

Revising Australian blowfly relationships using morphology and genomics

Liam Foley

Supervisors: Dr Nikolas Johnston
& Prof. James Wallman



Blowflies are important!

Ecological roles

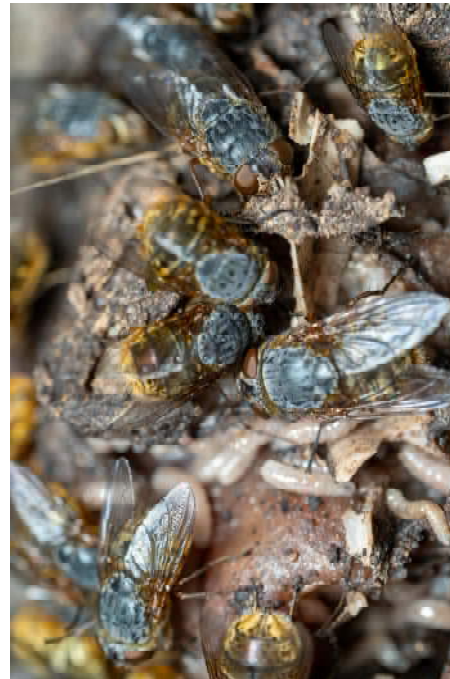
- Decomposition & nutrient recycling
- Pollination

Agricultural pests

- Flystrike (myiasis)

Forensic science

- Time since death



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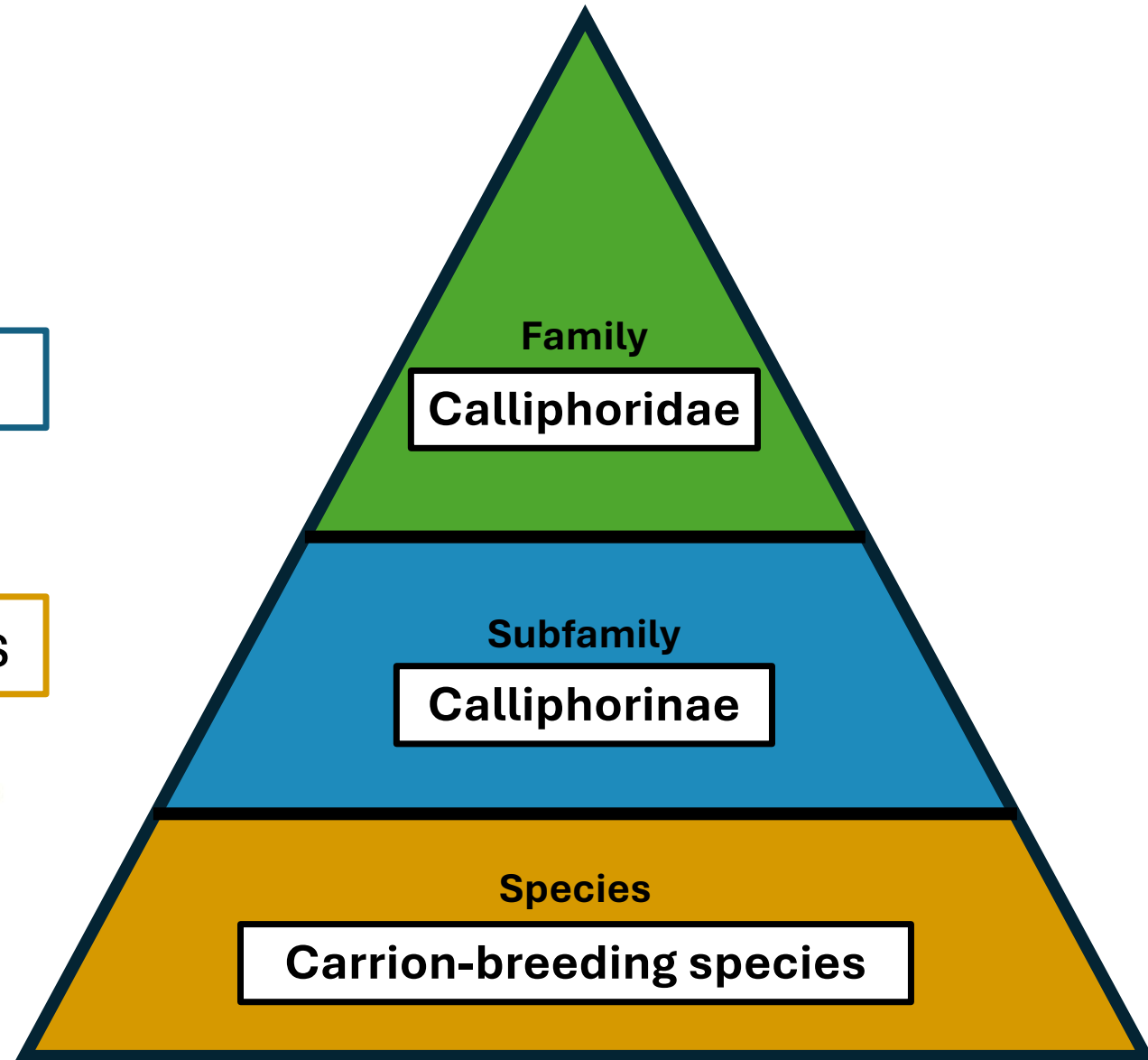
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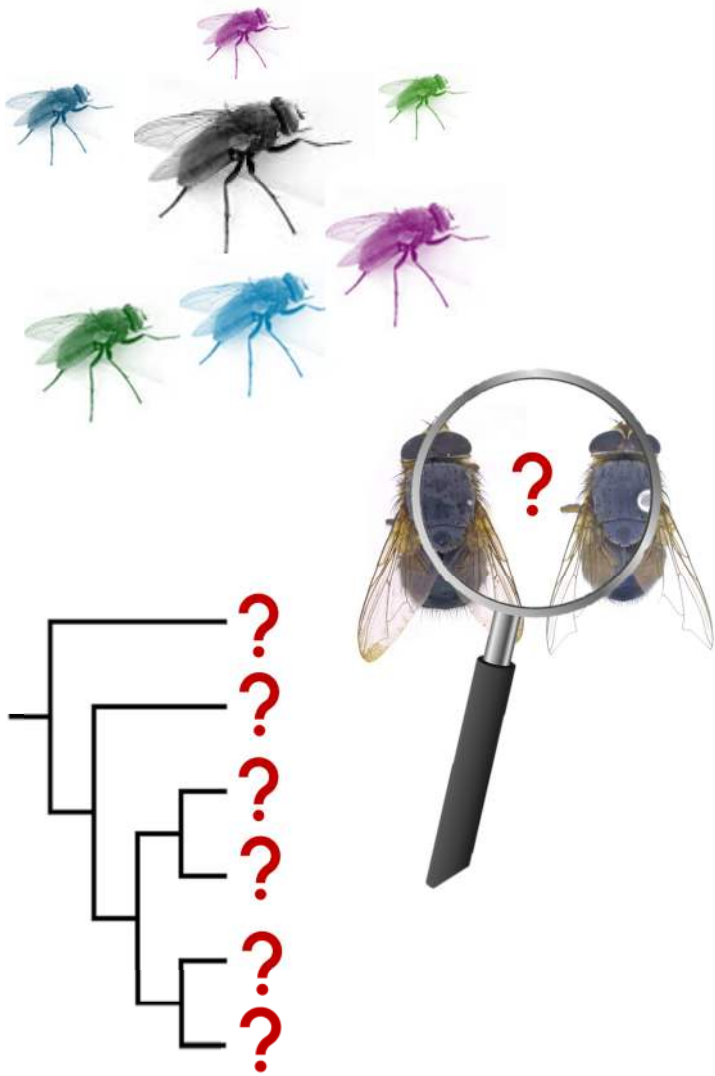
Project Focus

Subfamily **Calliphorinae**

Carrion-breeding species

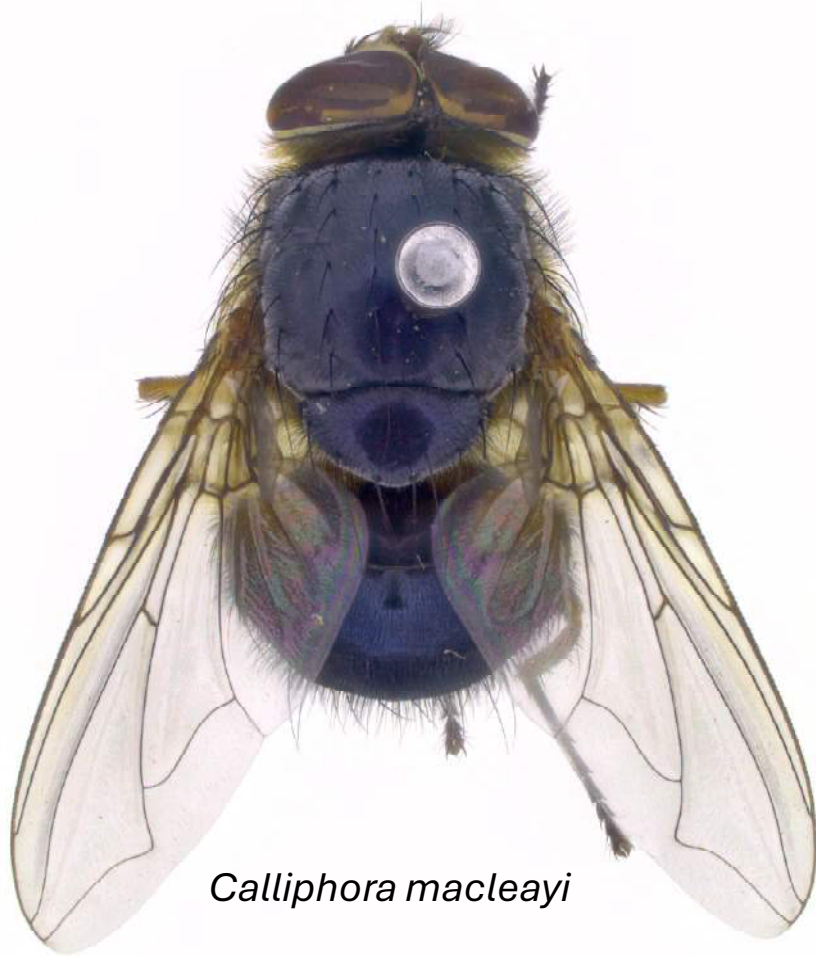


The problem: Australian blowfly relationships are a mess!



- **Extreme diversity:** subfamily Calliphorinae includes ~50 species & possibly more
- **Cryptic morphology:** Species are difficult to identify – morphology is subtle & inconsistent
- **Uncertain relationships:** No study has ever analysed all species together

Cryptic morphology

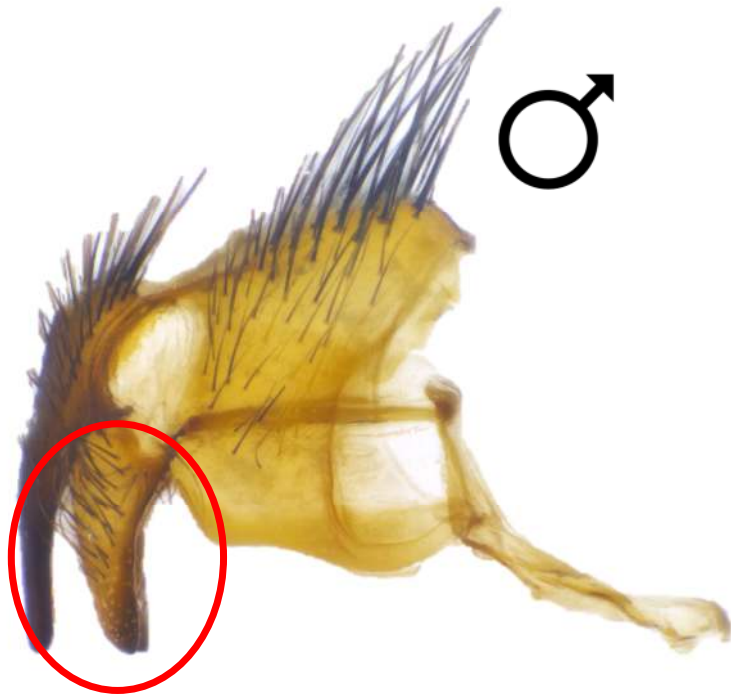


Calliphora macleayi

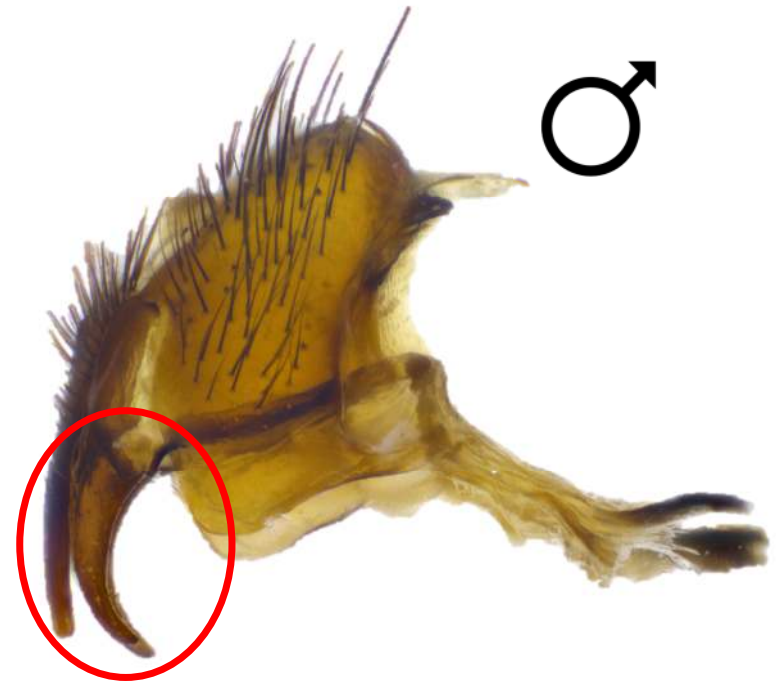


Calliphora centralis

The solution: genitalia characters

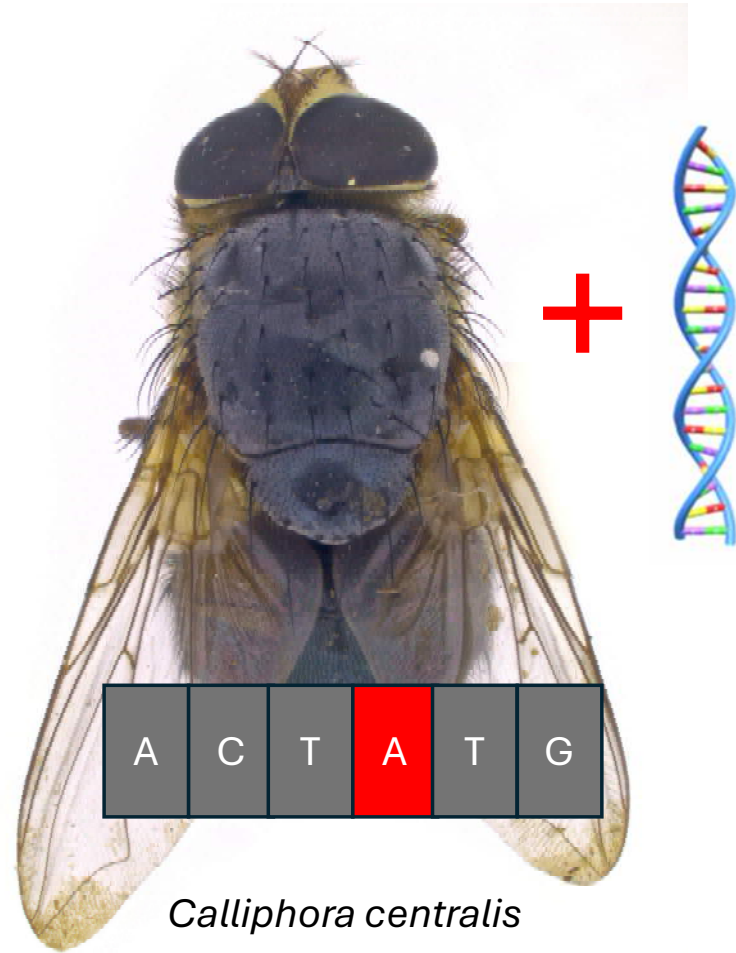
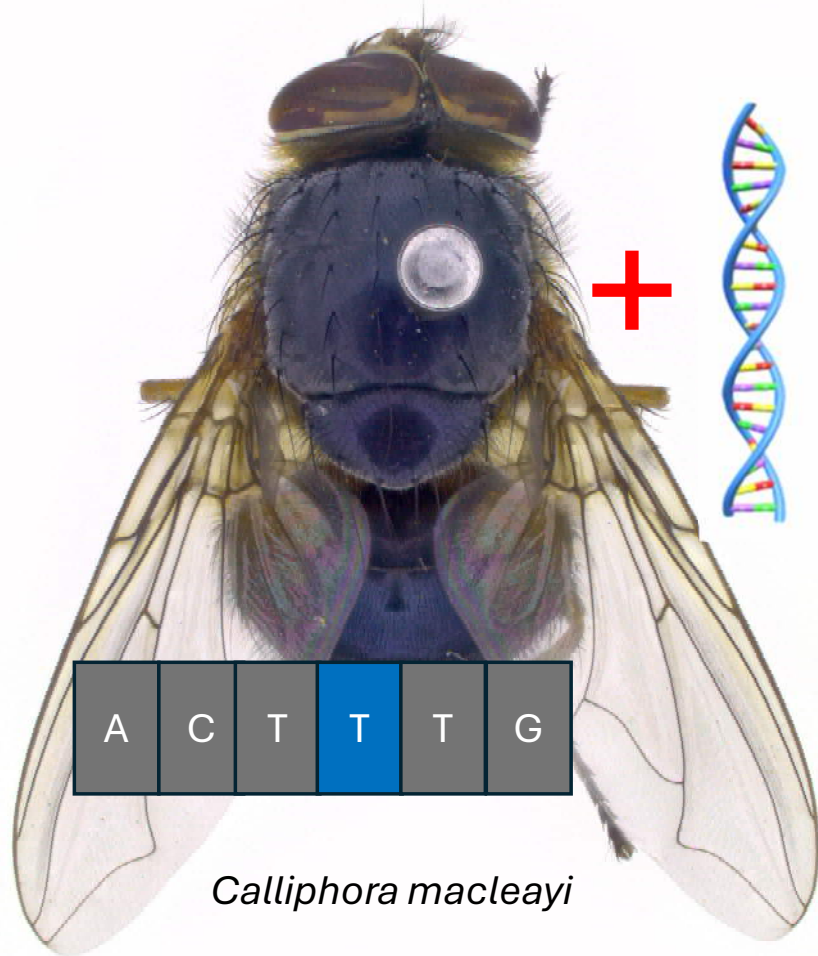


Calliphora macleayi



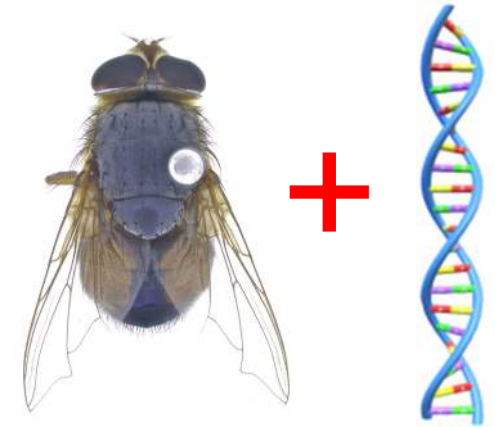
Calliphora centralis

The solution: molecular data

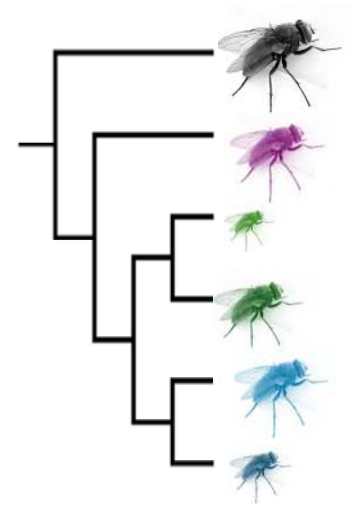


Project Aims

1. Integrate morphology & molecular data to determine the **species boundaries** in the Australian carrion-breeding Calliphorinae



2. Use phylogenetic analysis to understand the **evolutionary relationships** in the Australian carrion-breeding Calliphorinae



Methods

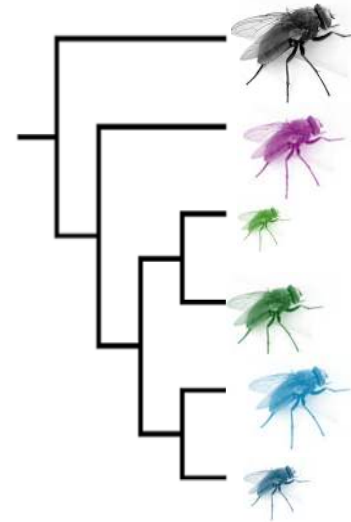
1. Morphological analysis



2. Molecular bioinformatics



3. Phylogenetic analysis



Morphological analysis

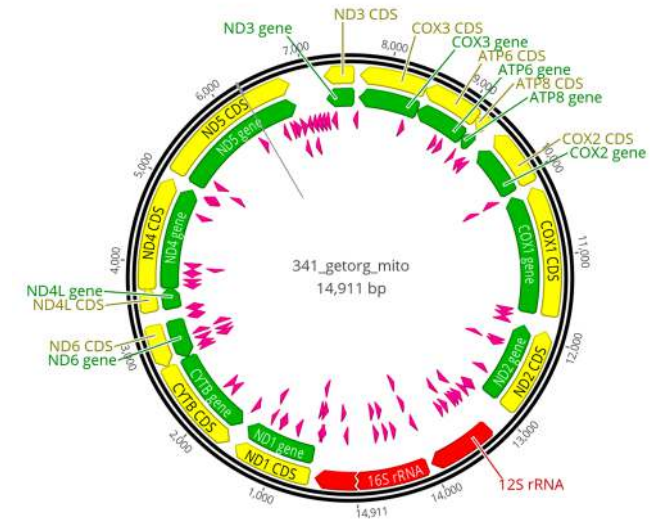
- Thousands of museum species & freshly collected specimens
- Adult specimens & genitalia analysed under microscopy
- Genitalia dissection & chemical processing
- Diagnostic images taken using focus stacking techniques



Over 20 characters described on the head alone

Molecular Bioinformatics

- Sequencing data quality control
- Whole mitogenome assembly
- Species delimitation using DNA barcodes (ASAP)

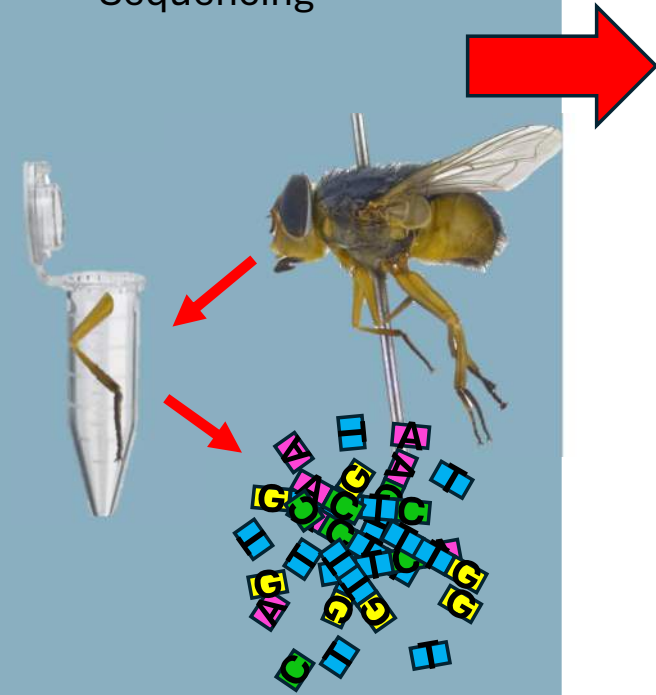


(Assemble Species by Automatic Partitioning)

Mitogenome Assembly Pipeline

DNA extraction

- 1 leg used from each specimen
- Library preparation
- Sequencing



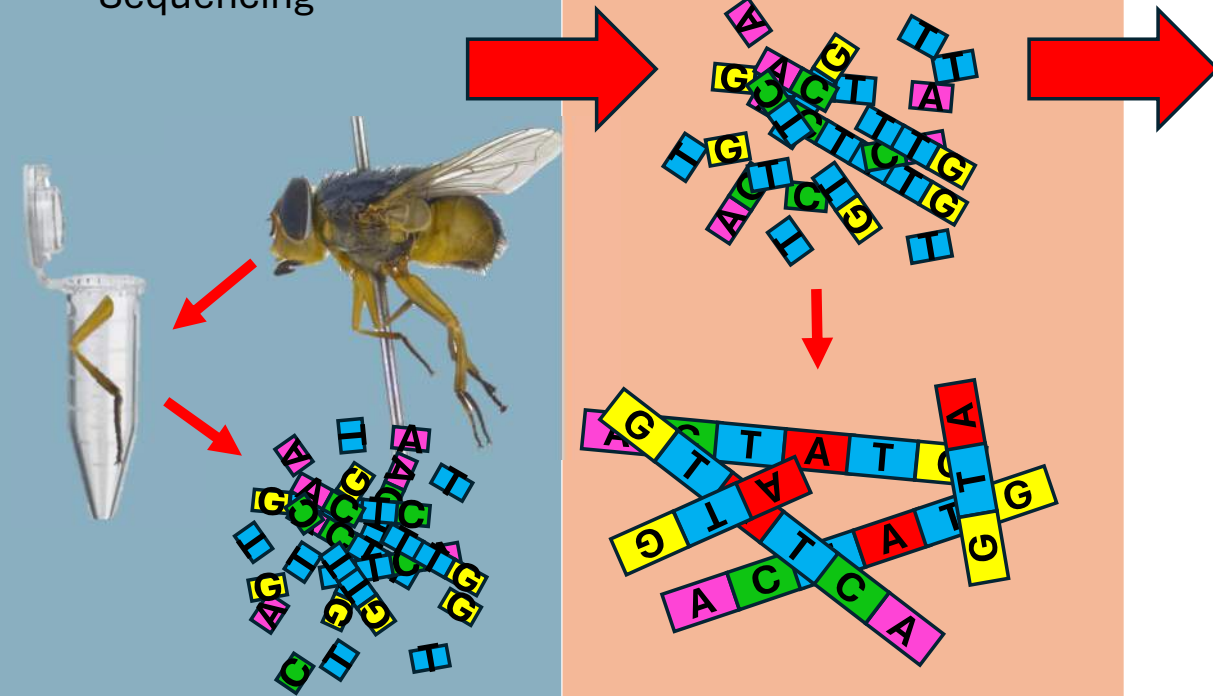
Mitogenome Assembly Pipeline

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Preprocessing

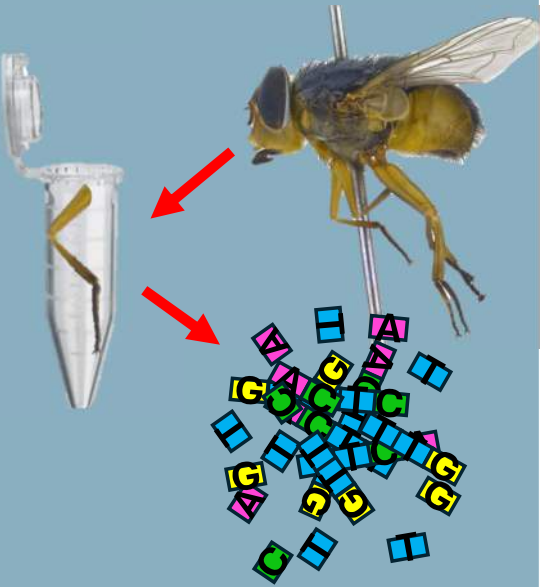
- Adapter trimming
- Quality control



Mitogenome Assembly Pipeline

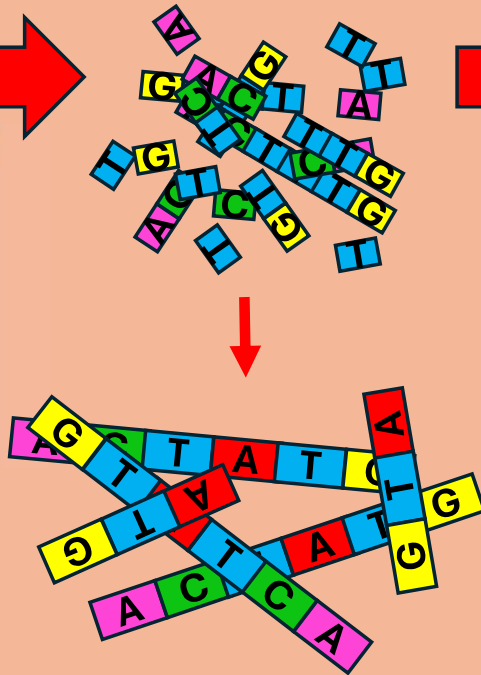
DNA extraction

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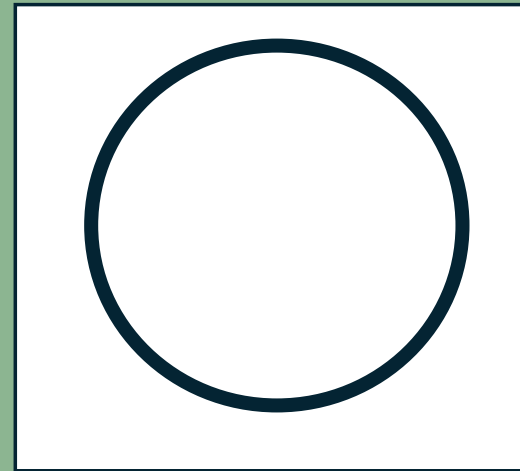
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Assembly

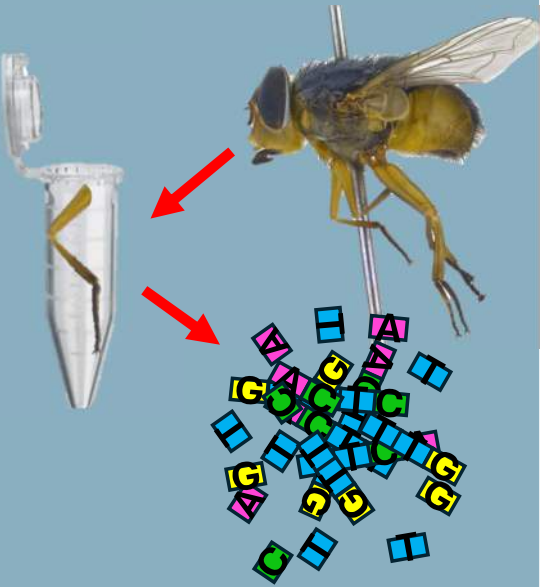
- Whole mitogenomes assembled
- (GetOrganelle + Genious Prime software)



Mitogenome Assembly Pipeline

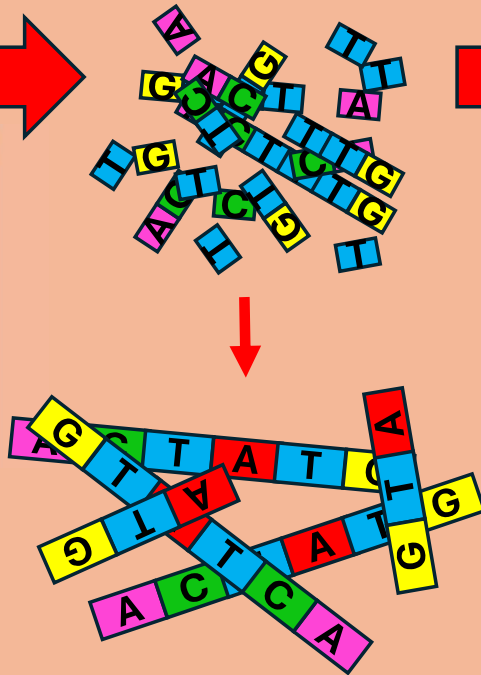
DNA extraction

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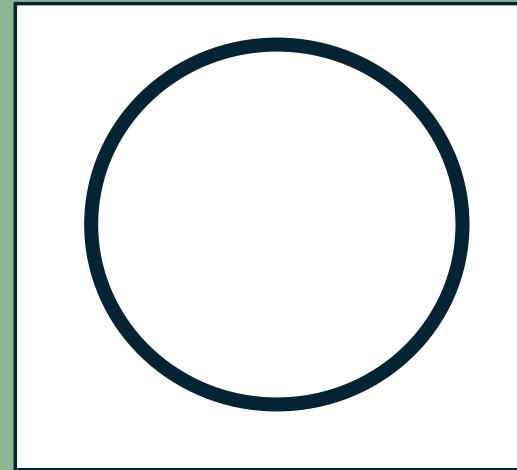
Preprocessing

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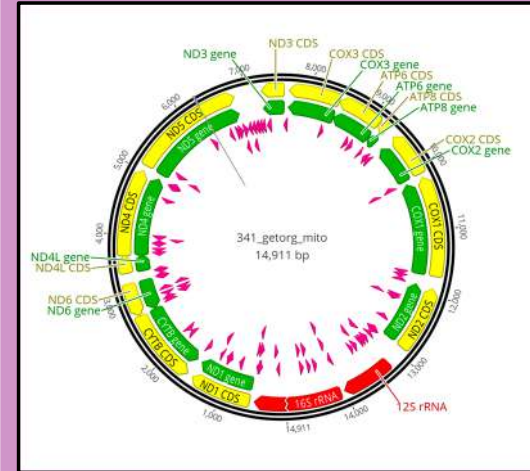
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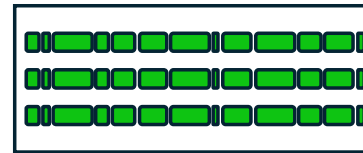
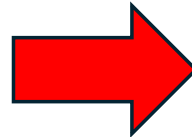
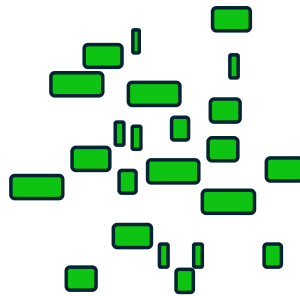
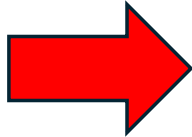
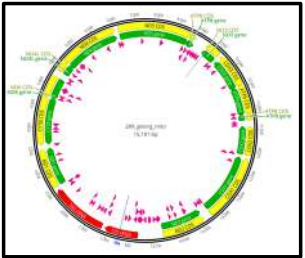
Mitogenome annotation

- Genes identified & annotated

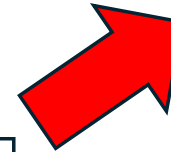


Gene Alignment

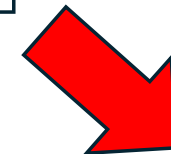
All 13 protein coding
genes (PCGs)
extracted



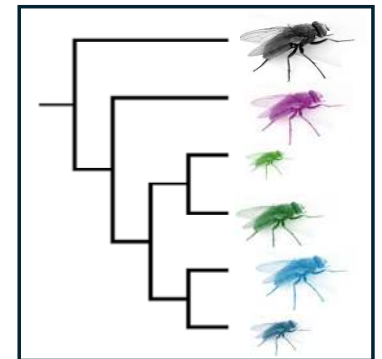
Genes aligned



Species delimitation

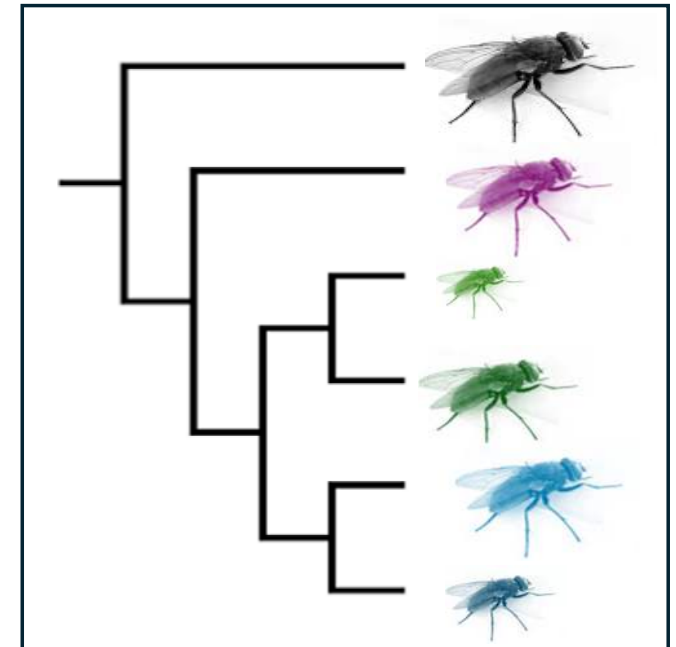


Phylogenetic analysis



Phylogenetic Analysis

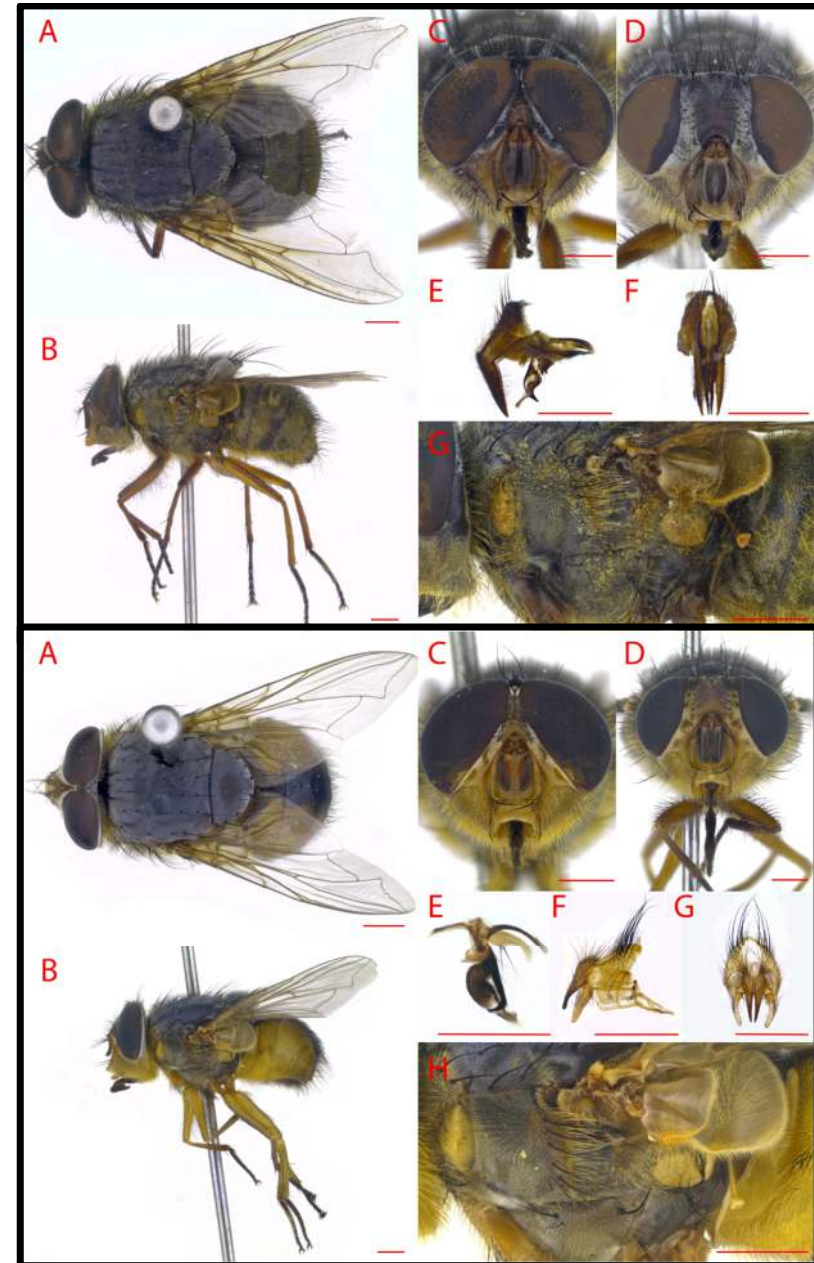
- Maximum likelihood analysis PCGs using an evolutionary model
- Partitioned by gene & codon position
- Included taxa from the northern hemisphere & New Zealand
- First phylogeny to include all Australian carrion-breeding species of Calliphoridae



Results

Morphological Analysis

- The first taxonomic key to all species of Australian carrion-breeding Calliphorinae
- Extensive morphological documentation of adults & male genitalia of each species
- Diagnostic photographs of each species



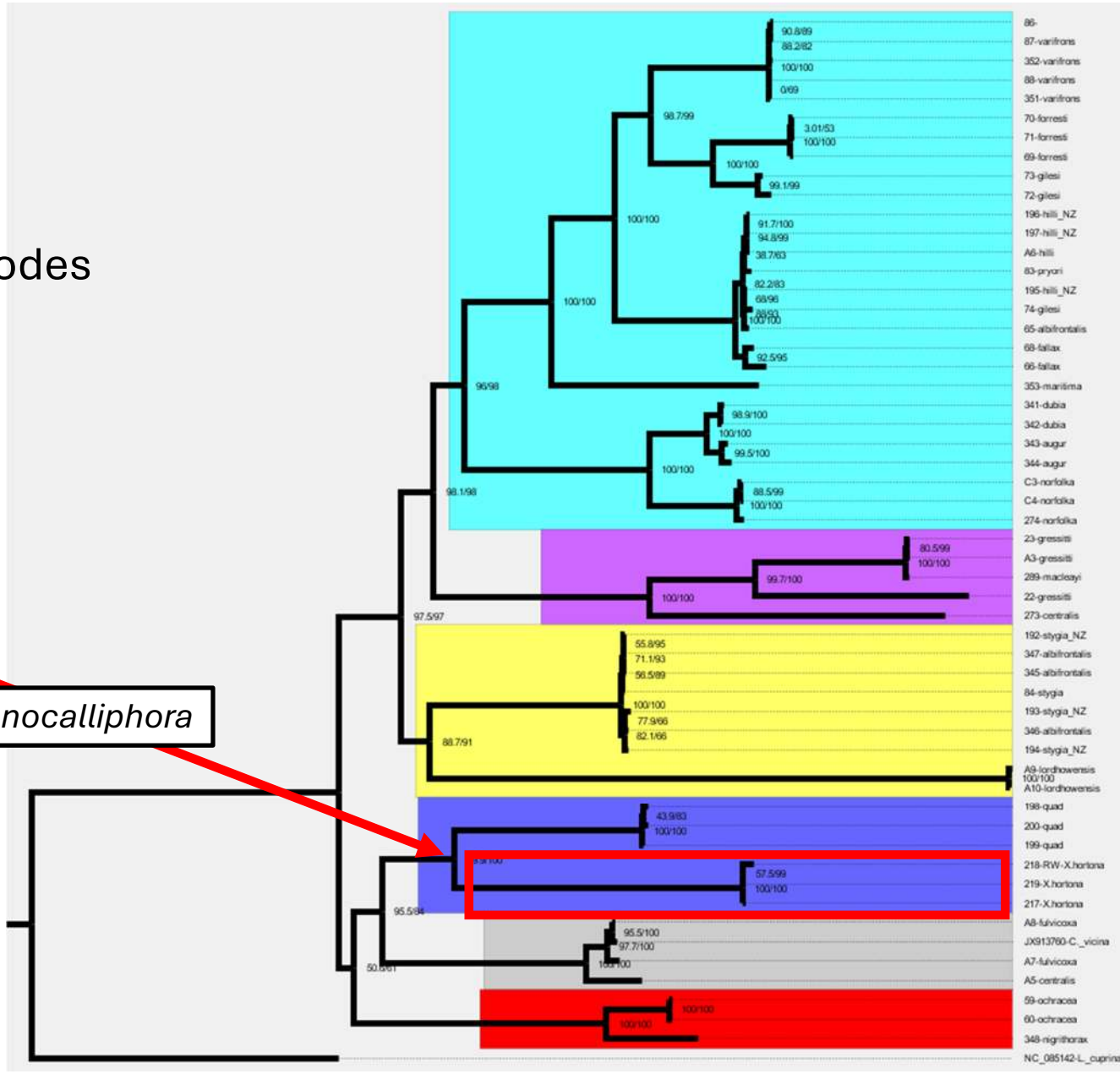
Results

Phylogenetic Analysis

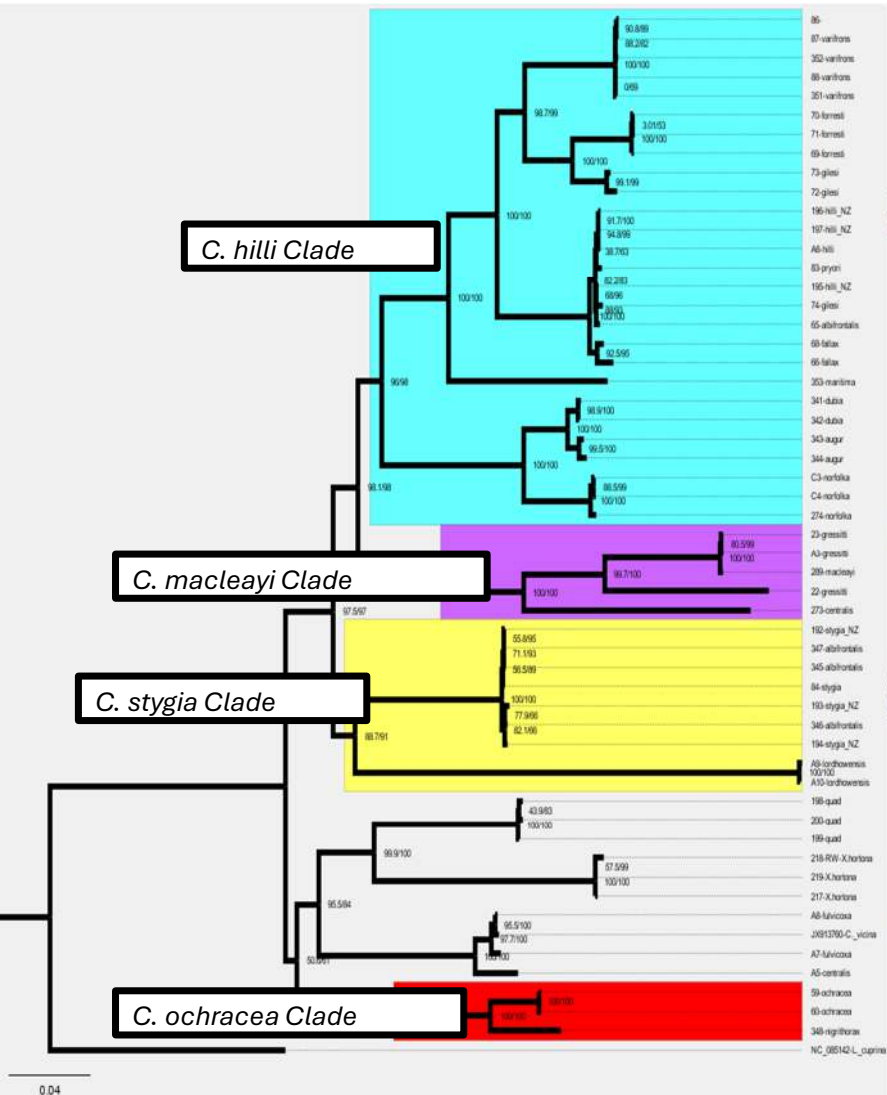
- Strong statistical support for key nodes
- Several clades
- Clades not monophyletic



Xenocalliphora



Clades



Clades cannot be easily defined using adult morphology

All flies are golden

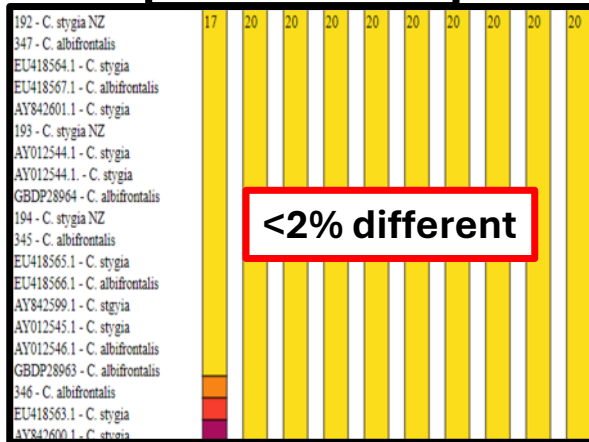


Genitalia morphology required for support

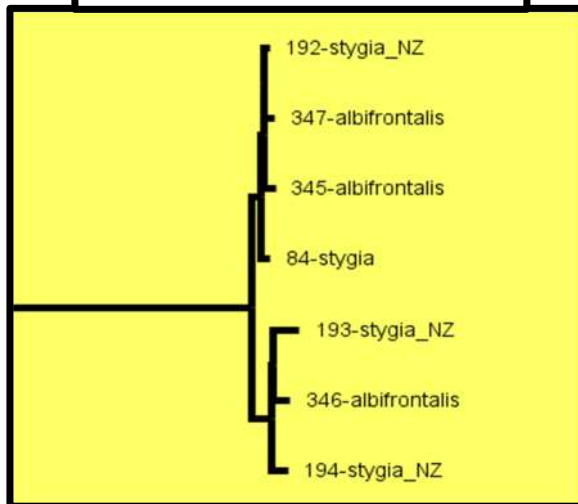


Unstable Species

ASAP analysis



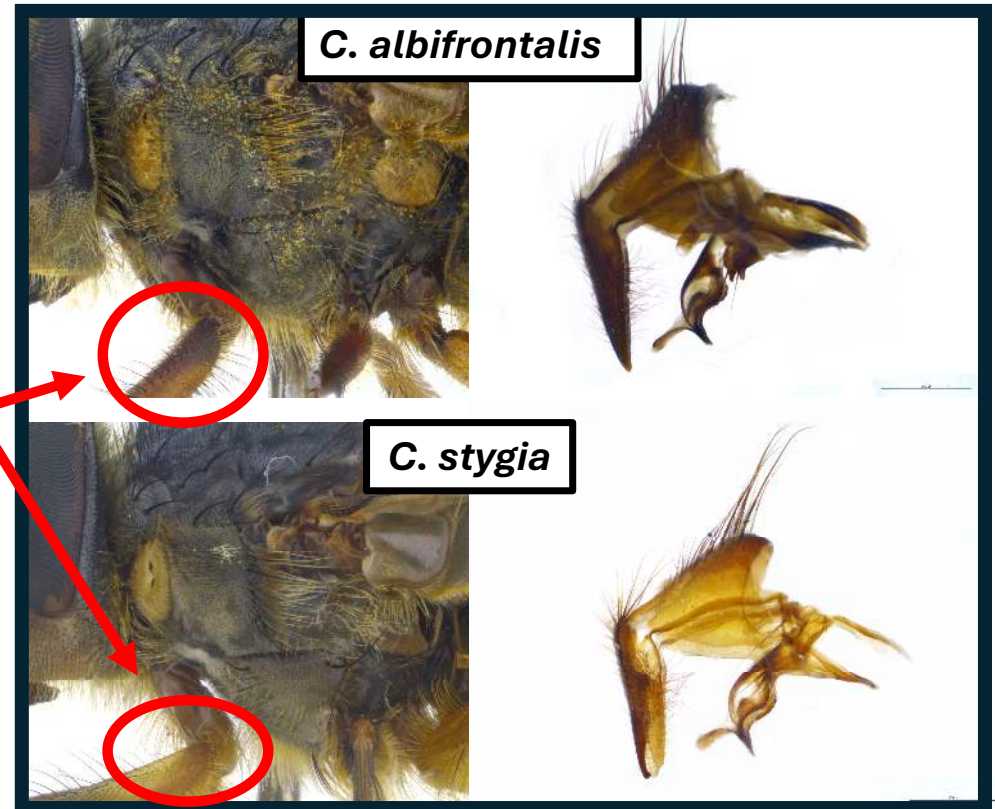
Phylogenetic analysis



Some species are **not well supported** molecularly

A solution could be **synonymisation**

Minor differences



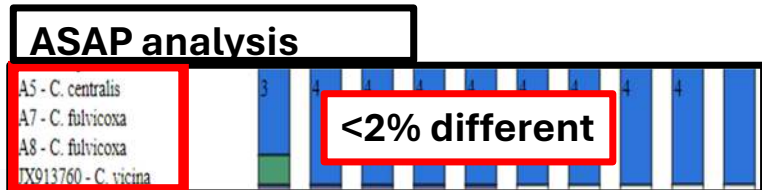
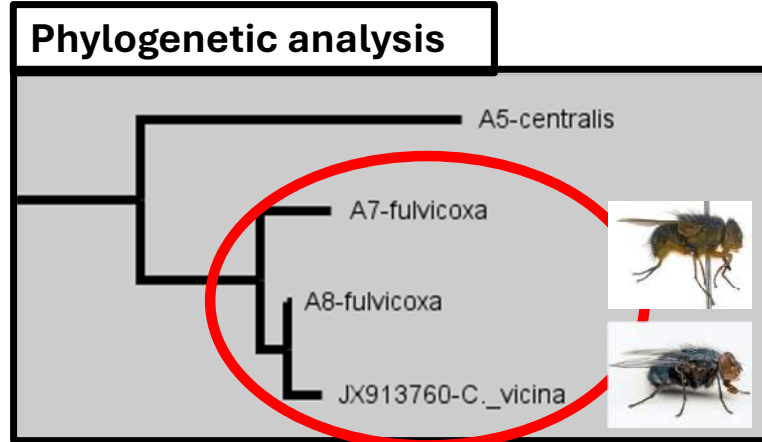
Hybridisation?

Morphology **does not always** support synonymisation

Some unstable species could suggest hybridisation

But hybrids undetectable using mitogenomes due to maternal inheritance

C. fulvicoxa may be a hybrid of *C. vicina*



Calliphora vicina



Calliphora fulvicoxa



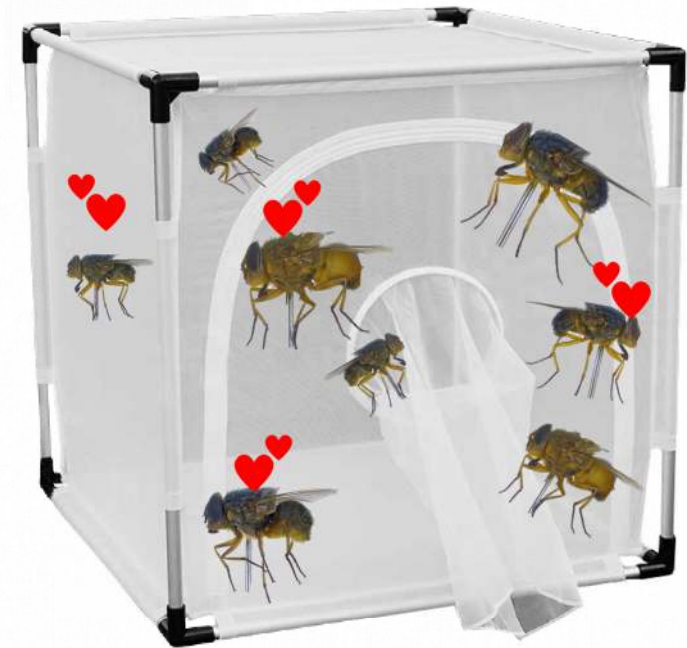
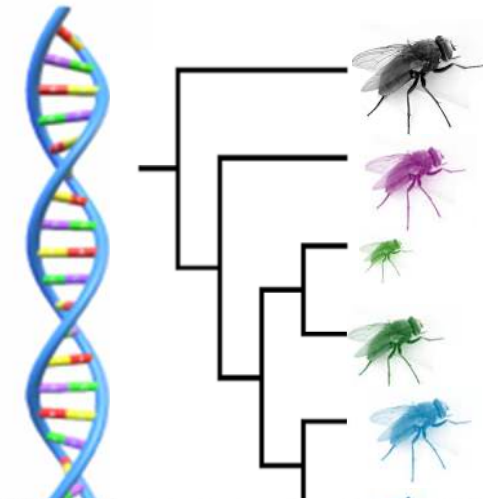
Future Directions

SNP population genetics studies

- Clarify species boundaries
- Determine population structures
- Reveal hybridisation events

Breeding experiments

- Investigate hybridisation within the Calliphorinae
- Which species are likely to hybridise?
- Understand hybrid morphology



The background of the slide is a collage. The top half features several close-up images of fly heads, showing their large, dark, textured eyes and various mouthparts. The bottom left corner shows a group of sheep grazing in a green field. The text boxes are overlaid on this background.

Conclusion

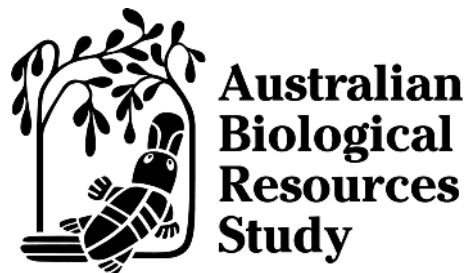
Australian species are divided into **several clades** & some **species may be the same**


Big implications for **forensically** & **agriculturally** important species

Future research should test **species boundaries** & **hybridisation**

Acknowledgements

- Dr Nikolas Johnston (UOW)
- Prof. James Wallman (UTS)
- Kelly Meiklejohn (NCSU)
- Australian Biological Resources Study (grant number: 4-G046X5I)
- Australian National Insect Collection (CSIRO)
- UTS eResearch High Performance Compute Facilities





Flies make up 10% of the
world's biodiversity!

(Marshall, 2012; Smith & Mayfield 2015)

Marshall, L. A. (2012). Natural History.

Smith, T. J., & Mayfield, M. M. (2015). Biological Conservation, 191, 436–443.



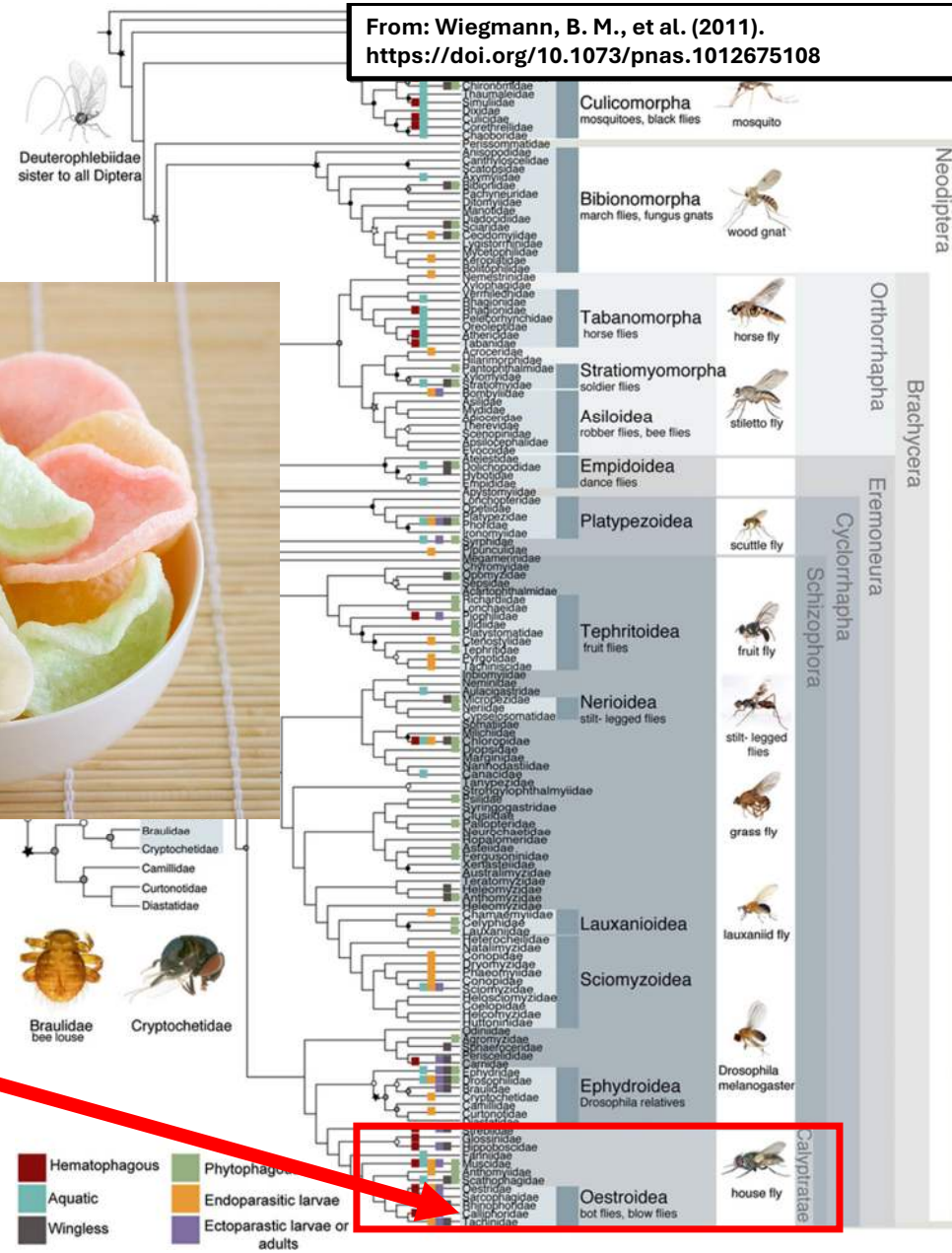
**~160,000 described
species**

~600,000 undescribed!

(Huang et al, 2022)

What are blowflies?

- Family **Calliphoridae**
- Calyptrate flies
- Prawn chip calypters



What are flies?

- Belonging to the insect order **Diptera**

Di + Ptero = two wings



What are flies?

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- Modified hindwings, “**halteres**”



What are flies?

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- **Modified mouthparts**

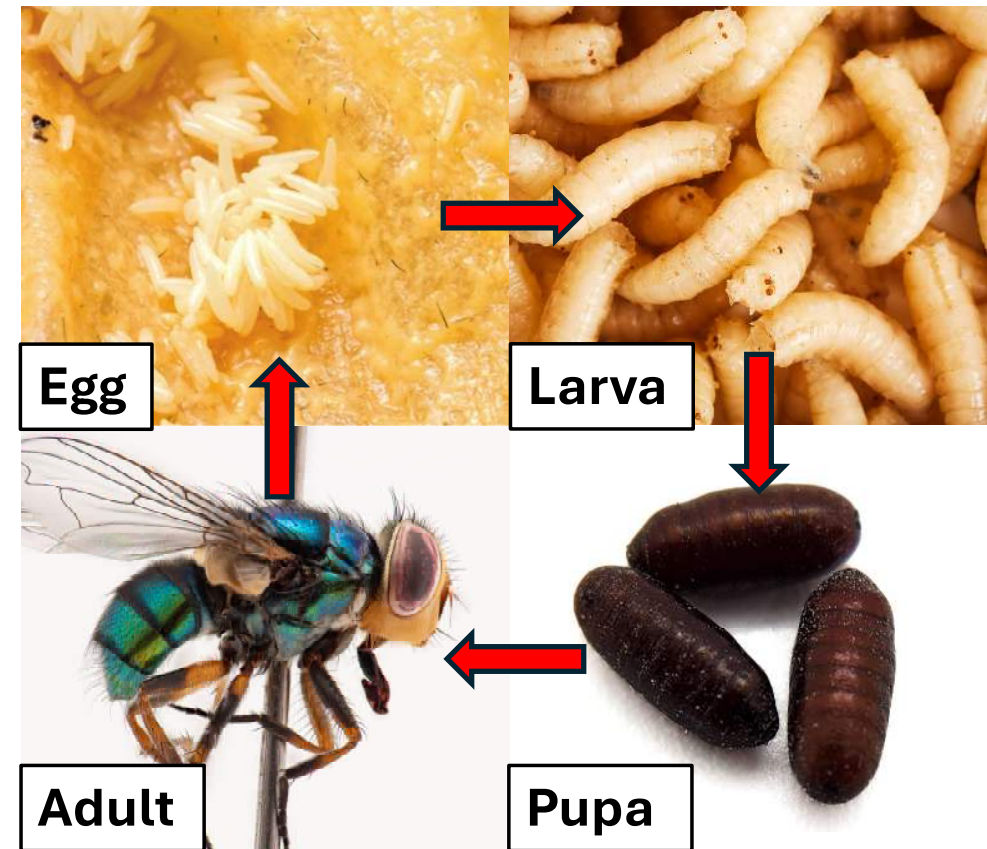


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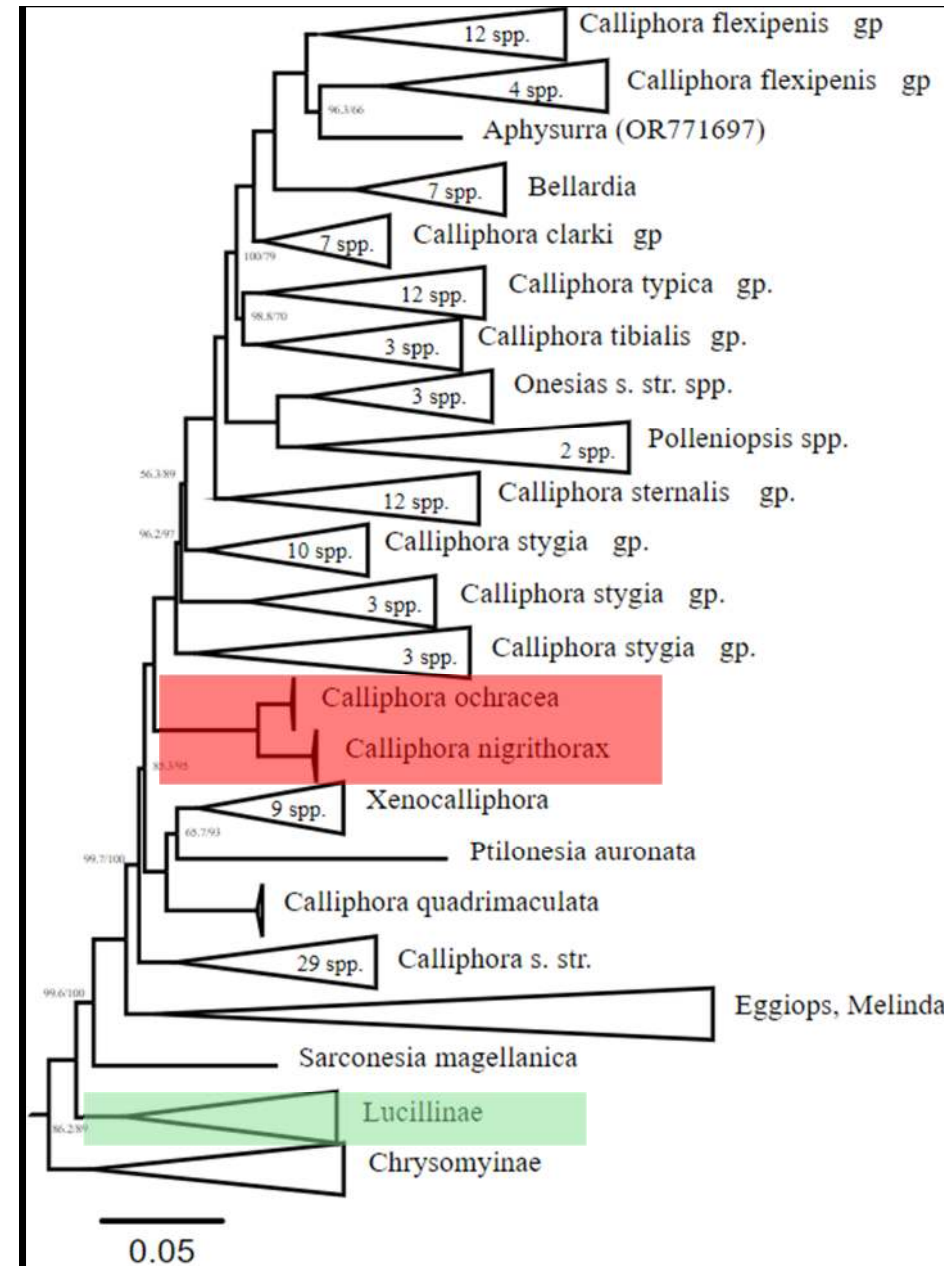
- Modified hindwings, “halteres”
- Modified mouthparts
- **Complete metamorphosis**



Even the genera are unstable

Broader taxon sampling supports my phylogenetic analysis

Resolves uncertain phylogenetic relationships

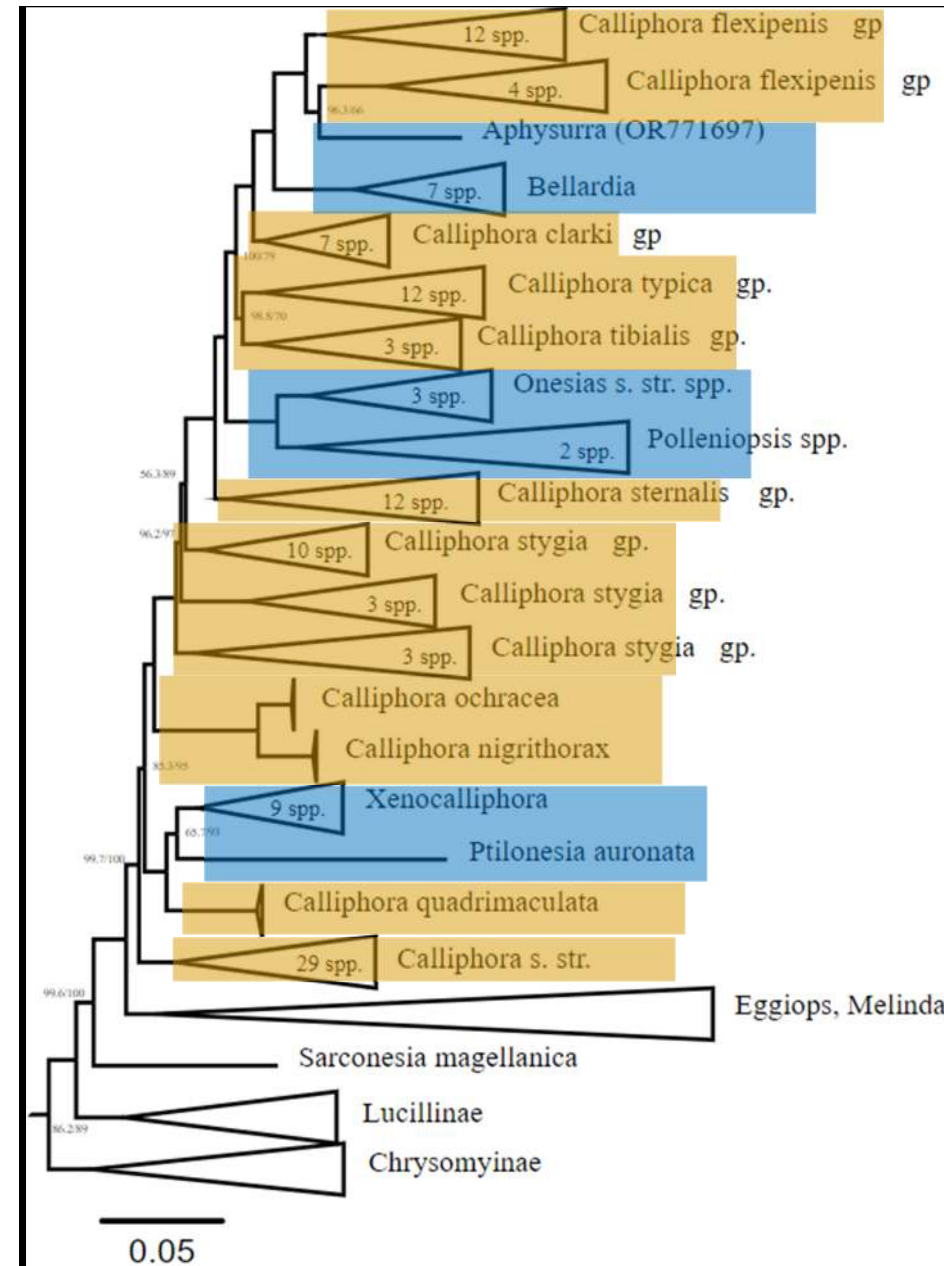


Even the genera are unstable

Broader taxon sampling supports my phylogenetic analysis

Resolves uncertain phylogenetic relationships

Confirms non-monophyly of the Australasian calliphora



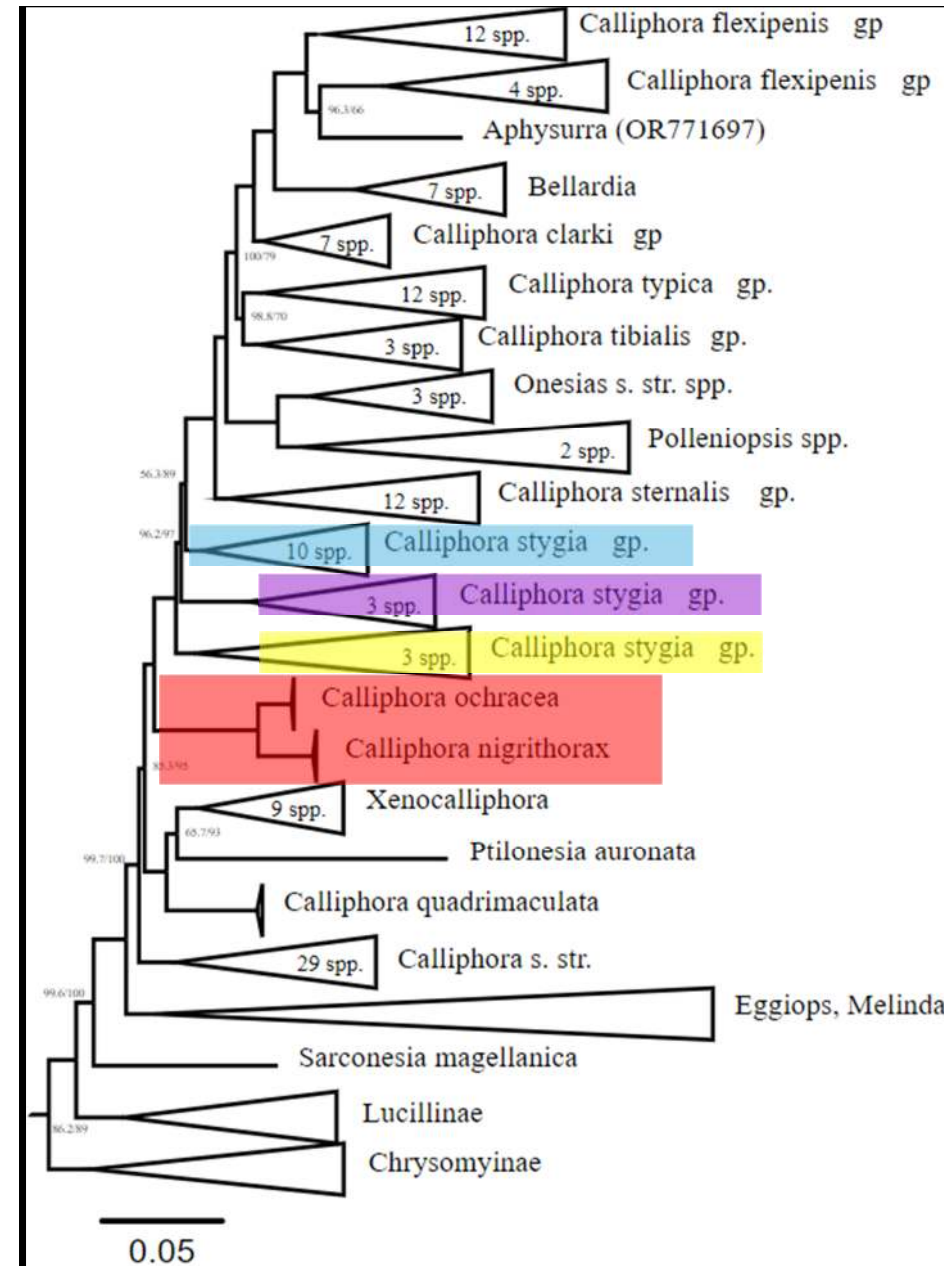
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Resolves uncertain phylogenetic relationships

Confirms non-monophyly of the Australasian Calliphora

Supports clades

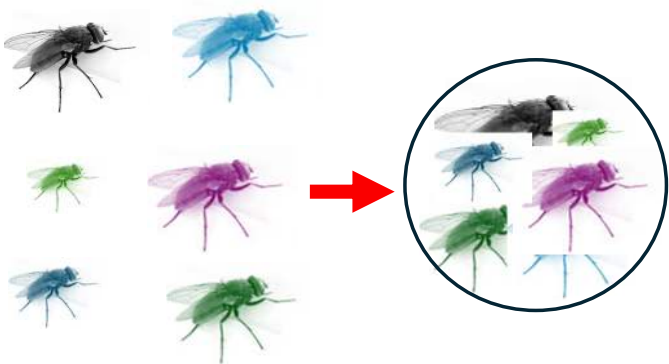


The problem

All species grouped
together



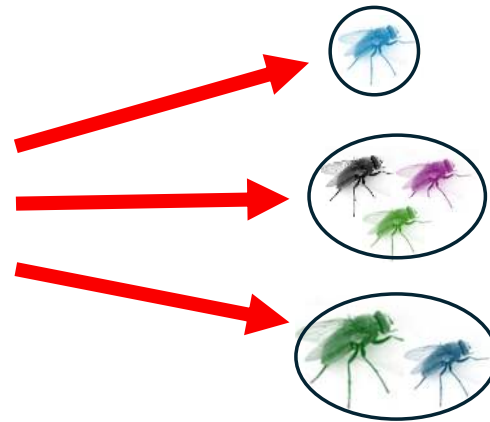
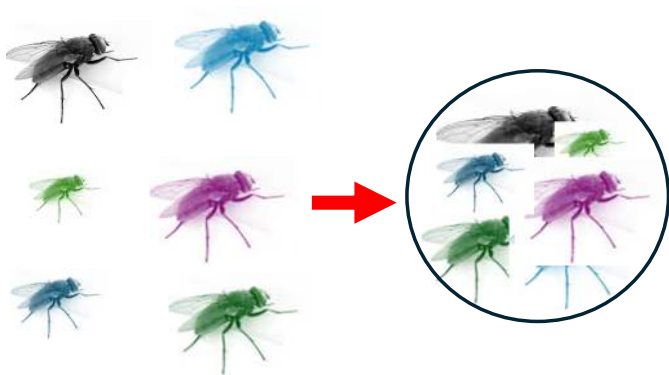
(Malloch, 1927)



The problem

All species grouped together

Species split up into sub-groups



The problem

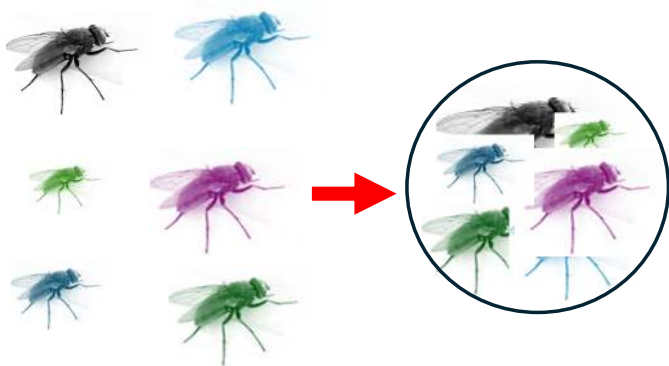
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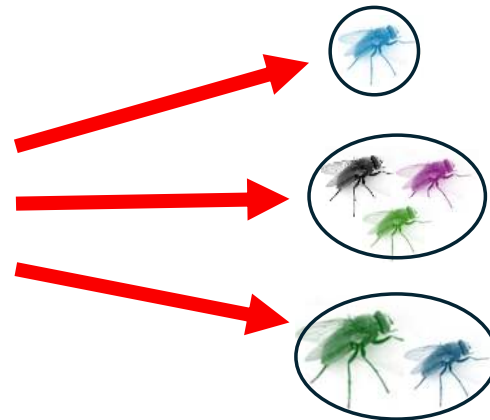
First molecular analyses



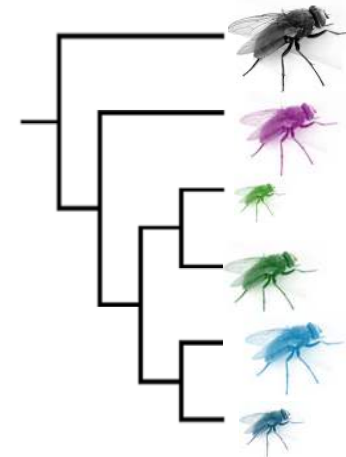
(Malloch, 1927)



(Kurahashi, 1971)



(Wallman & Donnellan, 2001)
(Wallman et al., 2005)



Malloch, J. R. (1927). Proceedings of the Linnean Society of New South Wales, 52.
Kurahashi, H., (1971). Pacific Insects, 13(1), 141-204.
Wallman, J. F., & Donnellan, S. C. (2001). Forensic Science International, 120(1-2).
Wallman, J.F., Leys, R., Hogendoorn, K. (2005). Invertebrate Systematics, 19(1-15)