subsequent
...ates.

# 1970s – Virus associated with natural epizootics

## In more insect species

### other Lepidoptera

JOURNAL OF INVERTEBRATE PATHOLOGY 20, 369–370 (1972)

**A Nonoccluded Virus of *Junonia coenia* (Nymphalidae: Lepidoptera)**

In 1968, during a general study of granulosis viruses, an attempt was made to infect larvae of *Aglais urticae* (Nymphalidae) with a granulosis virus of the Buckeye caterpillar, *Junonia coenia*. This sample was received by the A.R.C. Virus Research Unit, Cambridge, from K. M. Hughes, Berkeley, California, in 1954 and had been stored at Cambridge at +3° from 1954 until 1968. One hundred first-instar *A. urticae* larvae

observed together with the granulosis virus capsules.

A further sample of *J. coenia* granulosis virus was then obtained from Y. Tanada which had been collected in 1955, again probably in the vicinity of Berkeley, but did not contain

Samples

rus which

were fed to

C. F. Rivers
J. F. Longworth
*Natural Environment Research Council Unit of Invertebrate Virology Commonwealth Forestry Institute, South Parks Road, Oxford, England*

*Received June 15, 1972*

### Other Diptera

*Reprinted from* PROCEEDINGS AND PAPERS OF THE FORTY-FOURTH ANNUAL CONFERENCE OF THE CALIFORNIA MOSQUITO CONTROL ASSOCIATION, INC., JANUARY 25 - 28, 1976
PUBLISHED July 27, 1976

**DENSONUCLEOSIS VIRUS AND CYTOPLASMIC POLYHEDROSIS VIRUS DISEASES IN LARVAE OF THE BLACKFLY, *SIMULIUM VITTATUM***

Brian A. Federici and Lawrence A. Lacey
University of California
Department of Entomology, Riverside, California 92502

ABSTRACT

A densonucleosis virus (DV) and cytoplasmic polyhed-

trophied to a lesser extent. All infected nuclei contained a

# 1980s – A new densovirus found in Crustacean

In shrimps cultures

JOURNAL OF INVERTEBRATE PATHOLOGY 45, 47–53 (1985)

## A Parvo-like Virus Disease of Penaeid Shrimp

D. V. LIGHTNER AND R. M. REDMAN

*Environmental Research Laboratory, University of Arizona, Tucson International Airport, Tucson, Arizona 85706*

Received May 3, 1984; accepted September 6, 1984

Cultured populations of four penaeid shrimp species (Crustacea, Decapoda) from four separate

# 12

## Parvoviridae of Invertebrates: Densonucleosis Viruses

SHIGEMI KAWASE

*Nagoya University, Chikusa, Nagoya, Japan*

EDOUARD KURSTAK

*University of Montreal, Montreal, Quebec, Canada*

# Persistent viruses can be beneficial for their hosts

OPEN ACCESS Freely available online

PLOS | PATHOGENS

## Densovirus Is a Mutualistic Symbiont of a Global Crop Pest (*Helicoverpa armigera*) and Protects against a Baculovirus and Bt Biopesticide

**Pengjun Xu[1,2], Yongqiang Liu[1], Robert I. Graham[3], Kenneth Wilson[3], Kongming Wu[1]***

1 State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, People's Republic of China, 2 Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao, People's Republic of China, 3 Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom

PNAS

## Densovirus induces winged morphs in asexual clones of the rosy apple aphid, *Dysaphis plantaginea*

**Eugene V. Ryabov[1], Gary Keane, Neil Naish, Carol Evered, and Doreen Winstanley**

Warwick HRI, University of Warwick, Wellesbourne, Warwick CV35 9EF, United Kingdom

Edited by David L. Denlinger, Ohio State University, Columbus, OH, and approved April 10, 2009 (received for review February 6, 2009)

**What is the diversity and the dynamics**

**of viruses In Insects?**

# How to explore virus diversity?

Viruses : absence of universal genetic marker !

- **With *a priori* (pathogen-driven)**
  - Persistent viruses in insect cells cultures, insect rearing
  - Epizootics
  - Zoonotics

⇒ PCR, Virus isolation

# How to search for viruses?

- **Without *a priori* (sequence-driven)**
  - Screening databases
  - Genomic based technologies

→ **Viral Metagenomics**

Concentrate viral particles from any environmental sample and amplify viral genetic material from all the members of the sampled communities (the metagenome)

# Viral Metagenomics

- ✓ **Sampling**
  - Methods
  - Scale (time and space)
  - Environments
- ✓ **Get "viromes"**
  - All Viral DNA and RNA
  - Multiplex samples
  - Whole Genome Sequencing
- ✓ **Analyze data**
  - Bioinformatics
  - Biostatistics

# The sampling

In crop

In rearing facilities

# The sampling

In crops

In prairies

# Processing insects for viral metagenomics

**Virus particle purification** → **Nucleic acid preparation** → **Next Generation Sequencing** → **Bioinformatic analyses**

**Virus particle purification**

- Filtration
- Ultracentrifugation
- DNase and RNase digestion

**Nucleic acid preparation**

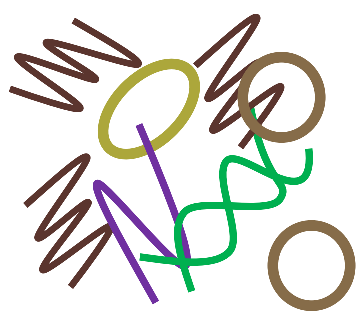
- RNA + DNA extraction
- cDNA synthesis
- DNA amplification
- DNA size selection
- Multiplexing

**Next Generation Sequencing**

- WGS

**Bioinformatic analyses**

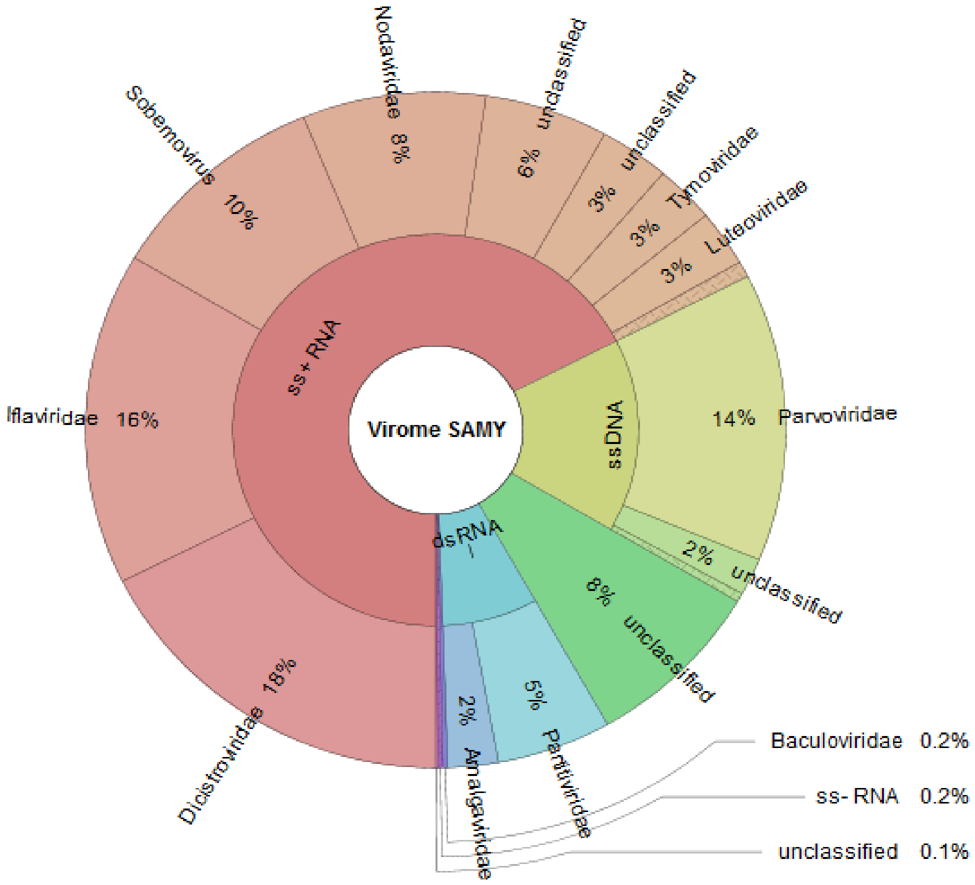
- Quality control
- de novo  assembly
- Taxonomic assignment



Candresse, PLOSone,  2014
Roossinck, Molecular Ecology, 2010

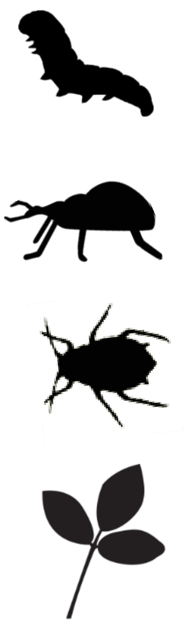
# Virus diversity in insect sampled in a crop

- Global virome of 23 arthropod species




- Large viral diversity (58 viral families)

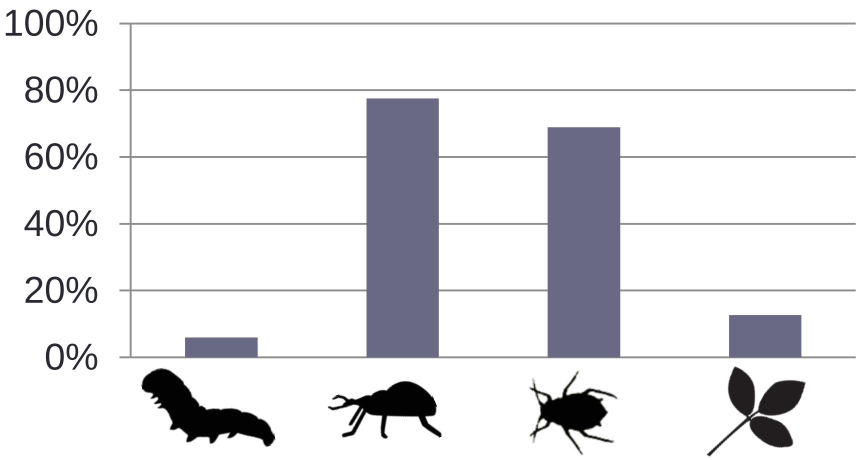
# Ex: Analysing Insect pests and their host plant



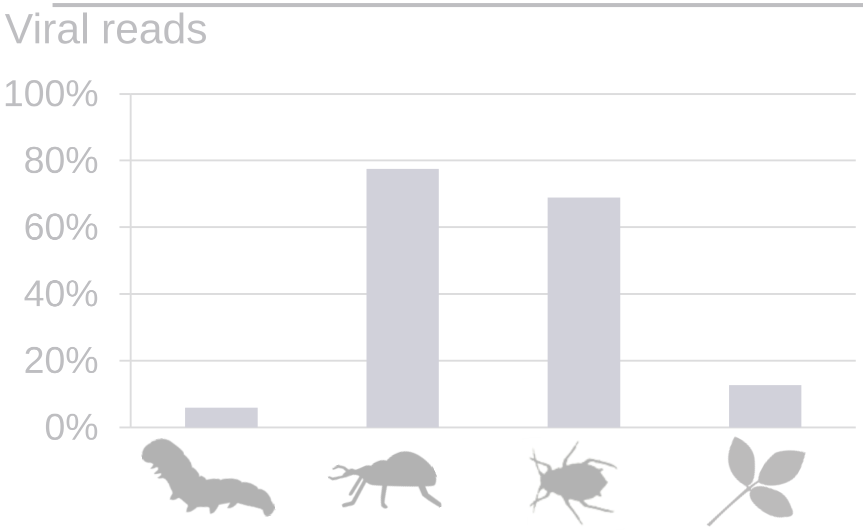
| Host | Number of samples | Number of individuals | Number of cleaned reads |
|---|---|---|---|
| *Helicoverpa armigera* | 132 | 1 590 | 9 318 409 |
| *Hypera postica* | 14 | ≈ 1400 | 2 928 803 |
| *Acyrthosiphon pisum* | 4 | ≈ 400 | 1 450 950 |
| *Medicago sativa* + grassland plants | 19 | 530 | 1 423 462 |
| **Total** | **169** | **≈ 4 000** | **15 121 624** |

# Viromes overview

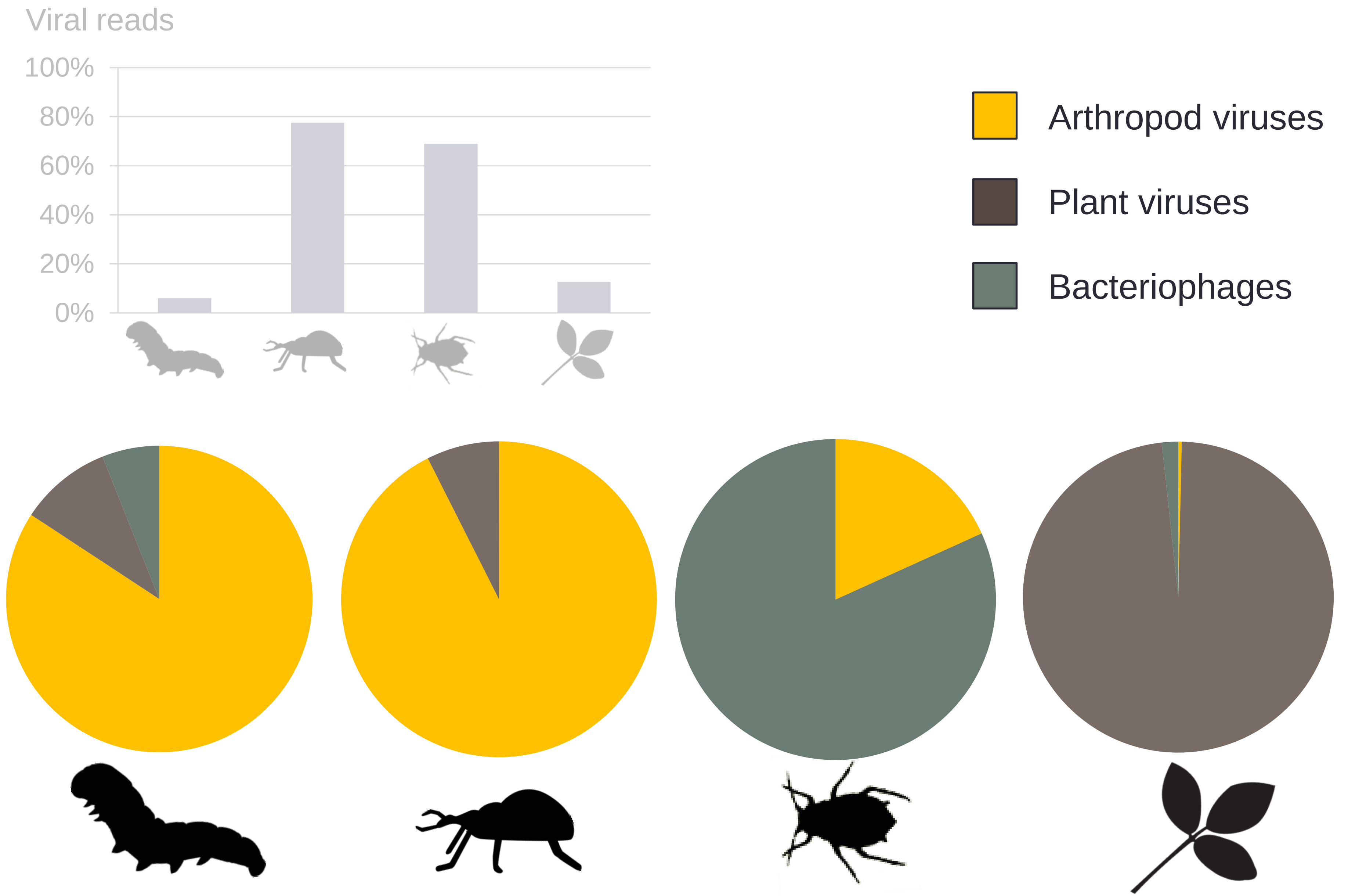
Viral reads



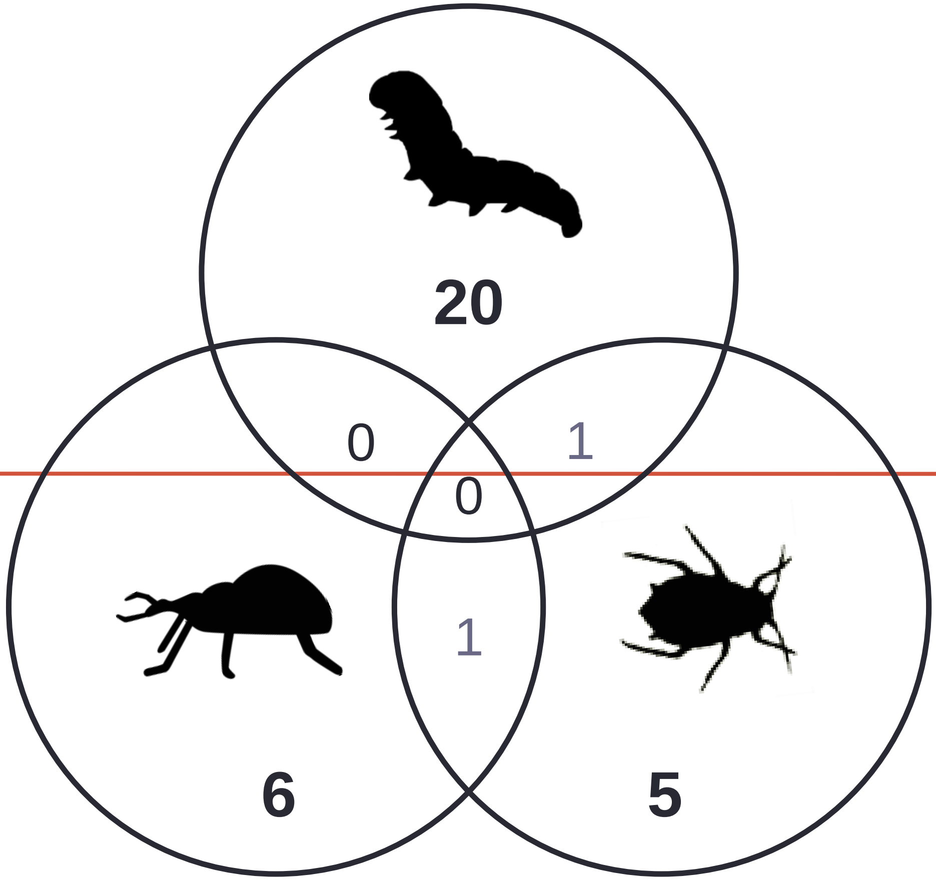
# Viromes overview



# Viromes overview



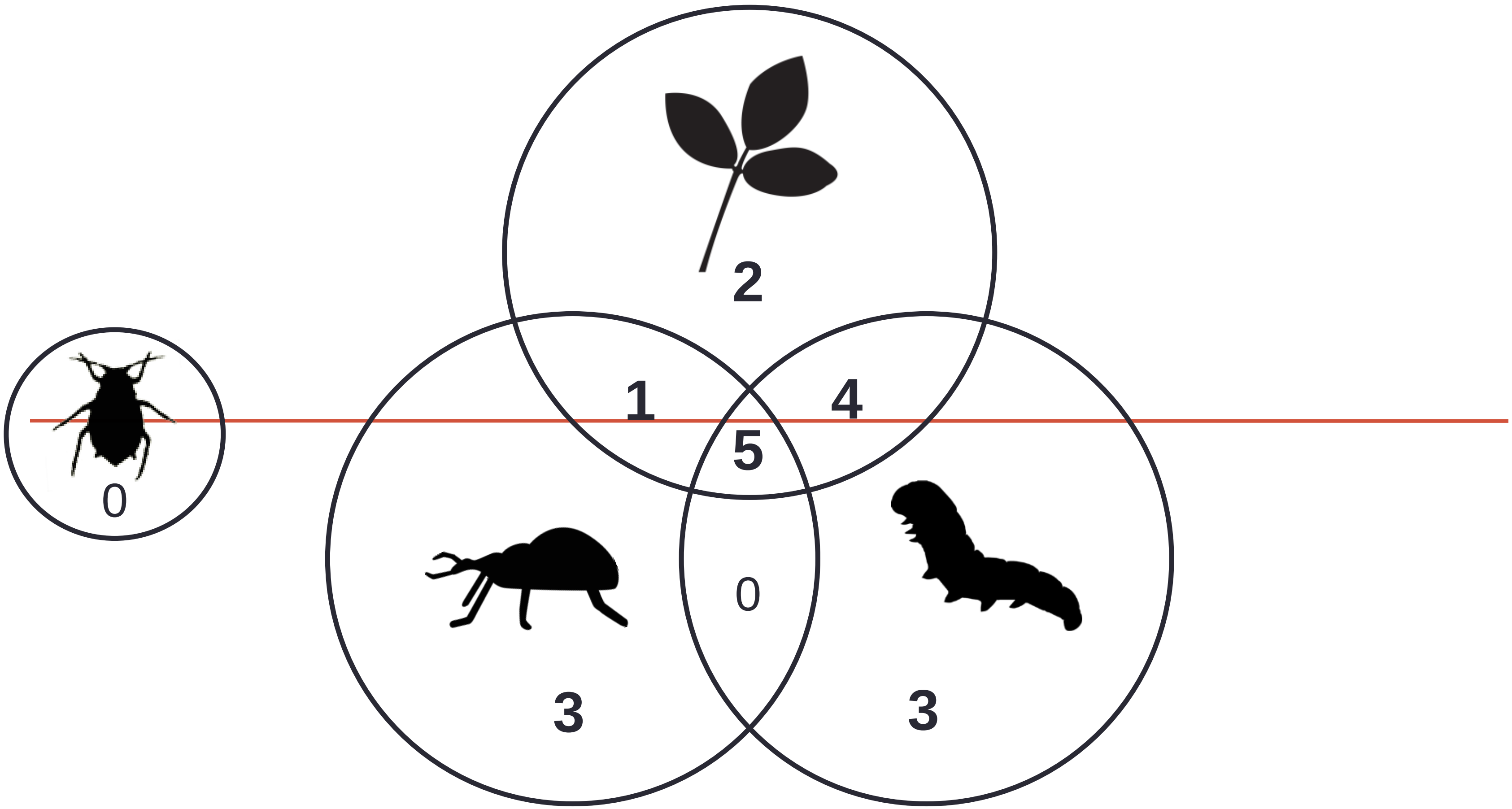
# Shared arthropod viruses between pests




Viruses shared : inter-sample contamination ?

**Viromes composition differs between pest species**

# Shared arthropod viruses between pests



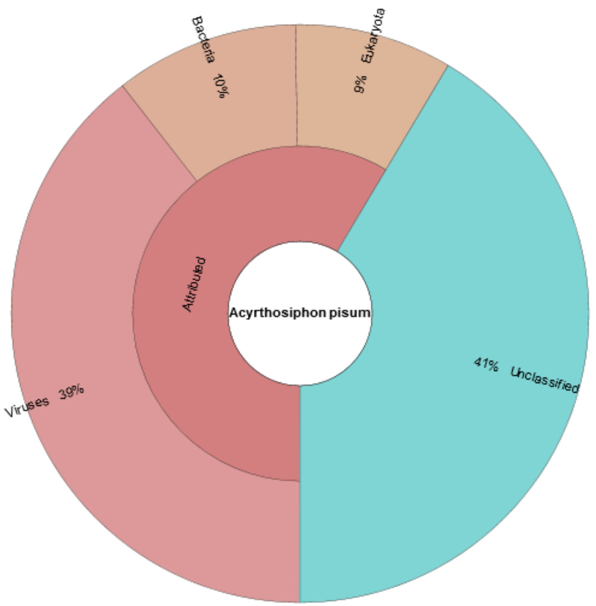

**Viruses shared in plants and arthropods**

**Viruses only found in arthropods**

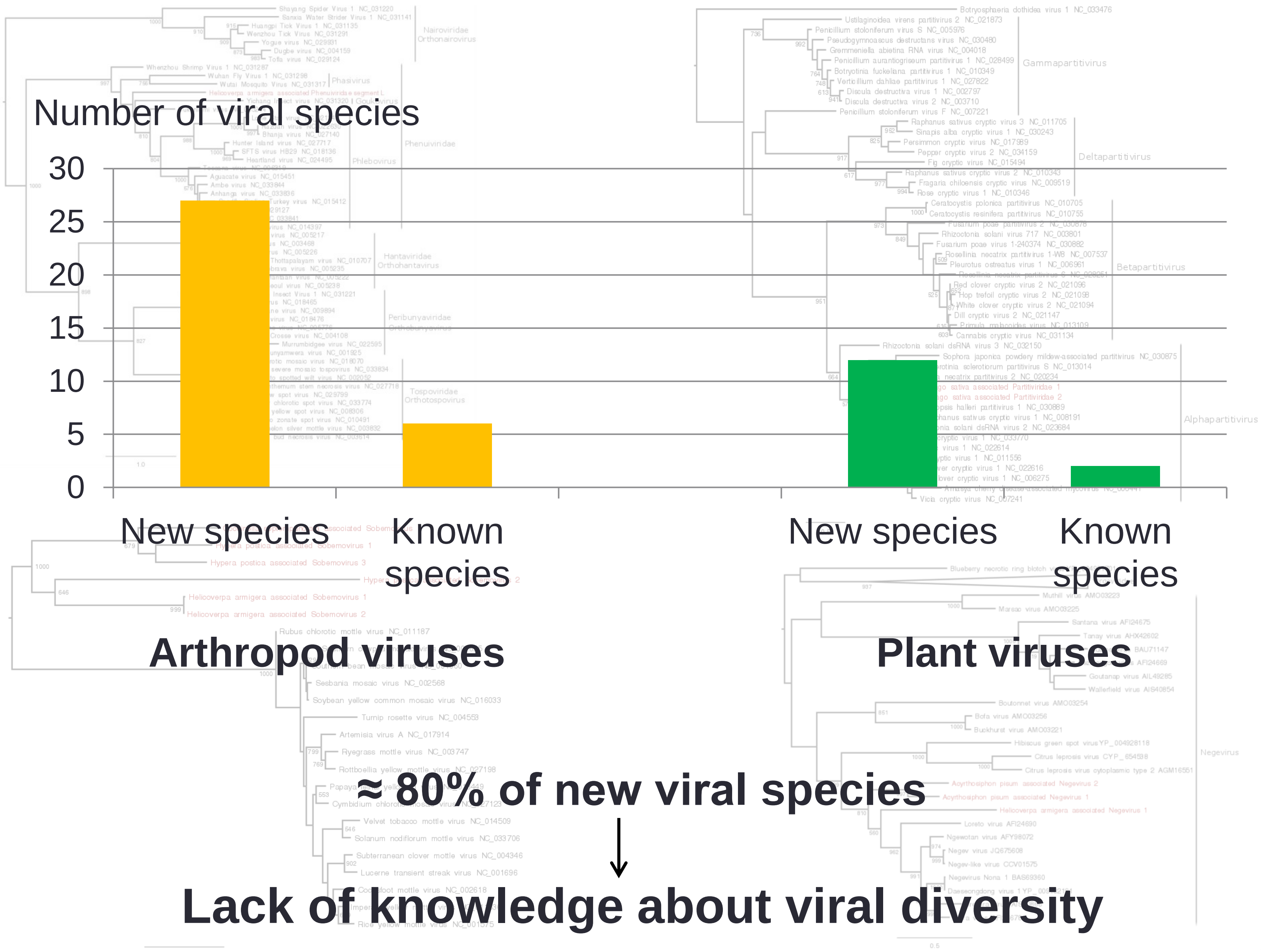
# Ex: the virome of *A. pisum*

- **For ≈ 100 000 reads :**
  - **>60% unclassified => dark matter (well, not so dark now → Francois et al. In prep)**
  - **40% attributed**
    - **15% host (ex A. pisum)**
    - **17% Symbionts**
    - **68% Viruses**
      - **Phages**
      - **Densoviruses**
      - **Iflaviruses**

**(1) Francois et al., in prep.**



# Virus discovery

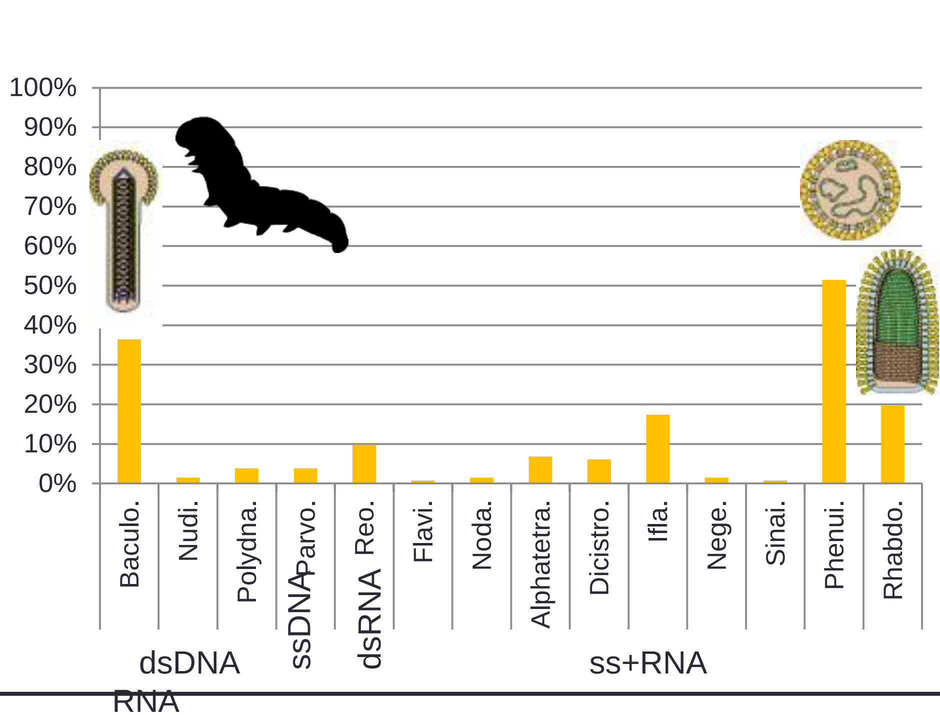


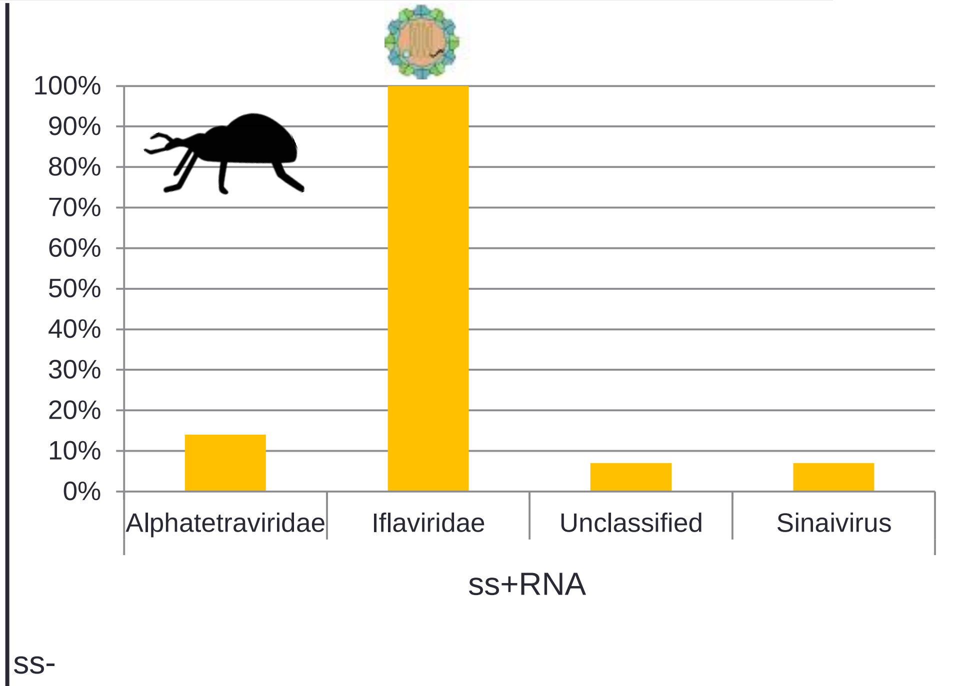

**≈ 80% of new viral species**

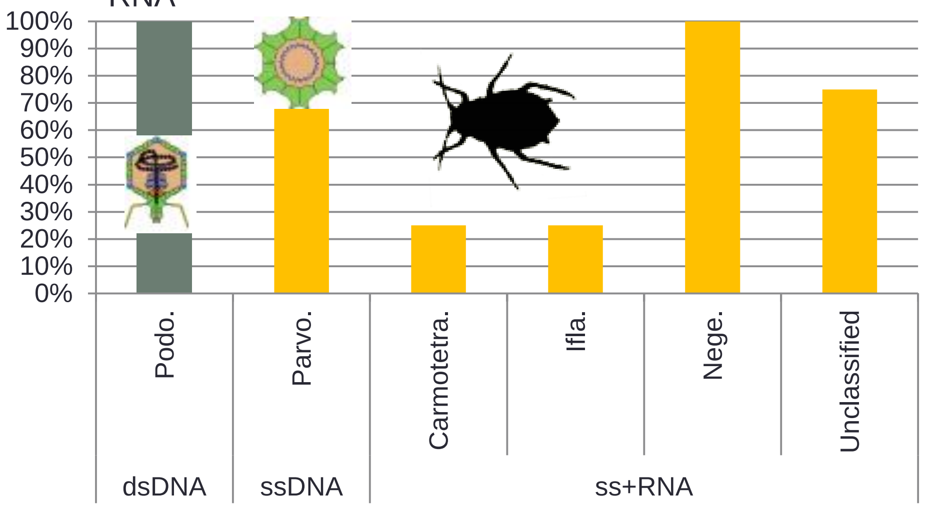
**↓**

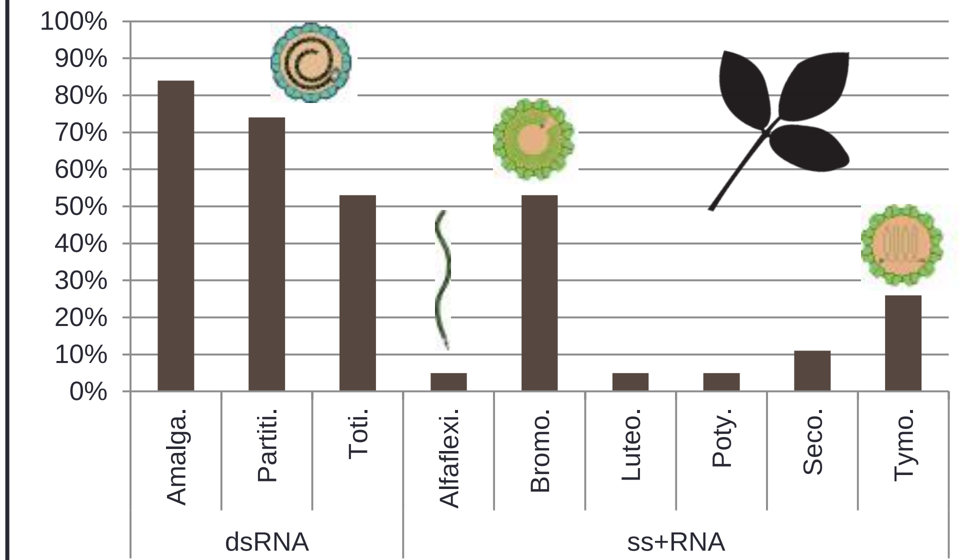
**Lack of knowledge about viral diversity**

# Virus prevalence



# Improve virus diagnosis in insect rearing

-

# Coupling NGS sequencing and PCR-based Methods

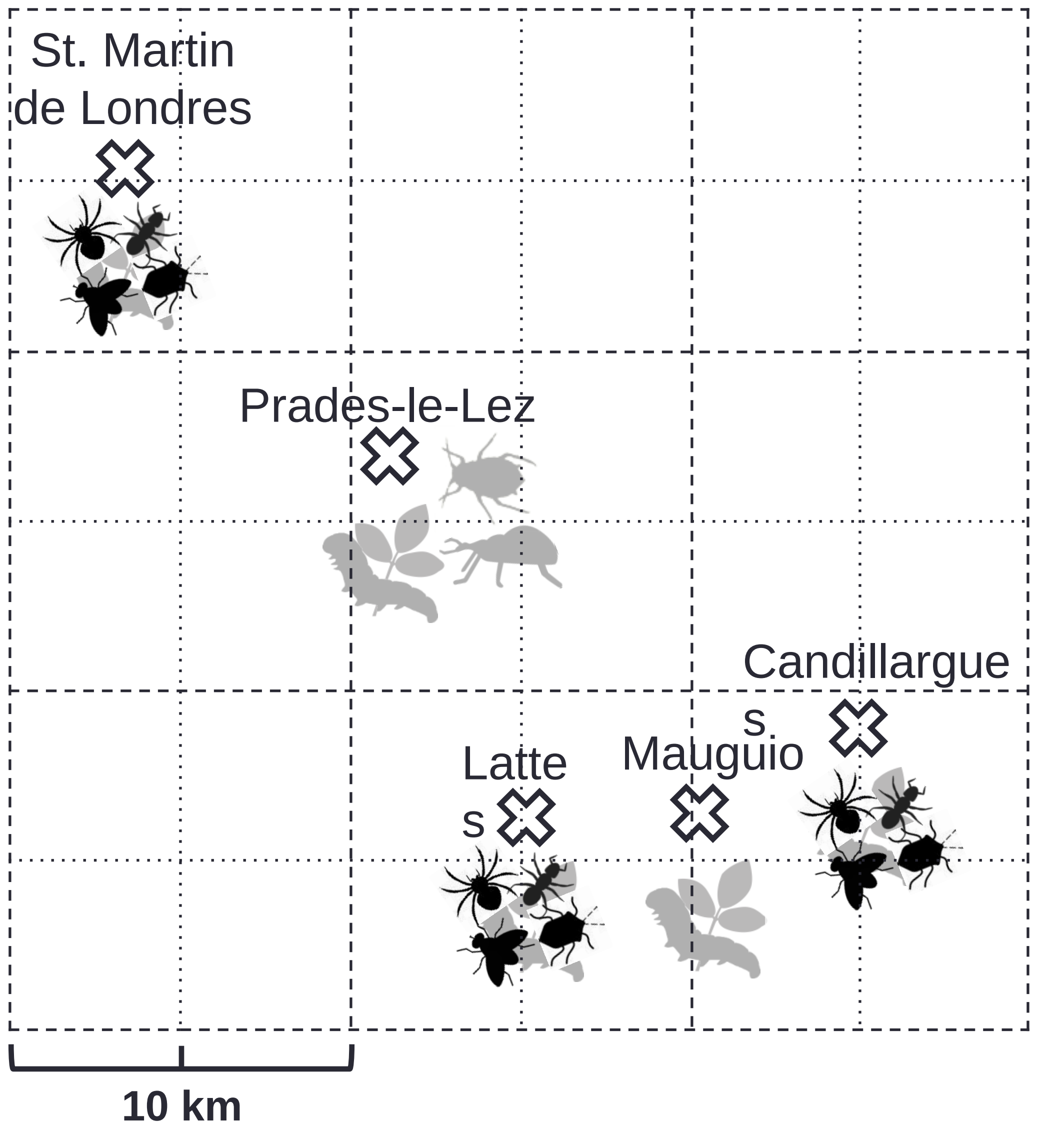
# Screening of the most prevalent viruses by PCR

## (13 viral species)

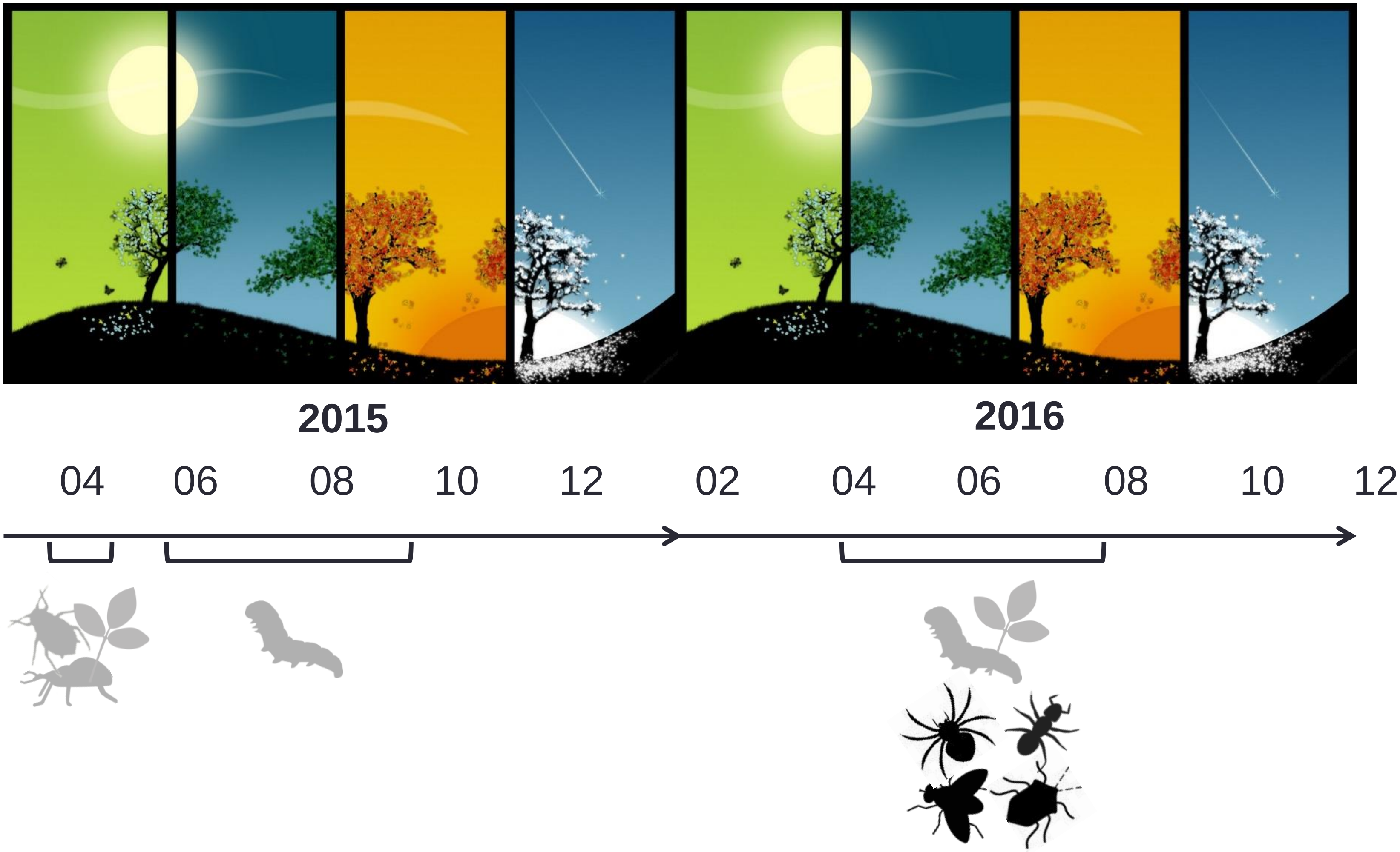
| Putative host | Baltimore classification | Viral family |
|---|---|---|
|  | ds DNA | Baculoviridae |
| | ds RNA | Reoviridae 1 |
| | | Reoviridae 2 |
| | ss+ RNA | Iflaviridae 2 |
| | | Nodaviridae |
| | | Alphatetraviridae |
|  | ss+ RNA | Iflaviridae 1 |
| | | Iflaviridae 2 |
|  | ds DNA | Podoviridae |
| | ss DNA | Parvoviridae |
| | ss+ RNA | Dicistroviridae |
| | | Negevirus |
| | | Unclassified |

# Sampling area

N



# A two years survey



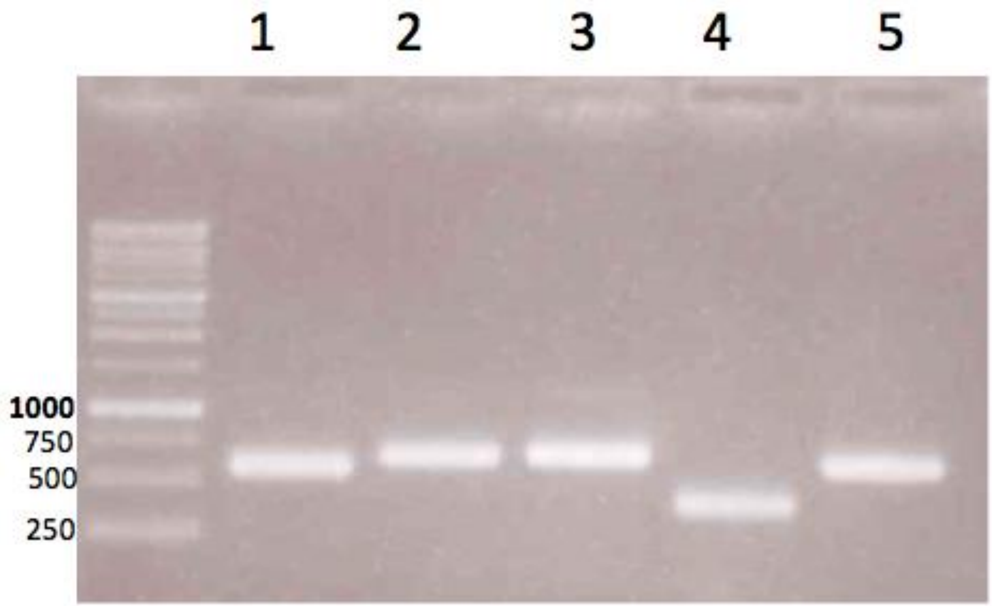
# Arthropod communities screening

**295 pools – 3 492 individuals**

| Host Taxonomy | | Pools (%) | Individuals (%) |
|---|---|---|---|
| Arthropods Hexapoda | Blattodea | 0% | 0% |
| | Coleoptera | 16% | 20% |
| | Dermaptera | 2% | 1% |
| | Diptera | 5% | 6% |
| | Hemiptera | 20% | 23% |
| | Hymenoptera | 5% | 23% |
| | Lepidoptera | 13% | 4% |
| | Mantoptera | 1% | 0% |
| | Neuroptera | 0% | 0% |
| | Orthoptera | 20% | 13% |
| Arthropods Chelicerata | Araneae | 12% | 7% |
| | Opiliones | 4% | 3% |
| **Total** | | **100 %** | **100%** |

# Arthropod communities screening

- Design specific primers, PCR, (RACE and cloning)




1: primers 61F-639R (amplicon 579 bp)
2. Primers 556F-1203R ( amplicon 648 bp)
3. Primers 1125F-1793R ( amplicon 669 bp)
4. Primers 1675f-2070R ( amplicon 395 bp)
5. Primers 20F- 639R( amplicon 619 bp)

Recover viruses from Insect samples

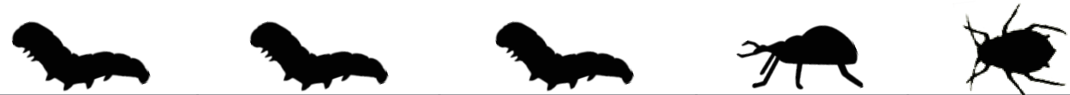
# PCR screening of viruses in Arthropod communities

## 295 pools – 3 492 individuals

| Host Taxonomy | | Pools (%) | Individuals (%) | Iflaviridae 2 | Nodaviridae 2 | Alphatetraviridae | Iflaviridae 1 | Dicistroviridae |
|---|---|---|---|---|---|---|---|---|
| Arthropods Hexapoda | Blattodea | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | Coleoptera | 16% | 20% | **15%** | 0% | 0% | **6%** | **17%** |
| | Dermaptera | 2% | 1% | 0% | 0% | 0% | 0% | 0% |
| | Diptera | 5% | 6% | **40%** | 0% | 0% | 0% | 0% |
| | Hemiptera | 20% | 23% | **22%** | **2%** | 0% | **2%** | **15%** |
| | Hymenoptera | 5% | 23% | **33%** | **13%** | 0% | **7%** | **33%** |
| | Lepidoptera | 13% | 4% | **18%** | 0% | 0% | 0% | 0% |
| | Mantoptera | 1% | 0% | **33%** | 0% | 0% | 0% | 0% |
| | Neuroptera | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | Orthoptera | 20% | 13% | **10%** | **2%** | 0% | 0% | **3%** |
| Arthropods Chelicerata | Araneae | 12% | 7% | **41%** | **9%** | **9%** | **3%** | **15%** |
| | Opiliones | 4% | 3% | **33%** | **42%** | **17%** | **8%** | **25%** |
| **Total** | | **100%** | **100%** | **21%** | **4%** | **2%** | **2%** | **11%** |

# PCR screening

**295 pools – 3 492 individuals**



| Host Taxonomy | | Pools (%) | Individuals (%) | Iflaviridae 2 | Nodaviridae 2 | Alphatetraviridae | Iflaviridae 1 | Dicistroviridae |
|---|---|---|---|---|---|---|---|---|
| Arthropods Hexapoda | Blattodea | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | Coleoptera | 16% | [illegible] | **15%** | 0% | 0% | **6%** | **17%** |
| | Dermaptera | 2% | 1% | 0% | 0% | 0% | 0% | 0% |
| | Diptera | 5% | 6% | **40%** | 0% | 0% | 0% | 0% |
| | Hemiptera | 20% | [illegible] | **22%** | **2%** | 0% | **2%** | **15%** |
| | Hymenoptera | 5% | [illegible] | **33%** | **13%** | 0% | **7%** | **33%** |
| | Lepidoptera | 13% | 4% | **18%** | 0% | 0% | 0% | 0% |
| | Mantoptera | 1% | 0% | **33%** | 0% | 0% | 0% | 0% |
| | Neuroptera | 0% | 0% | 0% | 0% | 0% | 0% | 0% |
| | Orthoptera | 20% | 13% | **10%** | **2%** | 0% | 0% | **3%** |
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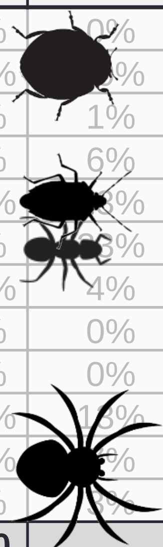
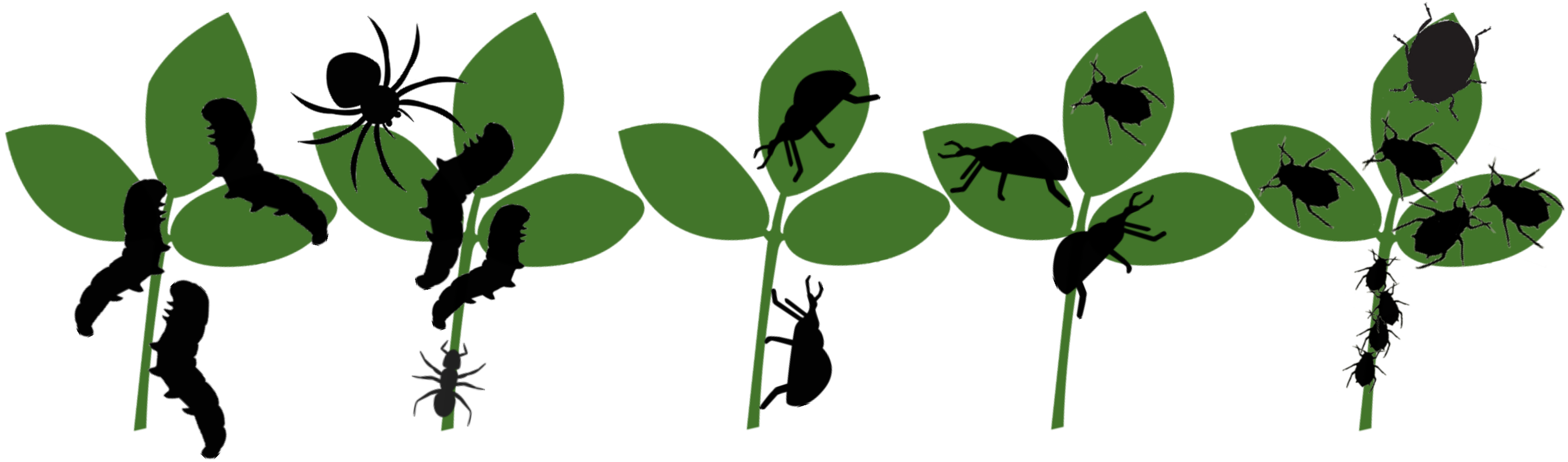


**Virus concentrated in predators → accumulation by trophic network**

# Virus circulation in ecosystems

Trophic network



# Virus circulation in ecosystems

Trophic network

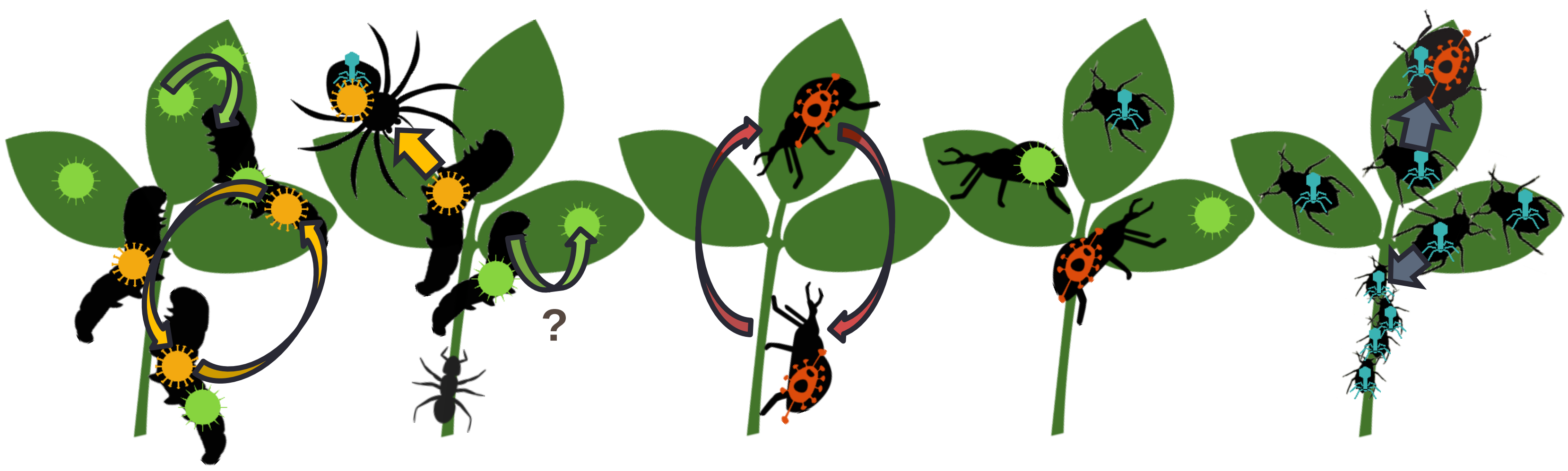
Viromes composition

# Virus circulation in ecosystems

Trophic network

Viromes composition

Virus circulation



# Conclusion

| **Viral metagenomics** | **PCR** |
|---|---|
| Without a priori ↓ Virus **discovery** | With a priori ↓ Virus **screening** |
| Up to 100 samples* per analysis (30-50€ sample) | Up to 396 samples* per analysis (0.2€ sample) |

* Samples can be pools of insects

Virus **diversity** and **prevalence**
in Insect communities

⇩

Improve management
At various levels

INRA SCIENCE & IMPACT

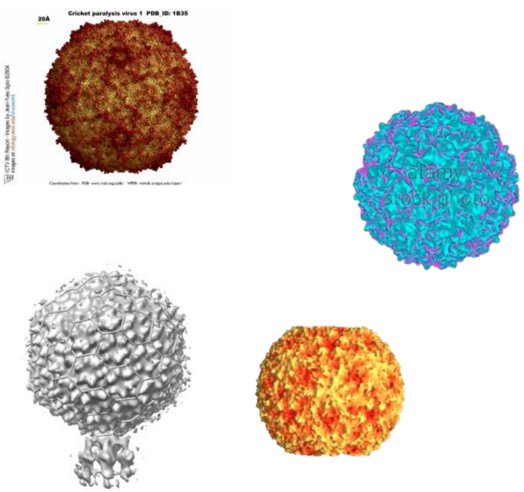
DGIMI Diversité, Génomes & Interactions Microorganismes Insectes


Anne-Sophie Gosselin
Guillaume Cambray
Sarah Francois
Doriane Mutuel
Fncs C
Marc Ravallec
Cécilia Multeau
Laetitia Pigeyre
Thierry Dupressoir
Marc Ravallec
Yuan Wang

P. Rouymagnac
D. Fillous
R. Froissart

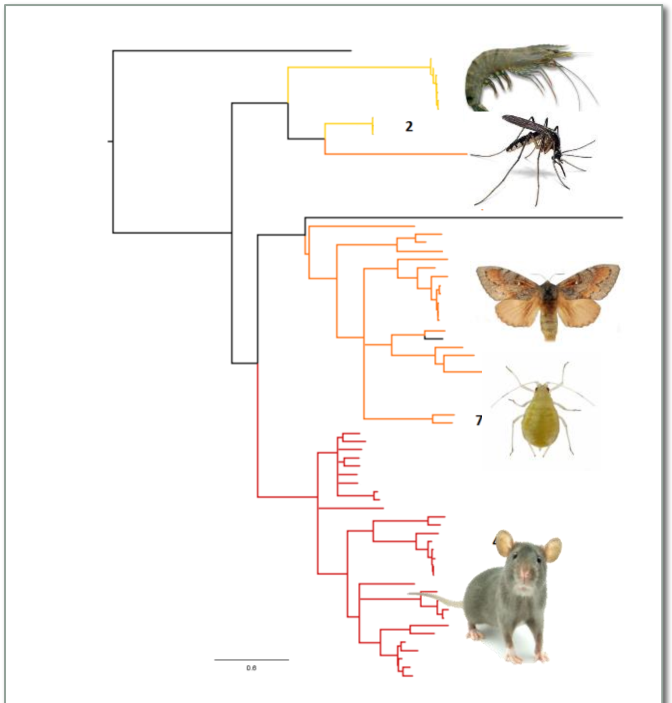
UMR BGPI Biologie et Génétique des Interactions Plante-Parasite


Thank You for your Attention!

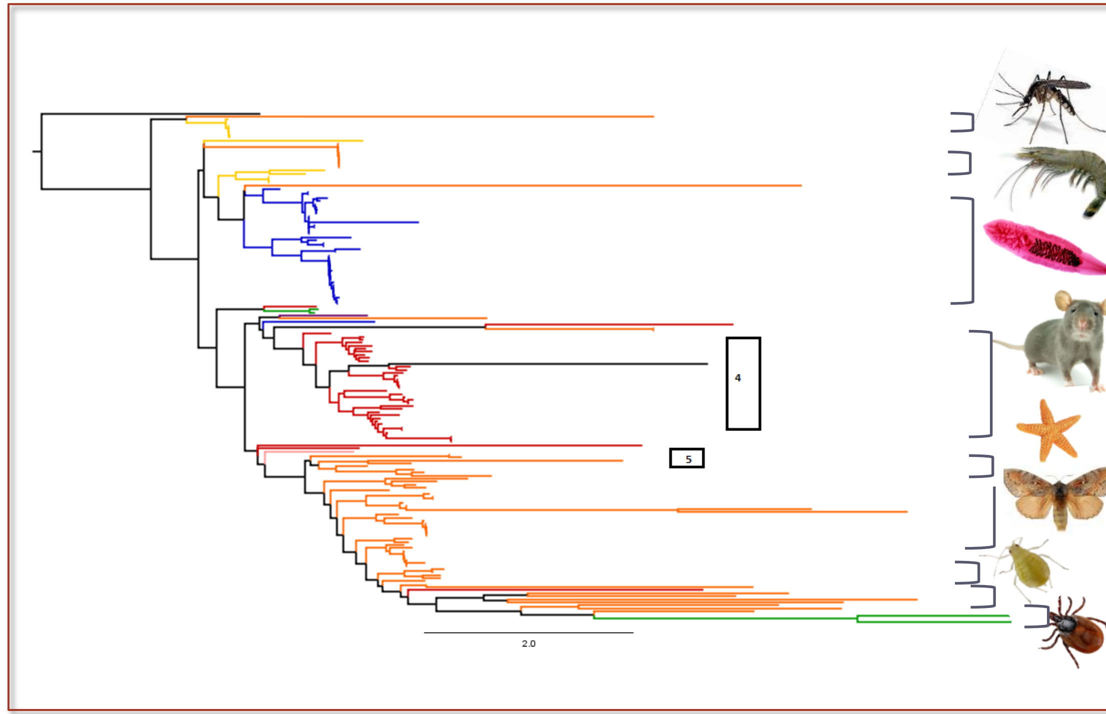



# Ex : Insect parvovirus diversity

Before 2016




Since 2016




- Cnidaires
- Annélides
- Plathelminthes
- Arachnides
- Crustacés
- Insectes
- Echinodermes
- Urochordés
- Vertébrés

**François et al., 2014, 2016, 2017**