

Phylogenomics and comparative genomics: The case of coevolution between the swallowtail butterflies (Papilionidae) and their host plants



GAS seminar
November 30th, 2021

Rémi Allio



Summary

PART 1 - Phylogenomics and comparative genomics in myrmecophagous mammals



PART 2 - The case of coevolution between the swallowtail butterflies (Papilionidae) and their host plants



Summary

PART 1 - Phylogenomics and comparative genomics in myrmecophagous mammals



1 – Genome wide approach

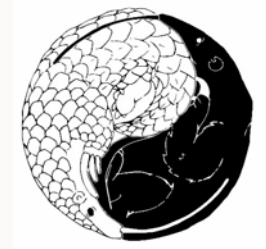
2 – Candidate gene family: Chitinases

Understanding the complex interplay between morphology, genome, and microbiome



Consolidator Grant
2016-2022

Morphology



Development



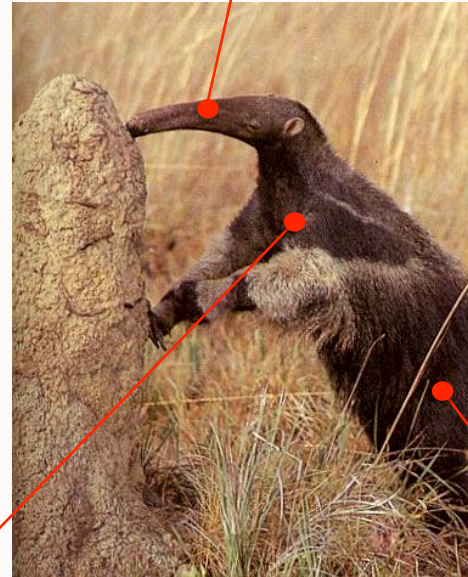
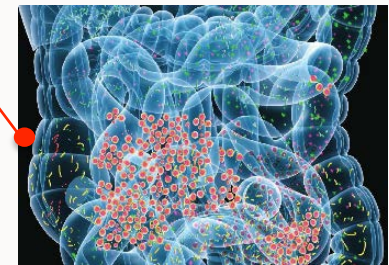
Genome



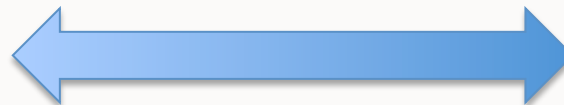
Gut anatomy



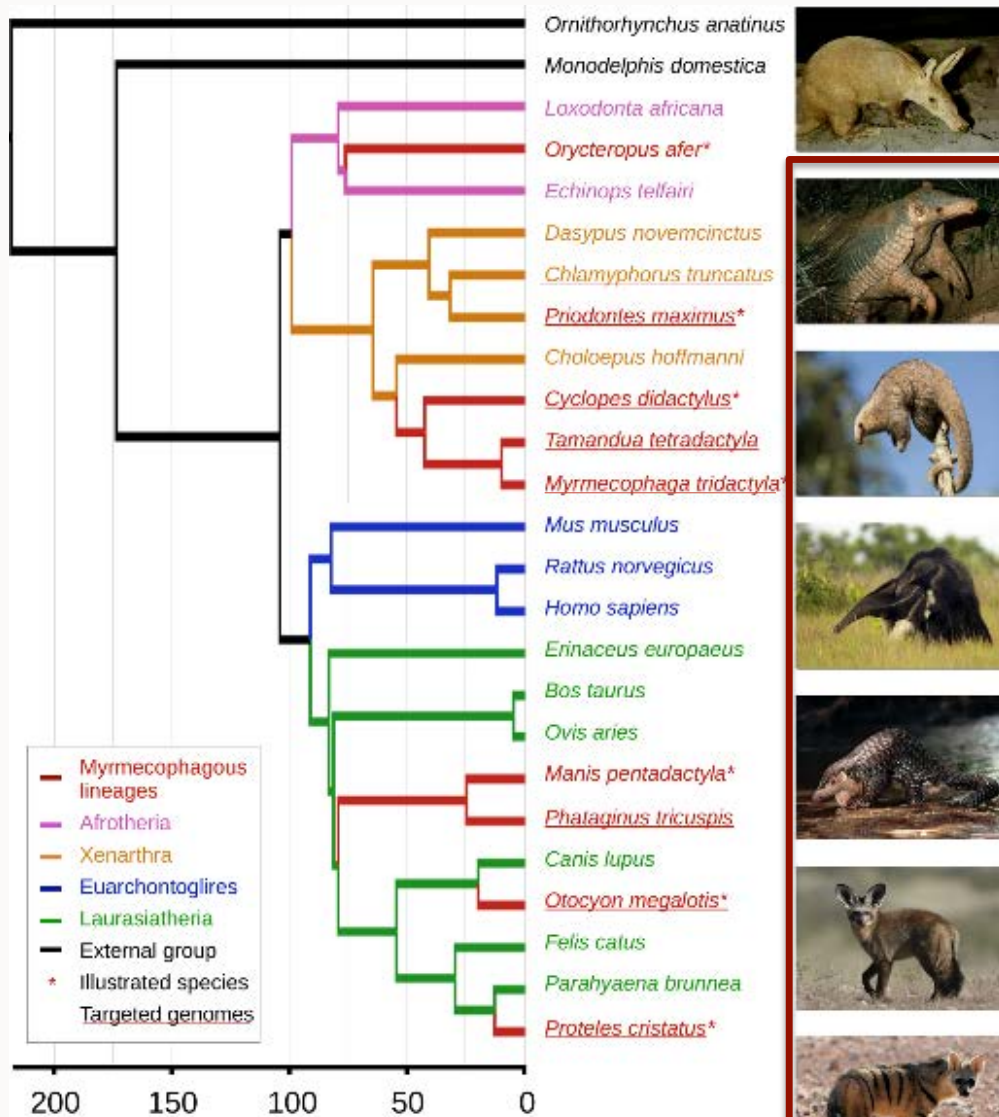
Microbiome



Host immunity



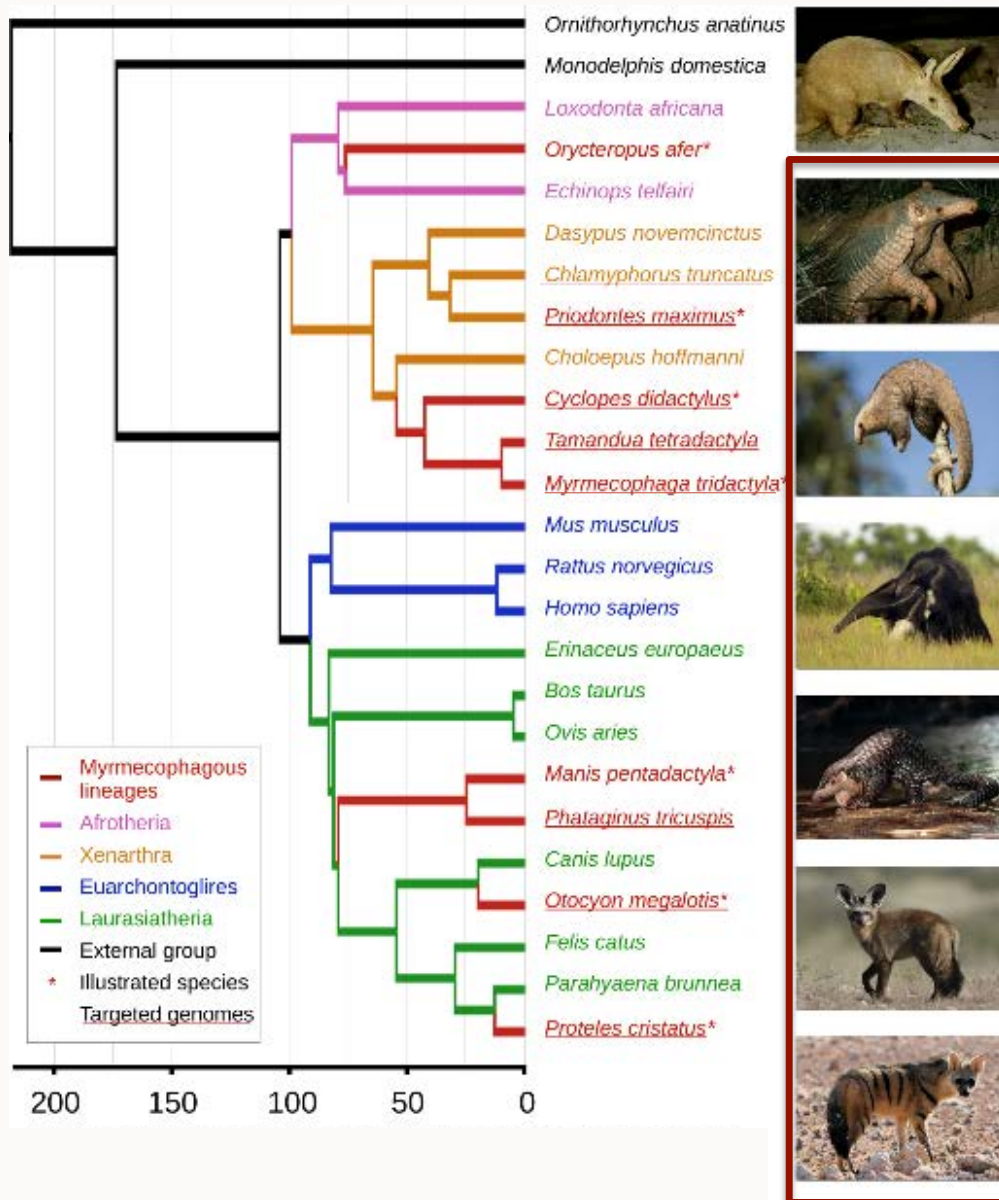
Genome assemblies



9 genomes
Genome size 2-5 Gb



Genome assemblies



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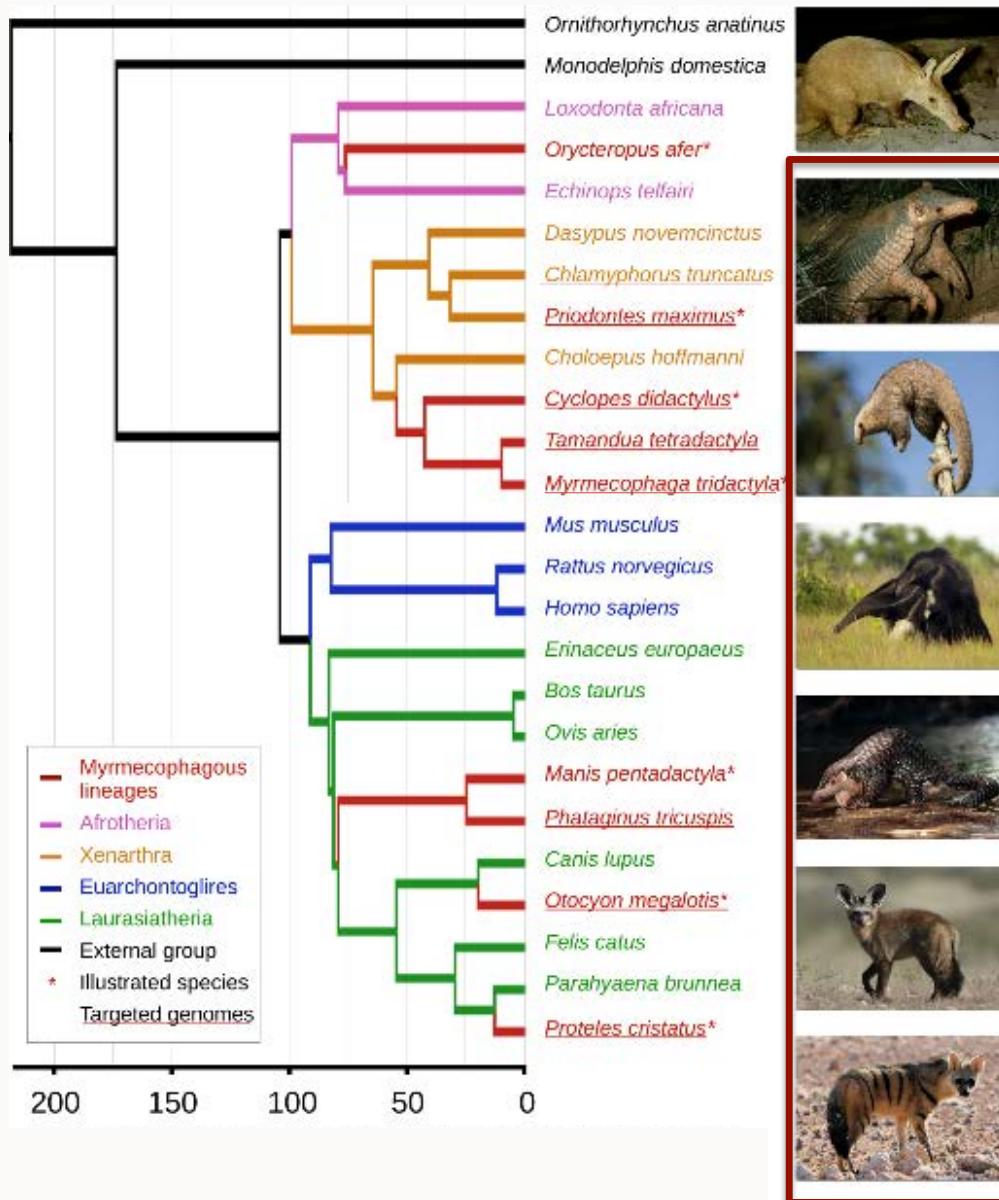


Short reads sequencing
(Illumina)
+
Long reads sequencing
(PacBio)



Hybrid Assembly

Genome assemblies



9 genomes
Genome size 2-5 Gb



Short reads sequencing
(Illumina)
+
Long reads sequencing
(PacBio)
(Minion)



Hybrid Assembly

Genome from road kills

Marie-Ka
Tilak



Roadkill samples



Sampling



DNA extraction



Library construction



Genome from road kills

Marie-Ka
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Roadkill samples



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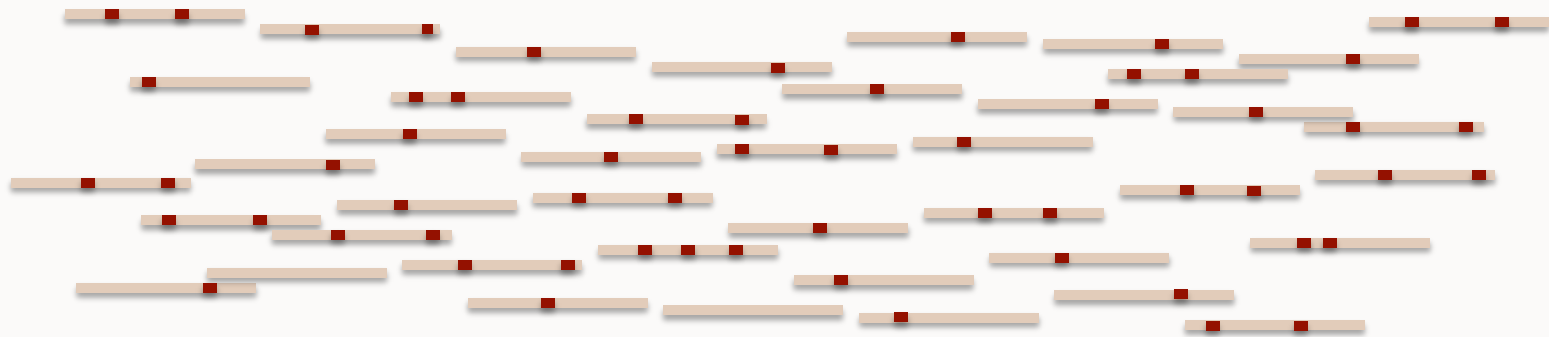
DNA extraction



Library construction



MinION®



Many **short** reads with **many errors**...

Genome from road kills

↳ An optimized protocol

Marie-Ka
Tilak



Roadkill samples

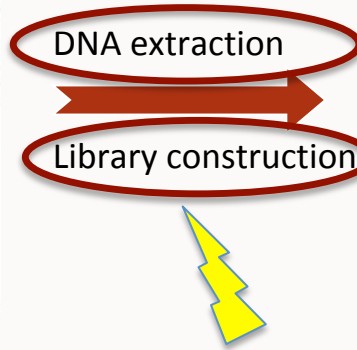


Sampling



DNA extraction

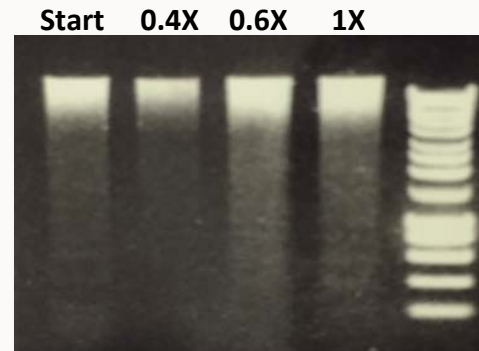
Library construction



Two keys



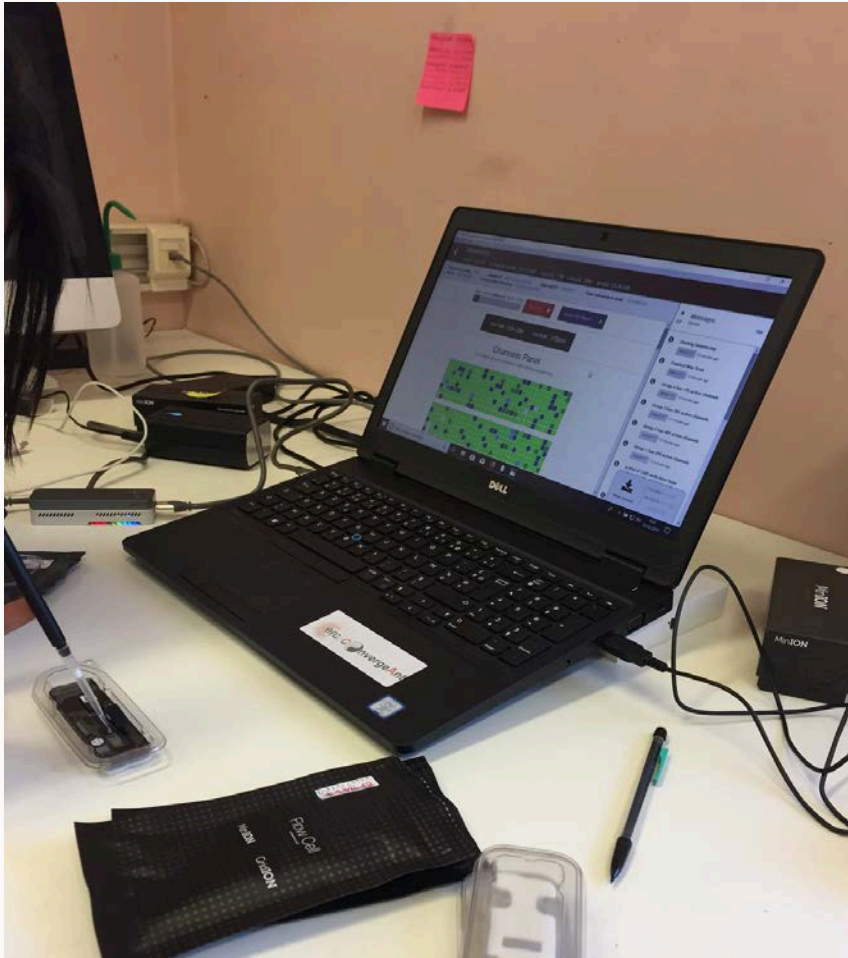
Tissues cleaning
DNA extraction check



DNA size selection
AMPure beads

It works!

Marie-Ka
Tilak

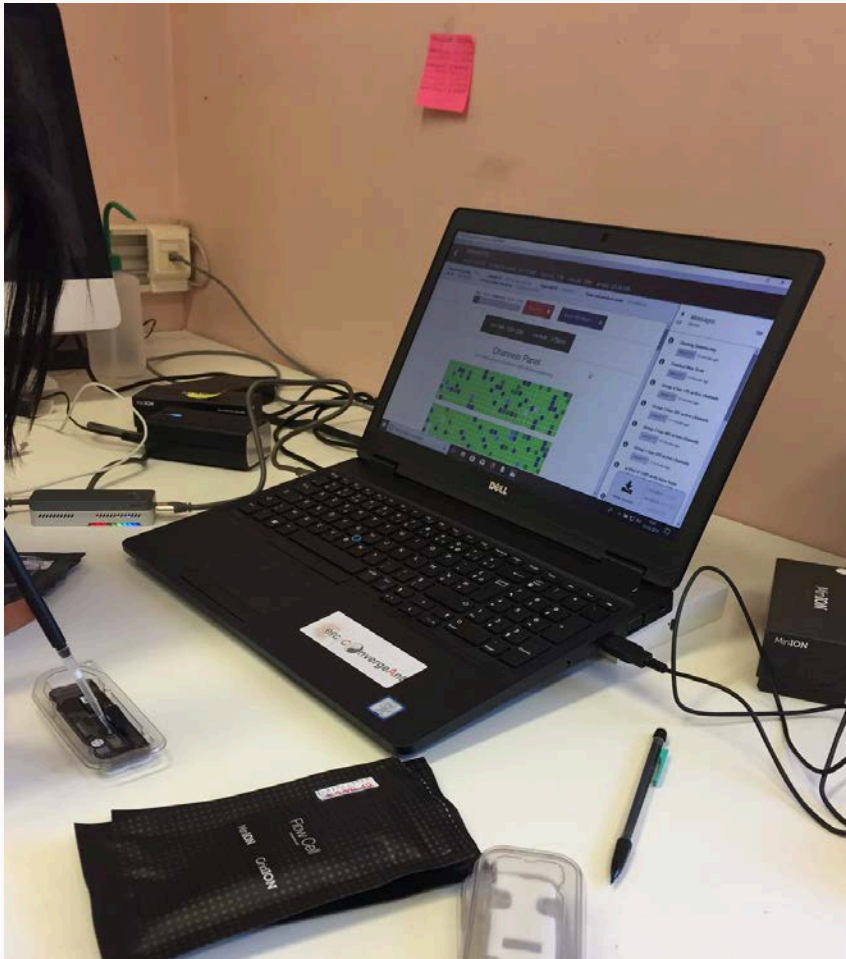


Read length distribution

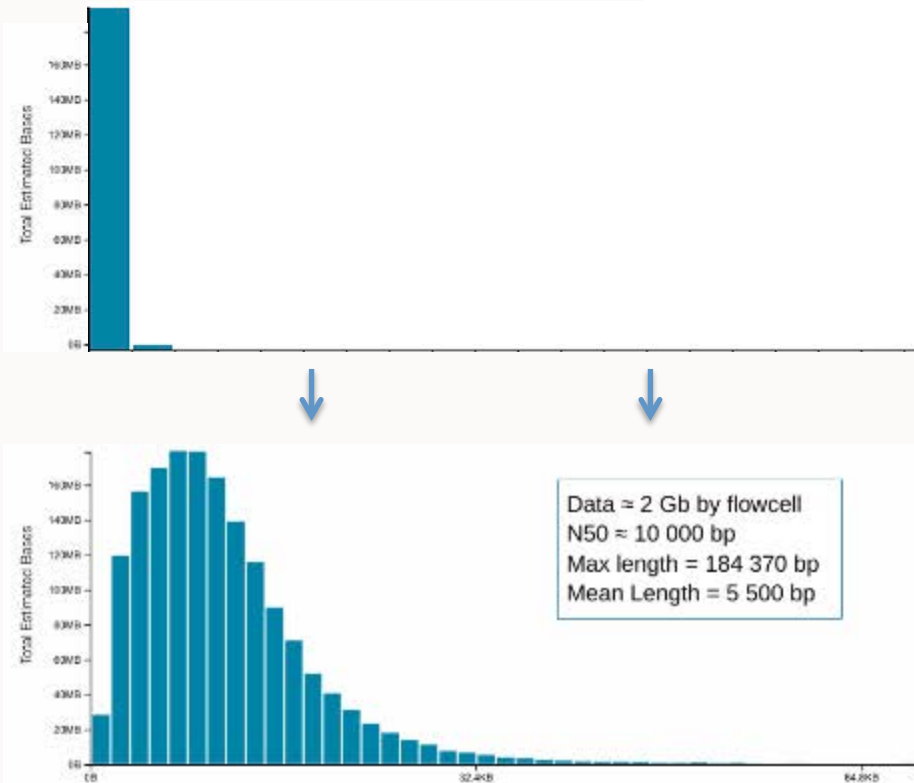


It works!

Marie-Ka
Tilak



Read length distribution



🌐 An optimized protocol for sequencing mammalian roadkill tissues with Oxford Nanopore Technology (ONT) V.2 ▼

Marie-Ka Tilak¹, Rémi Allio¹, Frédéric Delsuc¹

¹Institut des Sciences de l'Evolution de Montpellier (ISEM), CNRS, IRD, EPHE, Université de Montpellier, Montpellier, France

It works!

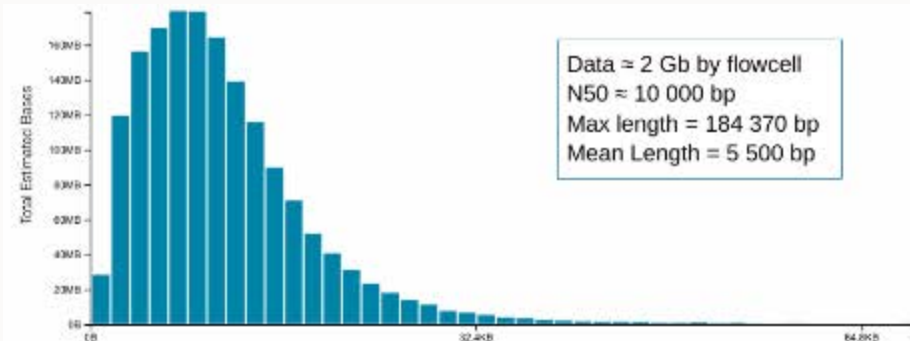
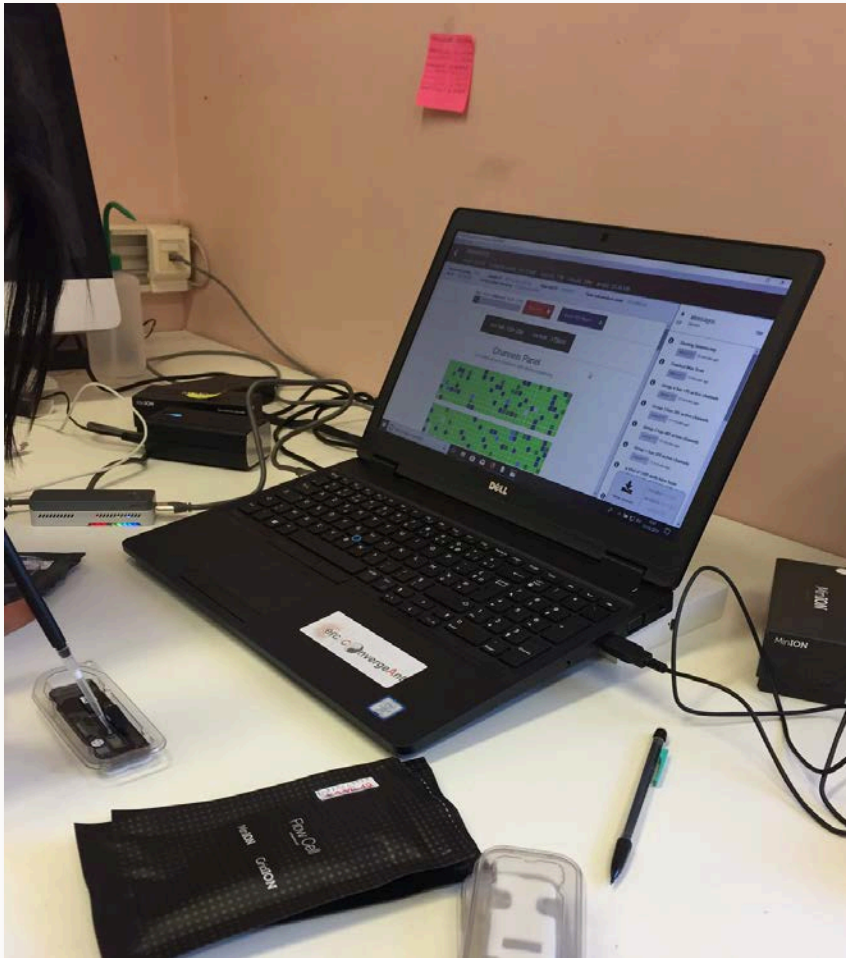
Marie-Ka
Tilak



Aardwolf (*Proteles cristatus*)

16 flow cells
27.5 Gb (~11x)

Scaffolds: 5,669
N50: 1.3 Mb



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All genomes

Marie-Ka
Tilak



Amandine
Magdeleine



Southern tamandua (*Tamandua tetradactyla*)

8 flow cells
51.3 Gb (~16x)



Scaffolds: 4,309
N50: 3.1 Mb

Giant anteater (*Myrmecophaga tridactyla*)

16 flow cells
50.3 Gb (~17x)



Scaffolds: 6,911
N50: 1.5 Mb

Silky anteater (*Cyclopes didactylus*)

21 flow cells
49.7 Gb (~15x)



Scaffolds: 14,869
N50: 795 Kb

Giant pangolin (*Smutsia gigantea*)

4 flow cells
29.1 Gb (~13x)



Scaffolds: 24,429
N50: 227 Kb

Pink fairy armadillo (*Chlamyphorus tr.*)

21 flow cells
32.9 Gb (~10x)



Scaffolds: 36,203
N50: 247 Kb

Bat-eared fox (*Otocyon megalotis*)

15 flow cells
33.0 Gb (~13x)



Scaffolds: 11,081
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Giant armadillo (*Priodontes maximus*)

15 flow cells
52.6Gb (~15x)



Scaffolds: 51,157
N50: 185 Kb

Pale-throated sloth (*Bradypus tridactylus*)

15 flow cells
41.7 Gb (~12x)



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BUSCO scores

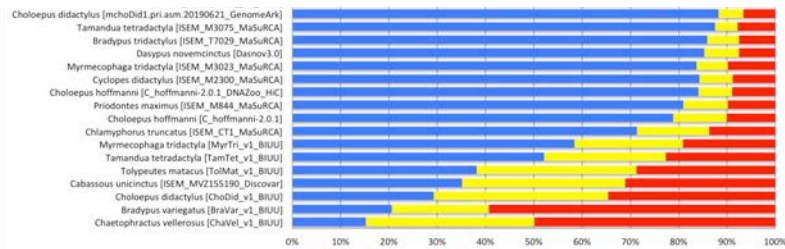
a) Carnivora



b) Pholidota



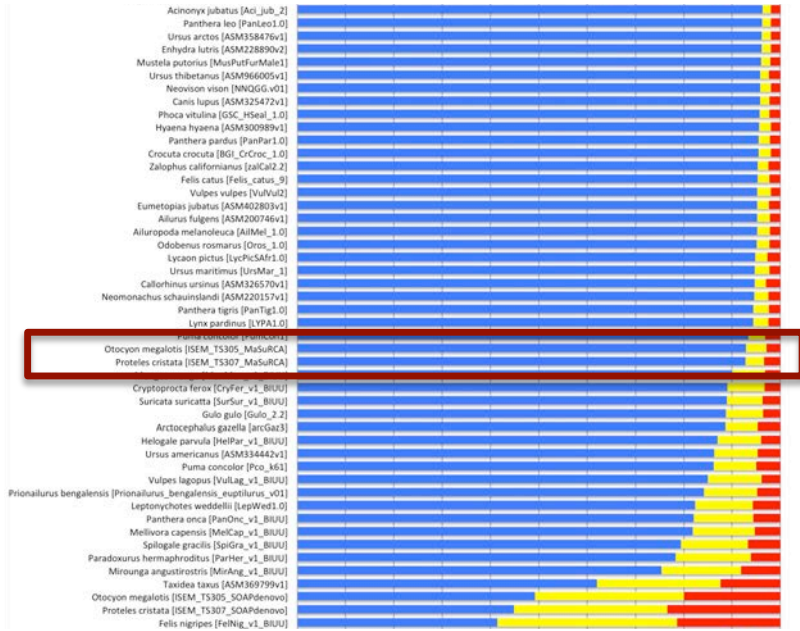
c) Xenarthra



Complete genes
 Fragmented genes
 Missing genes

BUSCO scores

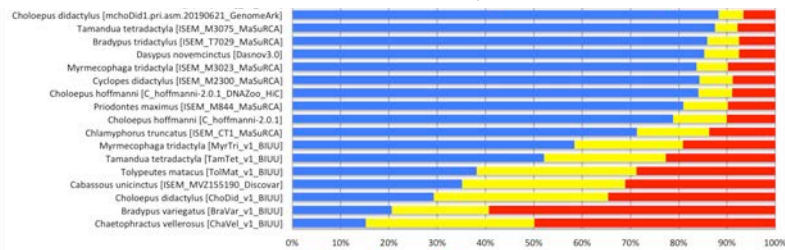
a) Carnivora



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c) Xenarthra



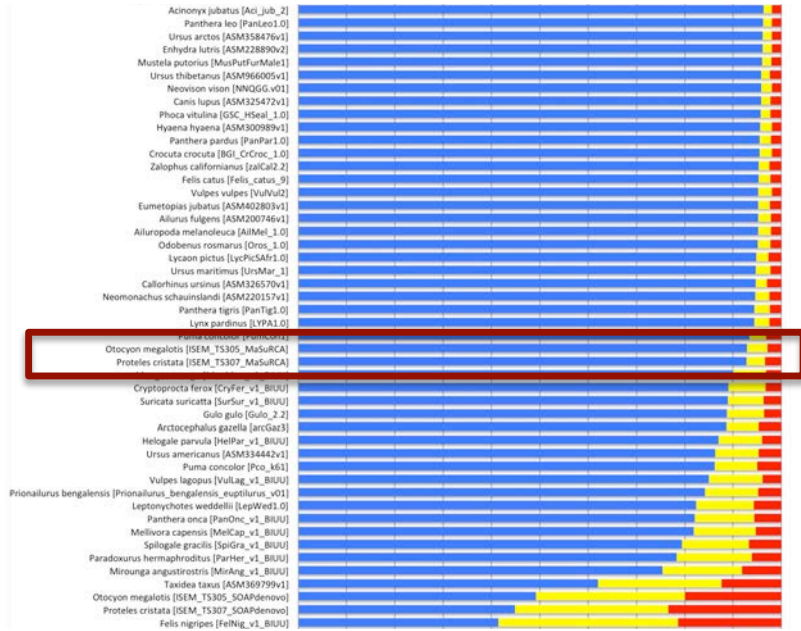
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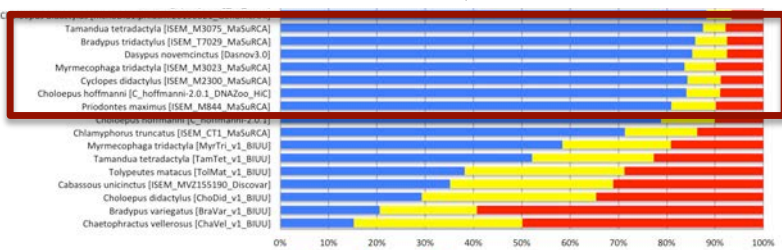
a) Carnivora



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c) Xenarthra



Complete genes
 Fragmented genes
 Missing genes

What are these genomes for?

What are these genomes for?

1- Species delineation analyses

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1- Species delineation analyses

In Carnivora



O. m.
megalotis

O. m.
virgatus



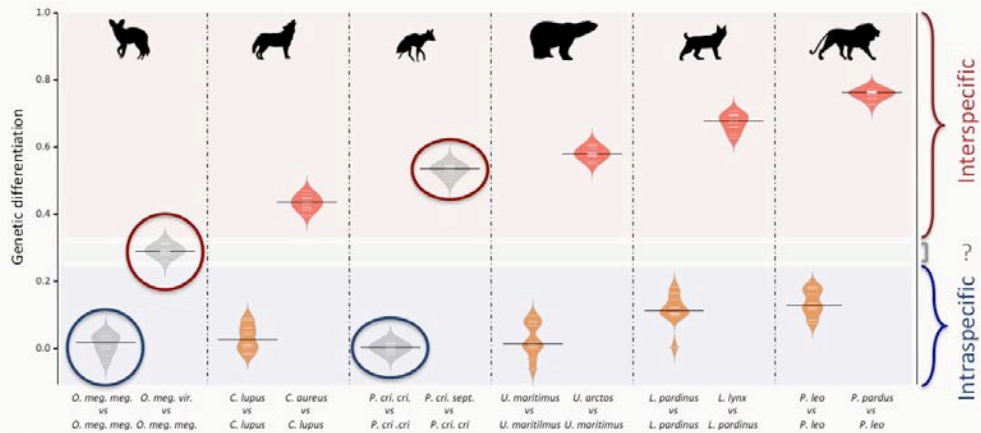
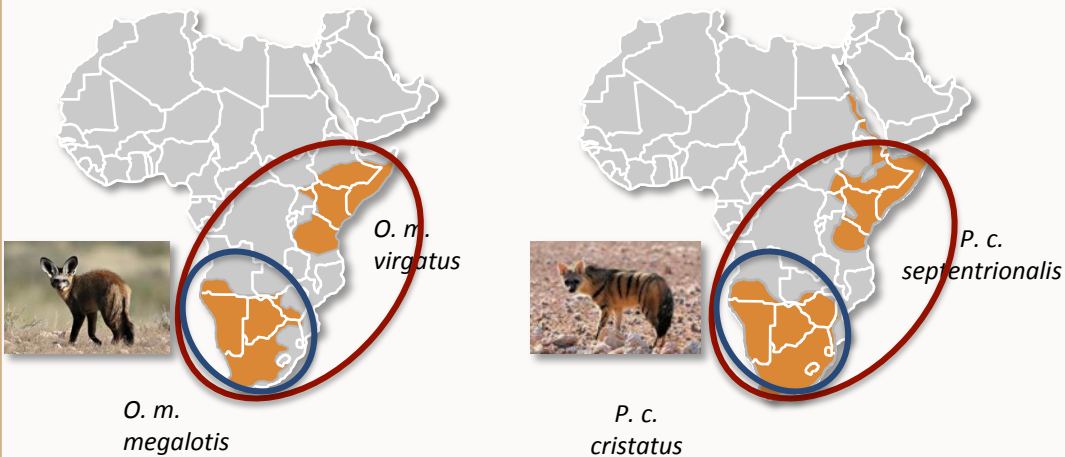
P. c.
cristatus

P. c.
septentrionalis

What are these genomes for?

1- Species delineation analyses

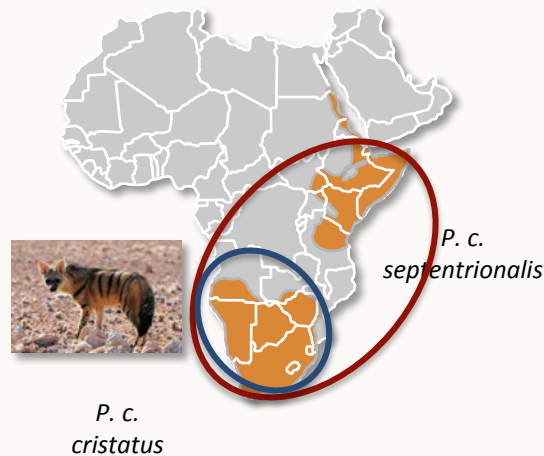
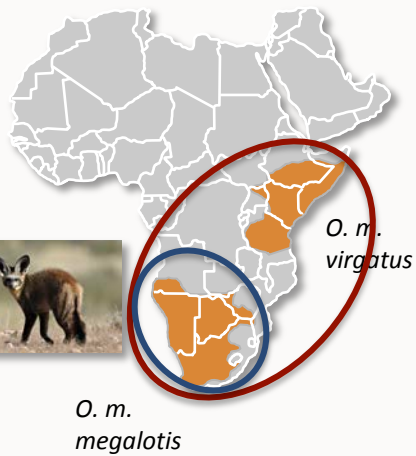
In Carnivora



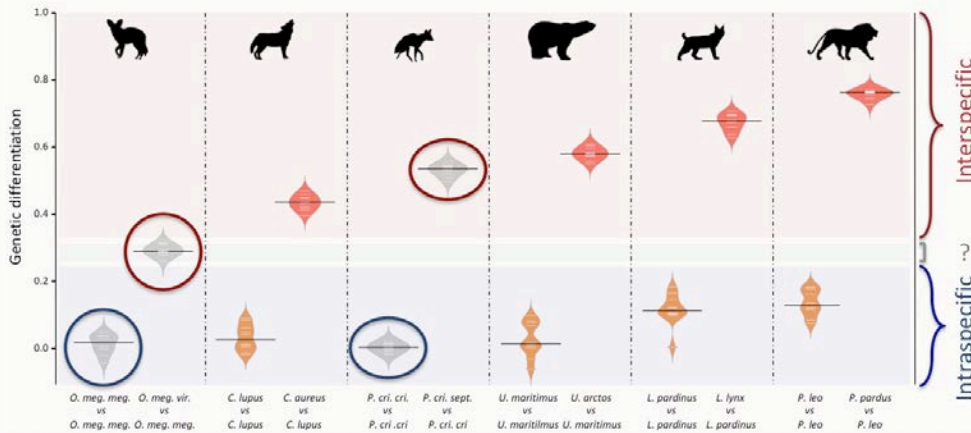
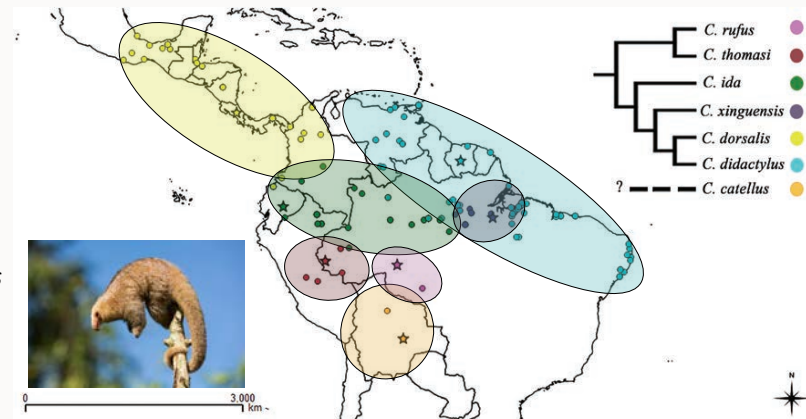
What are these genomes for?

1- Species delineation analyses

In Carnivora



In Xenarthra



In progress!

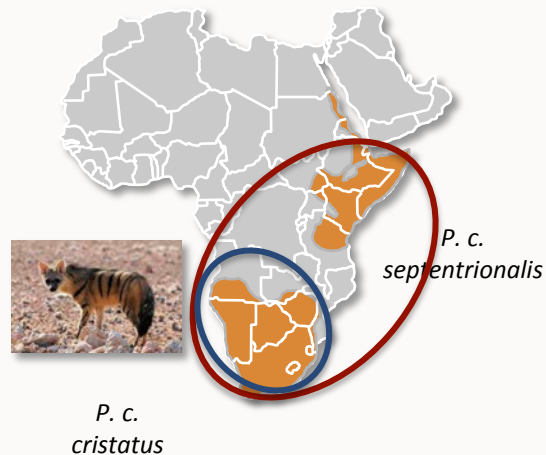
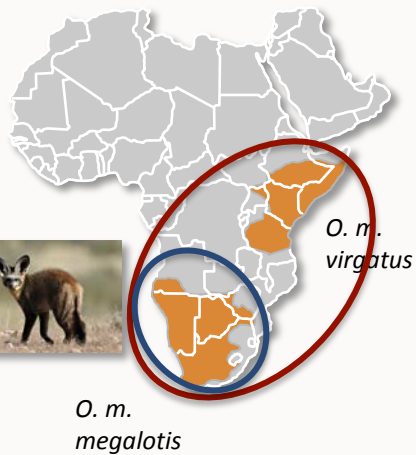


Mathilde Barthe

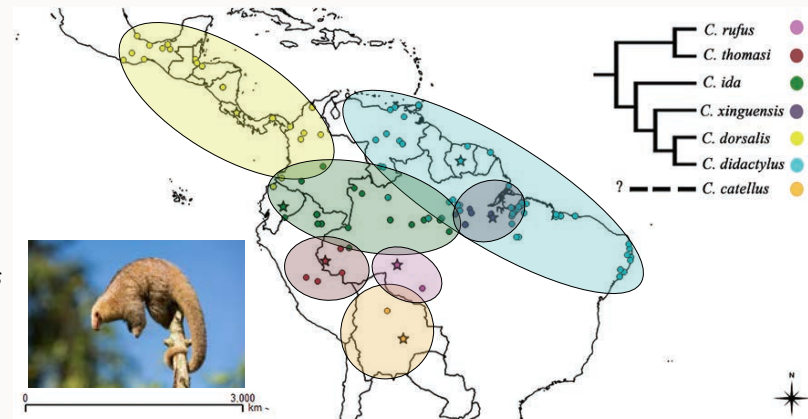
What are these genomes for?

1- Species delineation analyses

In Carnivora



In Xenarthra

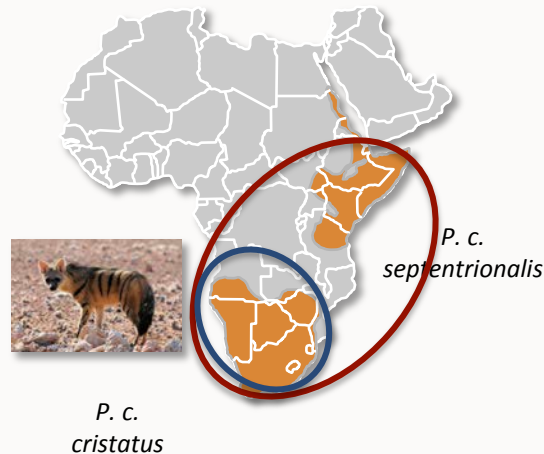
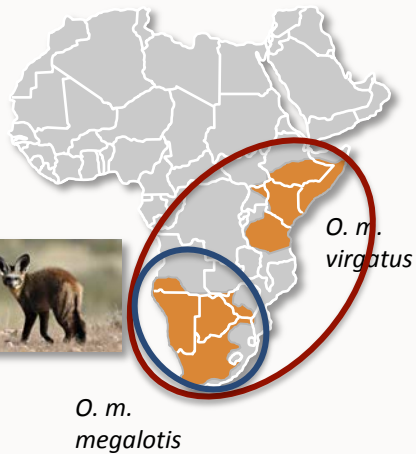


2- Genome-wide signatures of convergent evolution?

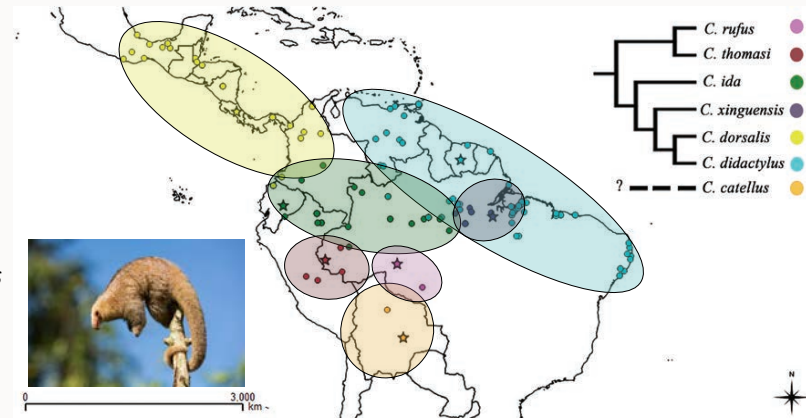
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1- Species delineation analyses

In Carnivora

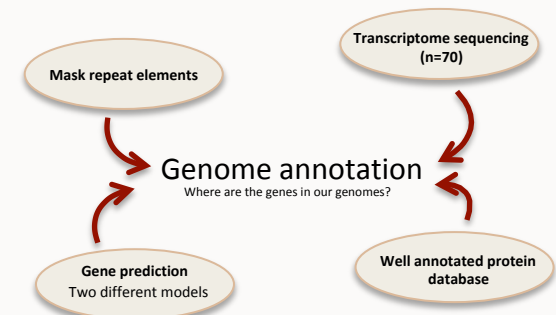


In Xenarthra



2- Genome-wide signatures of convergent evolution?

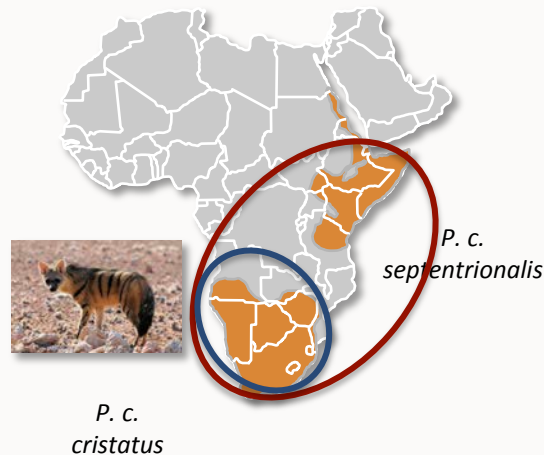
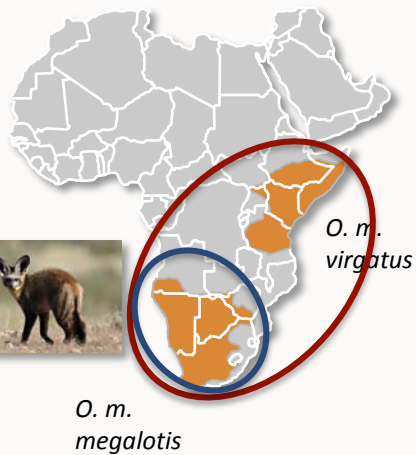
Genome annotation is done
Dataset construction in progress...



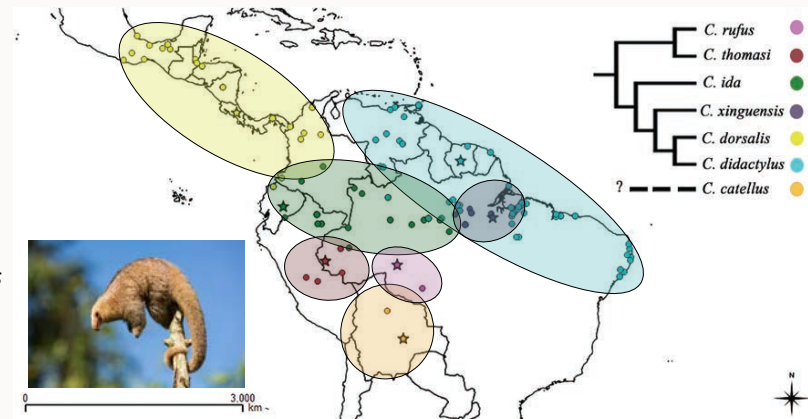
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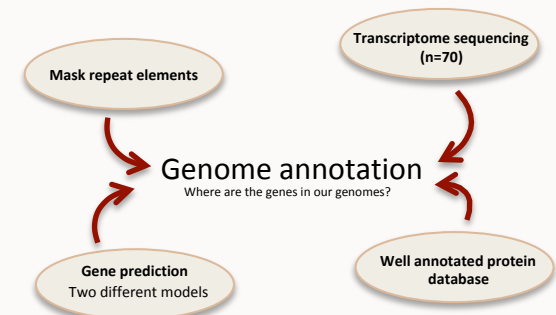


In Xenarthra



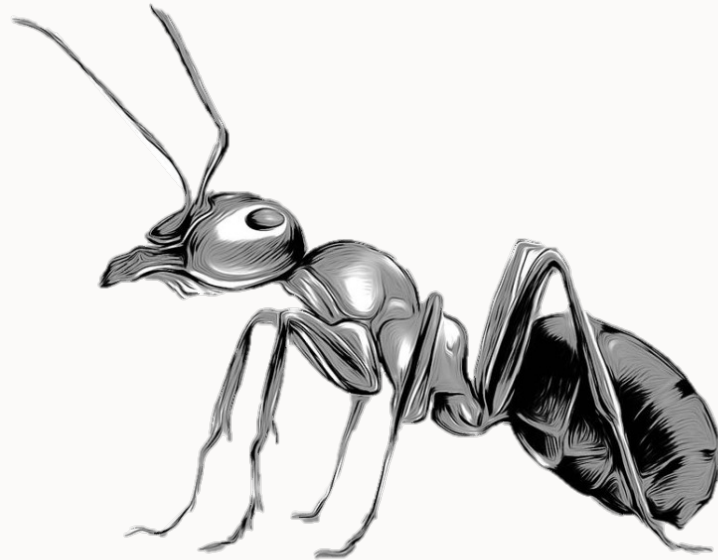
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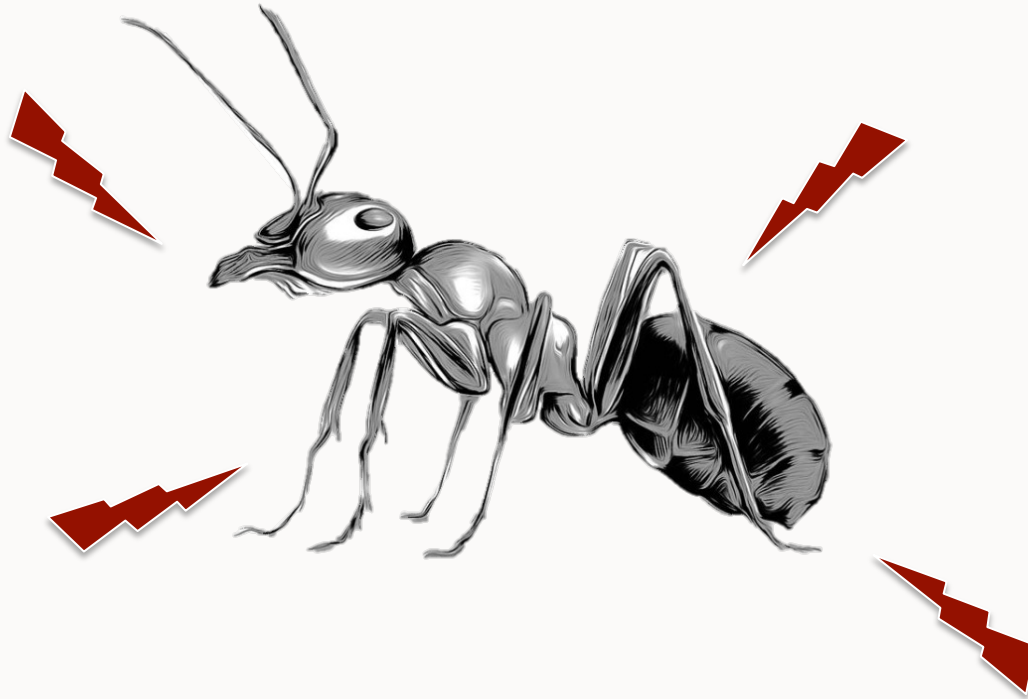
↳ **Candidate gene family: Chitinases**

Why the chitinase gene family?



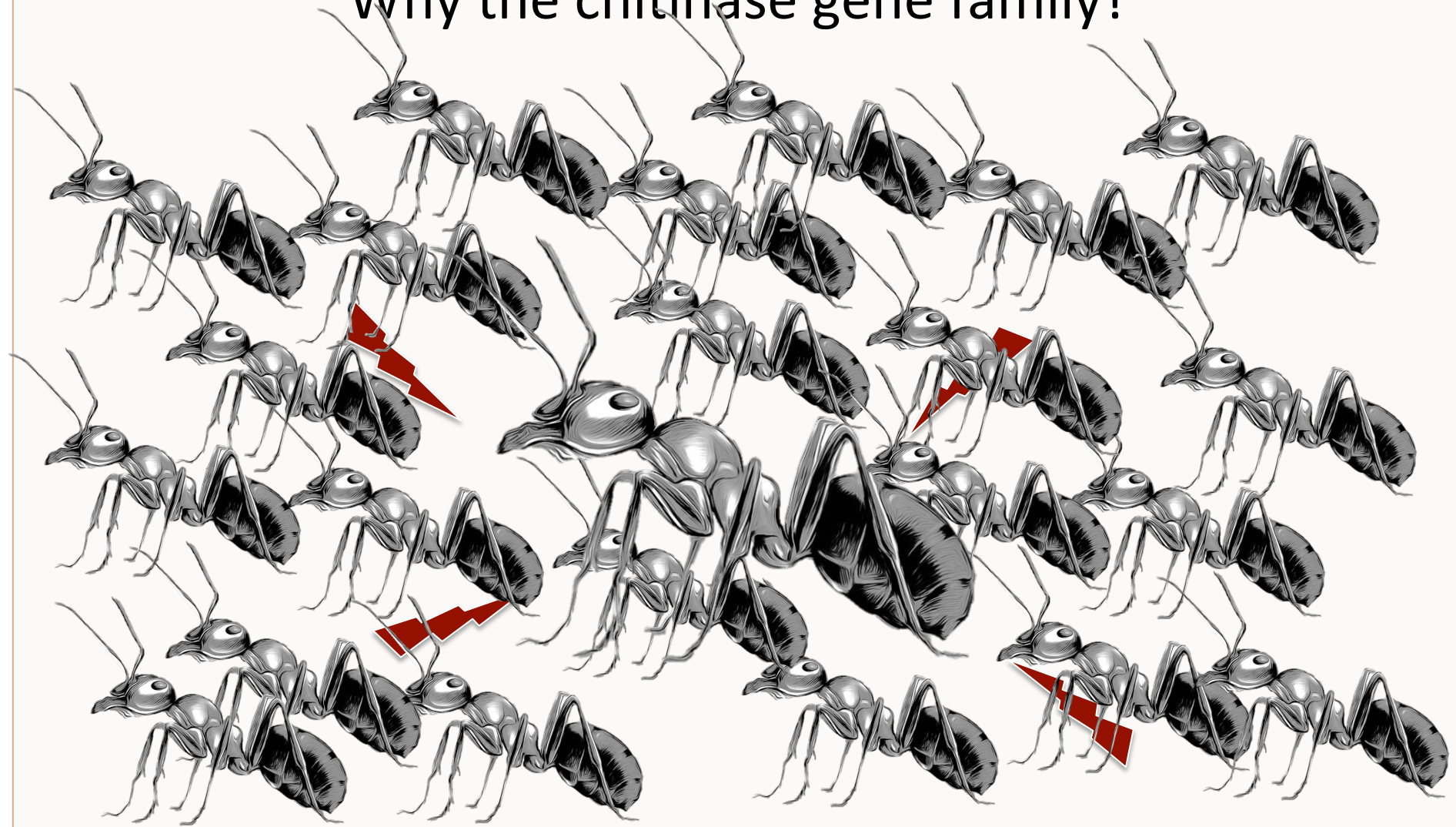
Chitin = insects' skeleton

Why the chitinase gene family?



Chitin = insects' skeleton

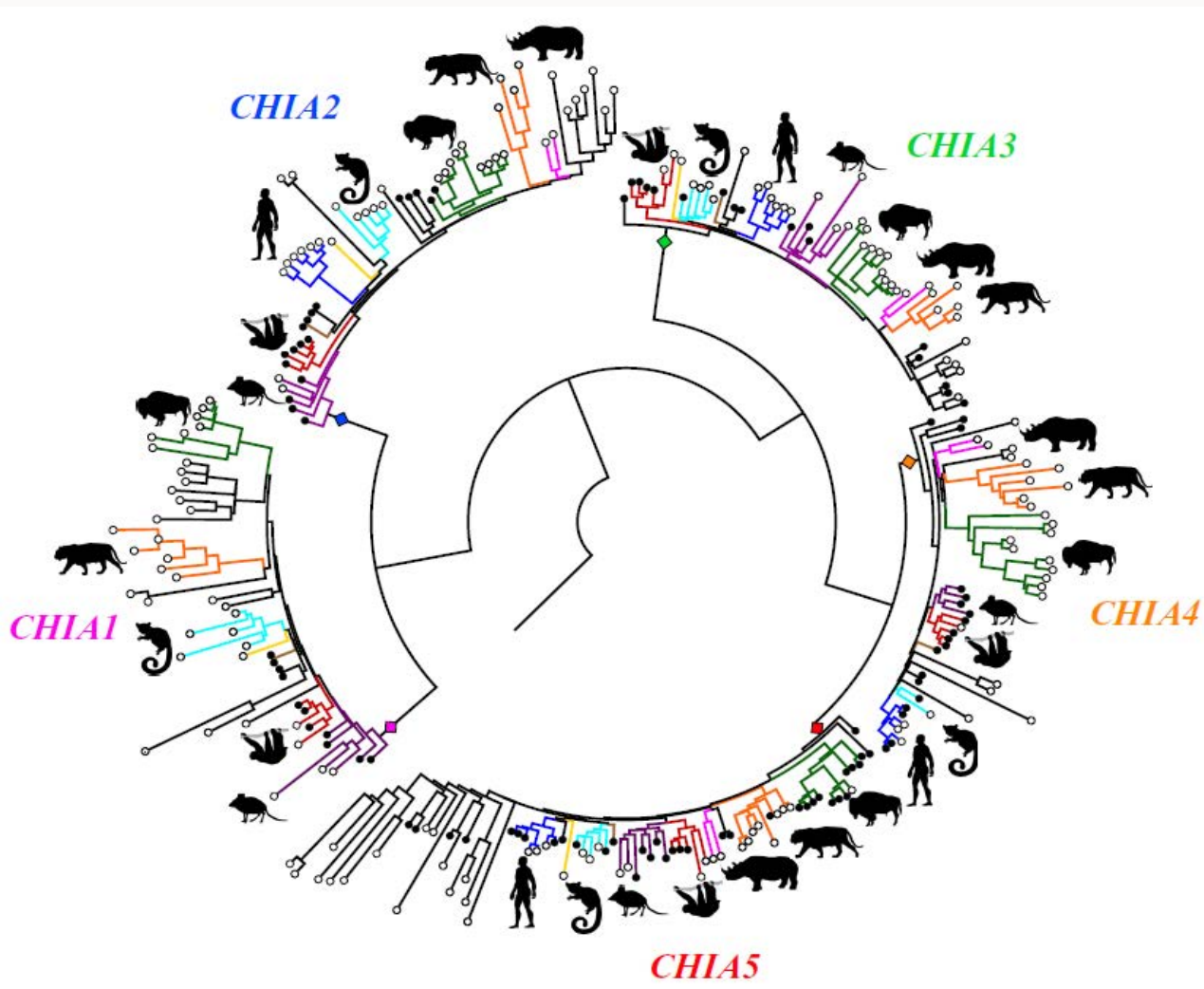
Why the chitinase gene family?



Chitin = insects' skeleton

The chitinase family

0-5 functional genes



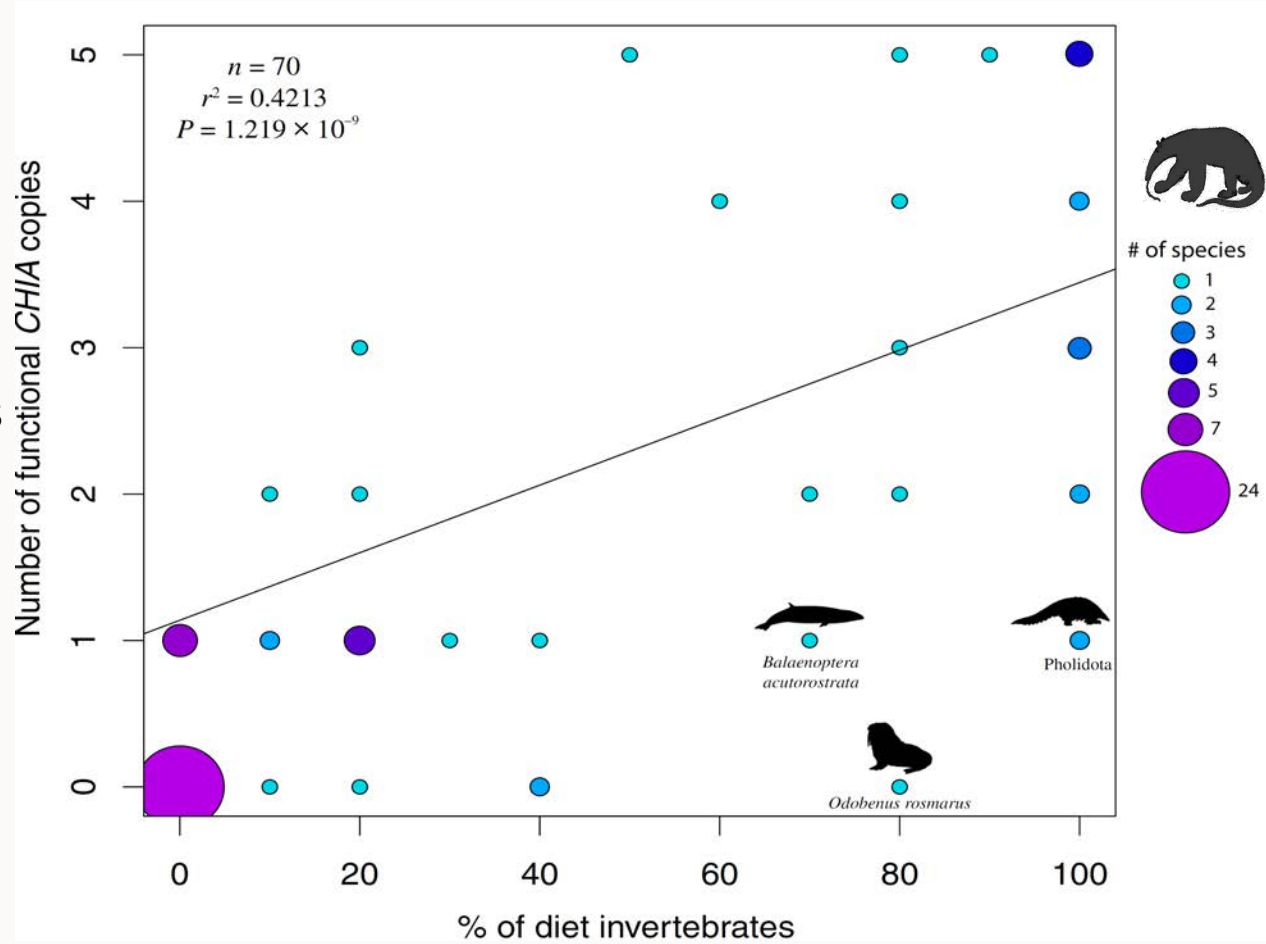
The chitinase family

0-5 functional genes

➤ % of diet invertebrates

=

➤ number of functional genes



The chitinase family

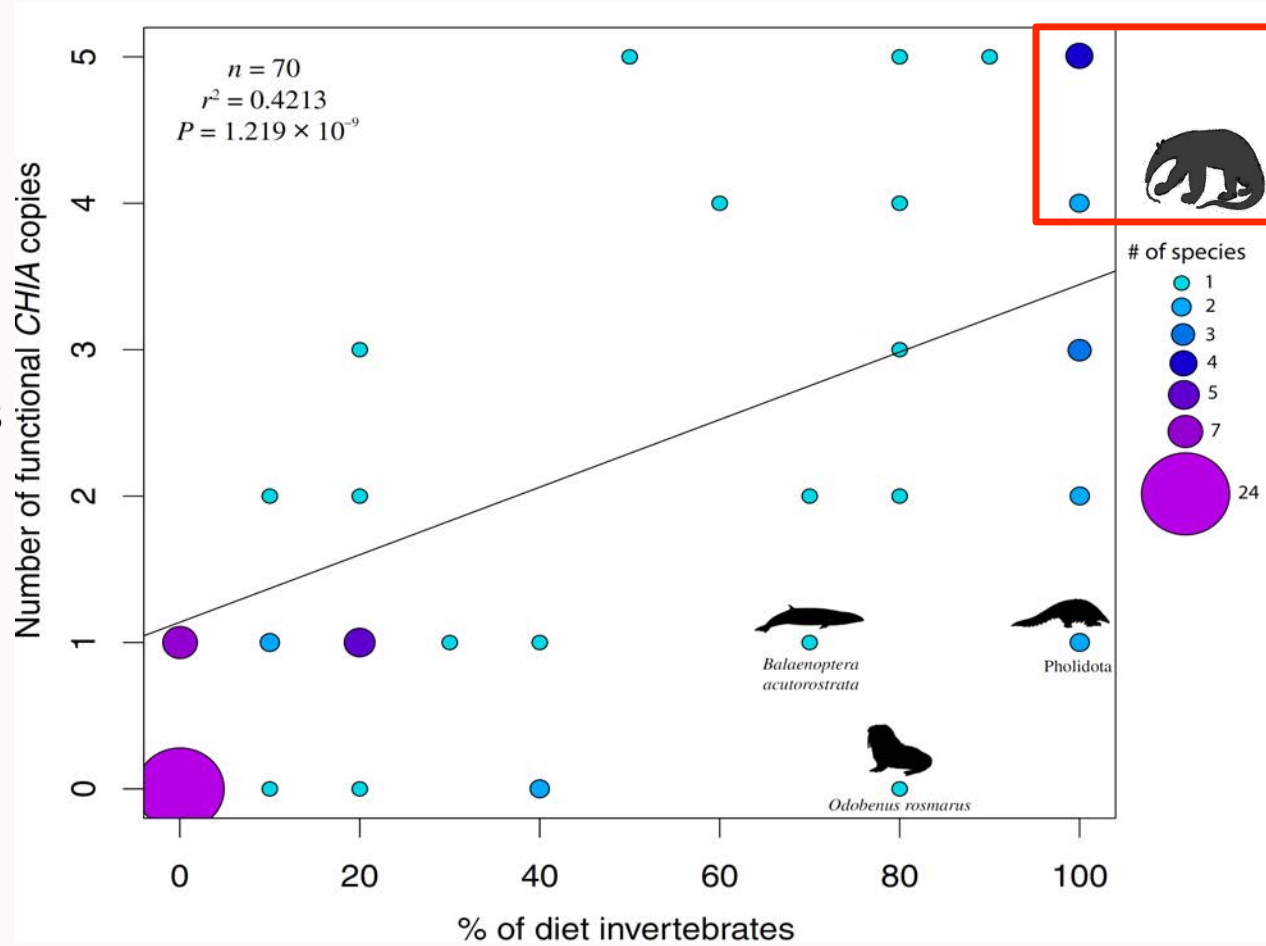
0-5 functional genes

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Myrmecophagous mammals
have several copies!



The chitinase family

0-5 functional genes

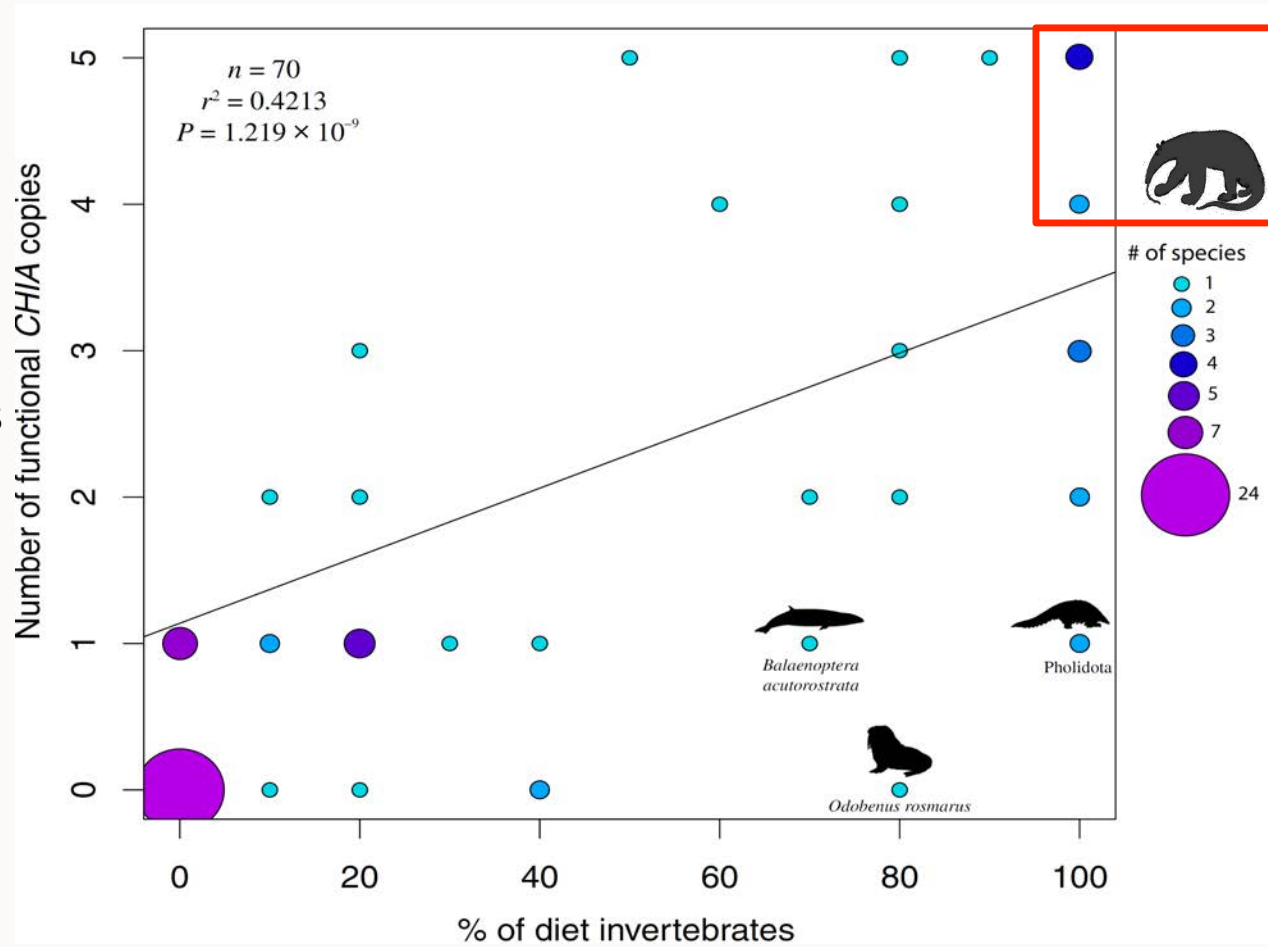
➤ % of diet invertebrates

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Myrmecophagous mammals
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except ...



The chitinase family

0-5 functional genes

➤ % of diet invertebrates

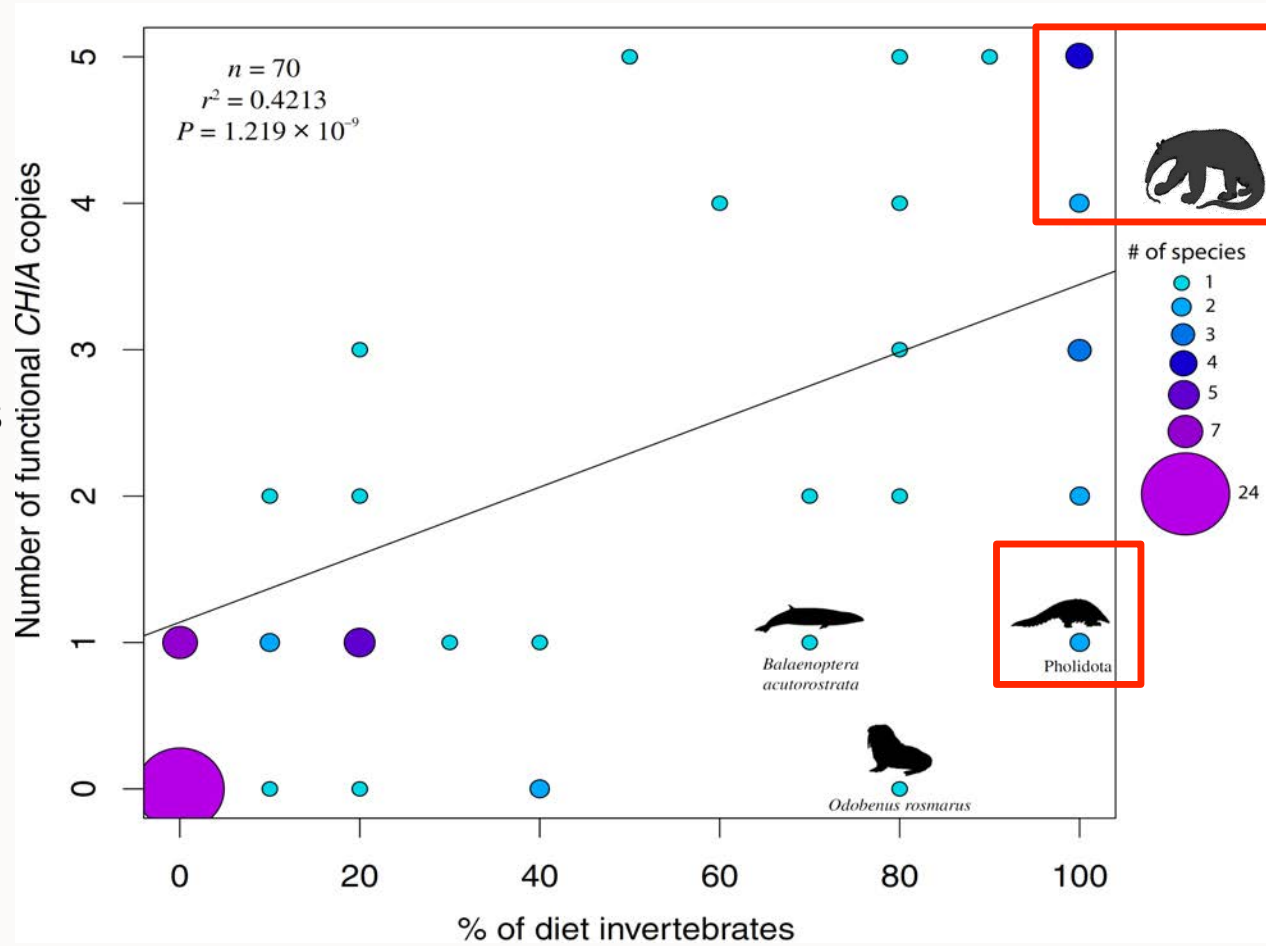
=

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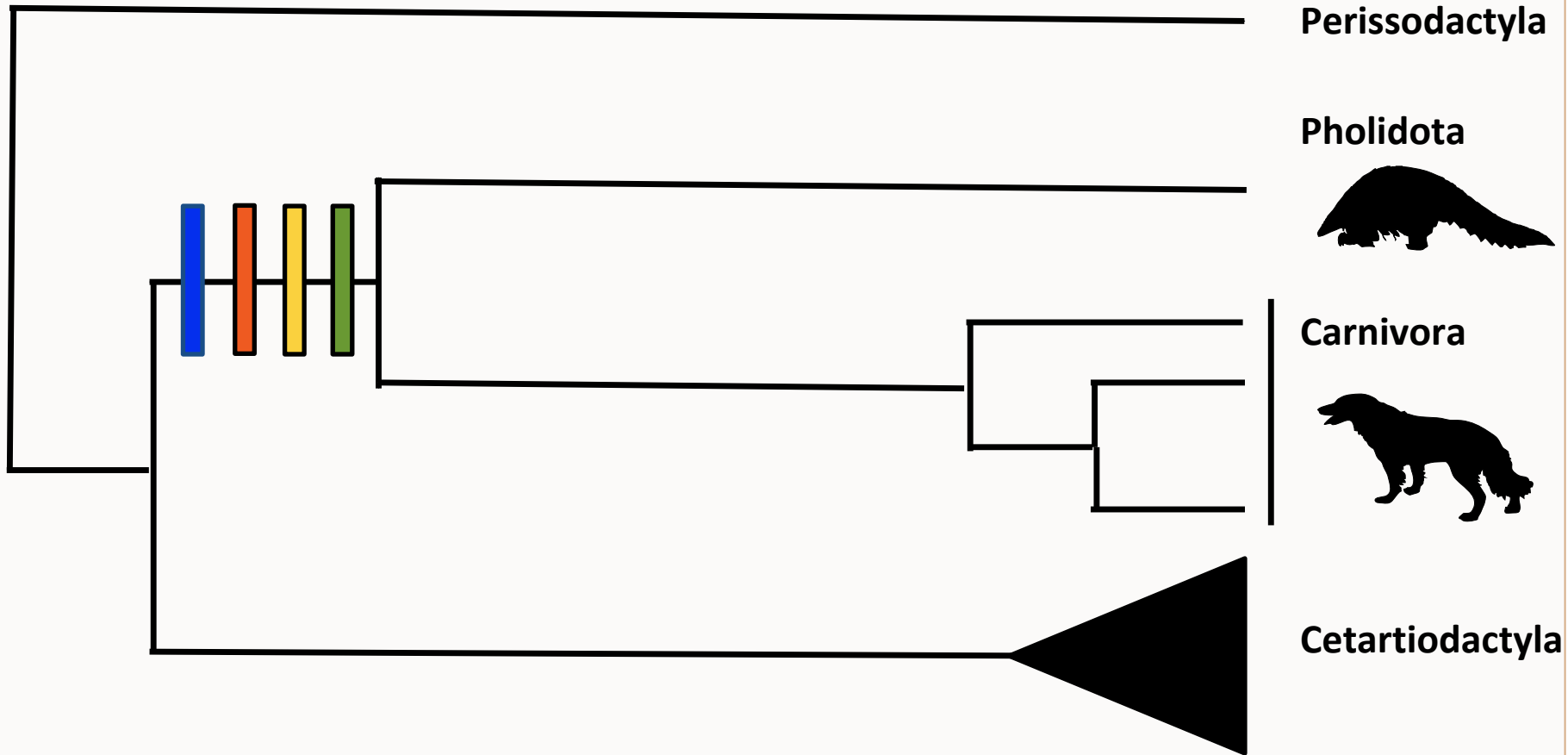
Myrmecophagous mammals
have several copies!

except ...

Manis javanica!



The chitinase family



The chitinase family

How does the pangolin digests chitin
with only one functional gene?

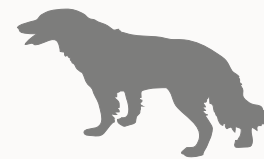


Perissodactyla

Pholidota



Carnivora



Cetartiodactyla

The chitinase family

How does the pangolin digests chitin
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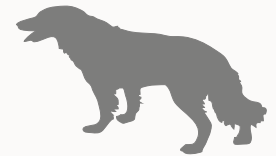
Does he over express this gene?

Perissodactyla

Pholidota



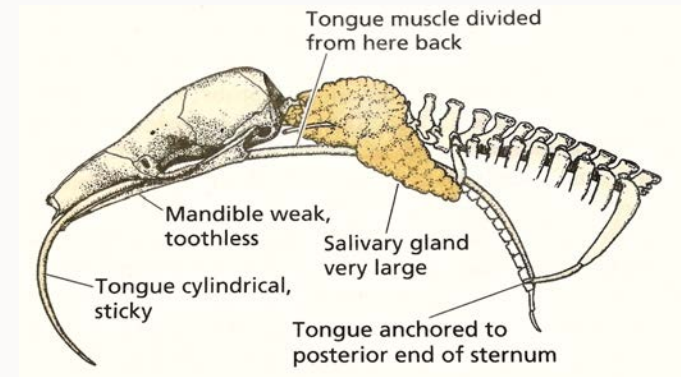
Carnivora



Cetartiodactyla

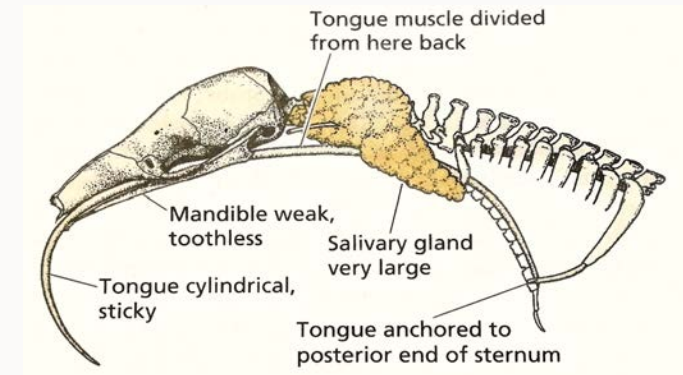
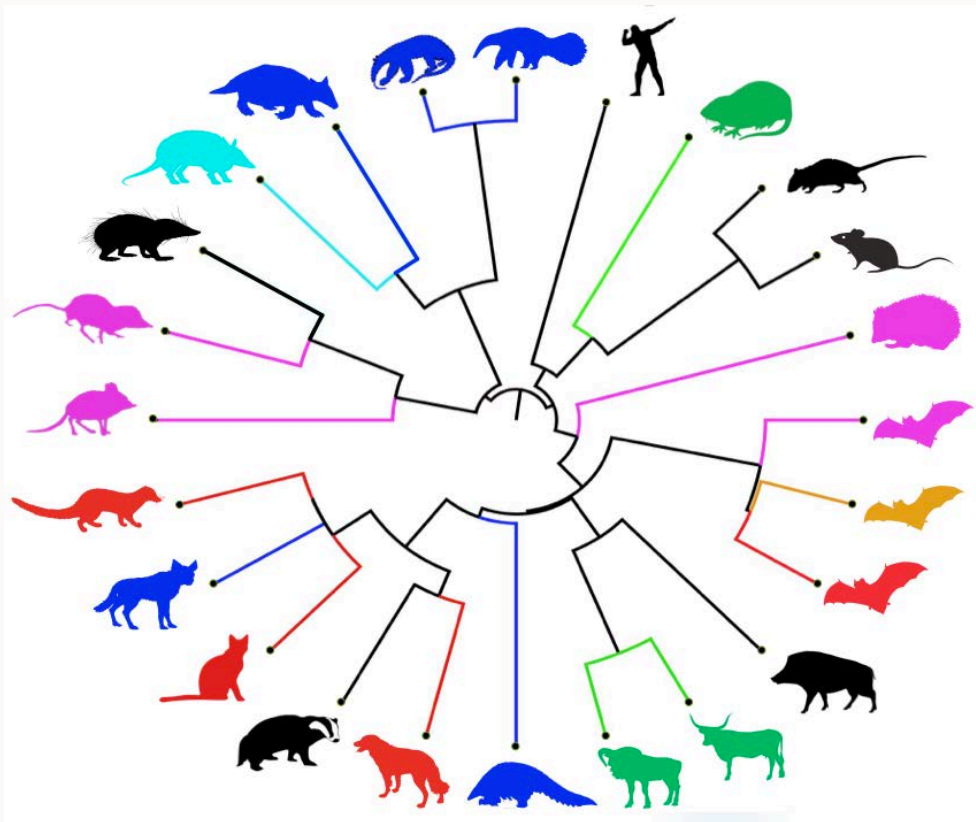
Gene expression in salivary glands

Gene expression in salivary glands



Gene expression in salivary glands

- 28 salivary gland transcriptomes
(24 species, 5 myrmecophagous)

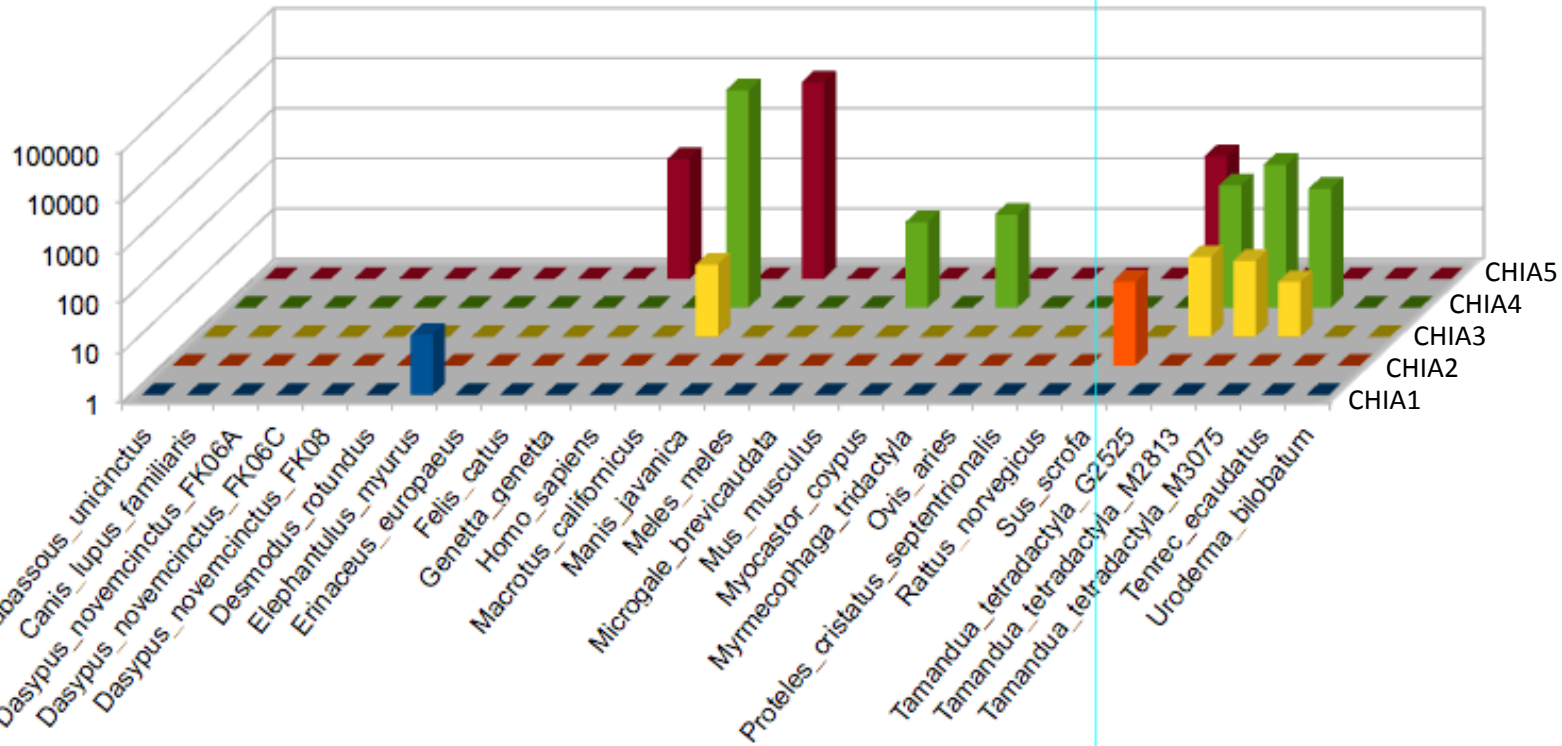
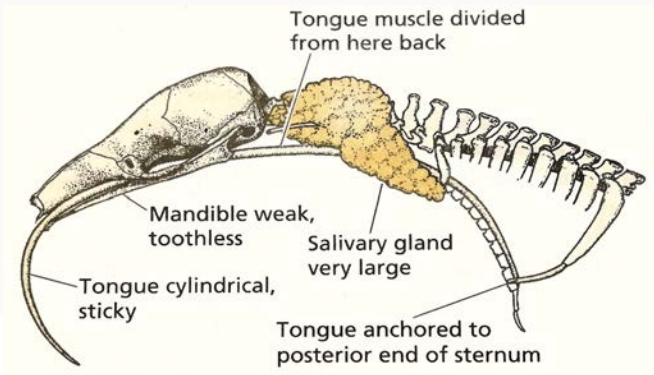


Myrmecophagous
90 -100%
75 -89%
Insectivore
Carnivore
Herbivore
Omnivore
Frugivore



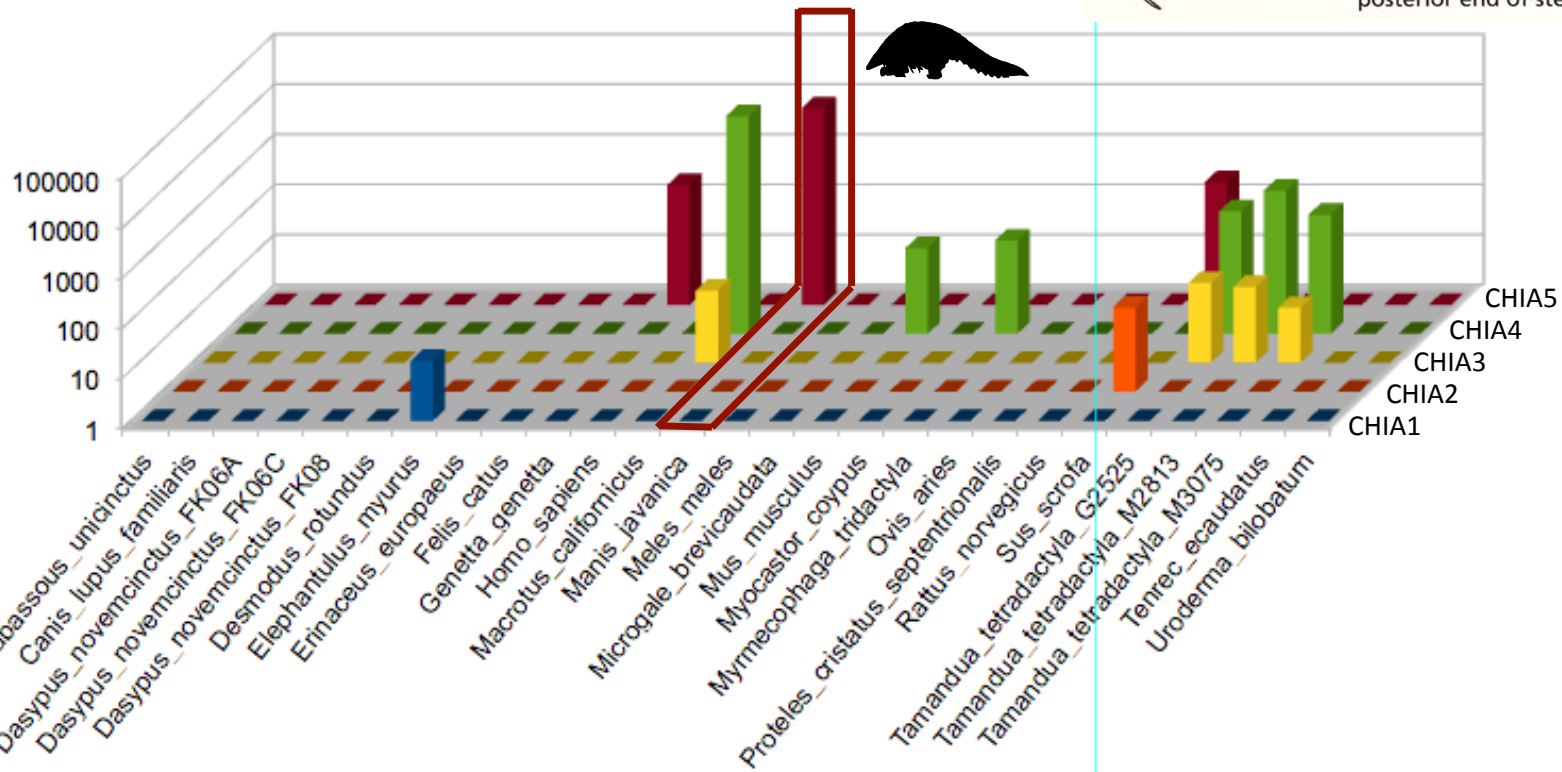
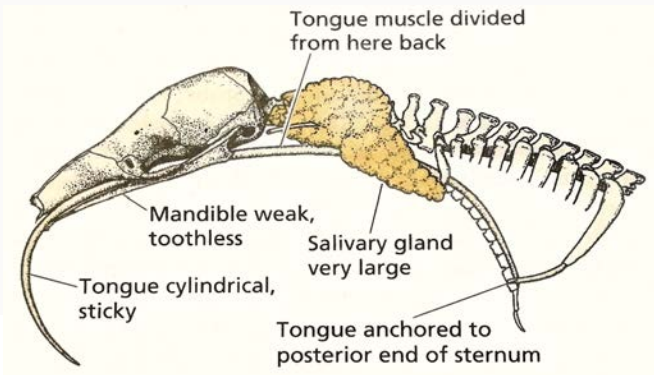
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(24 species, 5 myrmecophagous)



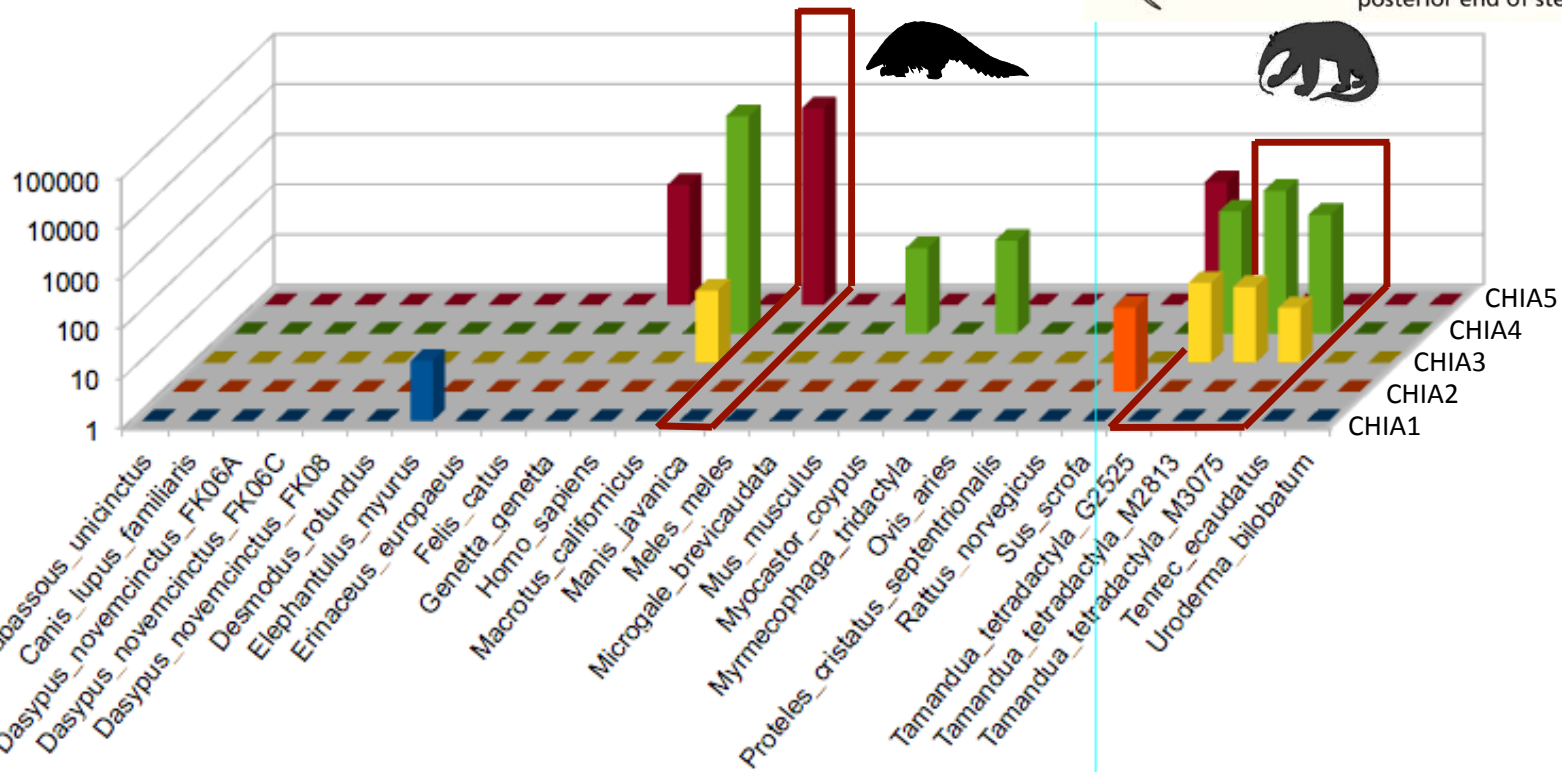
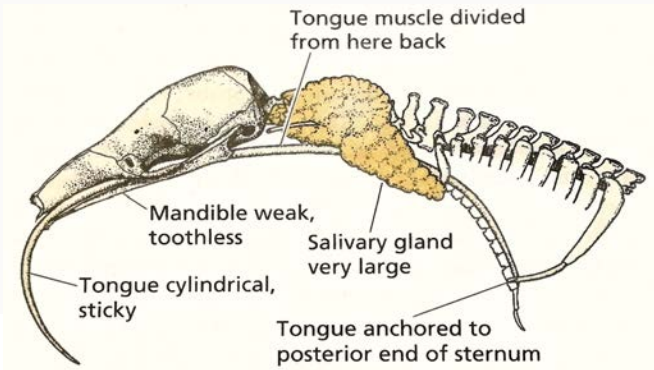
Gene expression in salivary glands

- 28 salivary gland transcriptomes
(24 species, 5 myrmecophagous)



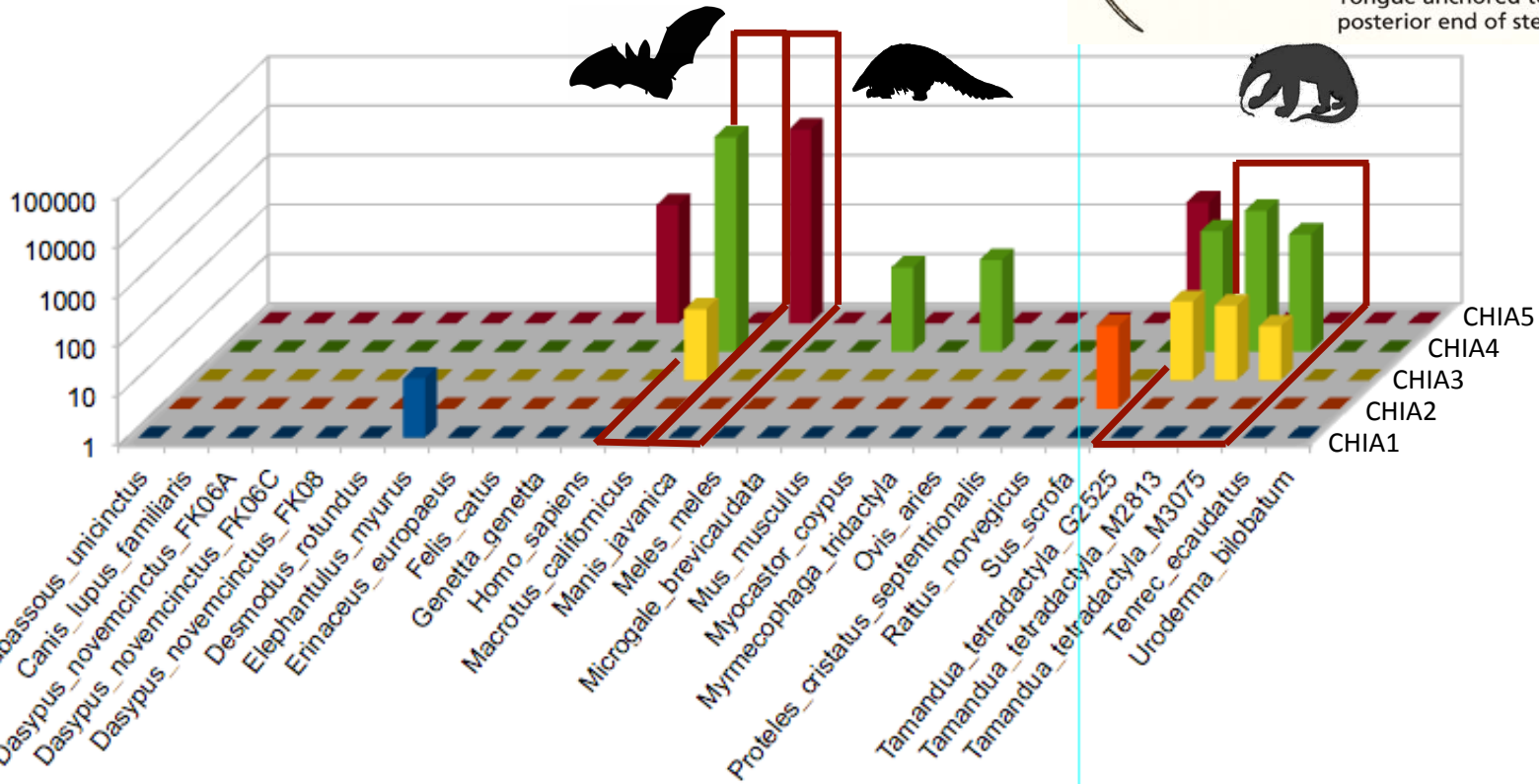
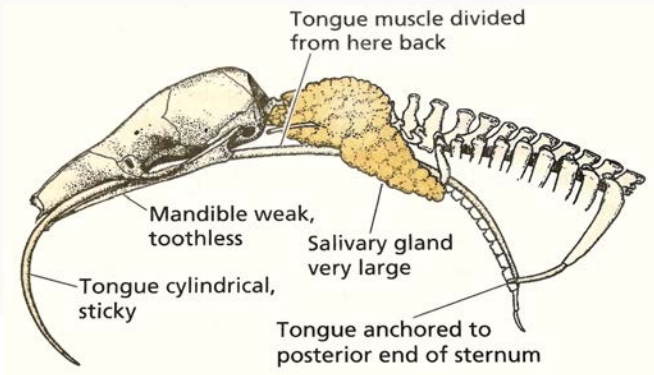
Gene expression in salivary glands

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Gene expression in salivary glands

- 28 salivary gland transcriptomes
(24 species, 5 myrmecophagous)



Gene expression in salivary glands

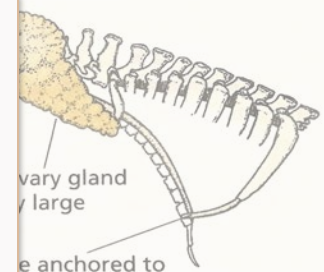
- 28 salivary gland transcriptomes

How does the pangolin digest chitin with only one functional gene?

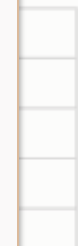
→ Overexpression of CHIA5 in salivary glands



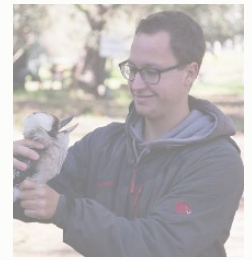
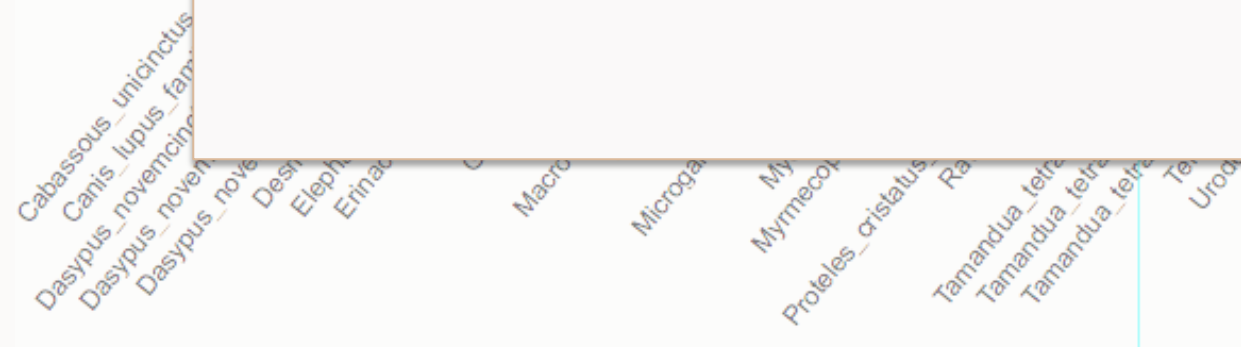
Tongue muscle divided from here back



Salivary gland (very large)



CHIA5
CHIA4
CHIA3
CHIA2



Gene expression in salivary glands

- 28 salivary gland transcriptomes

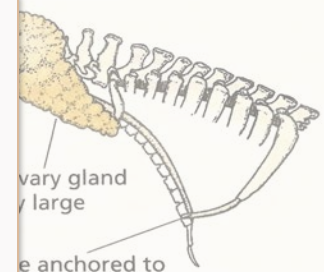
How does the pangolin digests chitin with only one functional gene?

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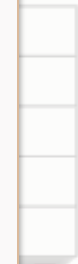


What about the other organs ?

Tongue muscle divided from here back



Tongue anchored to anterior end of sternum



CHIA5
CHIA4
CHIA3
CHIA2

100000
10000
1000
100
10
1

Cabassous_unicinctus
Canis_lupus_fam
Dasypus_novemcinctus
Dasypus_nove
Dasypus_nove
Desm
Eleph
Erinac
Macro
Microga
My
Myrmecop
Proteles_cristatus
Ra
Tamandua_tetra
Tamandua_tetra
Tamandua_tetra
Te
Urood



Gene expression in salivary glands

- 28 salivary gland transcriptomes

How does the pangolin digests chitin with only one functional gene?

→ Overexpression of CHIA5 in salivary glands



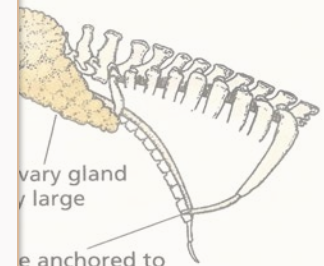
What about the other organs ?

New dataset with 3 myrmecophagous mammals and several organs

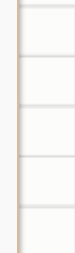


Sophie Teullet (2020) *Master thesis*

Tongue muscle divided from here back



Salivary gland anchored to anterior end of sternum



CHIA5
CHIA4
CHIA3
CHIA2



Dave Lutgen (2019) *Master thesis*

100000
10000
1000
100
10
1
Cabassous_unicinctus
Canis_lupus_fam
Dasypus_novemcinctus
Dasypus_nove
Dasypus_nove
Desm
Eleph
Erinac
Macro
Microga
Myrmecop
Proteles_cristatus
Ra
Tamandua_tetra
Tamandua_tetra
Tamandua_tetra
Te
Urod

Comparative transcriptomics across different organs

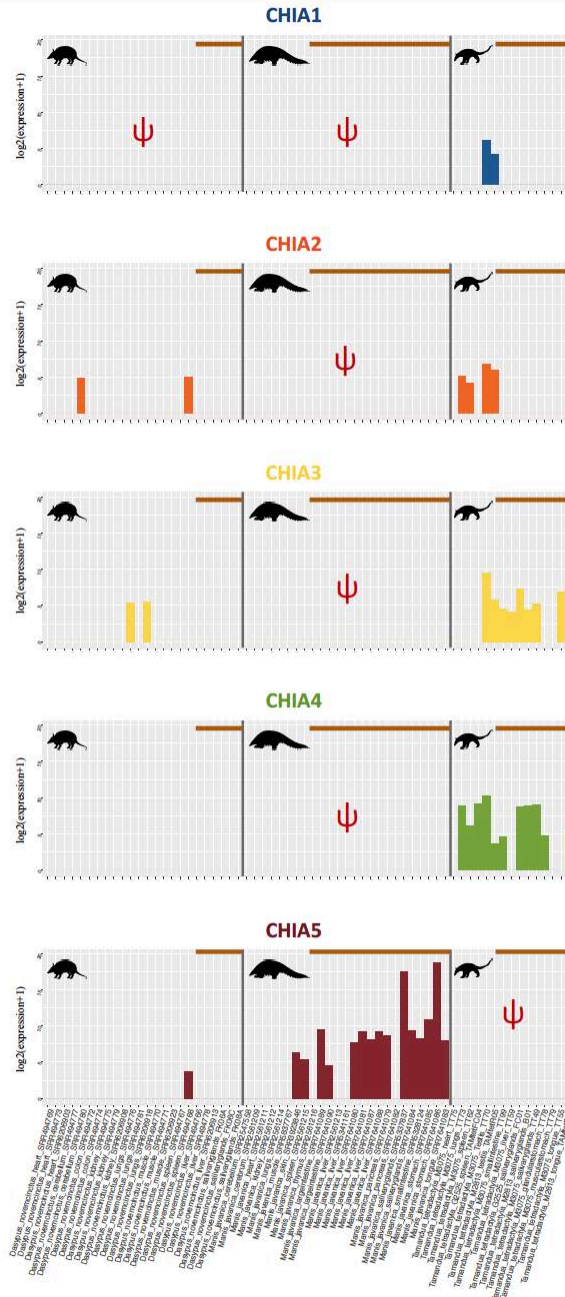
Digestive organs

Manis javanica



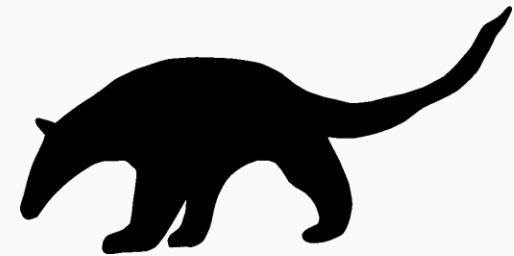
CHIA1
 CHIA2 Ψ CHIA5 \checkmark
 CHIA3
 CHIA4

Many digestive organs:
 Salivary glands + stomach:
CHIA5 + + +



Ψ Pseudogenes

Tamandua tetradactyla



CHIA1
 CHIA2 \checkmark CHIA5 Ψ
 CHIA3 \checkmark
 CHIA4

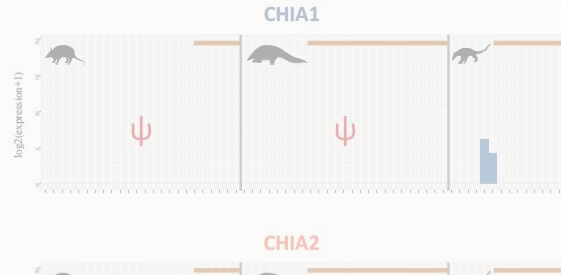
Salivary glands, tongue, liver,
 intestine: **CHIA3 and CHIA4**



Allio*, Teullet*, Lutgen* et al. (in prep.)

Comparative transcriptomics across different organs

Digestive organs



ψ Pseudogenes

Manis javanica



CHIA1
 CHIA2 ψ
 CHIA3 ψ
 CHIA4
 CHIA5 ✓

Many digestive organs:
 Salivary glands + stomach
CHIA5 + + +

Conclusion

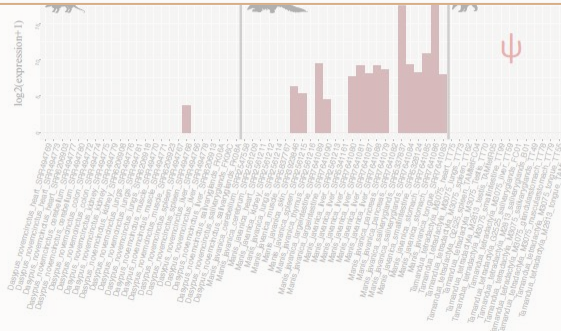
- Chitinase gene family
- ✓ Over expression of CHIA5 in pangolins
- ✓ Impact of historical contingency
- ✓ Different strategy

Tamandua tetradactyla



A1
 A2 ✓
 A3 CHIA5 ψ
 A4

Salivary glands, tongue, liver,
 intestine: **CHIA3 and CHIA4**



Summary

PART 1 - Phylogenomics and comparative genomics in myrmecophagous mammals



Take Home Message

Roadkill tissues are suitable for long DNA sequencing



Species delineation possible with few individuals



High quality genomes for study convergence



Impact of historical contingency



Summary

PART 1 - Phylogenomics and comparative genomics in myrmecophagous mammals



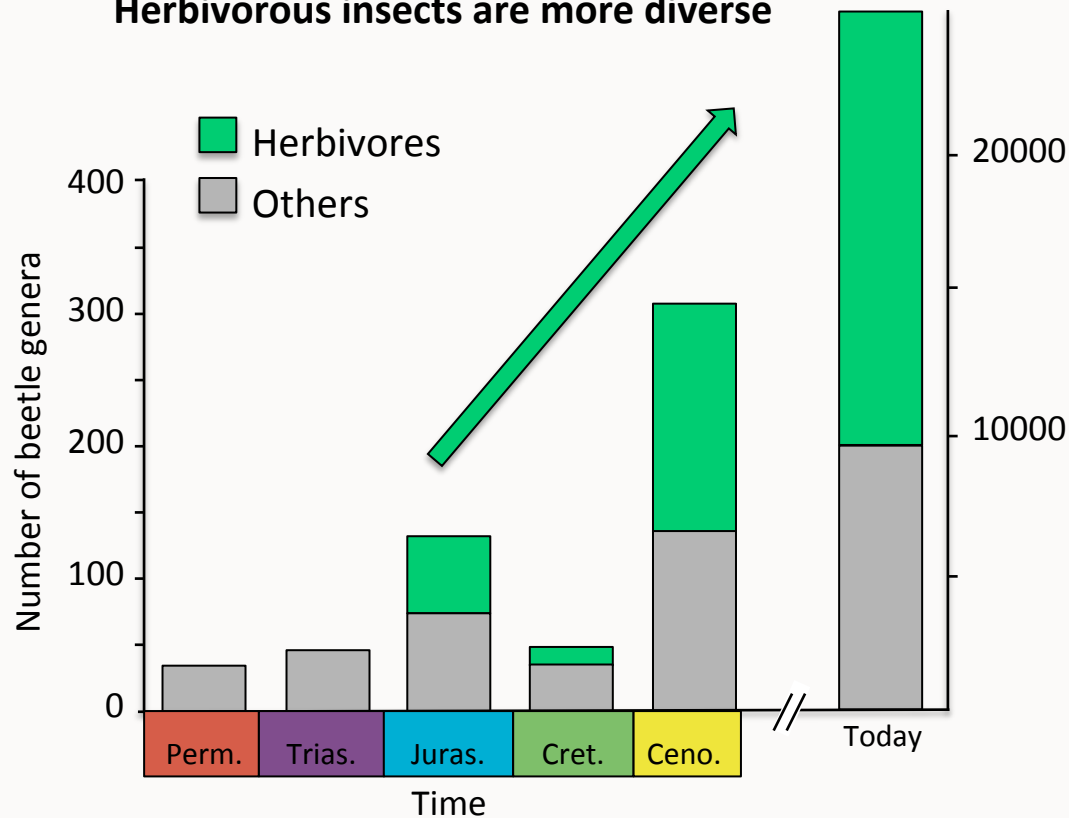
PART 2 - The case of coevolution between the swallowtail butterflies (Papilionidae) and their host plants



CONTEXT

- 1 million insect species
- 300,000 plant species

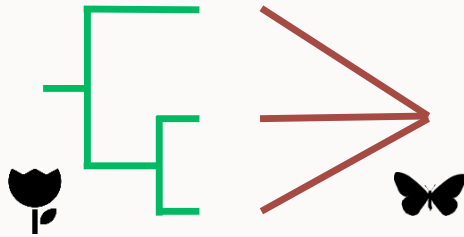
Herbivorous insects are more diverse



What is the role of interaction in diversification?

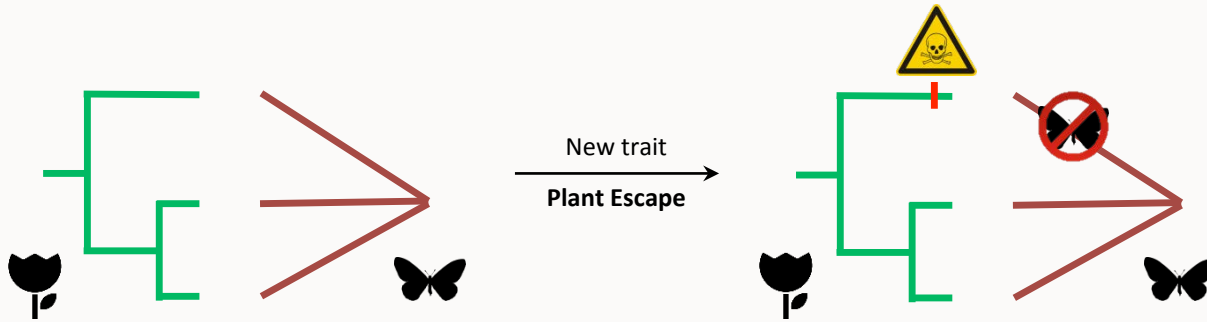
CONTEXT

“Escape and Radiate” hypothesis



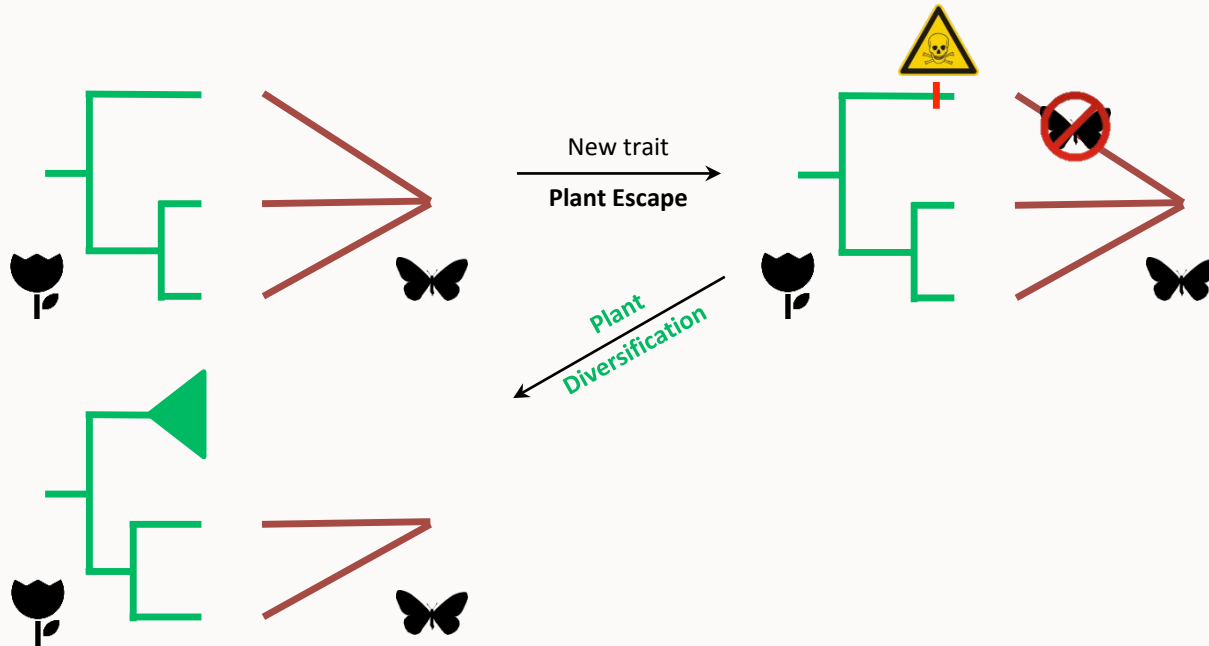
CONTEXT

“Escape and Radiate” hypothesis



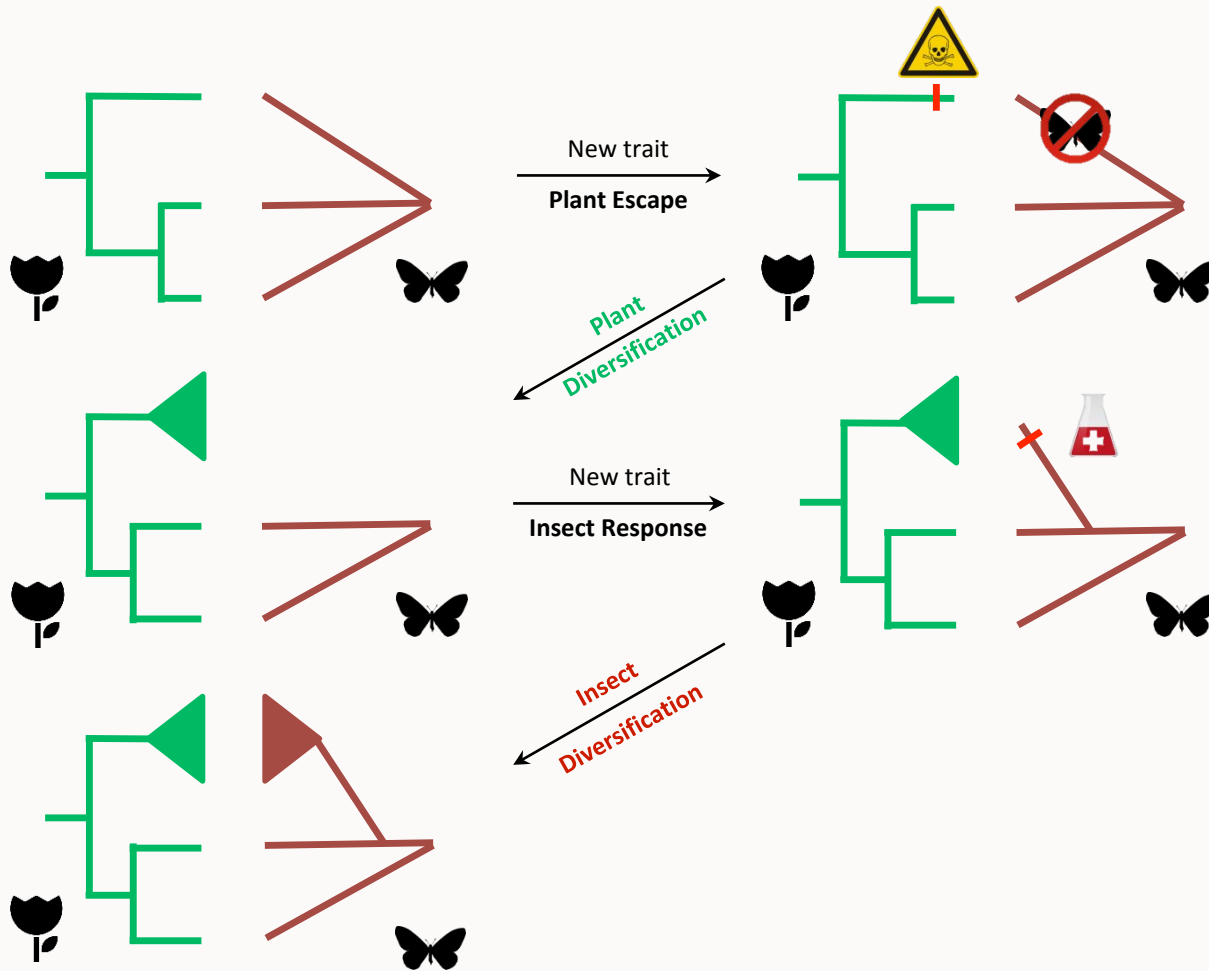
CONTEXT

“Escape and Radiate” hypothesis



CONTEXT

“Escape and Radiate” hypothesis



The swallowtail butterflies (Papilionidae)



Allancastris



Archon



Atrophaneura



Baronia



Battus



Bhutanitis



Byasa



Cressida



Euryades



Eurytides



Graphium



Hypermnestra



Iphiclides



Lamproptera



Luehdorfia



Meandrusa



Mimoides



Ornithoptera



Pachliopta



Papilio



Parides



Parnassius



Pharmacophagus



Protesilaus



Protographium



Sericinus



Teinopalpus



Troides



Trogonoptera



Zerynthia

- Well known family
- High morphological diversity
- 32 genera in 570+ species
- Feed on 12 families of flowering plants



Aristolochiaceae



Rutaceae ...



Annonaceae

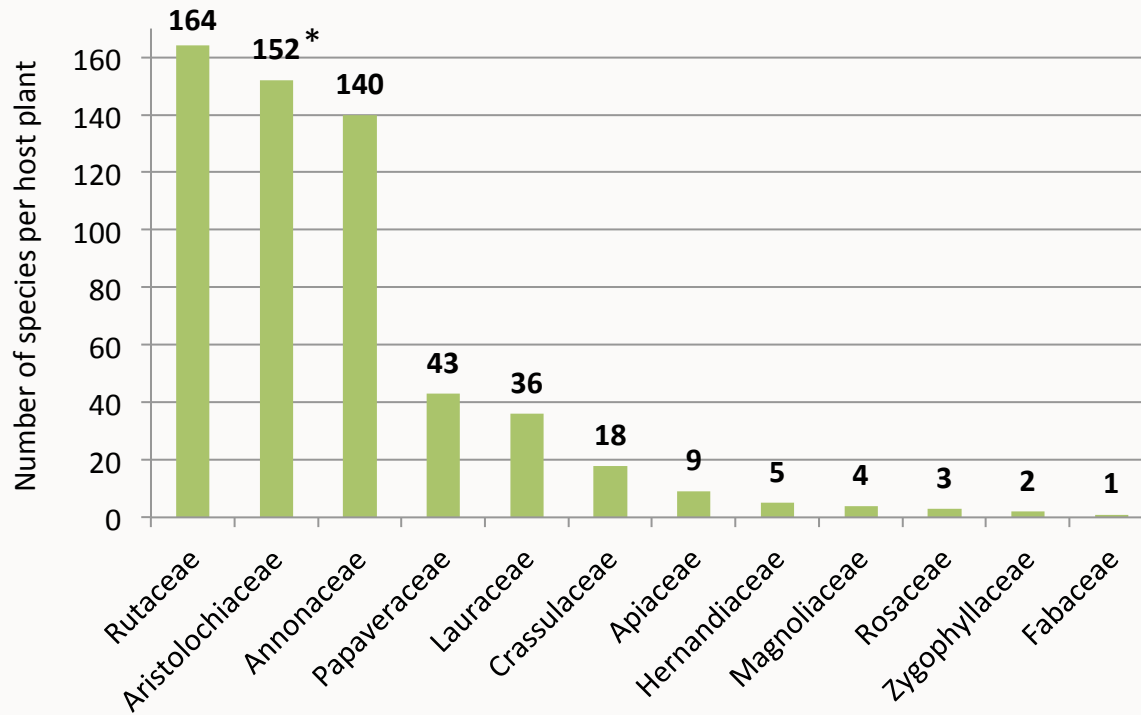


Apiaceae

- Many species are monophagous
= specialists

Swallowtail butterflies and their host plants

Highly **unbalanced associations** between papilionid diversity and host plants



* Three groups are **specialized on Aristolochiaceae** (highly toxic plants, Aristolochic acid)

Sequestration of toxic compounds → **Defense** against predators

Hypothesis of **coevolution** between Papilionidae and their host plants

Swallowtails on:

Rutaceae



Annonaceae



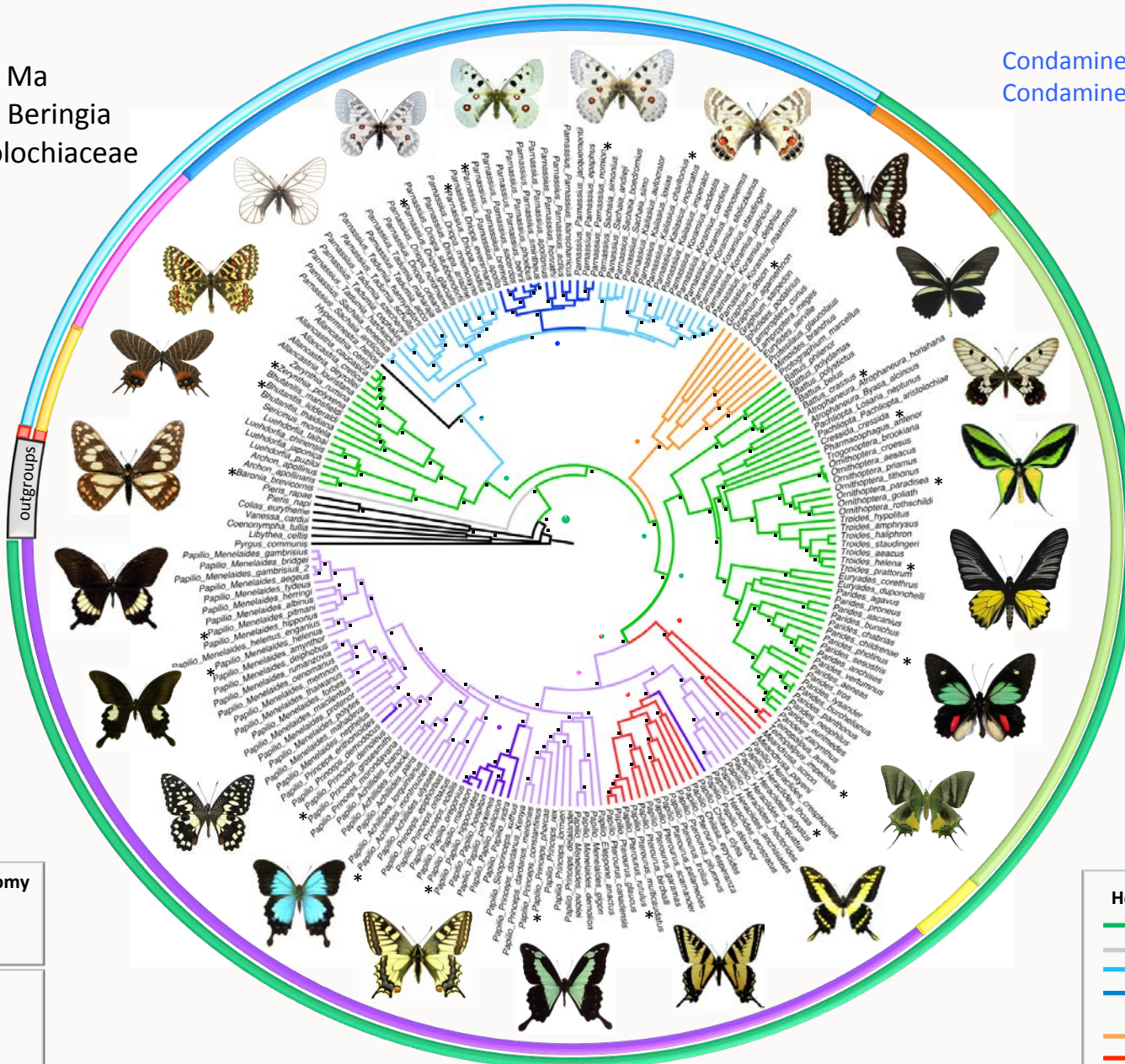
Aristolochiaceae



Papilionidae:

- appeared 52 Ma
- originated in Beringia
- fed on Aristolochiaceae

Condamine *et al.* 2012 – Ecol. Lett.
 Condamine *et al.* 2013 – J. Biogeog.



Swallowtail taxonomy

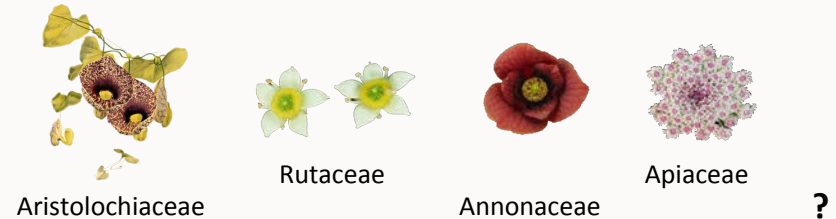
Green	Papilioninae
Light Blue	Parnassiinae
Red	Baroniinae
Purple	Papilionini
Yellow	Teinopalpini
Light Green	Troidini
Orange	Leptocircini
Dark Blue	Parnassiini
Pink	Zerynthiini
Yellow	Luehdorfiini

Host plant association

Green	Aristolochiaceae
Grey	Fabaceae (<i>Acacia</i>)
Light Blue	Papaveraceae
Dark Blue	Crassulaceae + Saxifragaceae
Orange	Annonaceae
Red	Lauraceae
Pink	Rutaceae
Purple	Apiaceae
Black	Zygophyllaceae

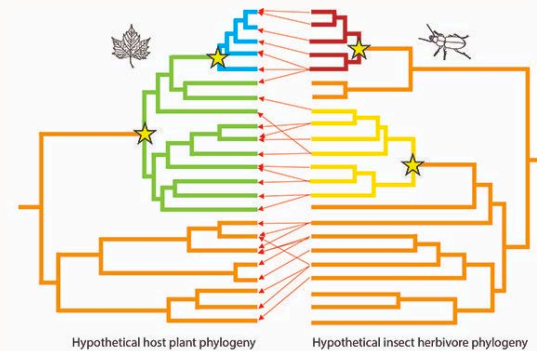
Main questions

1. What are the ancestral host plants of Papilionidae?



2. Temporal congruence?

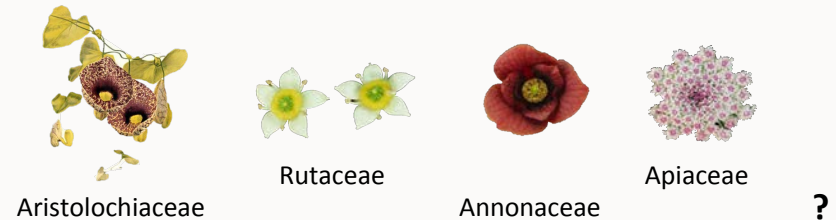
3. Biogeographic congruence?



4. Signatures of genomic adaptations and on speciation rates?

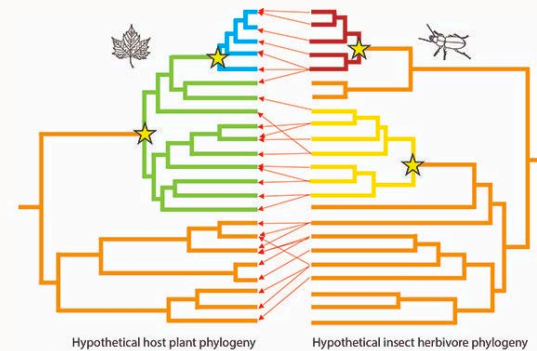
Main questions

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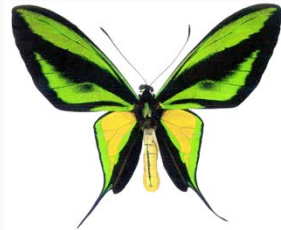
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4. Signatures of genomic adaptations and on speciation rates?

Inferring the most comprehensive and robust phylogeny with Sanger data

Sanger sequencing for **408 species (72% of the diversity)**



DNA extractions

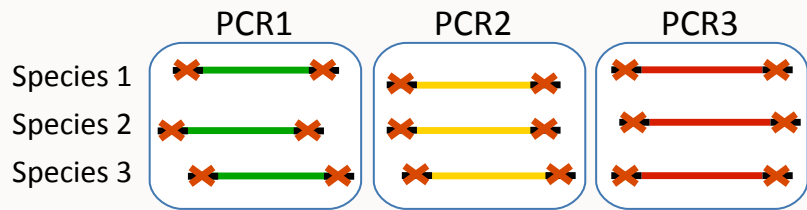


PCR

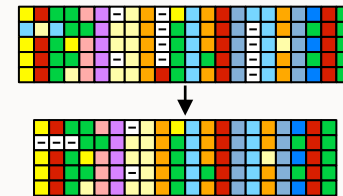


Sequence cleaning

Alignments



Geneious

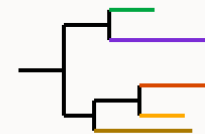


MAFFT

Mesquite

Phylogenetic inferences

Dataset: - 408 species (+ 20 outgroups)
- 7 genes ; 5 873 nucleotides



IQ-TREE

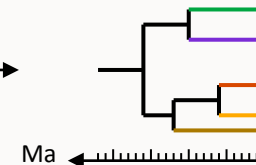
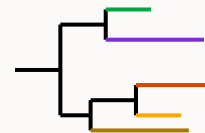
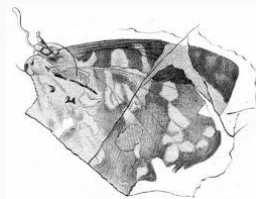
MrBayes

Molecular dating

4 fossils

4 ingroup fossils


Outgroups excluded



BEAST

Inferring the most comprehensive and robust phylogeny with Sanger data

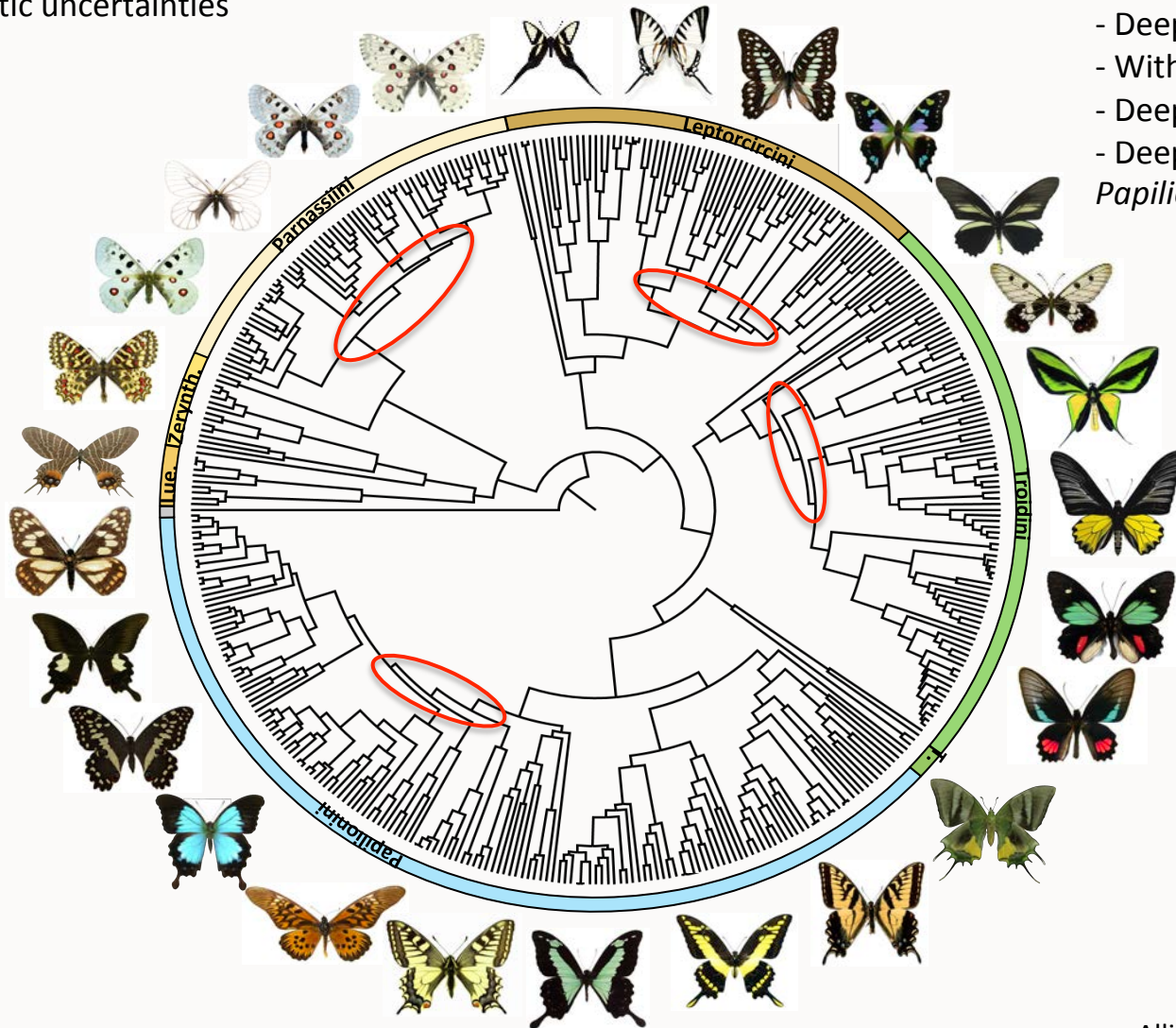
MrBayes: 100 M. MCMC generations

 Phylogenetic uncertainties

85% of nodes had PP \geq 0.95

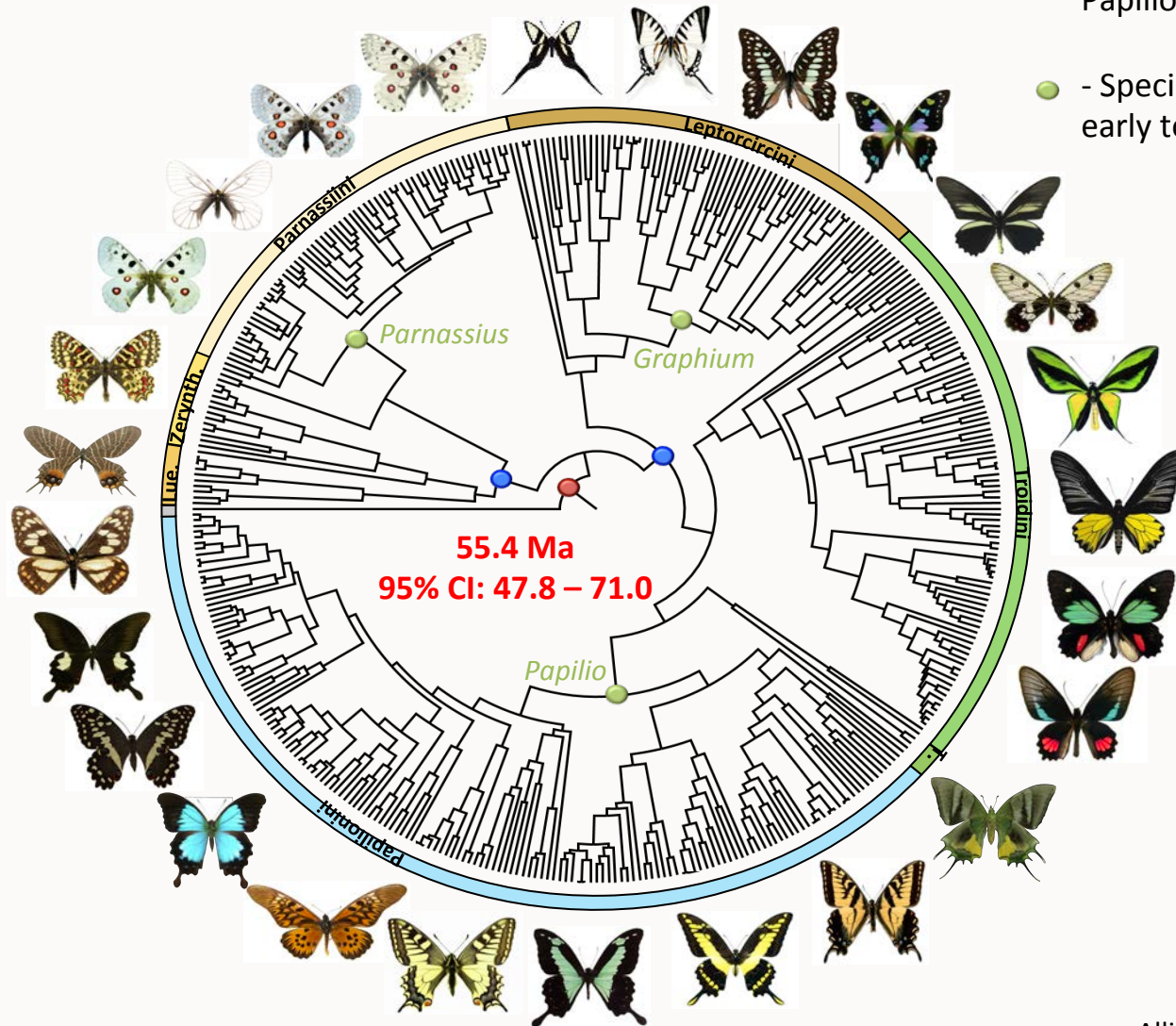
Not well resolved in rapid radiations such as:

- Deep nodes in *Parnassius*
- Within *Graphium*
- Deep nodes in Troidina
- Deep nodes in Old World *Papilio*



Inferring the most comprehensive and robust phylogeny with Sanger data

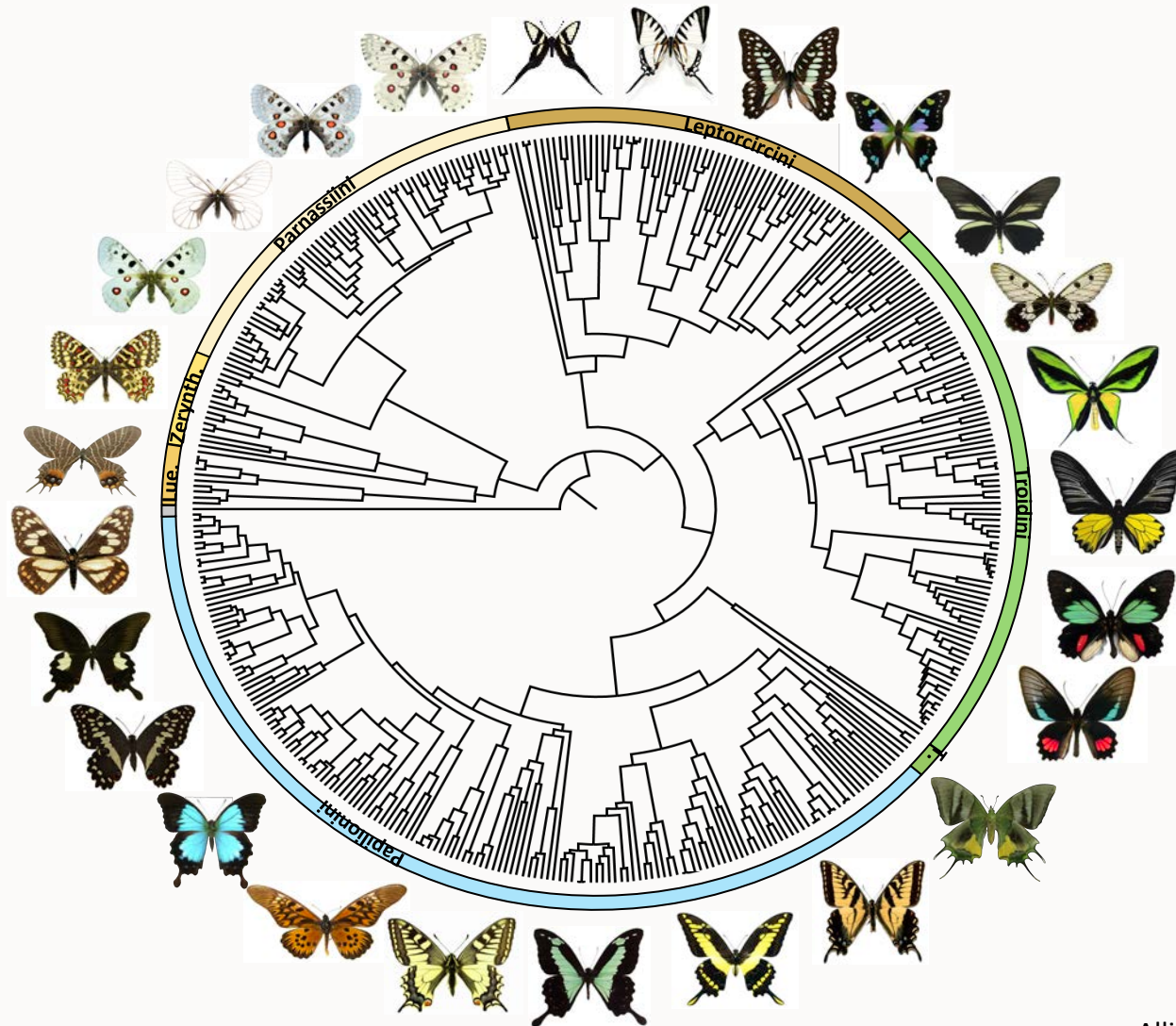
BEAST: fix topology / 200 M. MCMC generations



- - Origin in the early Eocene
- - Parnassiinae and Papilioninae are mid-Eocene
- - Species-rich genera are early to mid-Miocene

55.4 Ma
95% CI: 47.8 – 71.0

What are the ancestral host plants Papilionidae fed on 55 Ma?



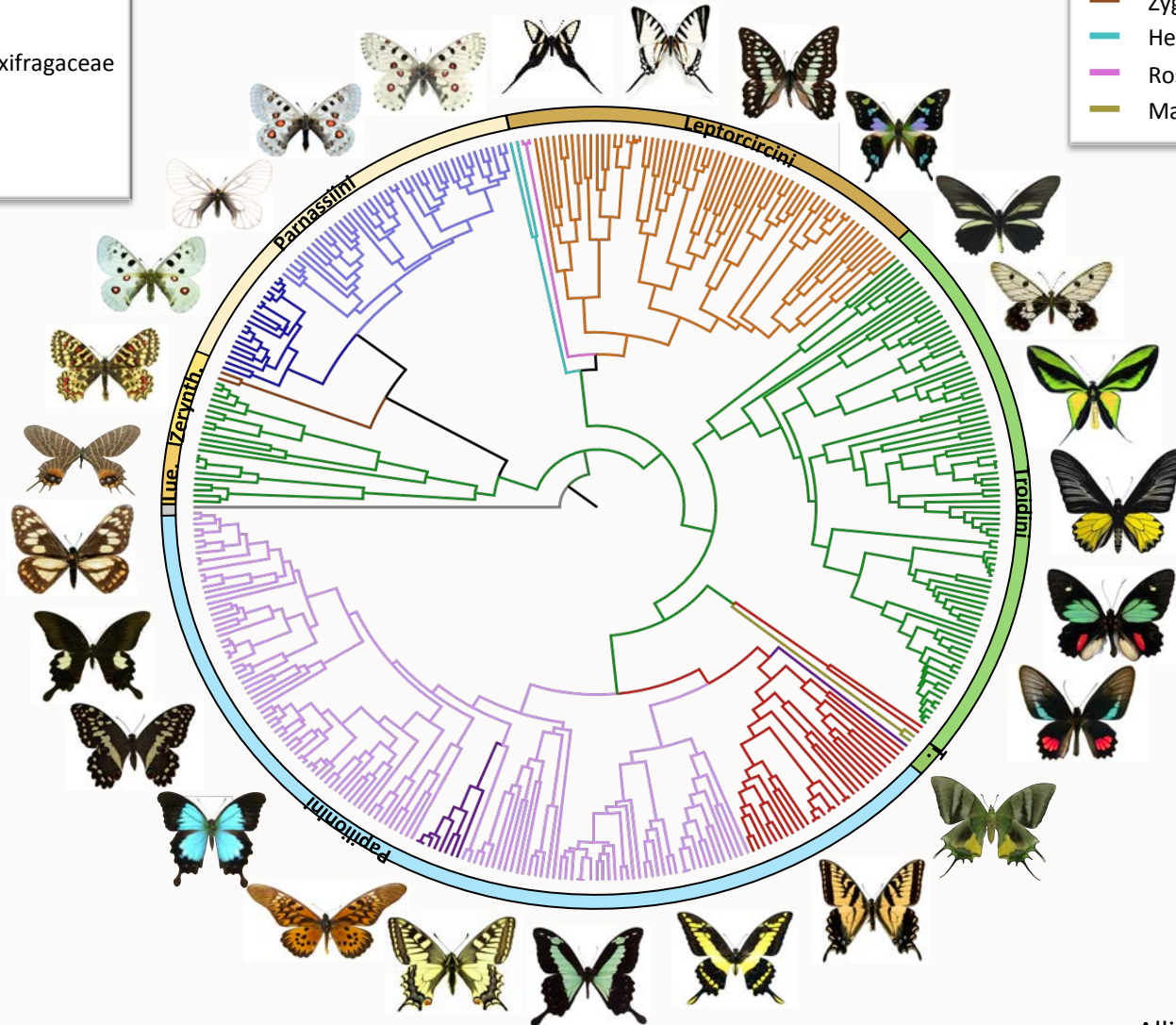
What are the ancestral host plants Papilionidae fed on 55 Ma?

Main host plants

- Aristolochiaceae
- Rutaceae
- Annonaceae
- Crassulaceae + Saxifragaceae
- Apiaceae
- Lauraceae
- Papaveraceae

Secondary host plants

- Fabaceae (*Acacia*)
- Zygophyllaceae
- Hernandiaceae
- Rosaceae
- Magnoliaceae



Back to the main questions

1. *What are the ancestral host plants of Papilionidae?*

 Fed on Aristolochiaceae

2. Temporal congruence?

3. Biogeographic congruence?

4. Signatures of genomic adaptations and on speciation rates?

Back to the main questions

1. What are the ancestral host plants of Papilionidae?

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Inferring the most comprehensive and robust phylogeny with Sanger data

Sanger sequences for 247 species



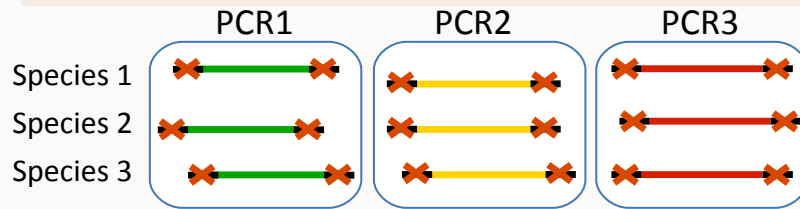
Collaborations
with botanists



GenBank data

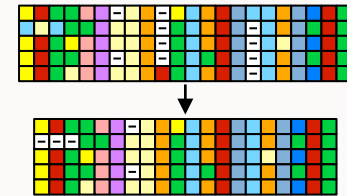


Sequence cleaning



Alignments

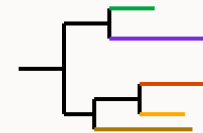
Geneious



MAFFT
Mesquite

Phylogenetic inferences

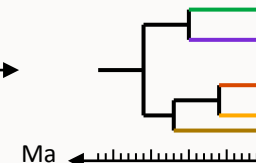
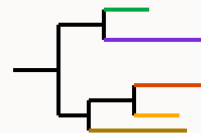
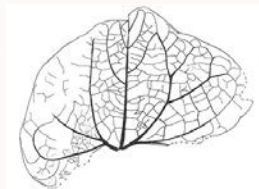
Dataset: - 247 species (+ 6 outgroups)
- 5 genes ; 4 377 nucleotides



IQ-TREE
MrBayes

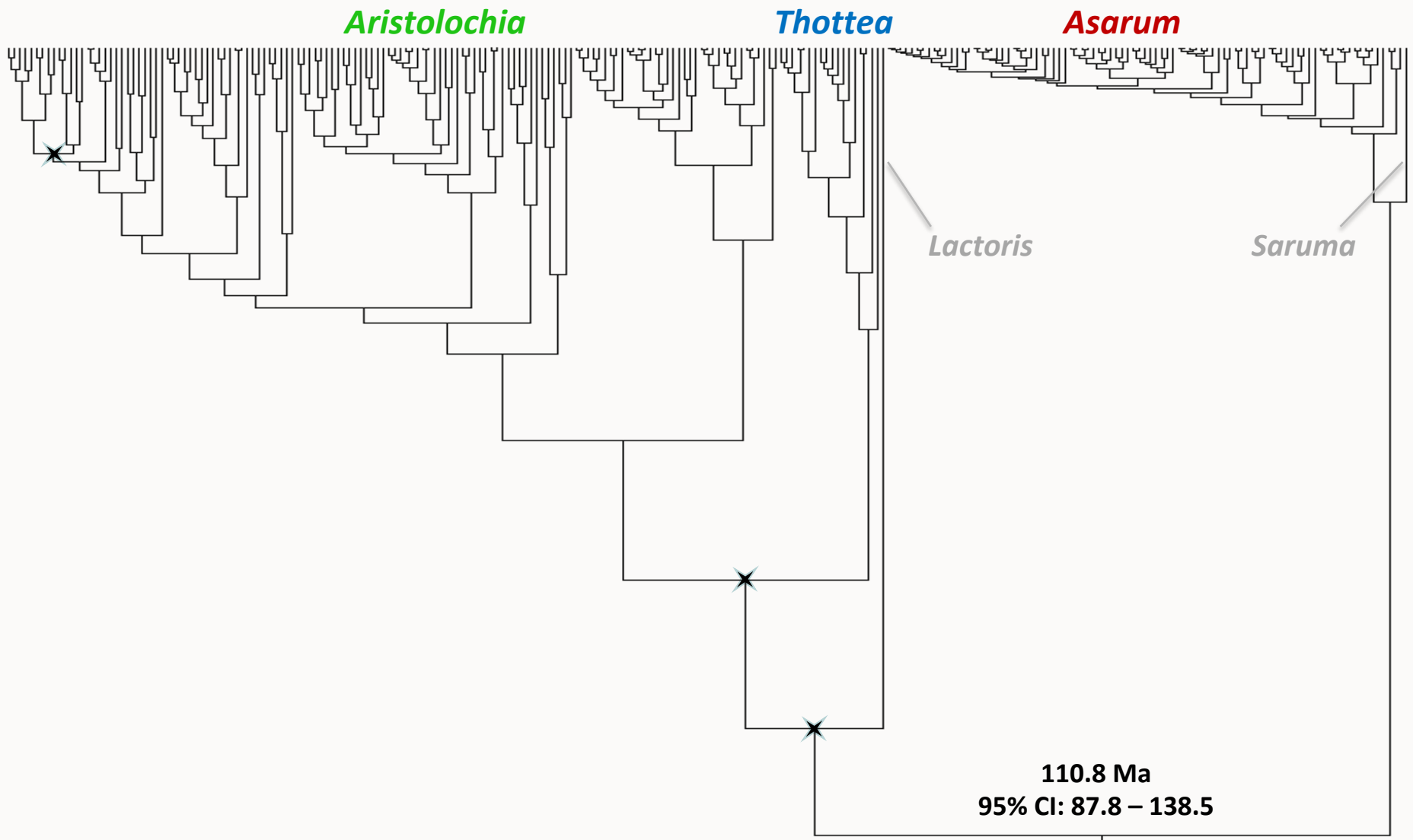
Molecular dating

4 fossils
3 ingroup fossils
1 outgroup fossil



BEAST

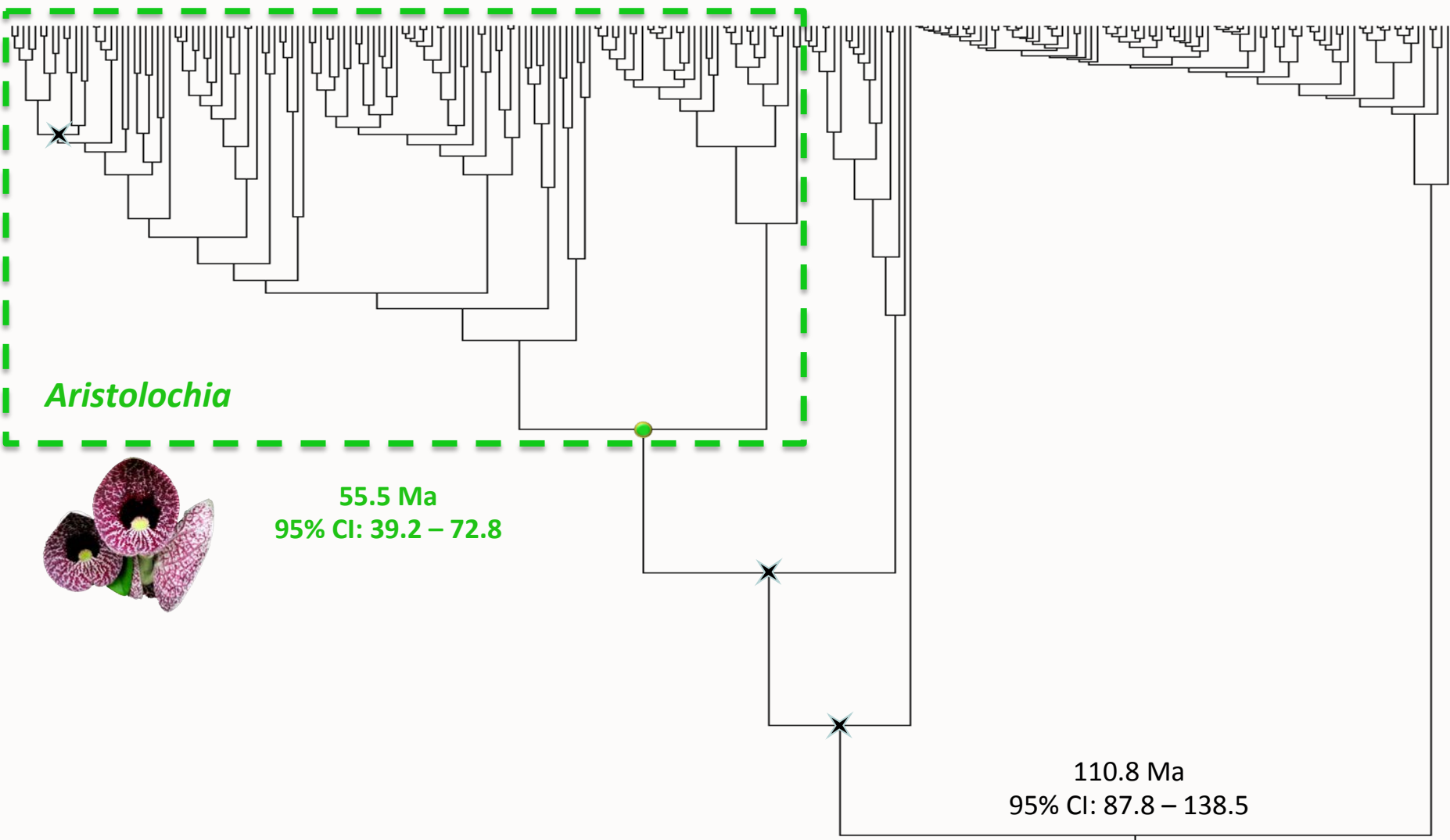
Time-calibrated phylogeny of Aristolochiaceae



BEAST 200 M. MCMC generations

✕ = Fossil calibrations

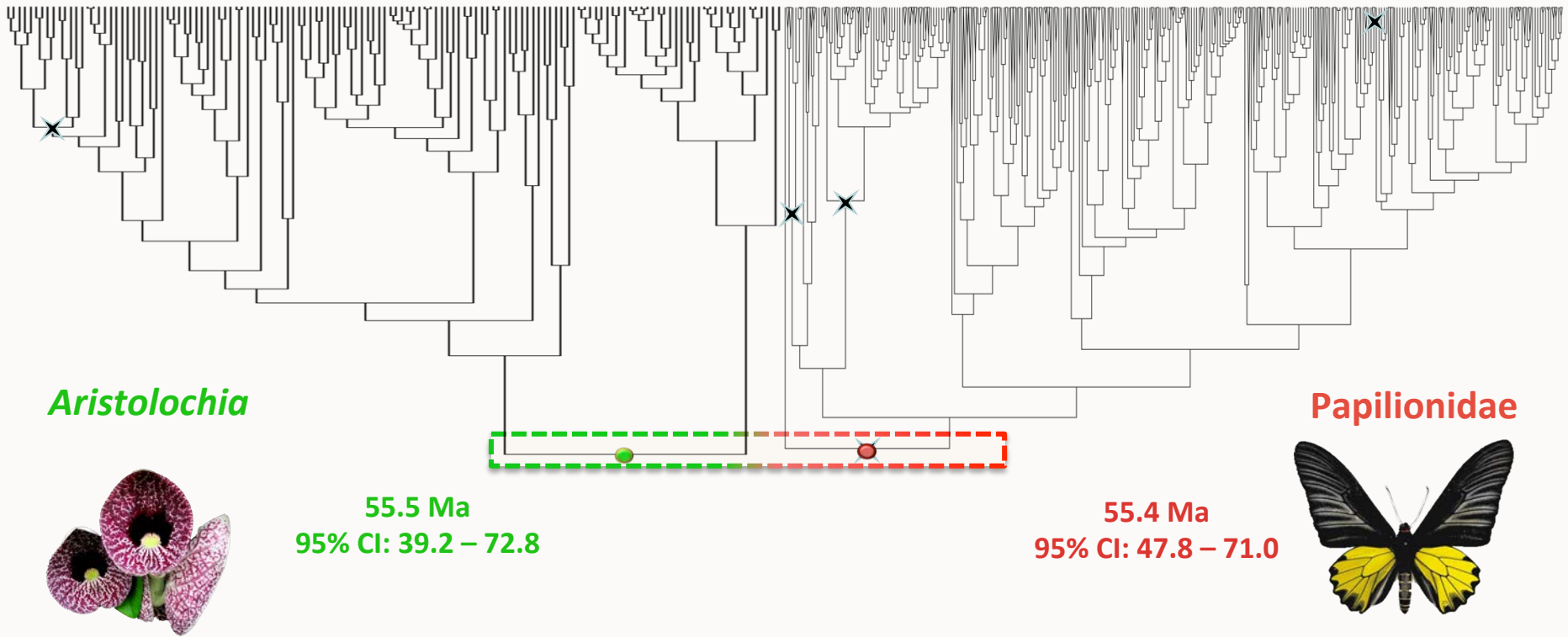
Time-calibrated phylogeny of Aristolochiaceae



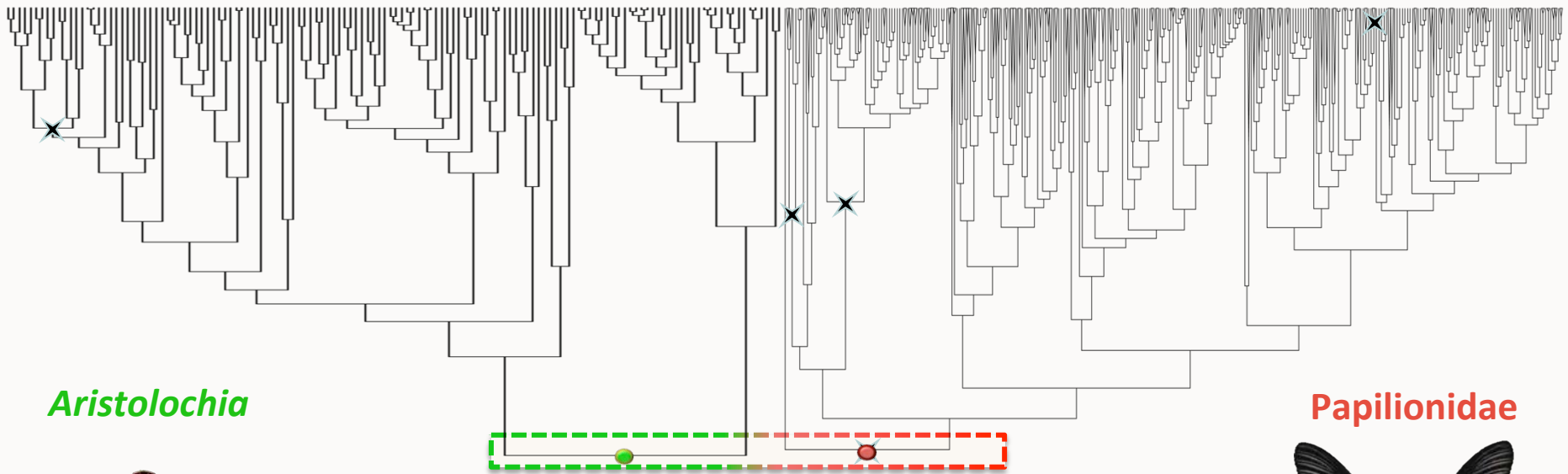
BEAST 200 M. MCMC generations

X = Fossil calibrations

Comparing herbivore and host plant origins



Comparing herbivore and host plant origins



Aristolochia

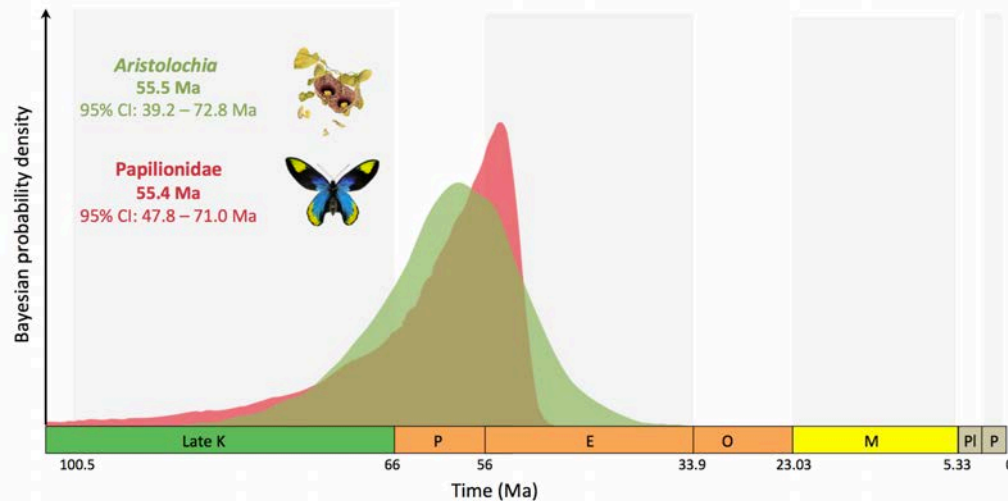


55.5 Ma
95% CI: 39.2 – 72.8

Papilionidae



55.4 Ma
95% CI: 47.8 – 71.0



Back to the main questions

1. What are the ancestral host plants of Papilionidae?

➡ Fed on Aristolochiaceae, very likely on the genus *Aristolochia*

2. Temporal congruence?

➡ Both Papilionidae and *Aristolochia* originated ≈55 Ma

3. Biogeographic congruence?

4. Signatures of genomic adaptations and on speciation rates?

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 Fed on Aristolochiaceae, very likely on the genus *Aristolochia*

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 Both Papilionidae and *Aristolochia* originated ≈55 Ma

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Inferring the biogeographic history of both groups

Distribution data for 408 spp. of Papilionidae and 247 spp. of Aristolochiaceae

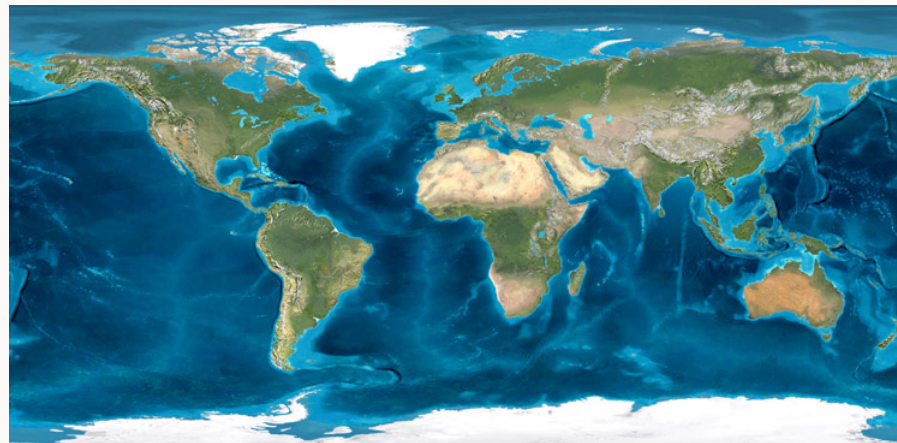


12 regions



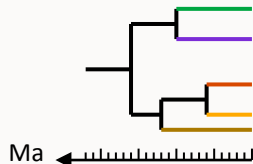
Time-stratified geographic model

- Present
- Miocene
- Oligocene
- Eocene
- Cretaceous-Tertiary boundary
- Late Cretaceous
- Middle Cretaceous
- Early Cretaceous



Adjacency matrices
and
Dispersal matrices

Biogeographic (DEC) analyses



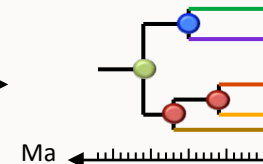
+

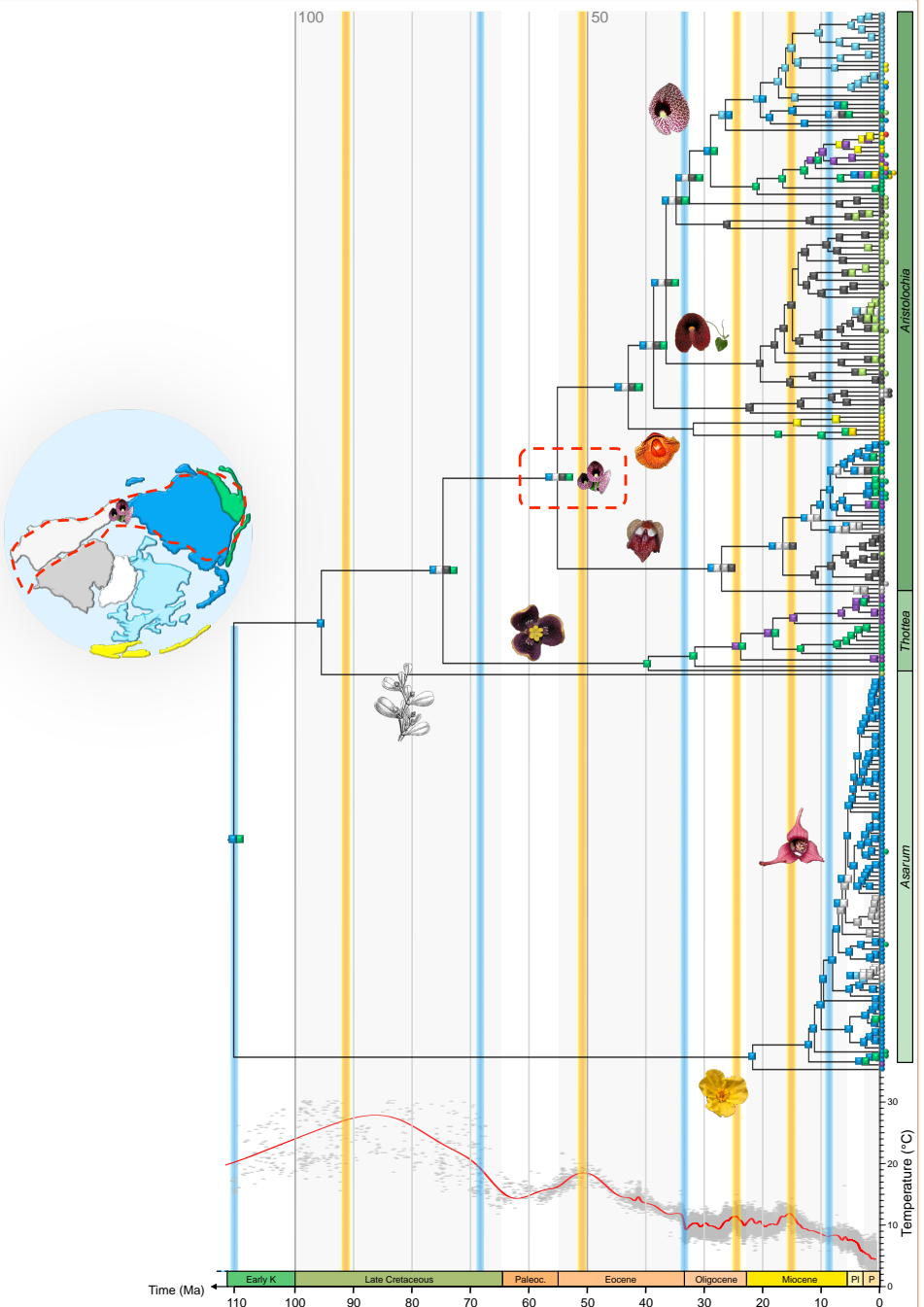
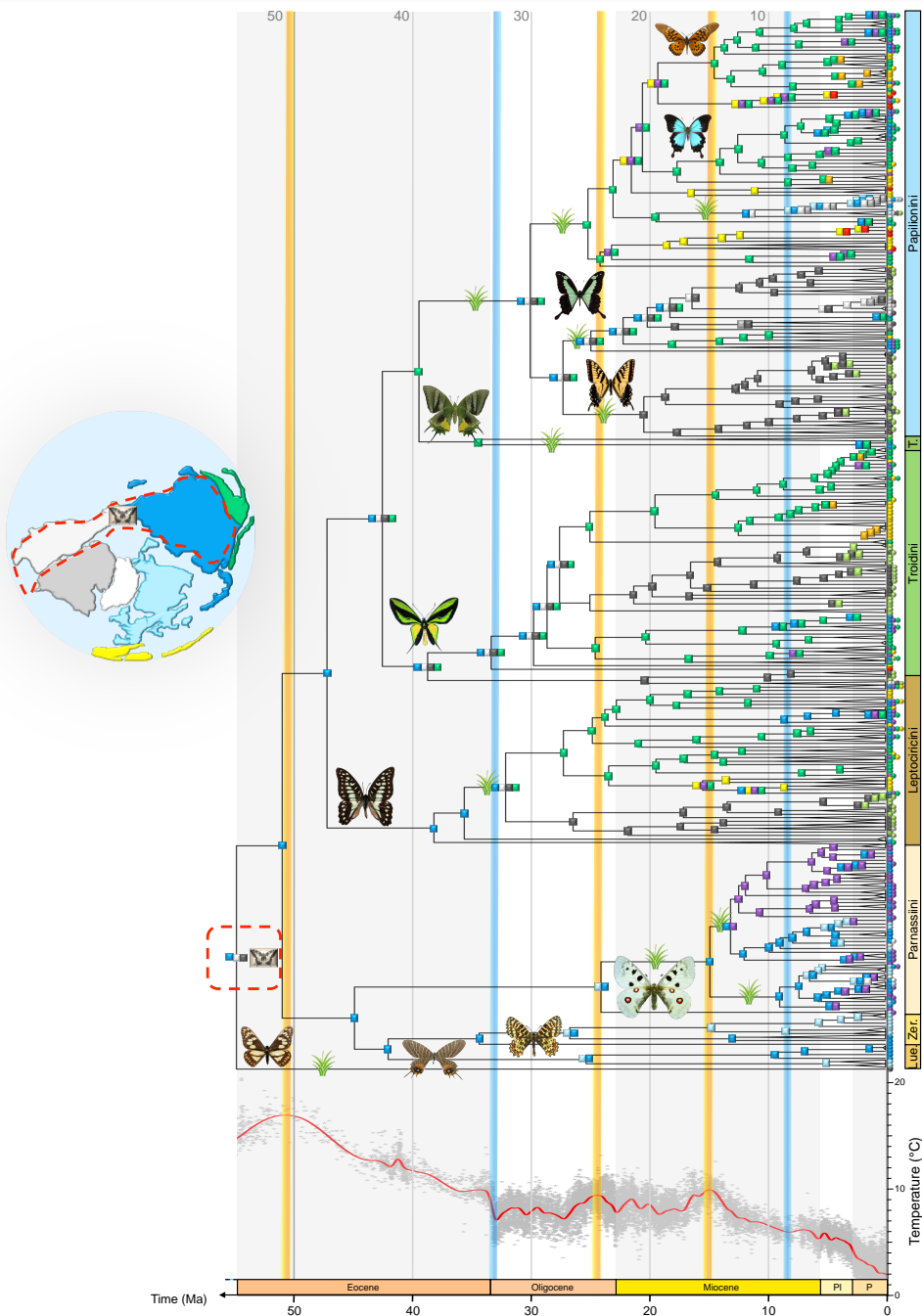
Species	S	D	F	A	B	C	G	E	H
2	1	1	0	0	0	0	0	0	0
10	1	0	1	1	0	0	0	0	0
8	1	0	0	1	1	0	0	0	0
7	0	1	1	0	1	0	0	0	0
9	0	1	1	0	0	0	0	0	0
4	1	0	0	1	0	1	0	0	0
1	0	0	1	1	0	1	0	0	0
5	0	0	0	1	0	0	0	0	0
6	0	0	0	1	0	0	0	1	1
3	0	0	0	0	1	0	1	1	1

+

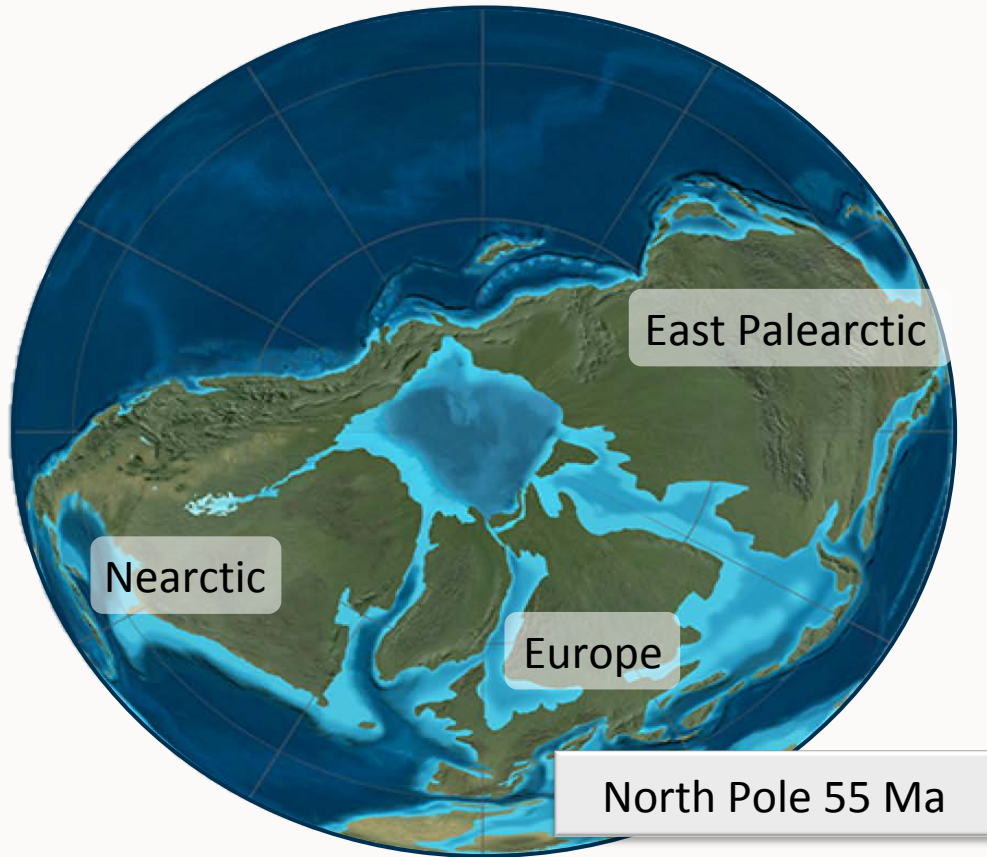
	S	D	F	A	B	C	G	E	H
2	1	1	0	0	0	0	0	0	0
10	1	0	1	1	0	0	0	0	0
8	1	0	0	1	1	0	0	0	0
7	0	1	1	0	1	0	0	0	0
9	0	1	1	0	0	0	0	0	0
4	1	0	0	1	0	1	0	0	0
1	0	0	1	1	0	1	0	0	0
5	0	0	0	1	0	0	0	0	0
6	0	0	0	1	0	0	0	1	1
3	0	0	0	0	1	0	1	1	1

DEC
models

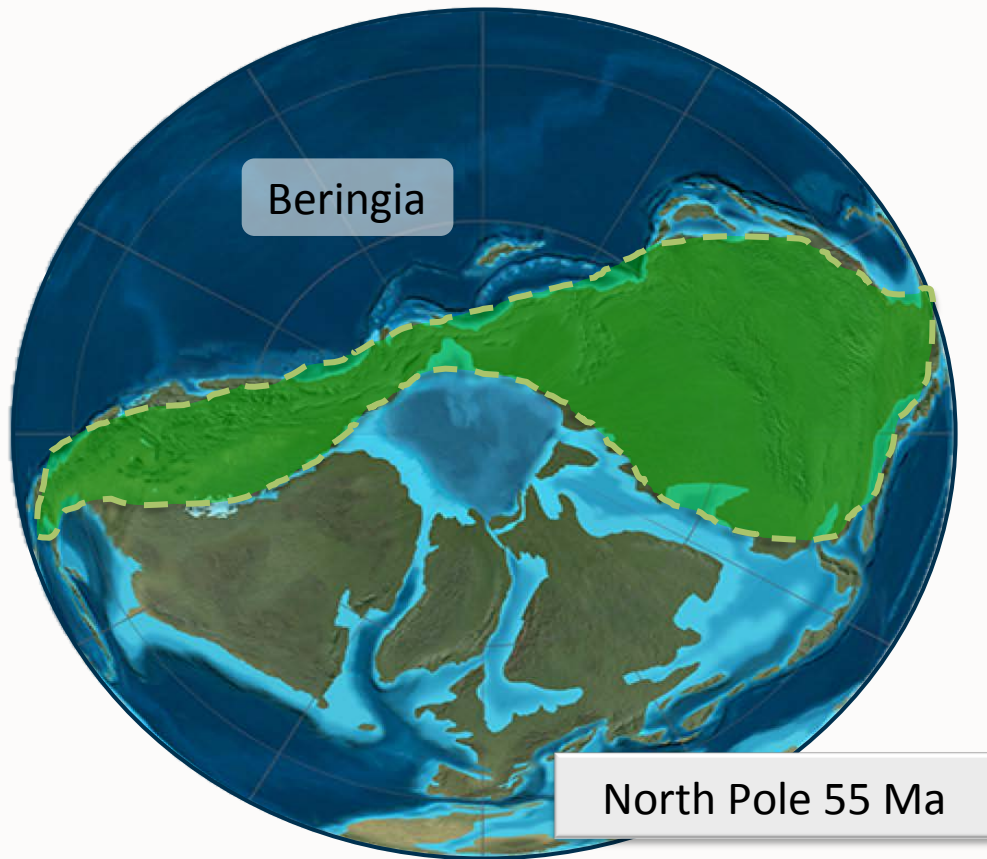




Both groups appeared at the same time and same place



Both groups appeared at the same time and same place

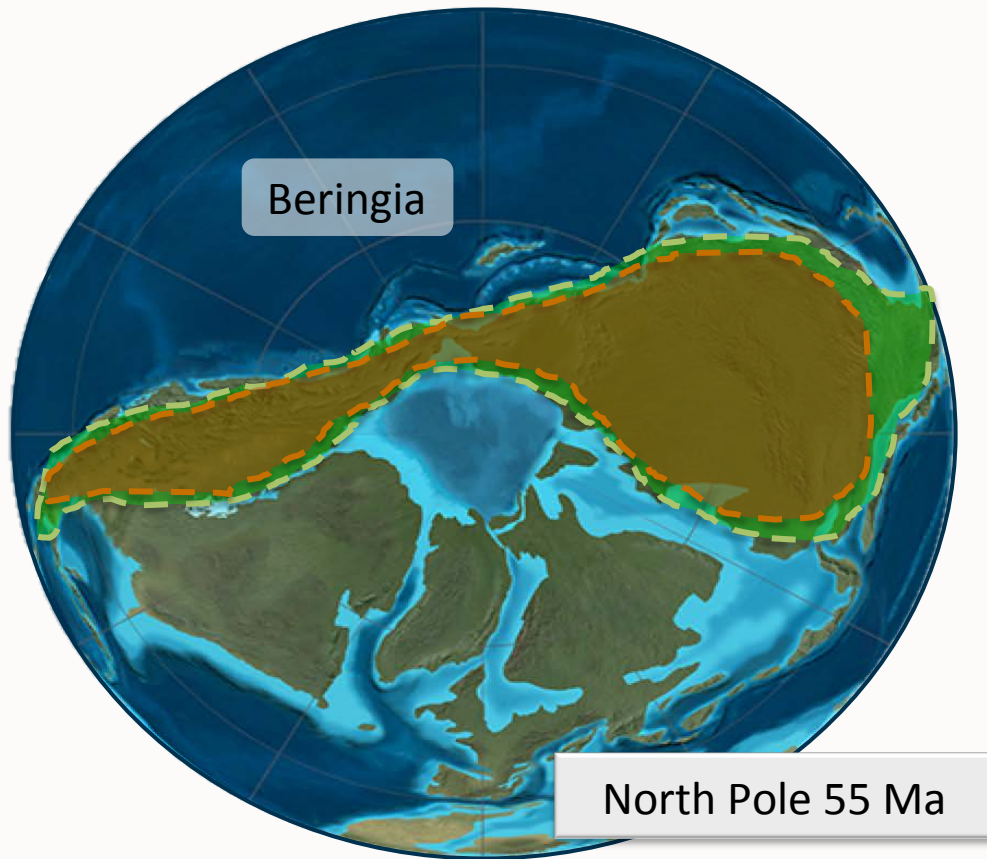


Ancestral area of *Aristolochia*



Central America + West Nearctic + East Palearctic +
North Indonesia

Both groups appeared at the same time and same place



Ancestral area of *Aristolochia*



Central America + West Nearctic + East Palearctic +
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Ancestral area of Papilionidae



Central America + West Nearctic + East Palearctic

Back to the main questions

1. What are the ancestral host plants of Papilionidae?

➡ Fed on Aristolochiaceae, very likely on the genus *Aristolochia*

2. Temporal congruence?

➡ Both Papilionidae and *Aristolochia* originated ≈55 Ma

3. Biogeographic congruence?

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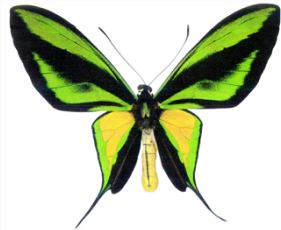
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➡ Both Papilionidae and *Aristolochia* originated ≈55 Ma in Beringia

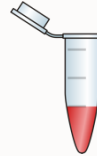
4. Signatures of genomic adaptations and on speciation rates?

Genomic dataset

Shotgun sequencing for 41 species



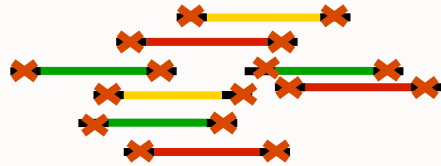
DNA extractions
Library preparations



Illumina NextSeq

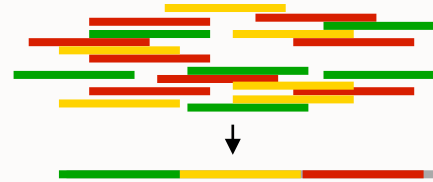


Reads cleaning



Trimmomatic

Shotgun assembly



SOAP-denovo

BLAST

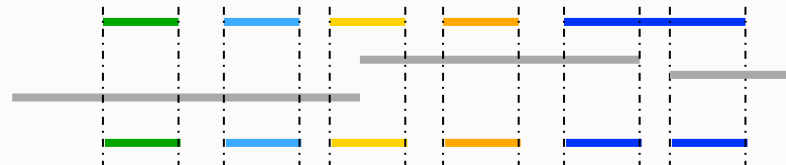
Alignment

Consensus

Reference proteins {

Scaffolds {

Annotated proteins {

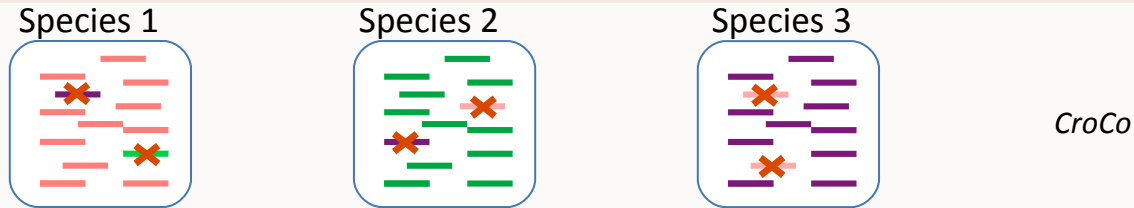


tblastn

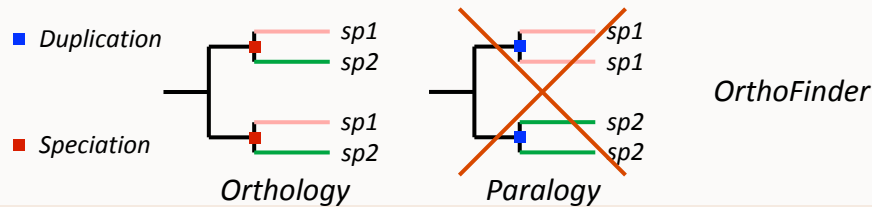
TranslatorX

Inferring a first phylogenomic backbone with NGS data

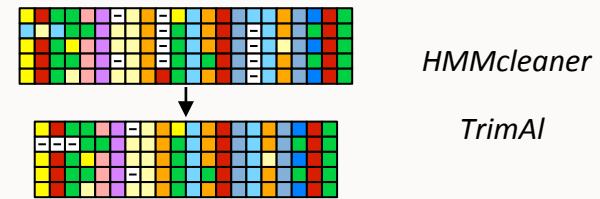
Check for cross-contamination



Detections of orthologous genes



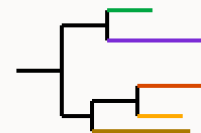
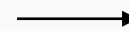
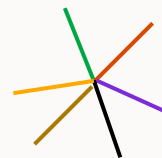
Alignment cleaning



Phylogenetic inferences

Dataset 1: 760 genes ; 288 446 AA
12% missing data

Dataset 2: 6621 genes ; 1 656 028 AA
32% missing data

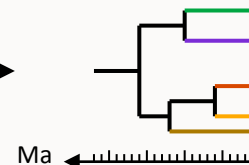
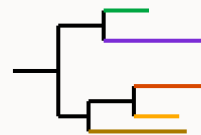
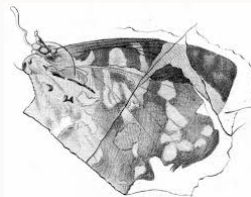


IQ-TREE
PhyloBayes
Supertree approach

Molecular dating

5 fossils

3 ingroup
2 outgroup



PhyloBayes

Inferring a first phylogenomic backbone with NGS data

IQ-TREE many tests with:

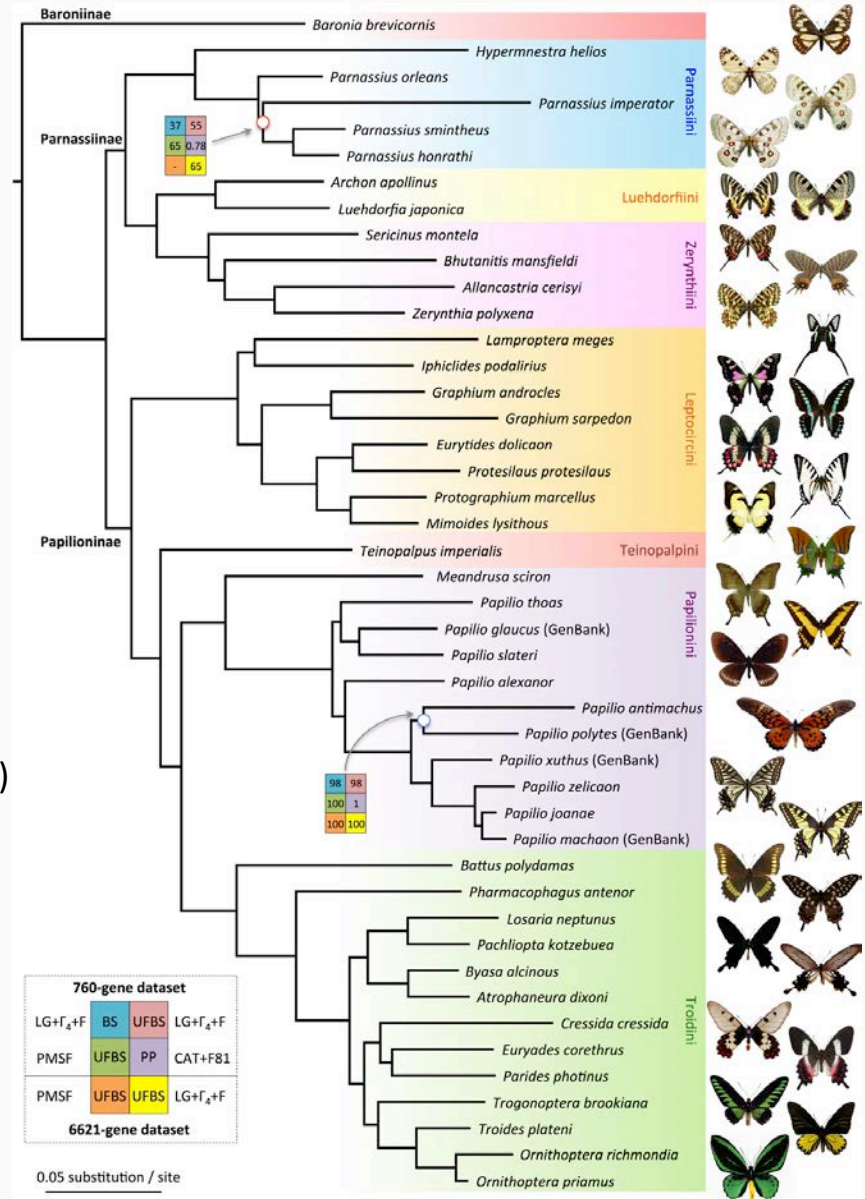
- 100 thorough bootstraps
- 1000 ultrafast bootstraps
- Protein models: LG+Γ₄+F / C50 / PMSF

PhyloBayes CAT model

All nodes but one had BV = 100 or PP = 1

All subfamilies, tribes, genera are monophyletic

Low-quality libraries are well placed (but long branches)



Taxon sampling = 45 Papilionidae + 16 outgroups

Inferring a first phylogenomic backbone with NGS data

IQ-TREE many tests with:

- 100 thorough bootstraps
- 1000 ultrafast bootstraps
- Protein models: LG+ Γ_4 +F / C50 / PMSF

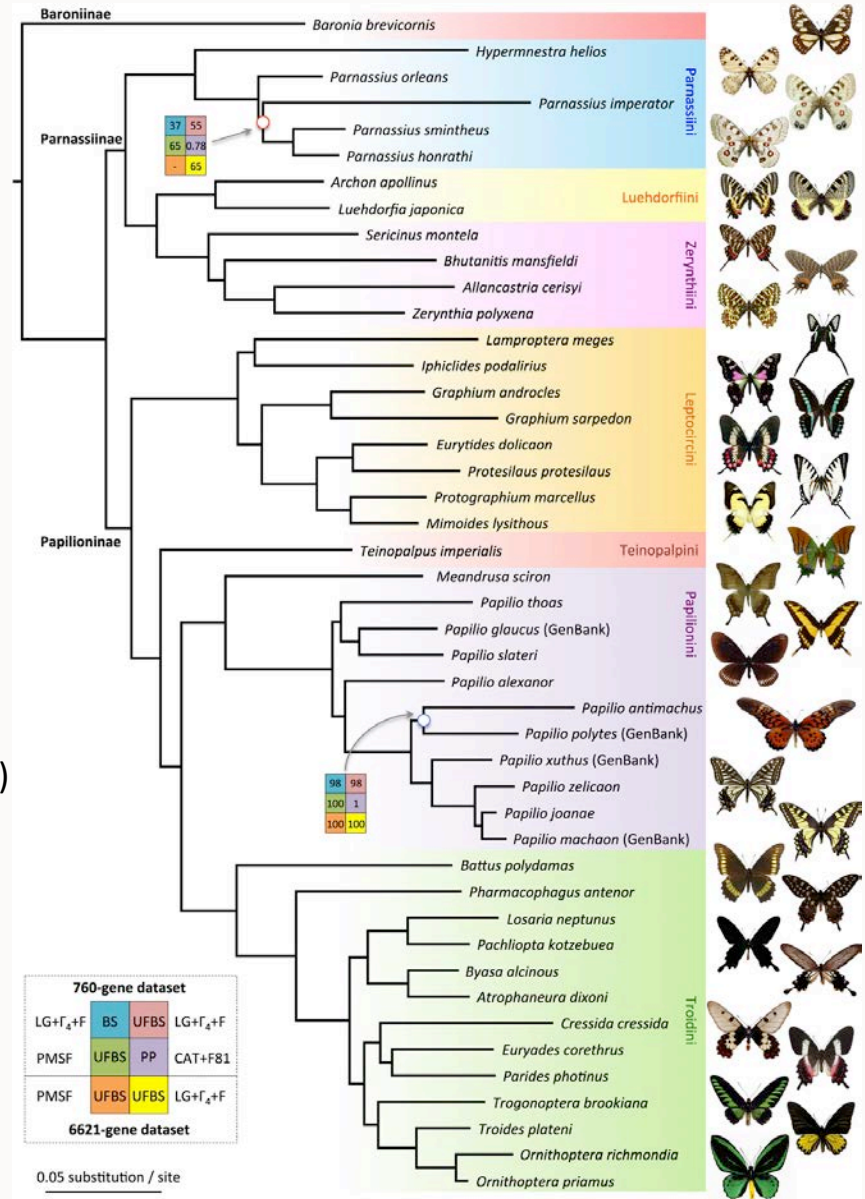
PhyloBayes CAT model

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All subfamilies, tribes, genera are monophyletic

Low-quality libraries are well placed (but long branches)

Signatures of genomic adaptations?



Taxon sampling = 45 Papilionidae + 16 outgroups

Are the differences between the sequences due to natural selection?

1. Studying the substitutions in the amino acid sequences with:

- **Synonymous substitutions** = distance between the sequences (ignoring selection)



dS = number of substitutions in synonymous codons

- **Non-synonymous substitutions** = either positive selection or drift



dN = number of substitutions in non-synonymous codons

Are the differences between the sequences due to natural selection?

1. Studying the substitutions in the amino acid sequences with:

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d_N = number of substitutions in non-synonymous codons

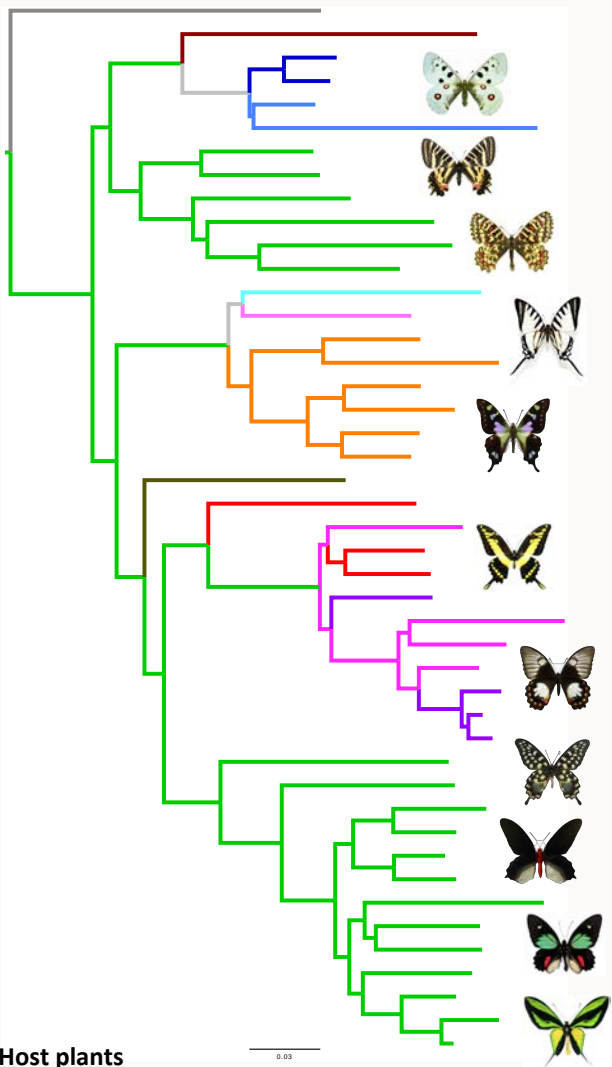
2. Ratio to infer the direction and magnitude of natural selection acting on protein-coding genes:

$$\omega = \frac{d_N}{d_S} = \frac{\textit{selection} + \textit{neutral}}{\textit{neutral}}$$

$\omega = 1$: no (neutral) selection

$\omega > 1$: positive selection, it implies positive or Darwinian selection (driving change)

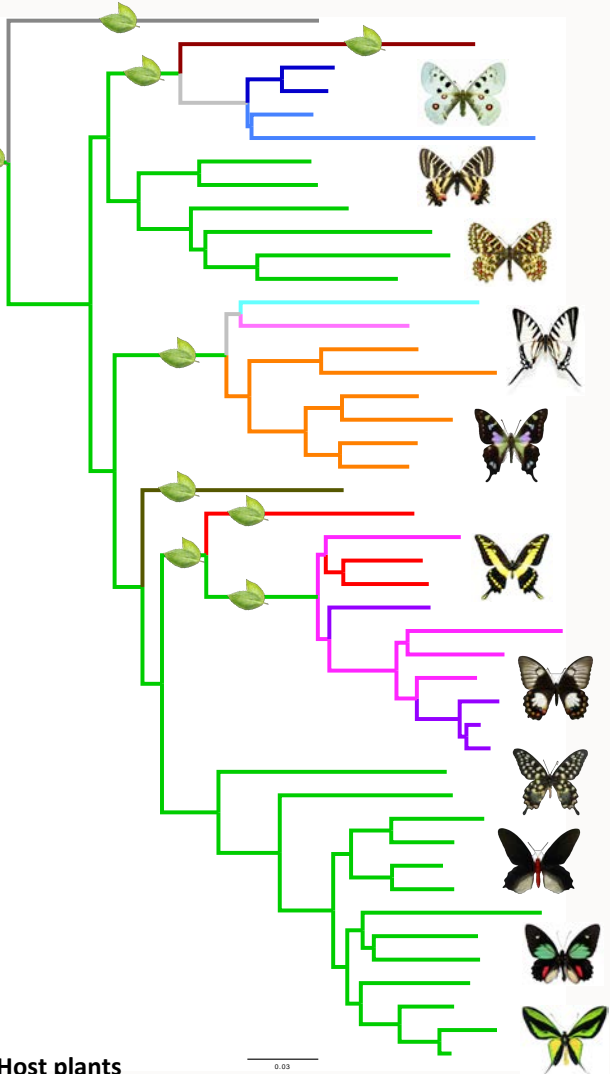
$\omega < 1$: negative selection, it implies purifying or stabilizing selection (acting against change)



Host plants

- █ Aristolochiaceae
- █ Rutaceae
- █ Annonaceae
- █ Lauraceae
- █ Apiaceae
- █ Crassulaceae + Saxifragaceae
- █ Papaveraceae
- █ Magnoliaceae
- █ Zygophyllaceae
- █ Fabaceae (*Acacia*)

 **Host-plant shift**

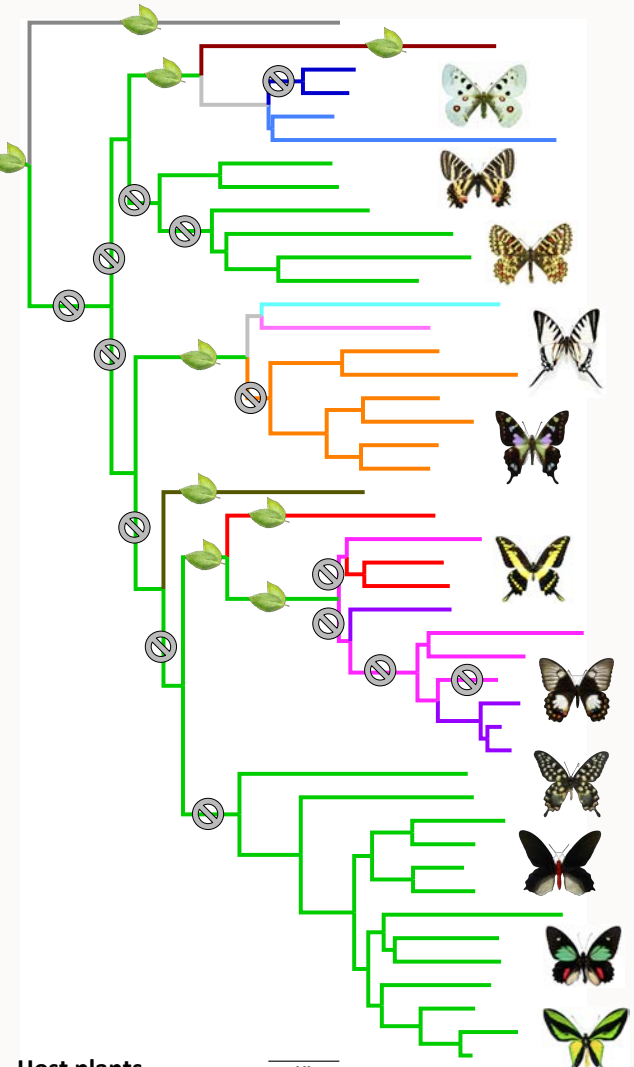


Host plants

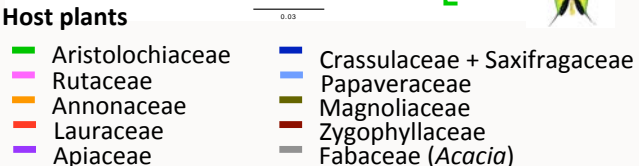
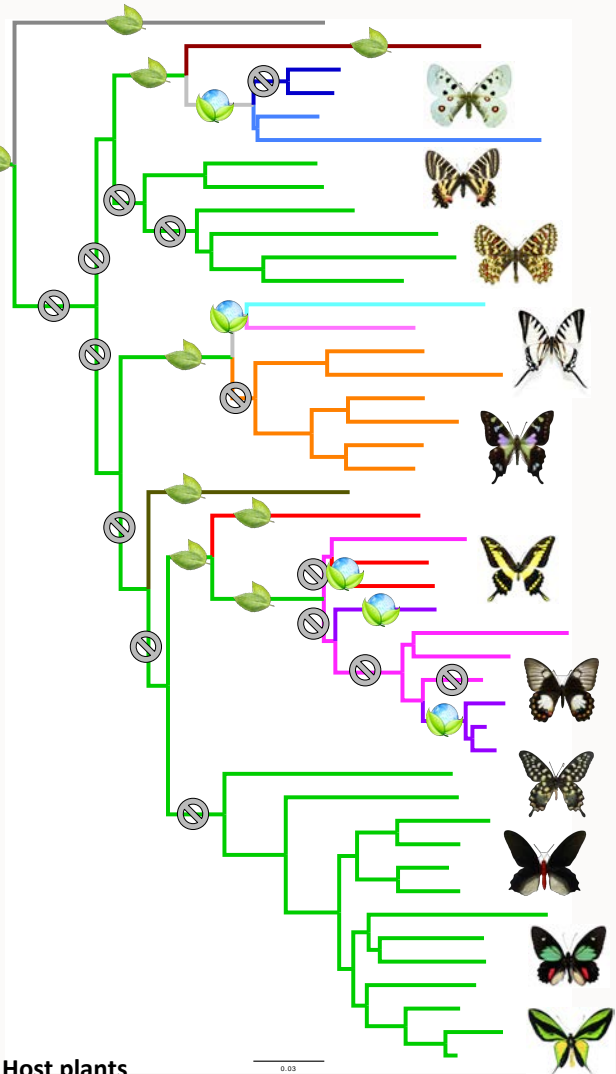
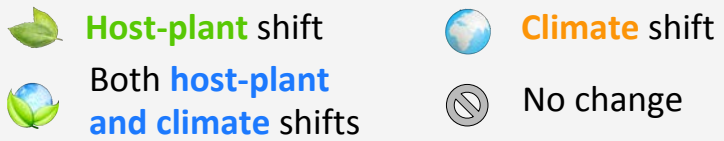
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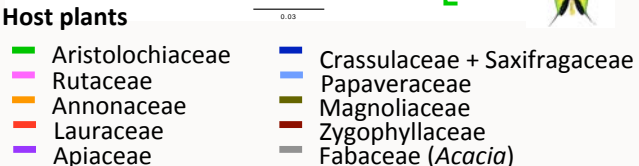
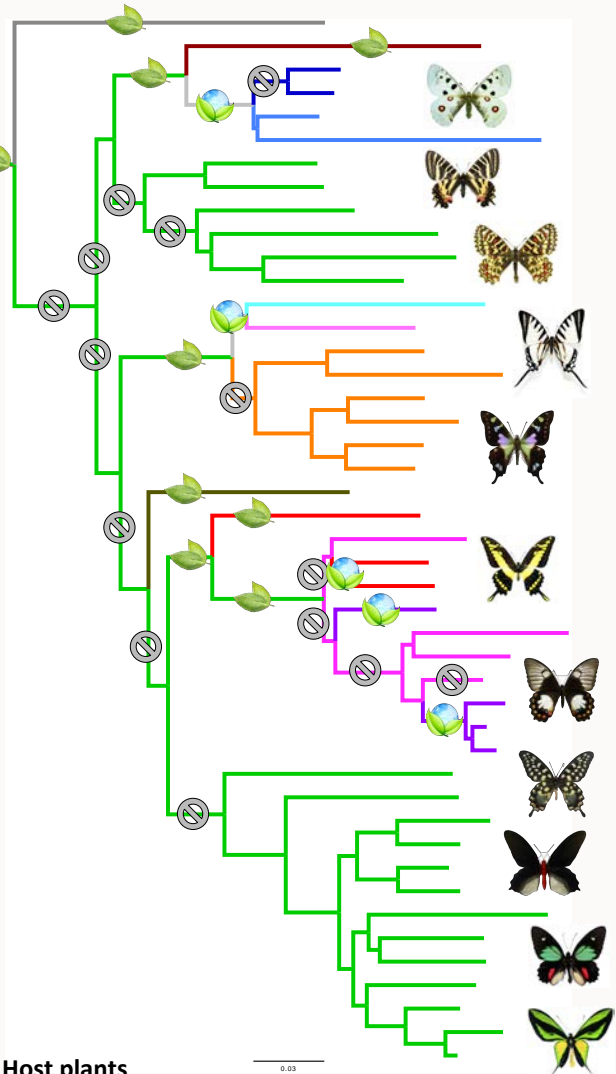
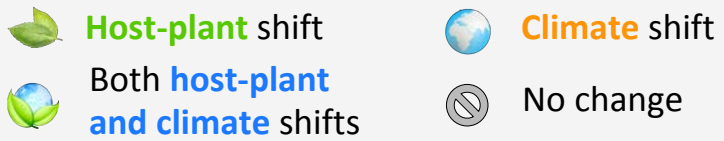
Host-plant shift

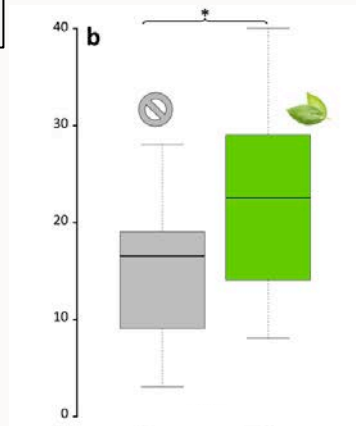
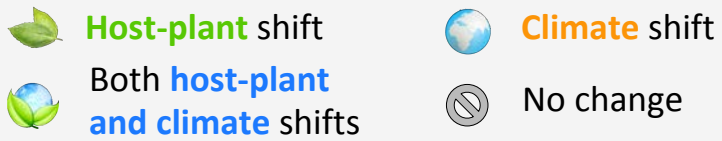
⊘ No change



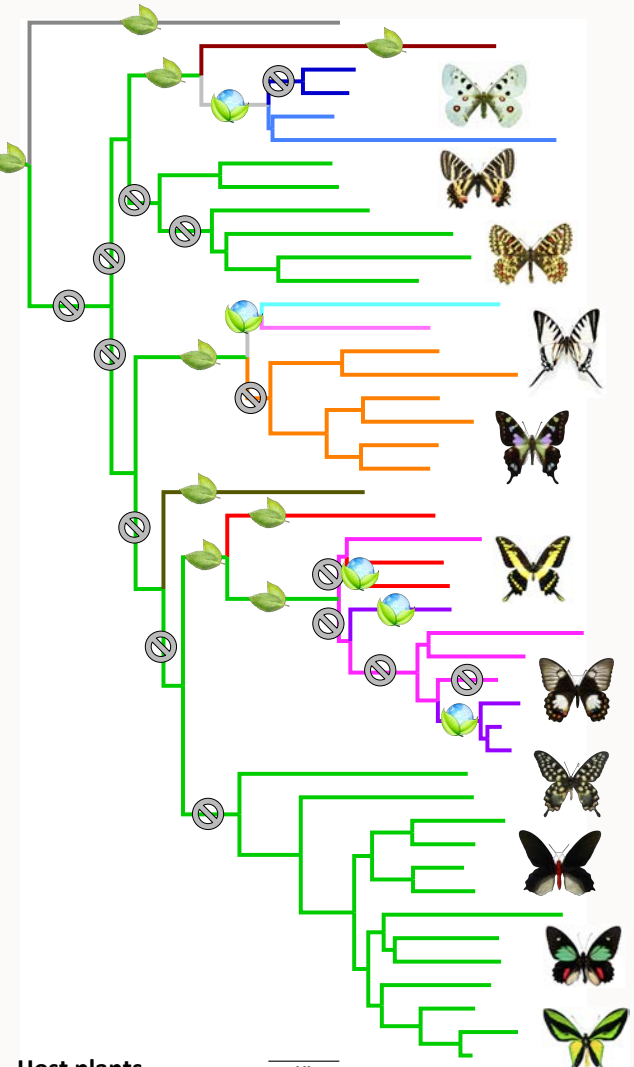
- Host plants**
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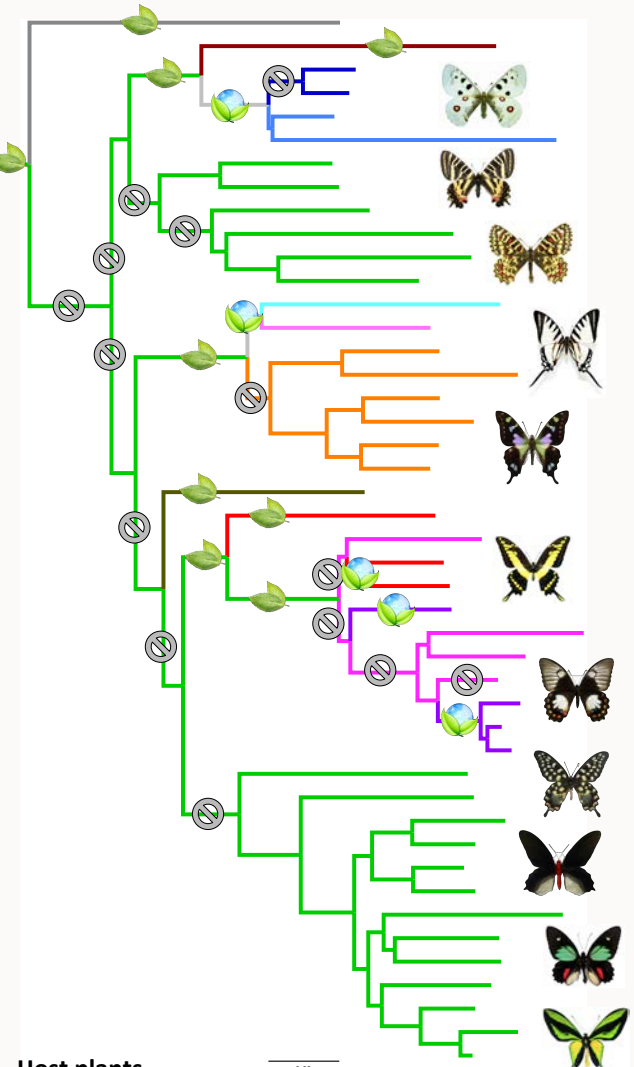
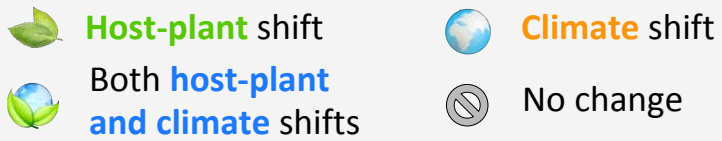




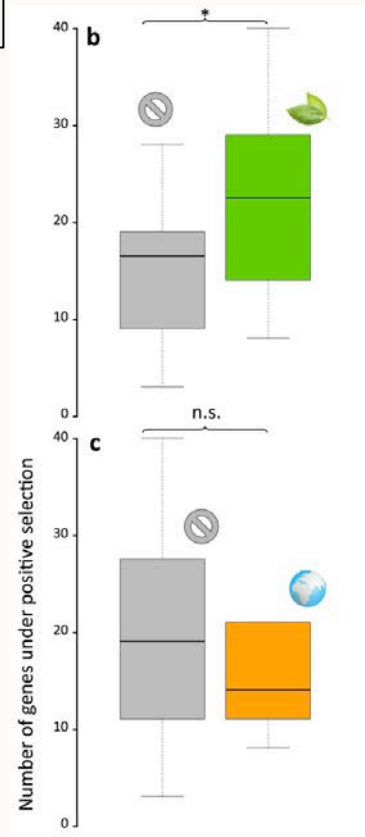
- Branches experiencing host shifts have **MORE** genes under positive selection



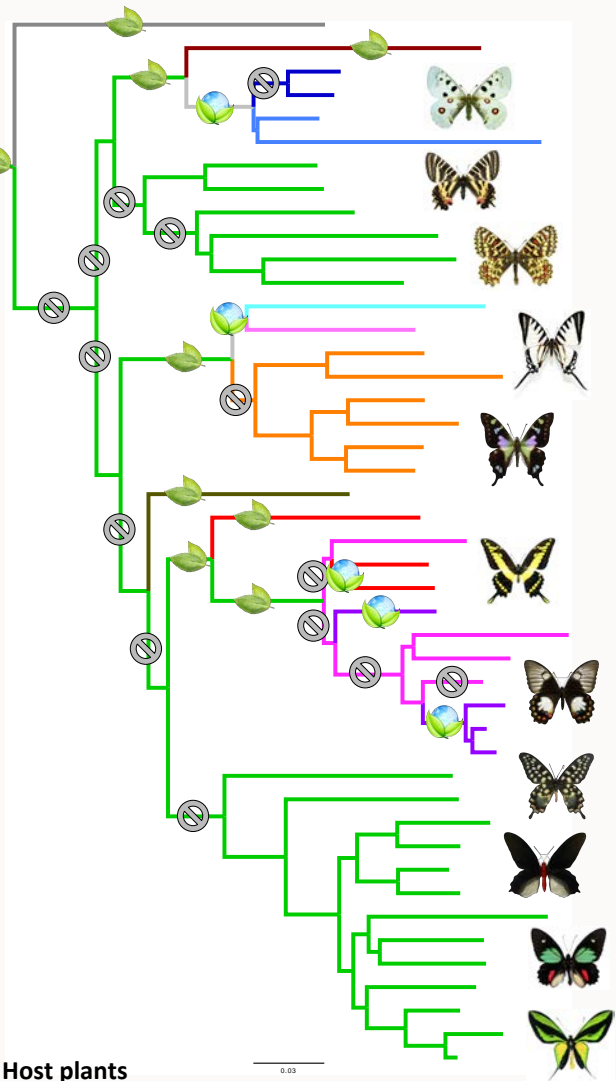
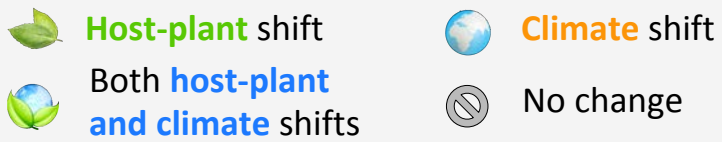
- Host plants**
- | | |
|--|---|
| <ul style="list-style-type: none"> ■ Aristolochiaceae ■ Rutaceae ■ Annonaceae ■ Lauraceae ■ Apiaceae | <ul style="list-style-type: none"> ■ Crassulaceae + Saxifragaceae ■ Papaveraceae ■ Magnoliaceae ■ Zygophyllaceae ■ Fabaceae (<i>Acacia</i>) |
|--|---|



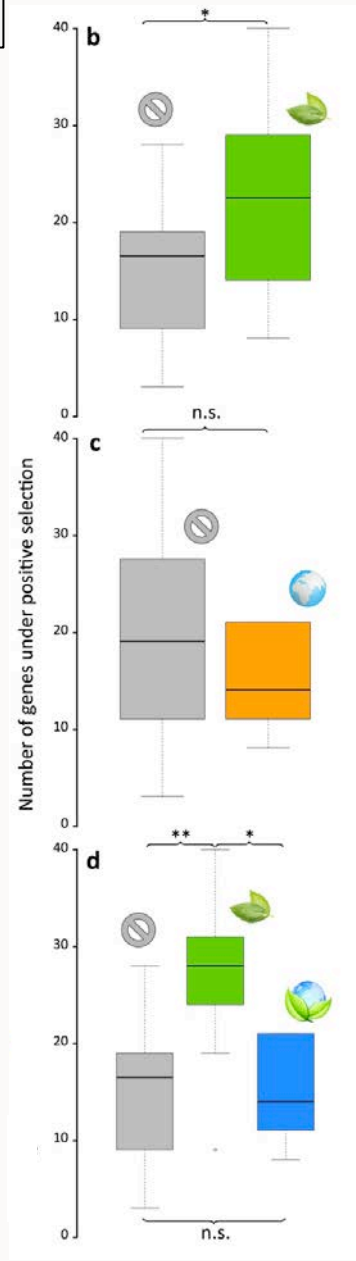
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- Branches experiencing host shifts have **MORE genes under positive selection**
- Branches experiencing environmental shifts have **NOT more genes under positive selection**



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- Branches experiencing host shifts have **MORE genes under positive selection**
- Branches experiencing environmental shifts have **NOT more genes under positive selection**
- Branches experiencing both host and environmental shifts have **NOT more genes under positive selection**

Back to the main questions

1. What are the ancestral host plants of Papilionidae?

➡ Fed on Aristolochiaceae, very likely on the genus *Aristolochia*

2. Temporal congruence?

➡ Both Papilionidae and *Aristolochia* originated ≈55 Ma

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➡ Both Papilionidae and *Aristolochia* originated ≈55 Ma in Beringia

4. Signatures of genomic adaptations and on speciation rates?

➡ Genome-wide adaptations

Back to the main questions

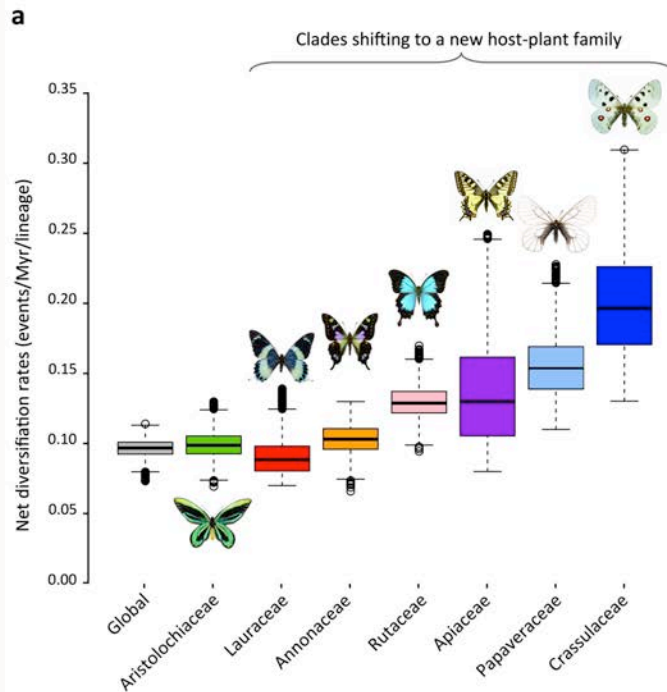
1. W

2. T

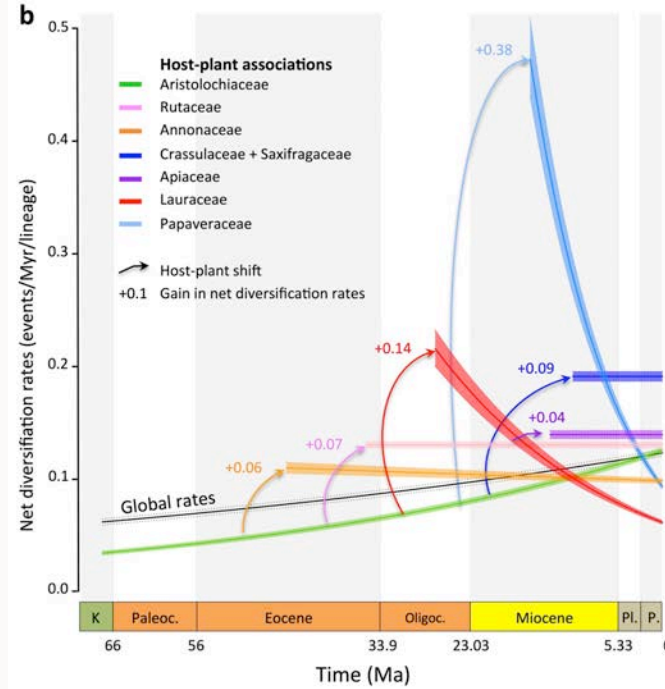
3. B

4. S

Trait-dependent constant-rate models



Clade-specific time-dependent models



es?

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4. Signatures of genomic adaptations and on speciation rates?

➡ Genome-wide adaptations and speciation rates increase with host-plant shifts

To summarize ...

- Papilionidae = excellent biological model to study insect-plant interactions:

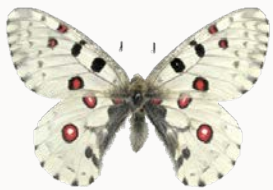


1. Ancestral host plant = Aristolochiaceae, highly toxic
2. Both groups originated at the same time and place
3. Strong niche conservatism of host-plant preferences
4. When butterflies shifted to new plants: increases of speciation rates and genome-wide signatures of positive selection



However... this is an ongoing work with many possibilities

1. Genomic data is huge: ontology of genes?
2. Increase the taxon sampling for whole genomes = more shifts
3. Transcriptomes



Acknowledgments

- People:

PI: Fabien Condamine

Entomologists: Felix Sperling, Adam Cotton, Gaël Kergoat

Botanists: Stephan Wanke, Oscar Pérez-Escobar, Guillaume Chomicki

Genomics: Benoit Nabholz

Molecular biology: Anne-Laure Clamens

- Funding:

Marie Curie IOF grant (BIOMME project)

ERC Starting Grant (GAIA project)



Thank you for your attention