

Exercises in Marine Ecological Genetics

11. Phylogenetic inference

- Perform and examine sequence alignments
- Infer phylogenies based on genetic distances and Maximum Likelihood
- Visualize phylogenetic trees

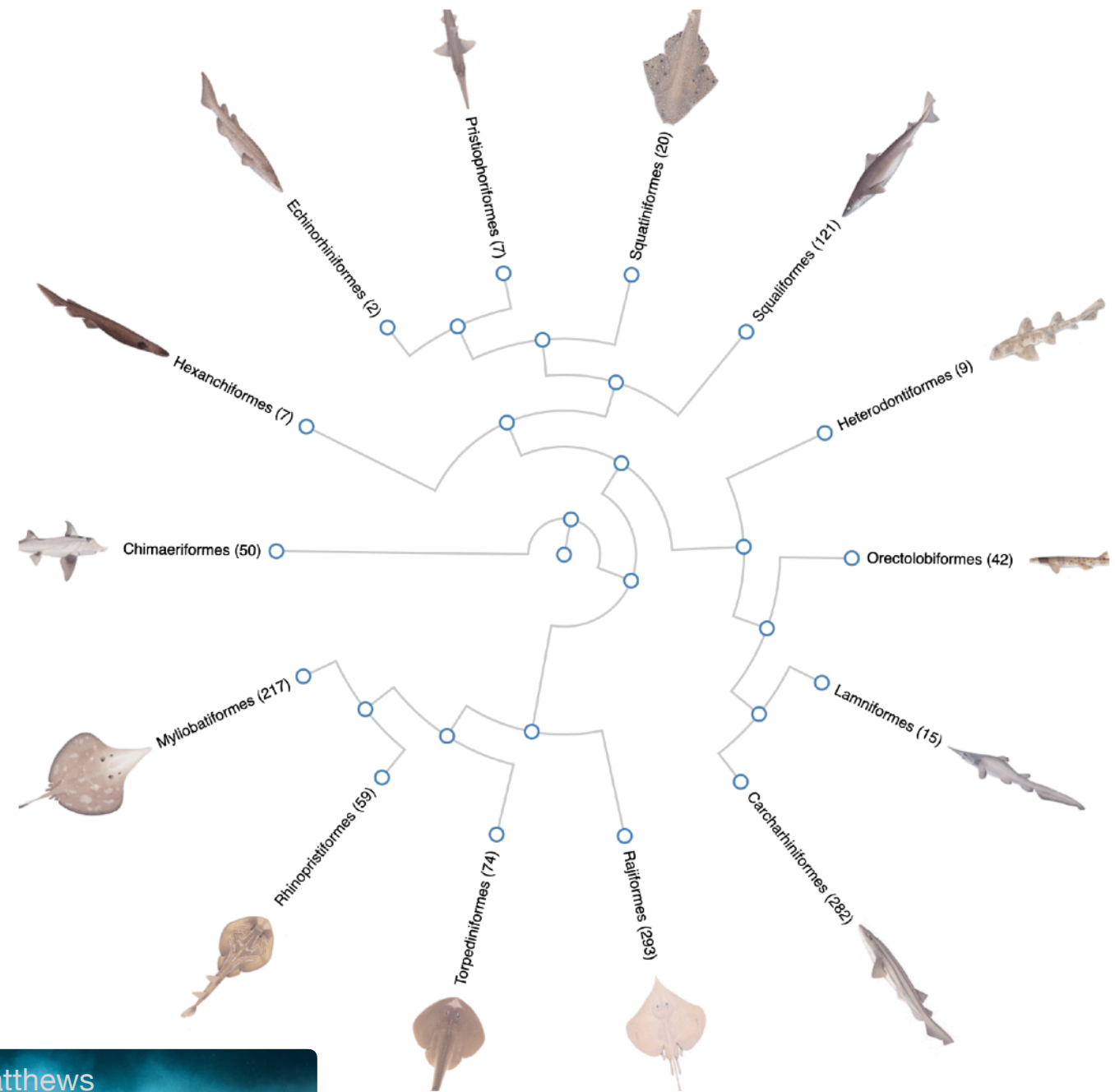
Martin Helmkamp

<https://github.com/mhelmkampf/meg25>



Rays and sharks

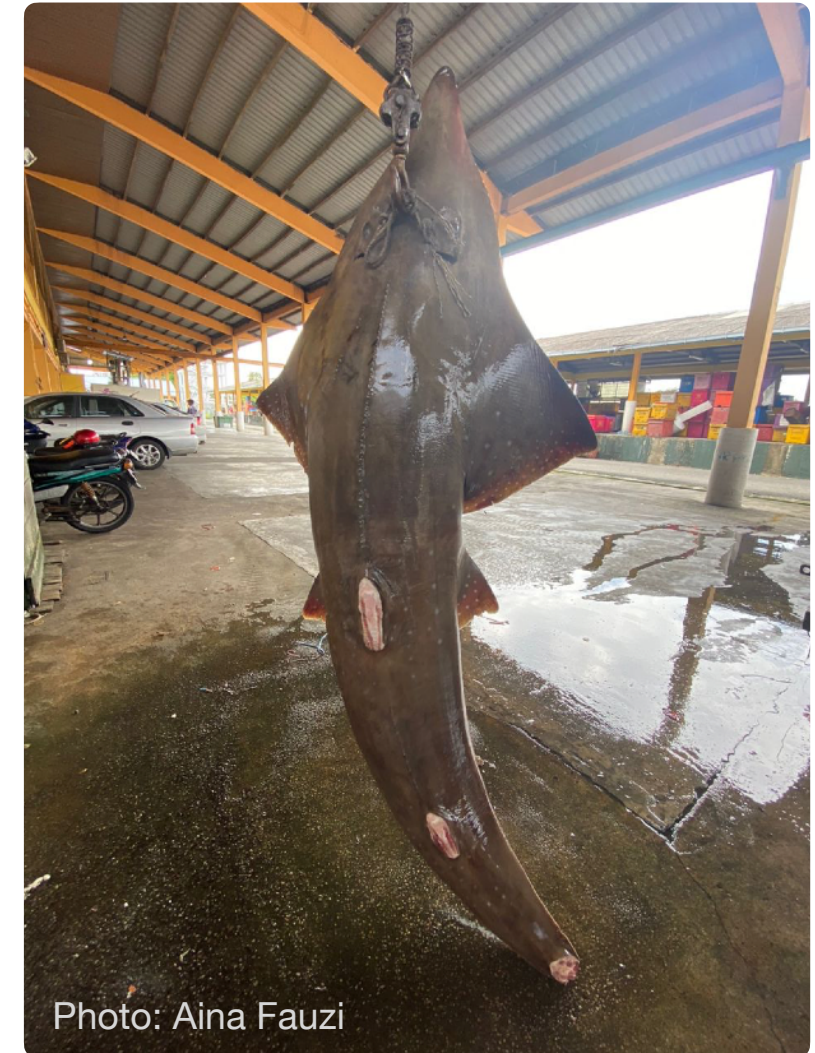
- ~ 1200 species
- Crucial in many marine ecosystems e.g. as top predators
- High economic value
- Every third species threatened with extinction



Sources: IUCN / Ocean Image Bank
Chondrichthyan Tree of Life

Threats

- Overfishing key driver of drastic decline
- Exacerbated by slow reproduction
- Lack of knowledge:
> 25 % species discovered in last 20 years



What is needed

- Improved management of fisheries and protected areas
- Better understanding of species distribution and genetic diversity of populations can help with spatial prioritization

Source: IUCN

Diversity of wedgefishes in Malaysia

- Wedgefishes are endangered but unprotected in Malaysia
- Limited knowledge of species distribution hinders effective management
- **Objective:** assess species diversity, distribution and genetic diversity across Malaysia



Photo: Ian Banks

Amy Yee-Hui Then



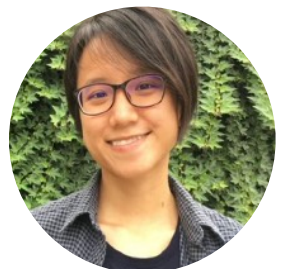
Kean Chong Lim



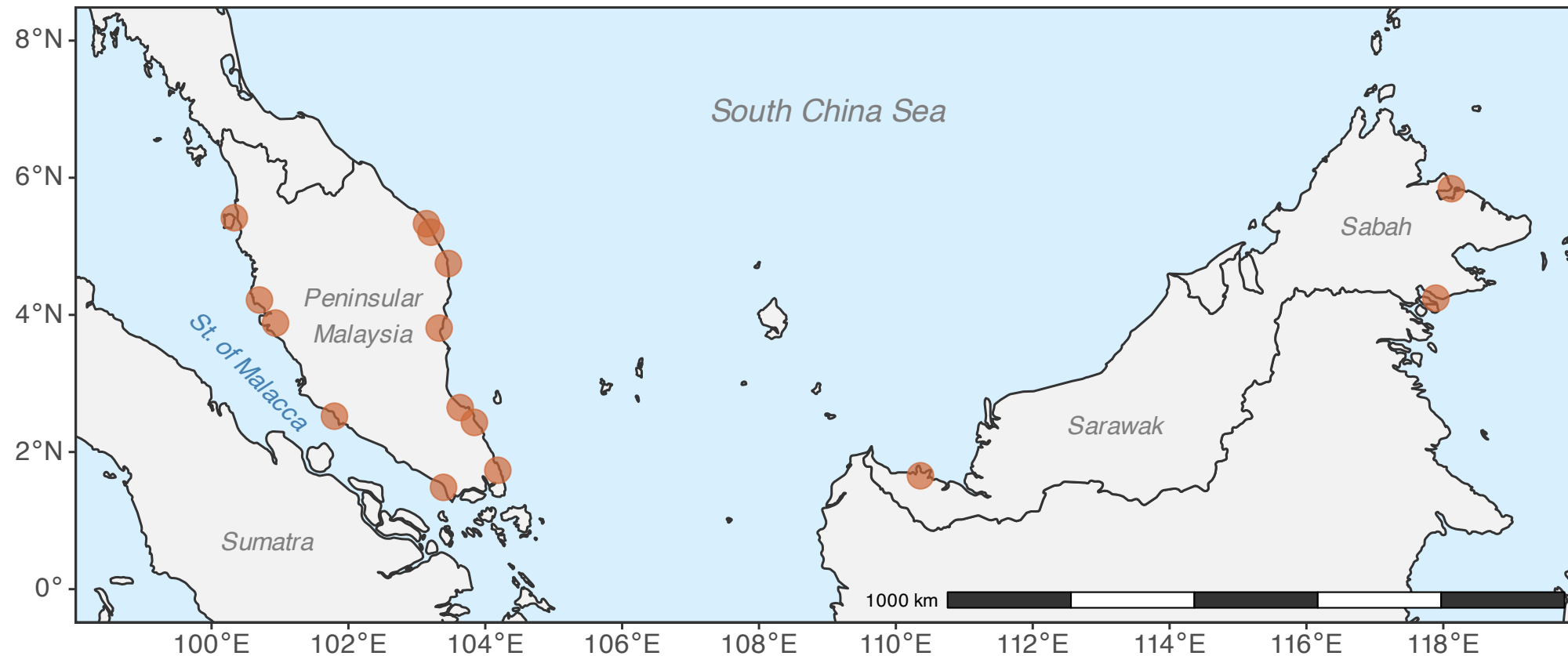
Amanda Jhu Xhin Leung



Serena Adam

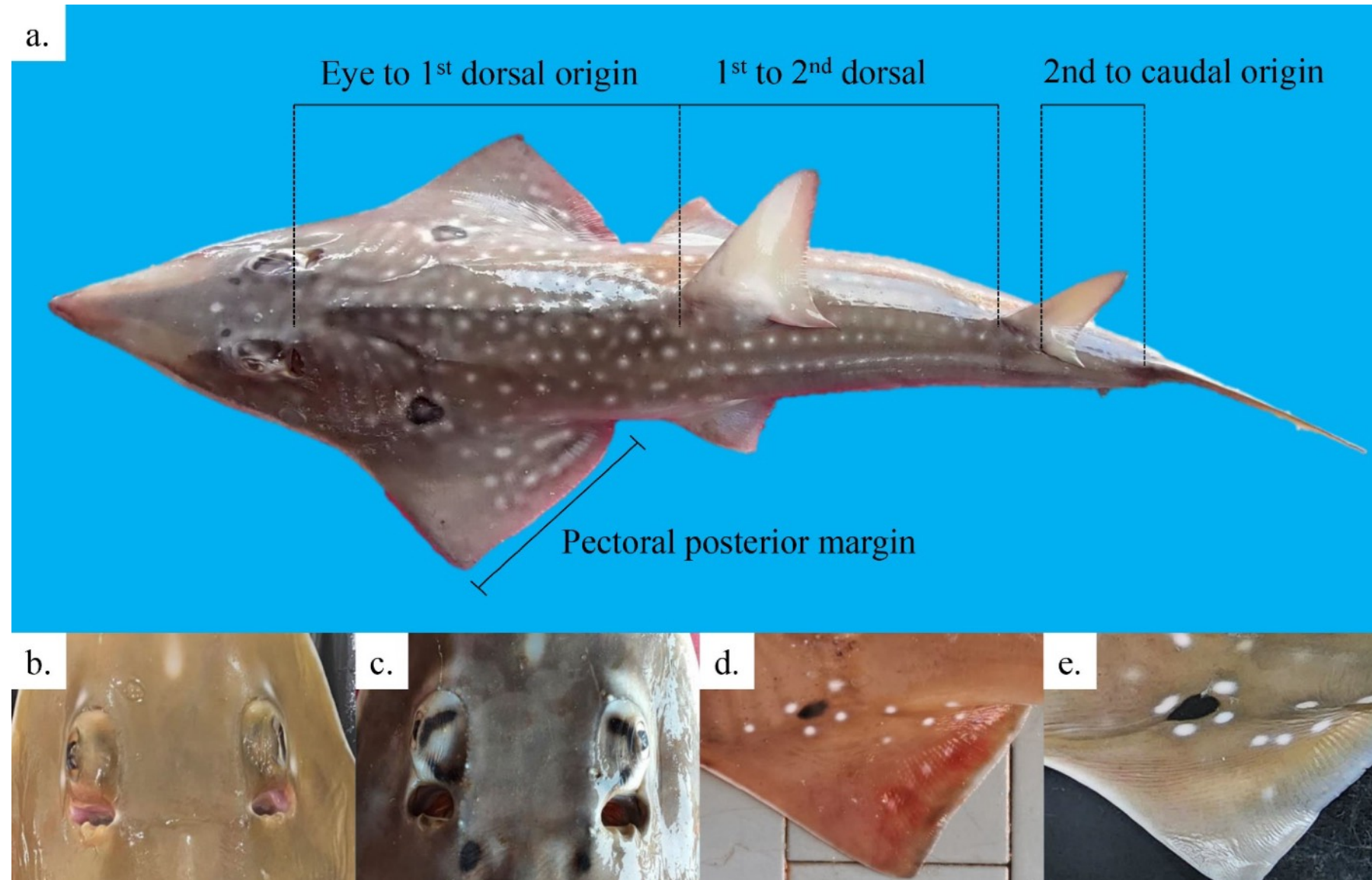


Sampling sites



- 85 specimens
- 15 landing sites and markets
- *In situ* identification, photographs
- Tissue samples
- Sequencing of COI and ND2

Morphological characters



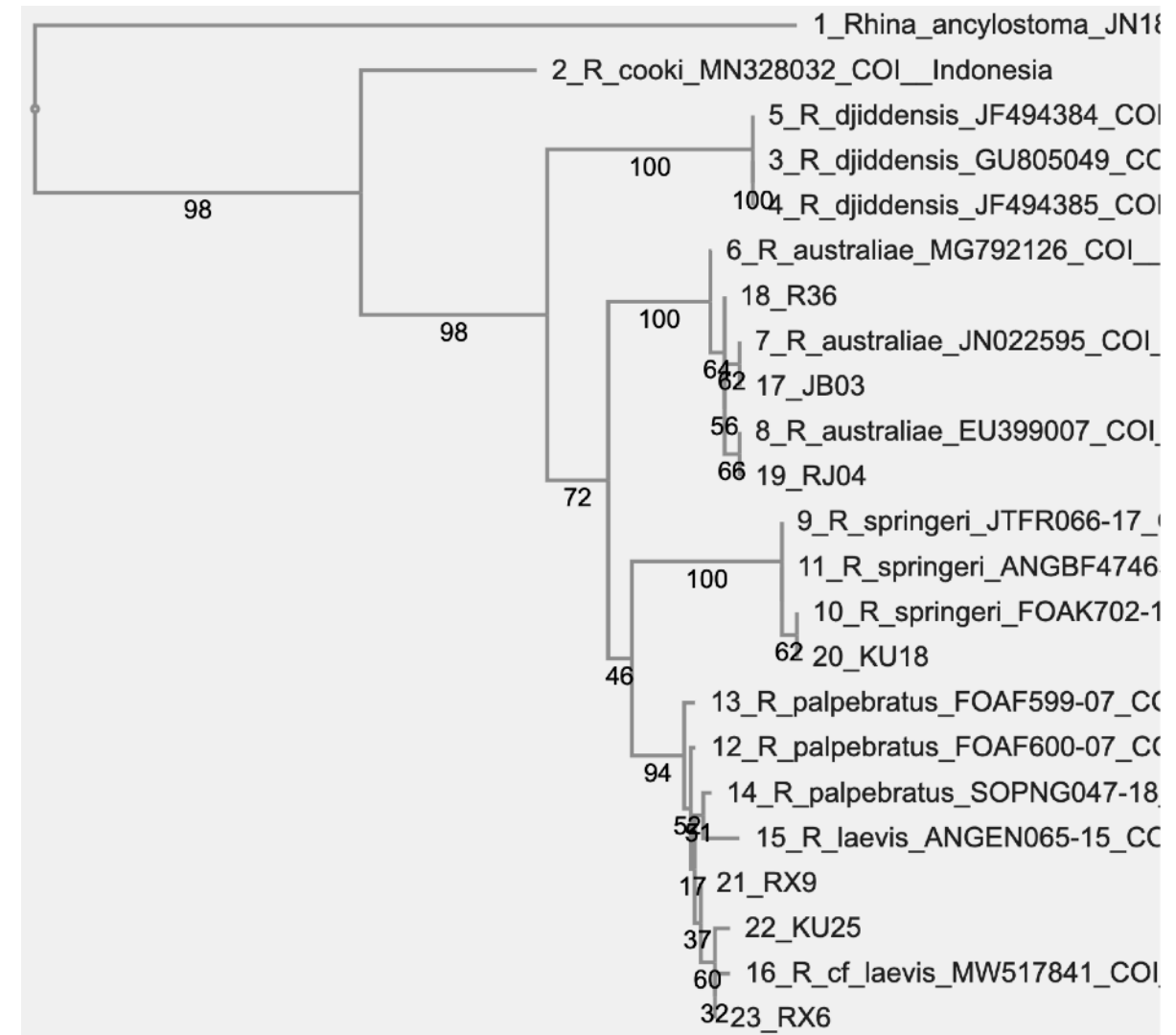
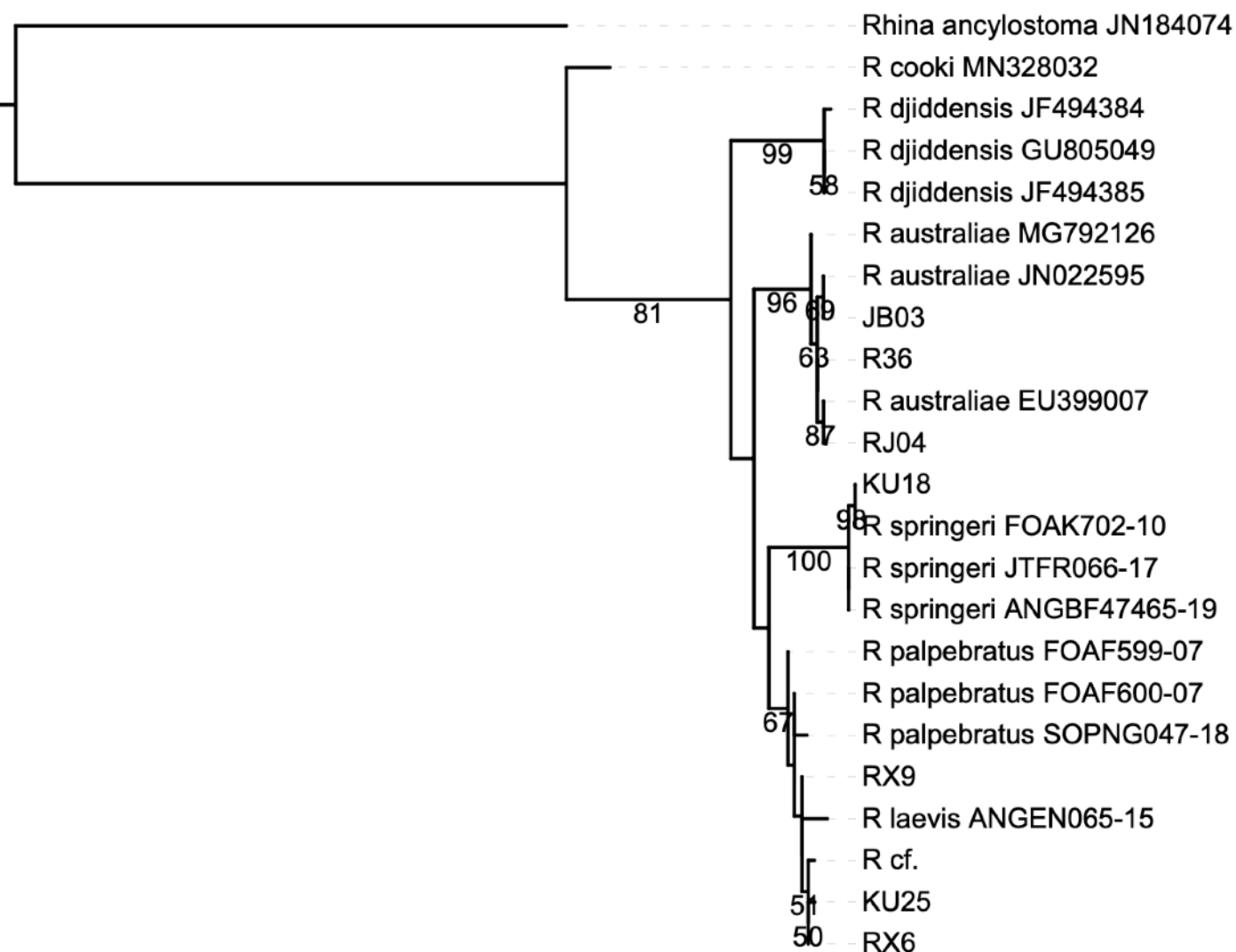
Eye / spiracle markings

Pectoral fin spot pattern

Tree comparison

How do the Neighbor-Joining (right) and Maximum Likelihood tree (left) differ?

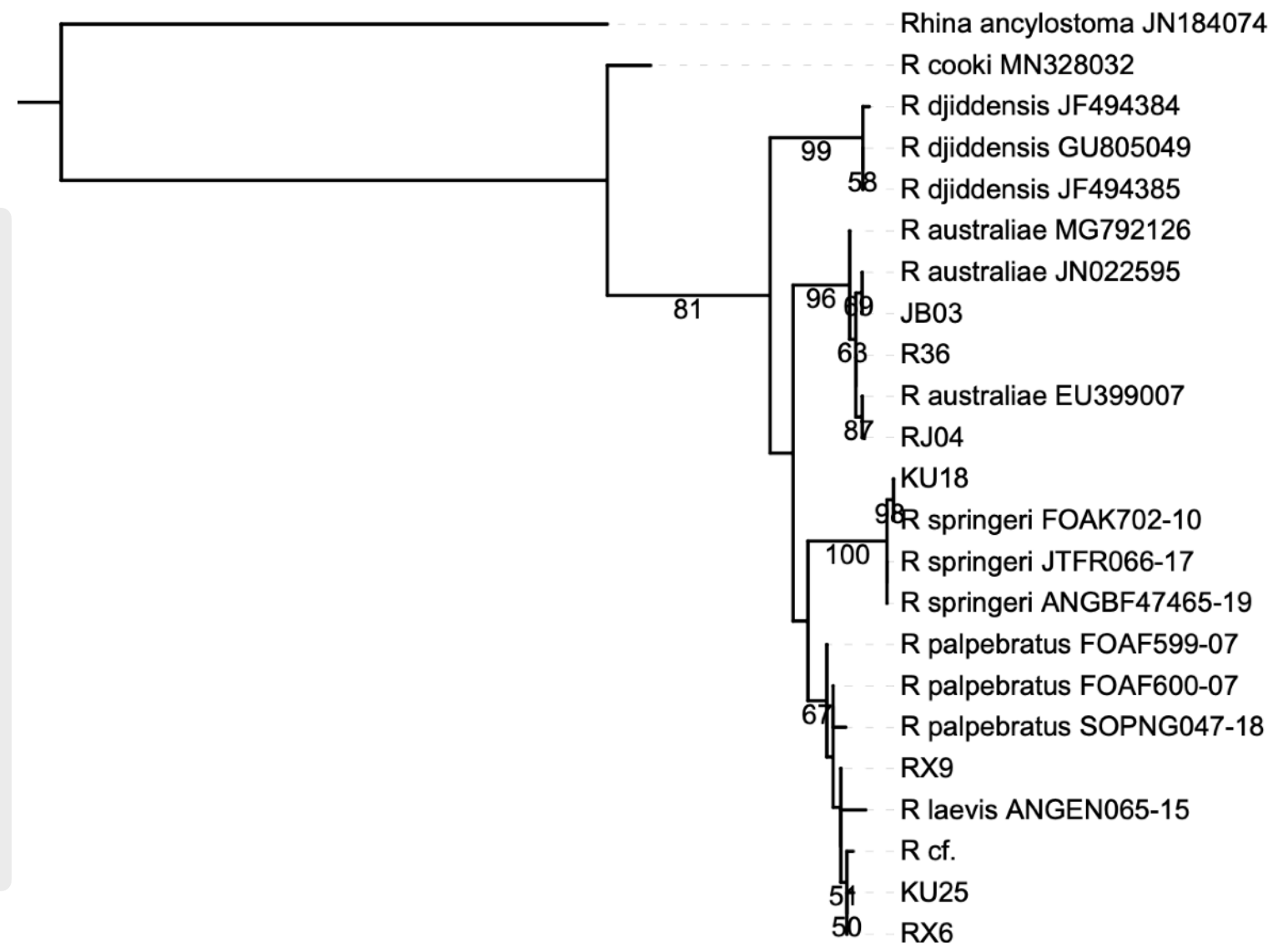
How are they the same?



Comparison with morphological species identification

Are samples assigned to different species according to the molecular phylogeny?

Sample	Morphological id
R36	<i>R. springeri</i>
JB03	<i>R. springeri</i>
RJ04	<i>R. australiae</i>
KU18	<i>R. springeri</i>
RX9	<i>R. australiae</i>
KU25	<i>R. palpebratus</i>
RX6	<i>R. australiae</i>

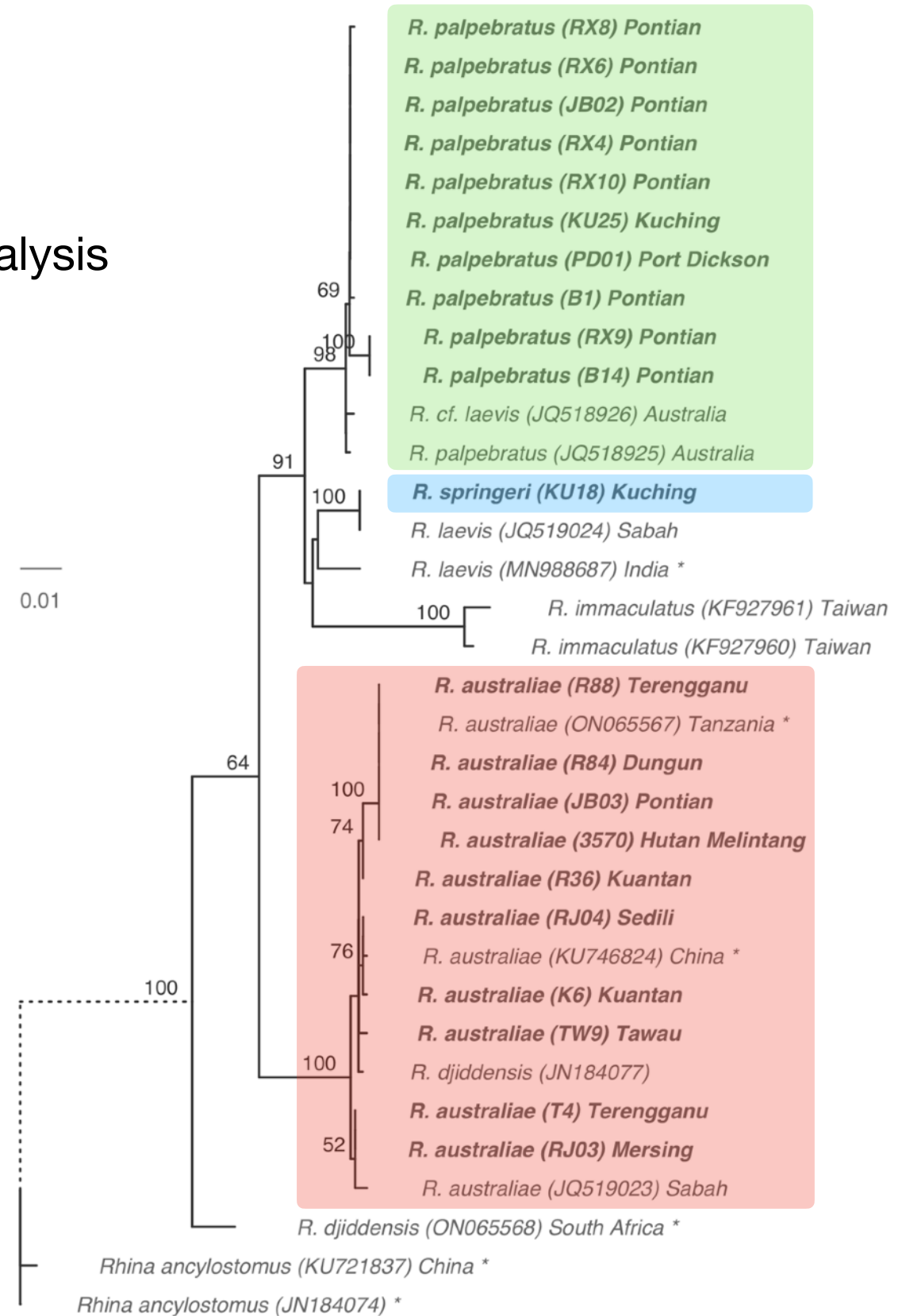


Genetic validation

Maximum likelihood-based phylogenetic analysis

ND2 gene

All specimens could be assigned to species with high confidence



Comparison of species identification methods

Species	Morphology	DNA barcoding
<i>R. australiae</i>	68	74
<i>R. palpebratus</i>	4	10
<i>R. springeri</i>	7	1
Unknown	6	0
Total		85

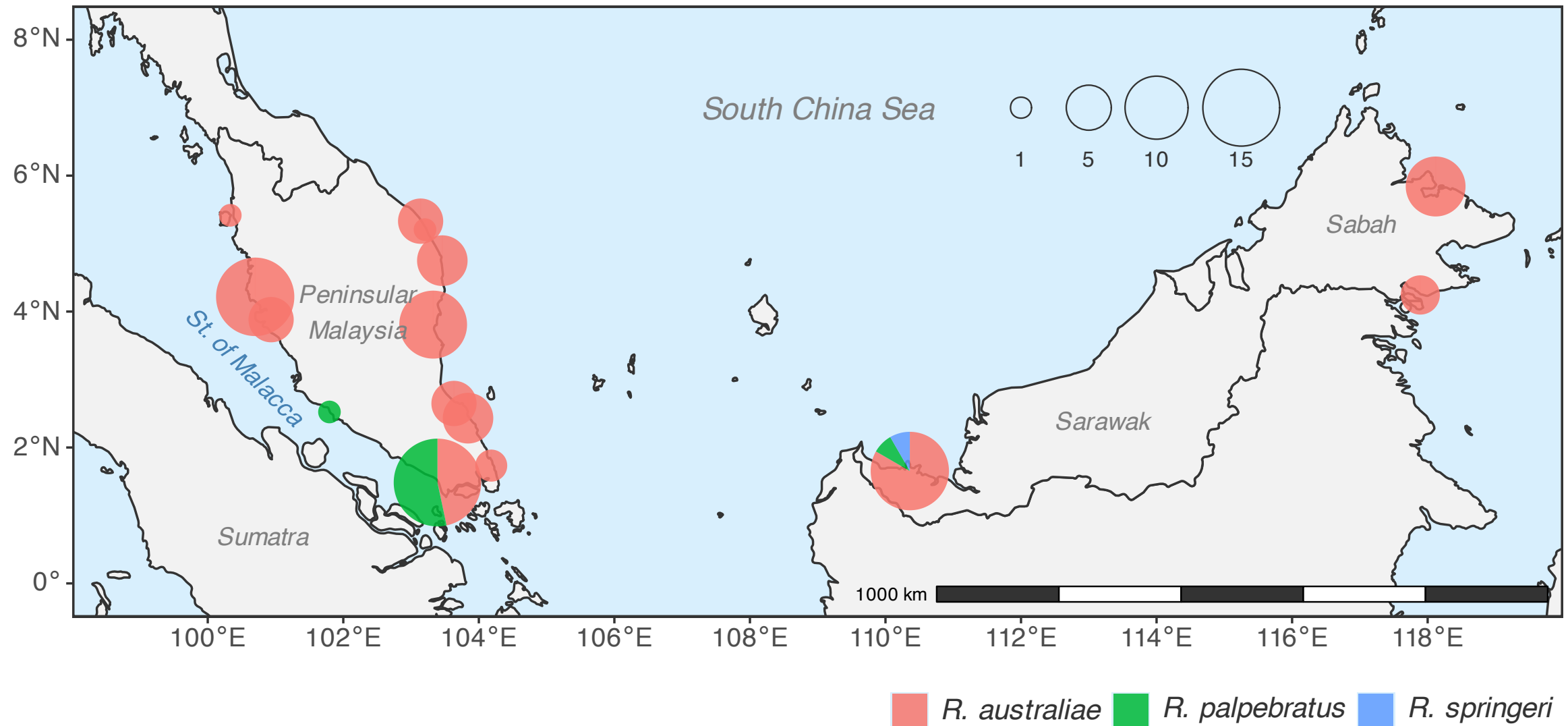
Underestimated by morphology

Overestimated by morphology



16 % of specimens were misidentified based on morphology alone

Species distribution



- *Rhynchobatus australiae* most common species
- First record of *R. palpebratus* in Malaysian waters


```
mafft input.fas > output.aln # Align sequences  
raxml-ng --all --msa input.aln --model MODEL --bs-trees n # ML + bootstrap  
# Visualize trees e.g. on https://itol.embl.de
```

- A **good sequence alignment** is your hypothesis of homology — accurate alignments are critical for reliable phylogenetic inference
- **Distance-based and Maximum Likelihood methods** rely on different models and assumptions to infer phylogenetic relationships, and have different use cases
- Visualizing and rooting your trees correctly helps you interpret the topology and branch lengths, and **compare trees**