Exercises in Marine Ecological Genetics

11. Phylogenetic inference

- Perform and examine sequence alignments

- Infer phylogenies based on genetic distances

  and Maximum Likelihood
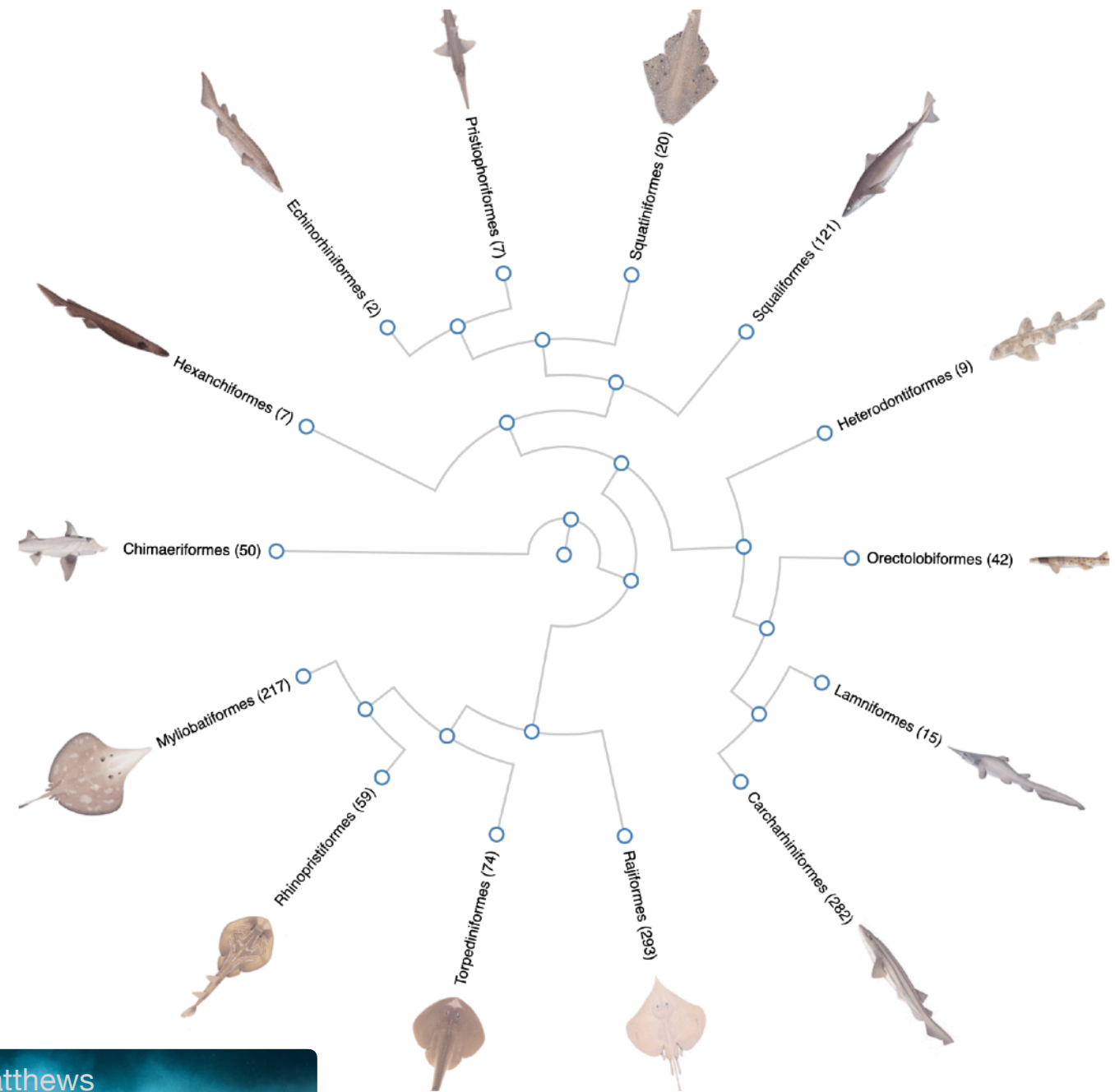
- Visualize phylogenetic trees

Martin Helmkampf

https://github.com/mhelmkampf/meg25

Jake Wilton

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11: Phylogenetic inference

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Universität
Oldenburg

Rays and sharks

- ~ 1200 species

- Crucial in many marine ecosystems e.g. as top predators

- High economic value

- Every third species threatened with extinction

Photo: Albert kok

Photo: Toby Matthews

Echinorhiniformes (2)
Pristiophoriformes (7)
Squatiniformes (20)
Squaliformes (121)
Hexanchiformes (7)
Heterodontiformes (9)
Chimaeriformes (50)
Orectolobiformes (42)
Lamniformes (15)
Myliobatiformes (217)
Carcharhiniformes (282)
Rhinopristiformes (59)
Torpediniformes (74)
Rajiformes (293)

Sources: IUCN / Ocean Image Bank

Chondrichthyan Tree of Life

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Threats

- Overfishing key driver of drastic decline

- Exacerbated by slow reproduction

- Lack of knowledge:

  > 25 % species discovered in last 20 years
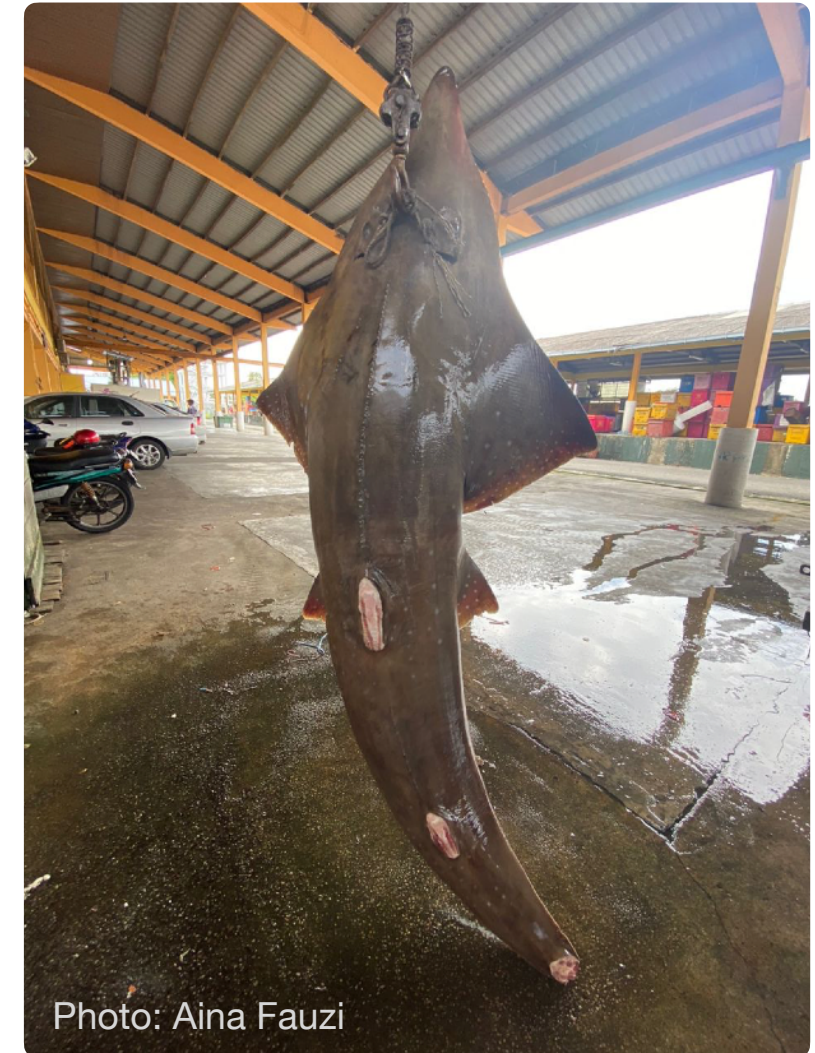
What is needed

- Improved management of fisheries and protected areas

- Better understanding of species distribution and genetic diversity of populations can help with spatial prioritization

Photo: Aina Fauzi

Source: IUCN

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Diversity of wedgefishes in Malaysia

Amy Yee-Hui Then

- Wedgefishes are endangered but unprotected in Malaysia

- Limited knowledge of species distribution hinders effective management

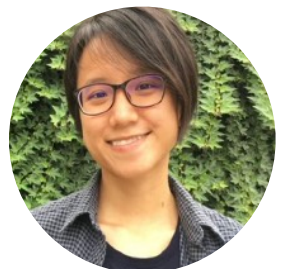- Objective: assess species diversity, distribution and genetic diversity across Malaysia

Kean Chong Lim

Amanda Jhu Xhin Leung

Serena Adam

Photo: Ian Banks

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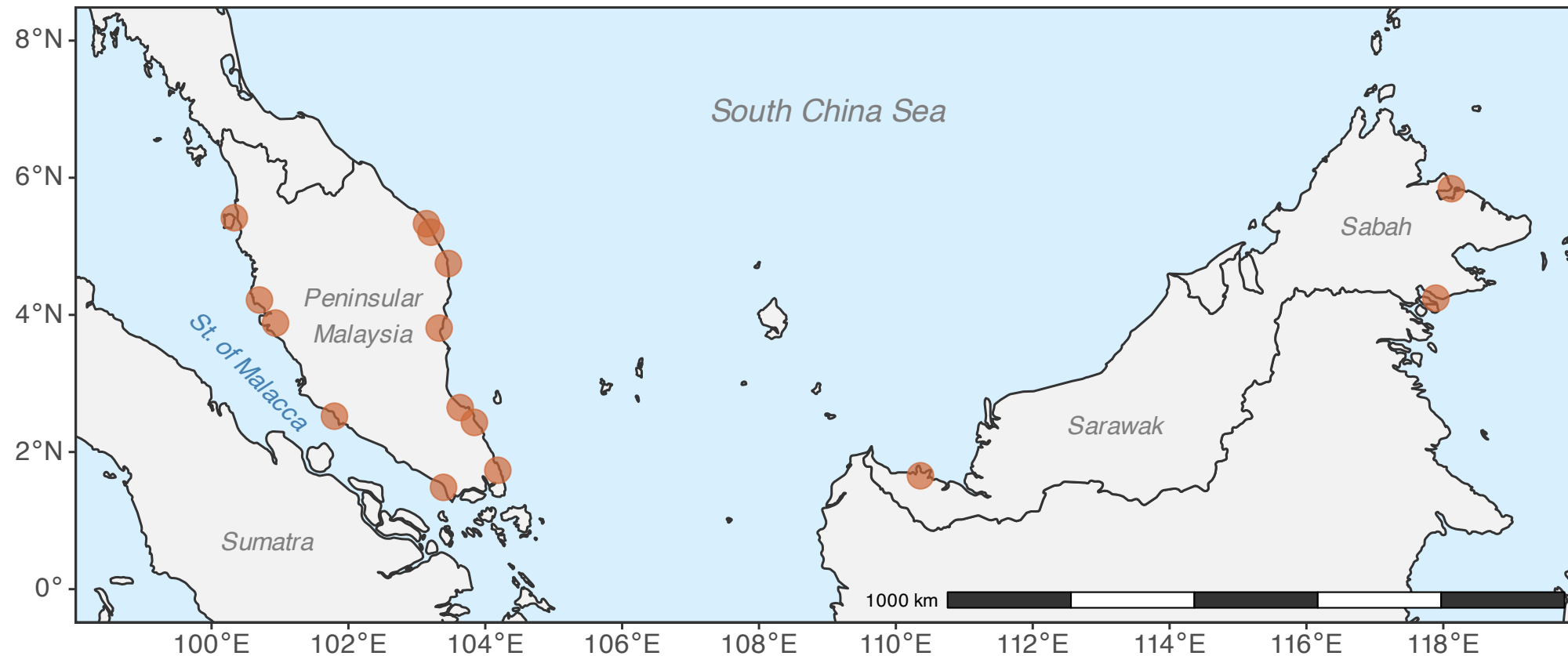
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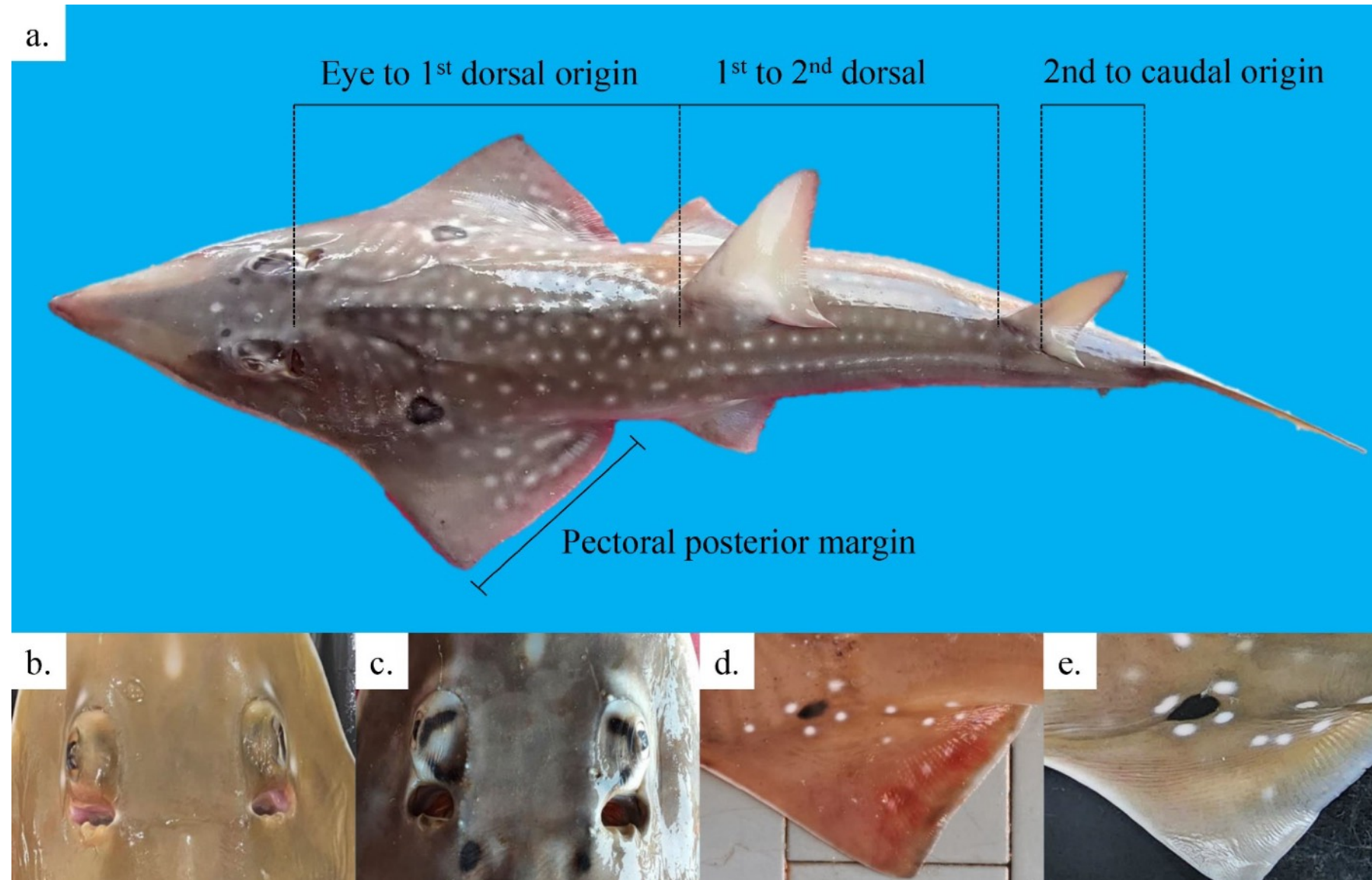# Sampling sites

- 85 specimens

- 15 landing sites and markets

- *In situ* identification, photographs

- Tissue samples

- Sequencing of COI and ND2

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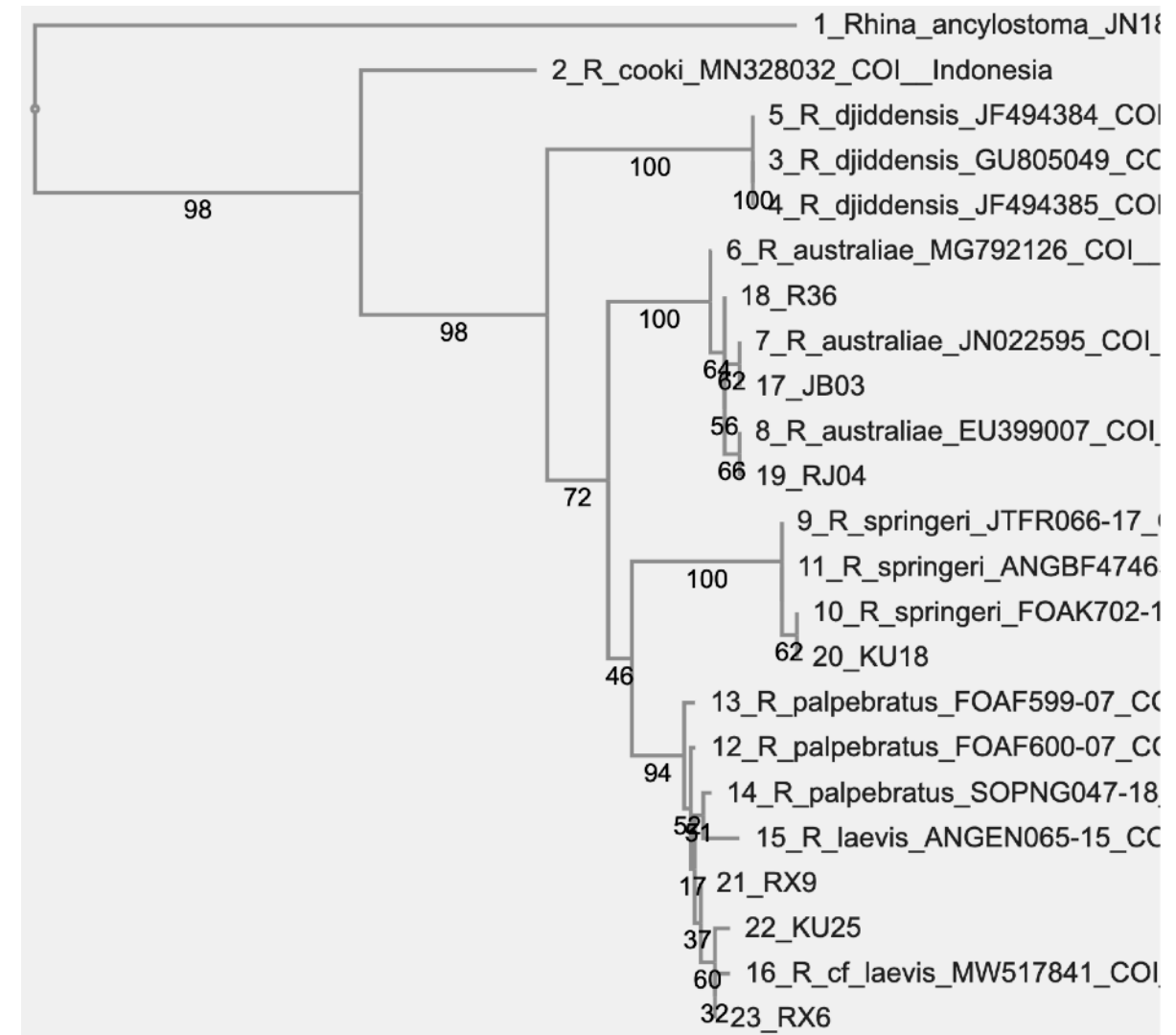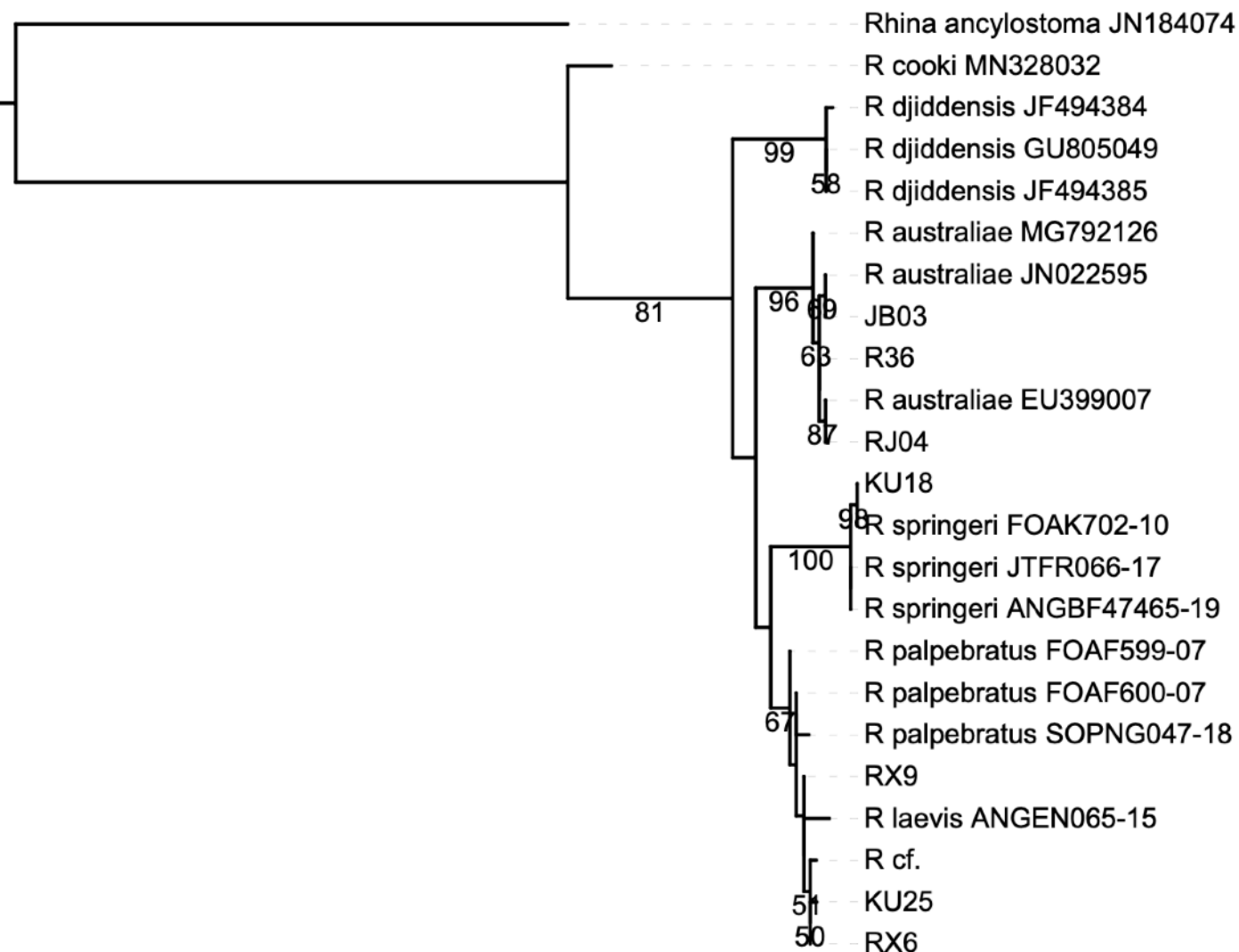Exercises in Marine Ecological Genetics
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Morphological characters

Eye / spiracle markings   Pectoral fin spot pattern

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Tree comparison

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

How are they the same?

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From barcoding to genomes: genetic approaches to shark and ray conservation
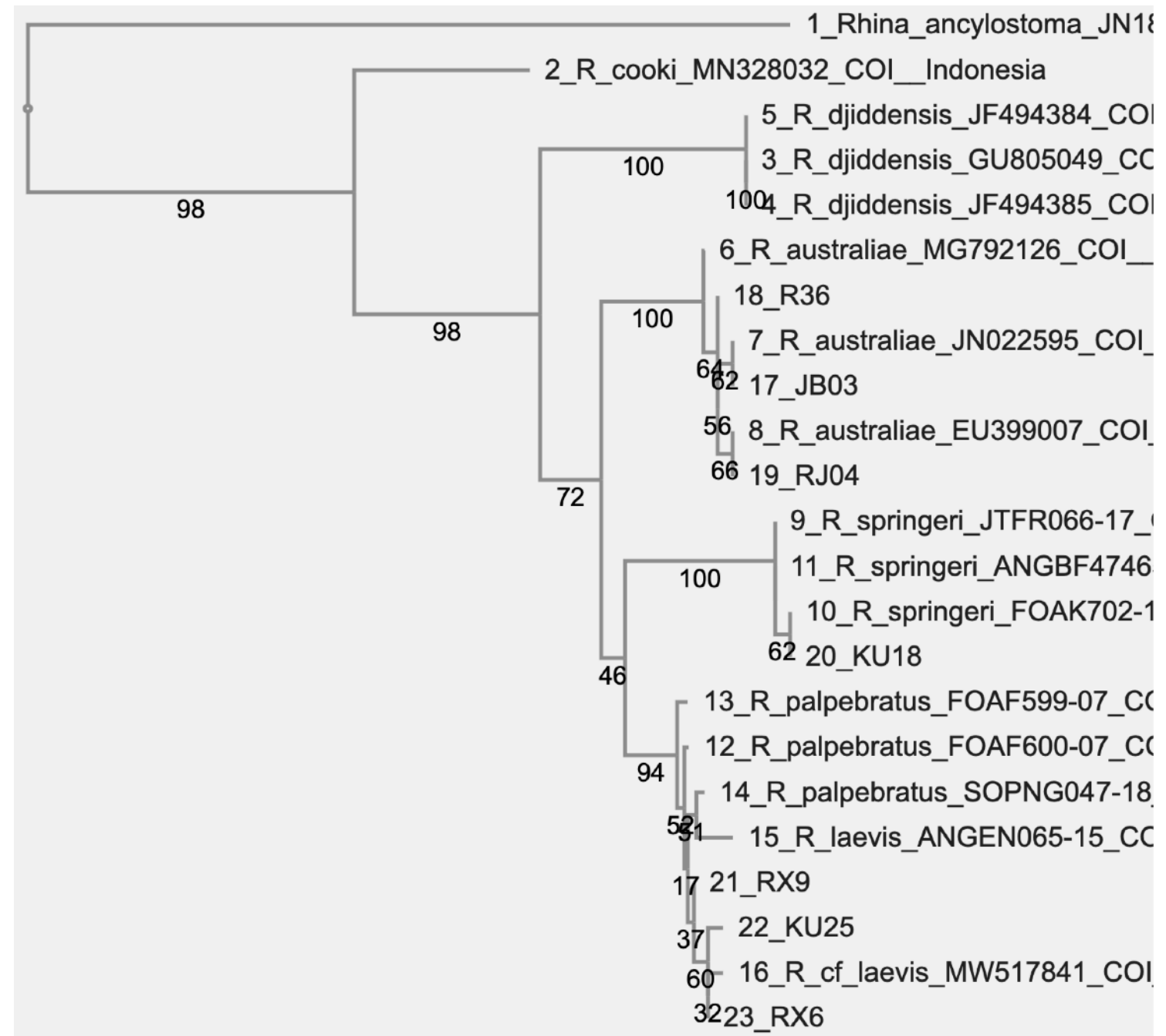
Comparison with morphological species identification

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

Sample	Morphological id
R36	*R. springeri*
JB03	*R. springeri*
RJ04	*R. australiae*
KU18	*R. springeri*
RX9	*R. australiae*
KU25	*R. palpebratus*
RX6	*R. australiae*

Martin Helmkampf
From barcoding to genomes: genetic approaches to shark and ray conservation
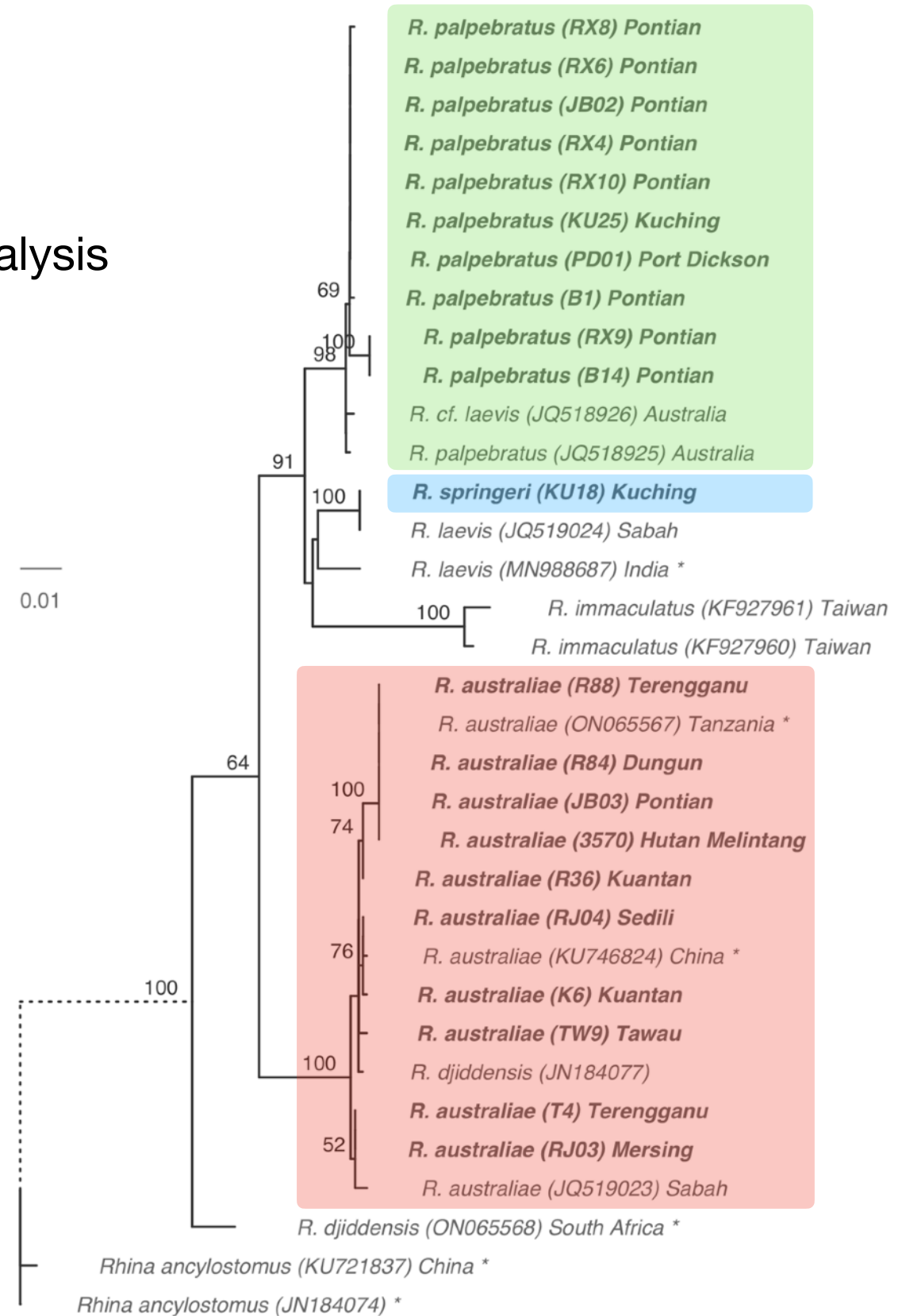
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Genetic validation

Maximum likelihood-based phylogenetic analysis

ND2 gene

All specimens could be assigned to species with high confidence

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From barcoding to genomes: genetic approaches to shark and ray conservation

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Comparison of species identification methods

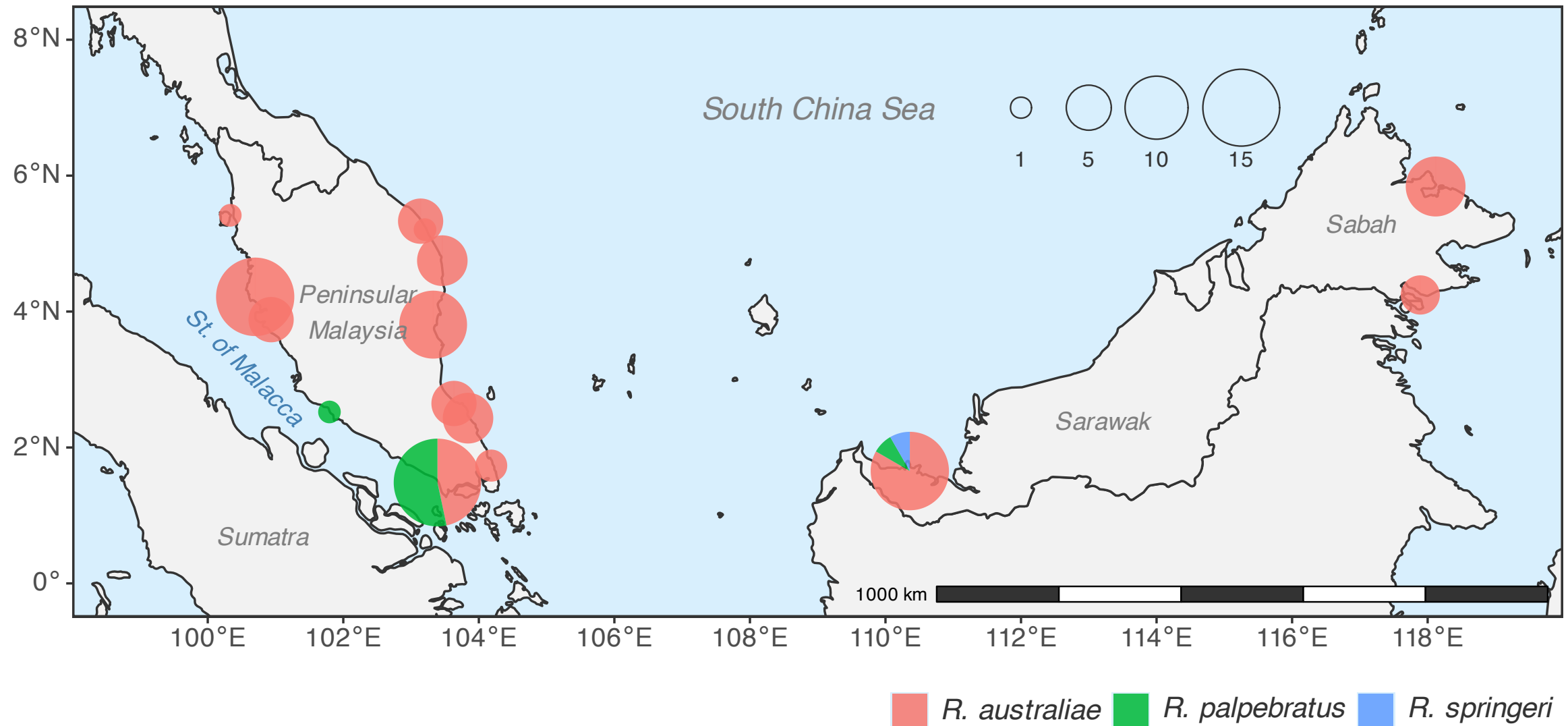| Species	Morphology	DNA barcoding
R. australiae	68	74
R. palpebratus	4	10
R. springeri	7	1
Unknown	6	0
Total		85

Underestimated by morphology

Overestimated by morphology

16 % of specimens were misidentified based on morphology alone

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Species distribution

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

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Phylogenetic inference

```
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**

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