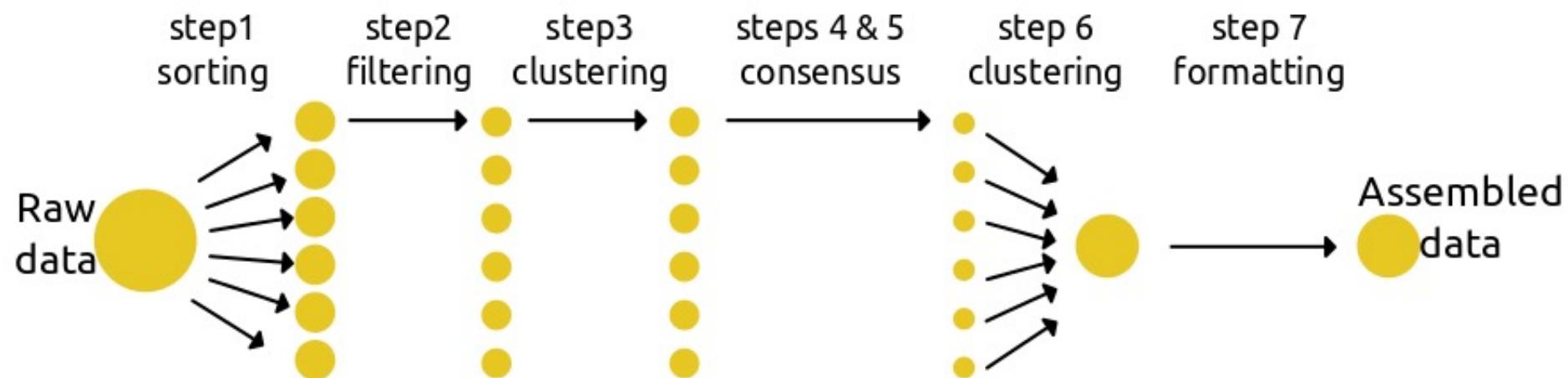
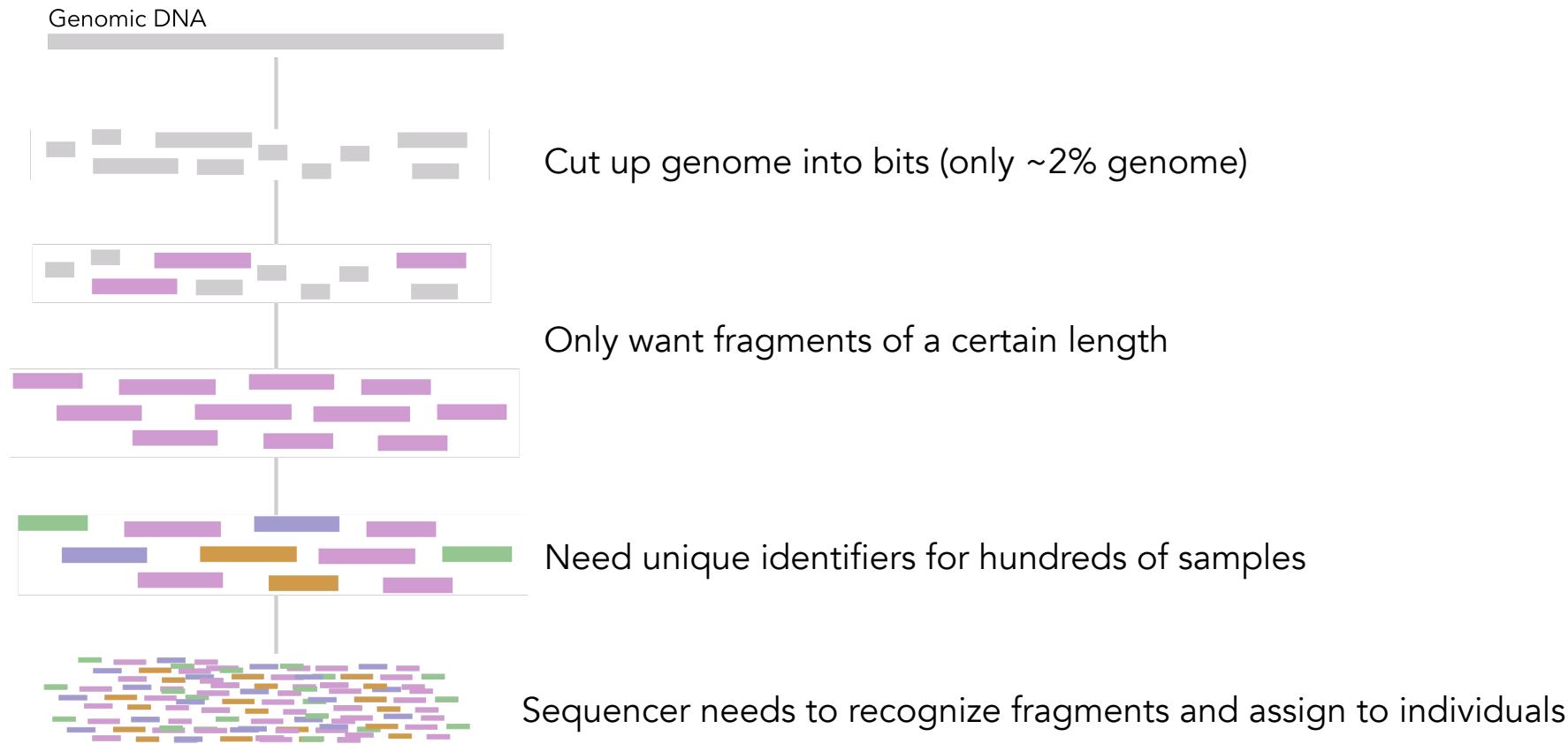


Using iPyrad for ddRAD data

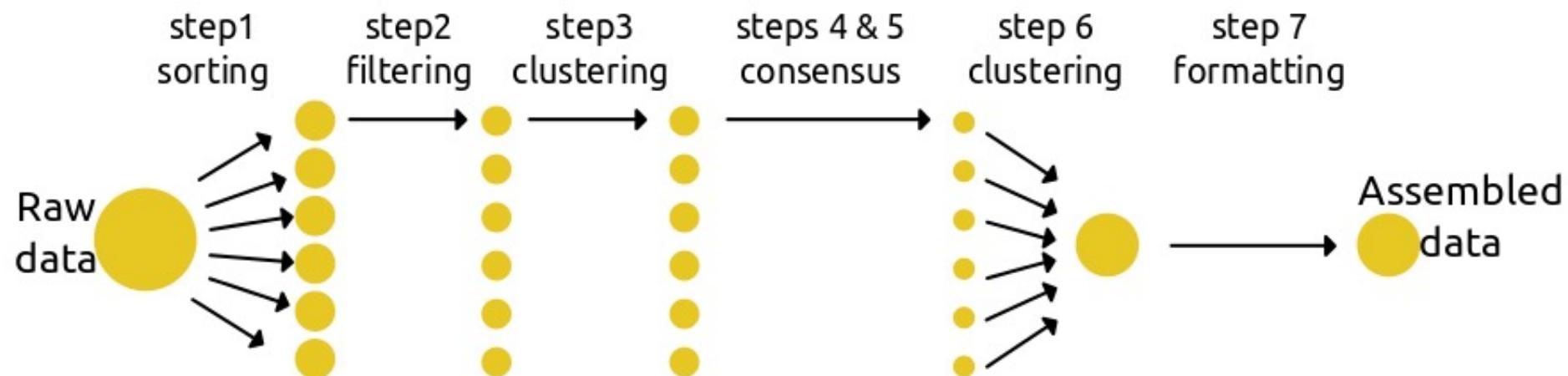


Library preparation: break down into problems

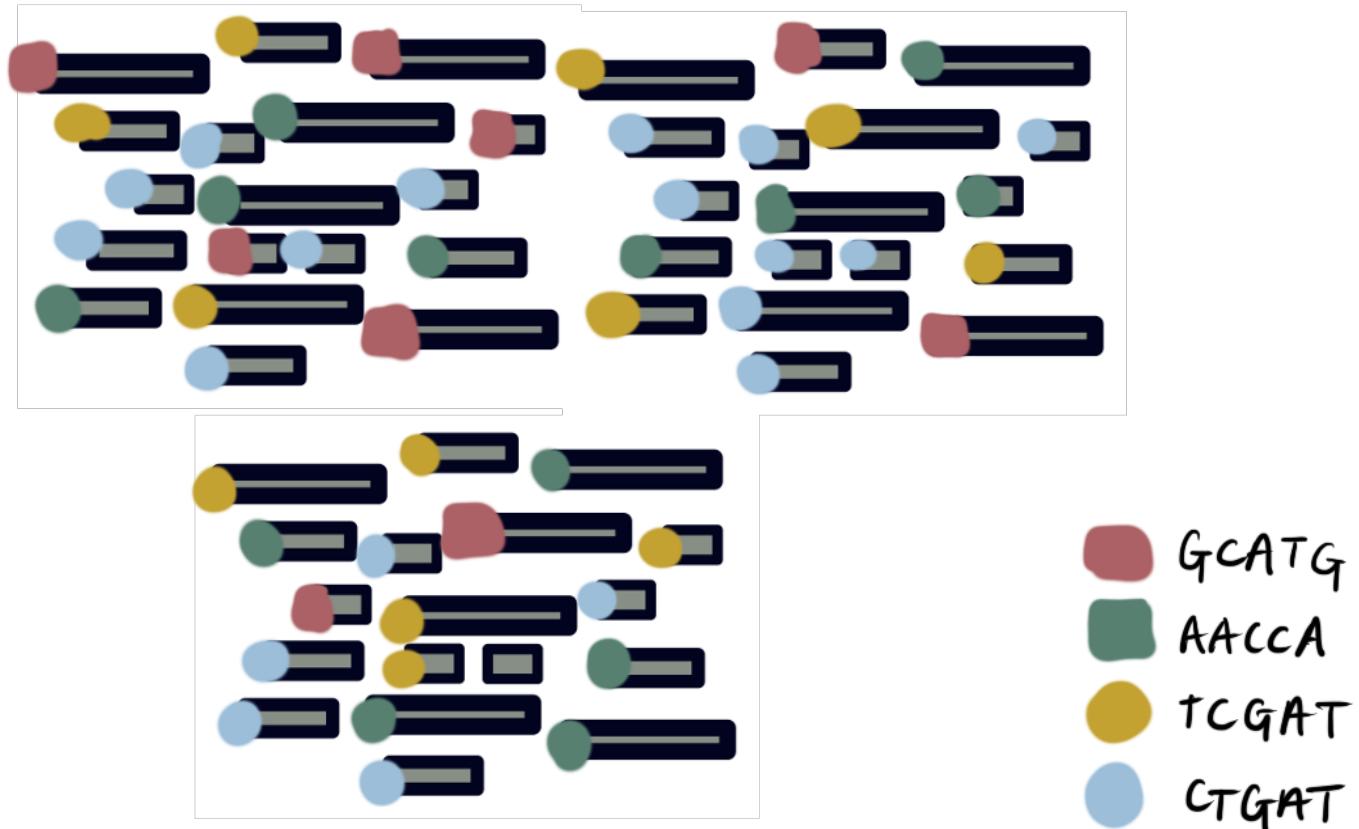


iPyrad: basic workflow

Seven sequential steps

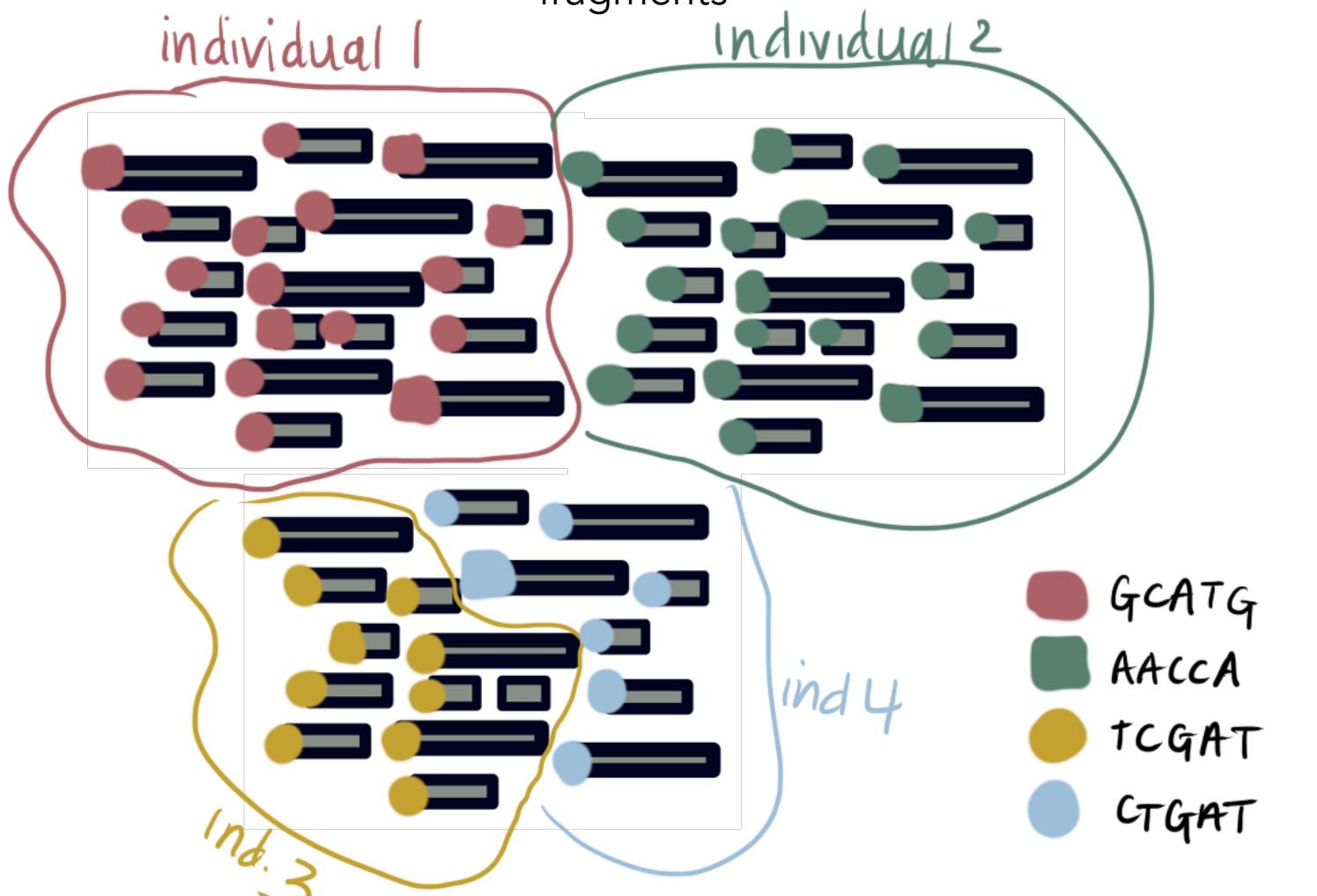


After sequencing is done, we have a bunch of reads from a bunch of fragments



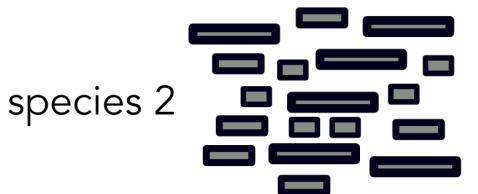
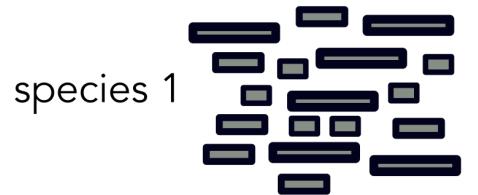
Step 1: sorting and demultiplexing

After sequencing is done, we have a bunch of reads from a bunch of fragments

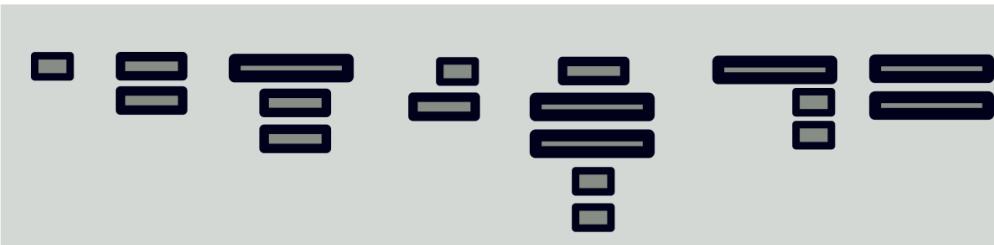


Step 1: sorting and demultiplexing

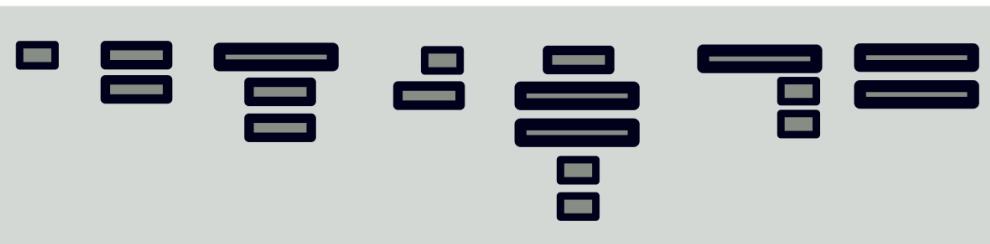
Step 1 (sorting)



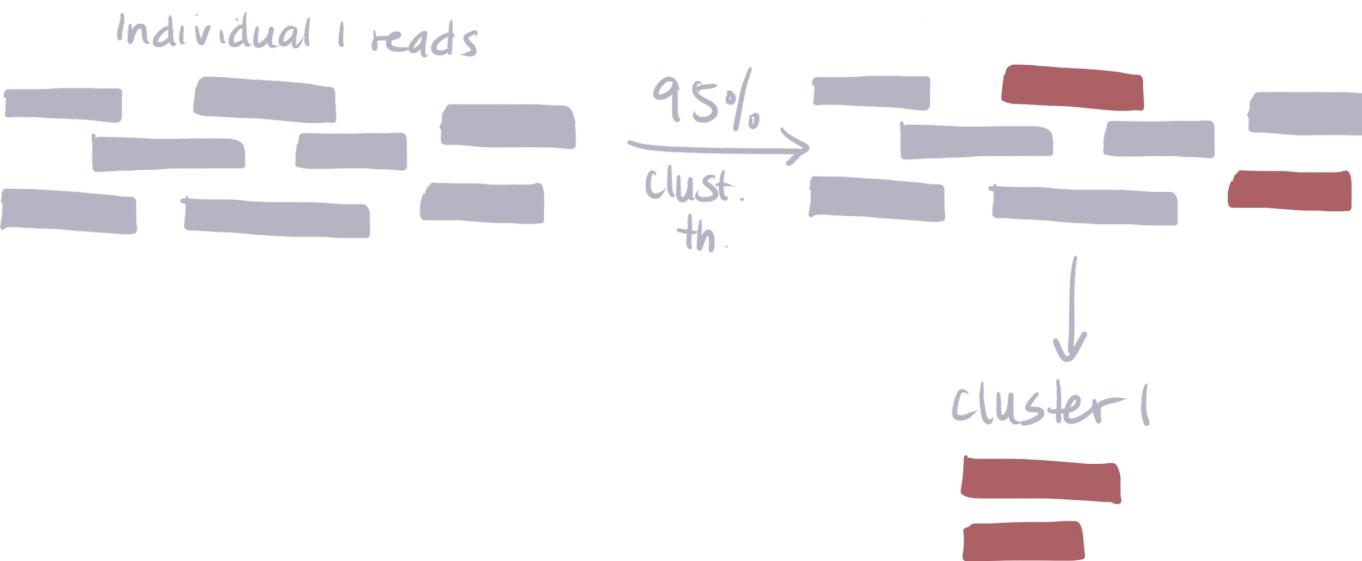
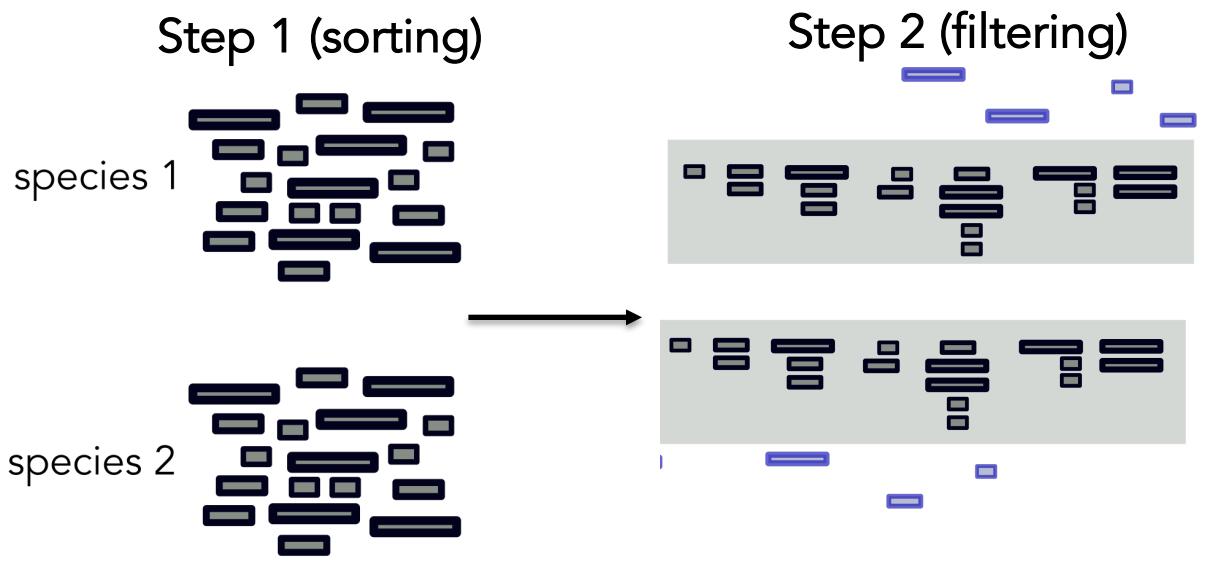
species 1



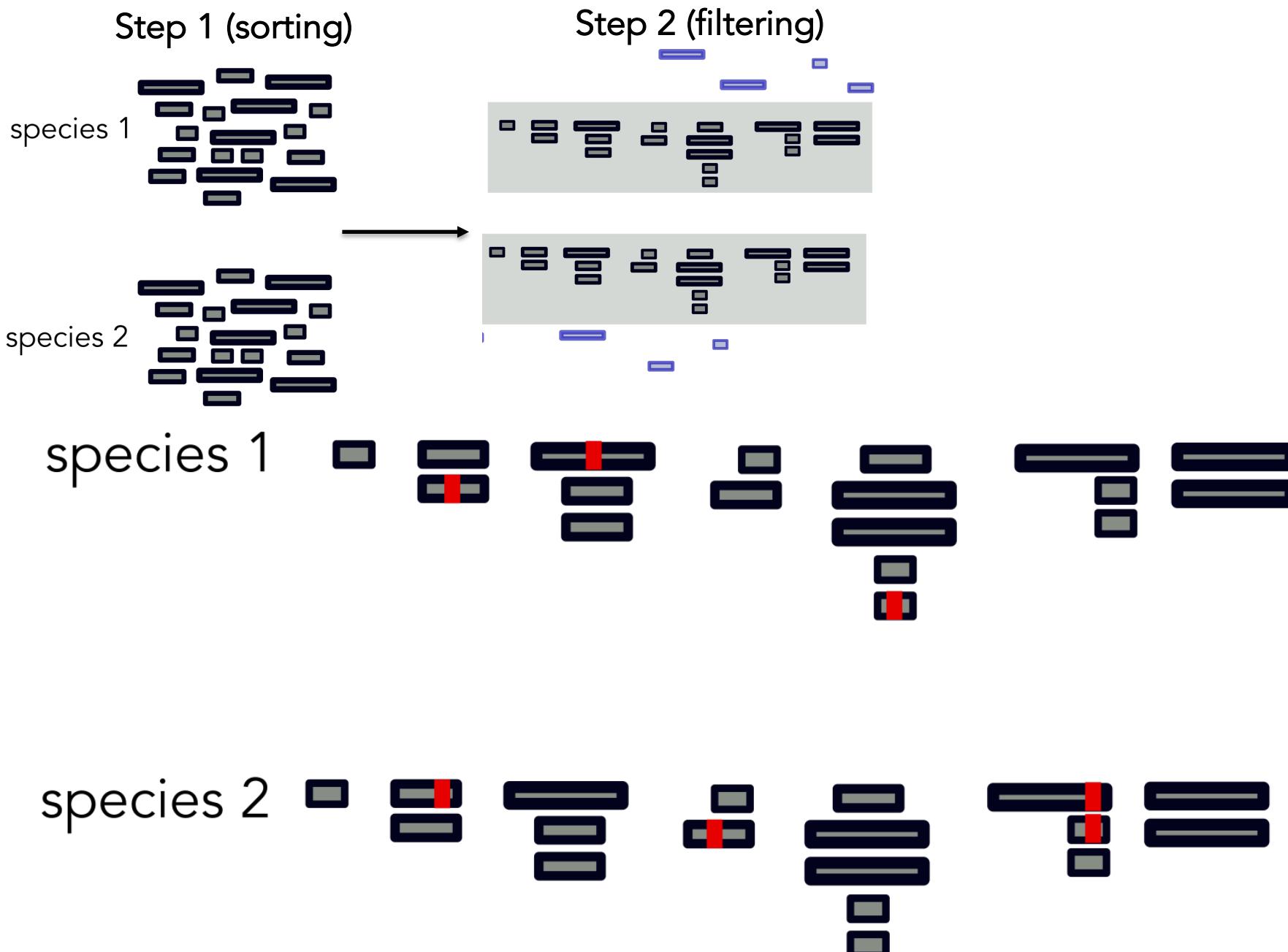
species 2



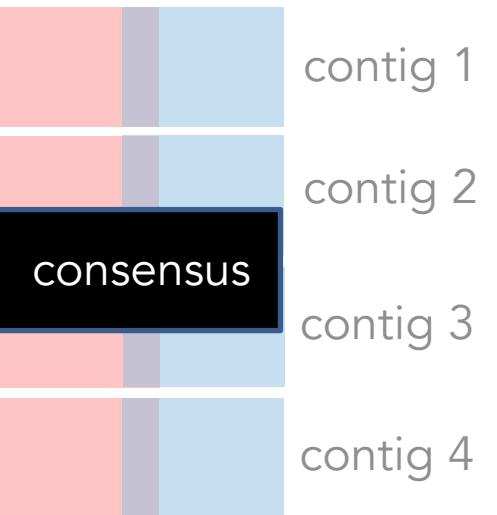
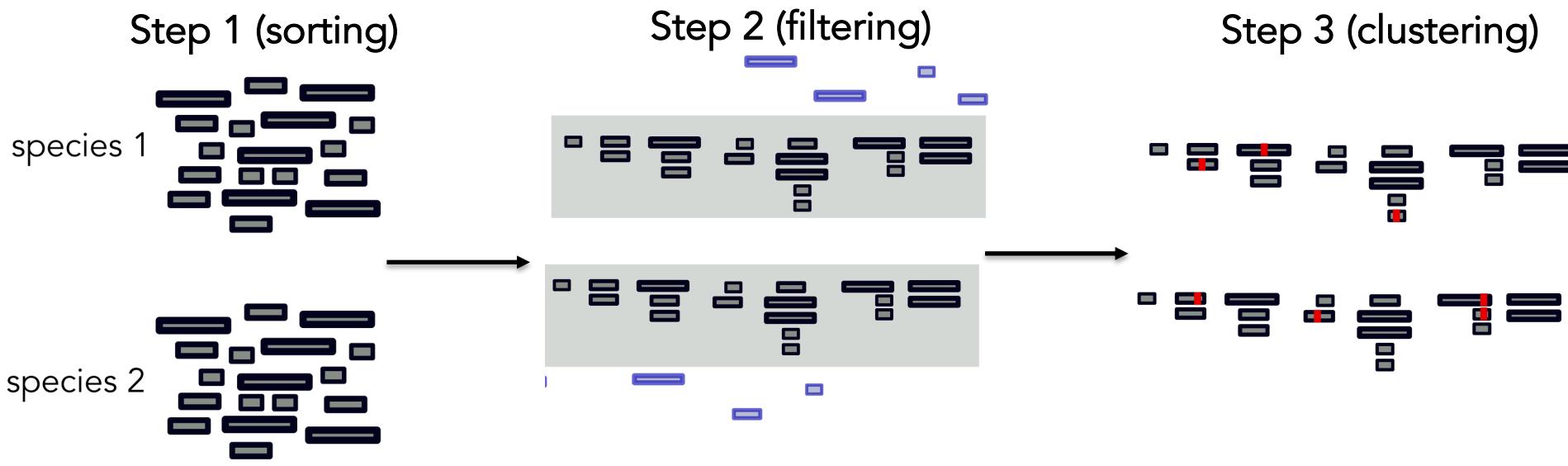
Step 2: filtering



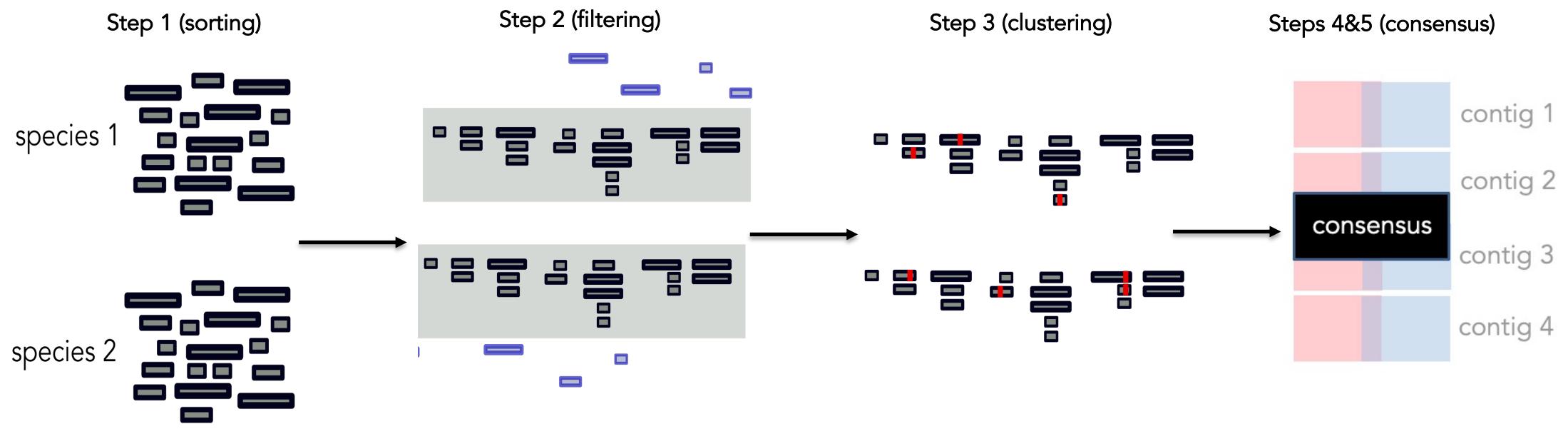
Step 3: clustering *within* inds (with clustering threshold)



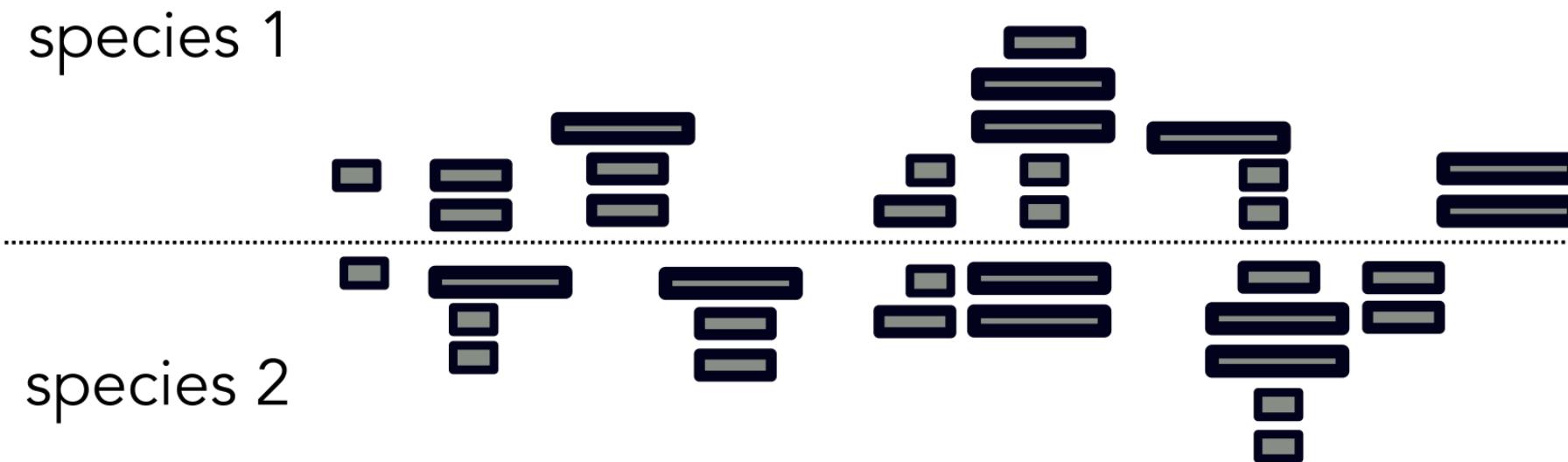
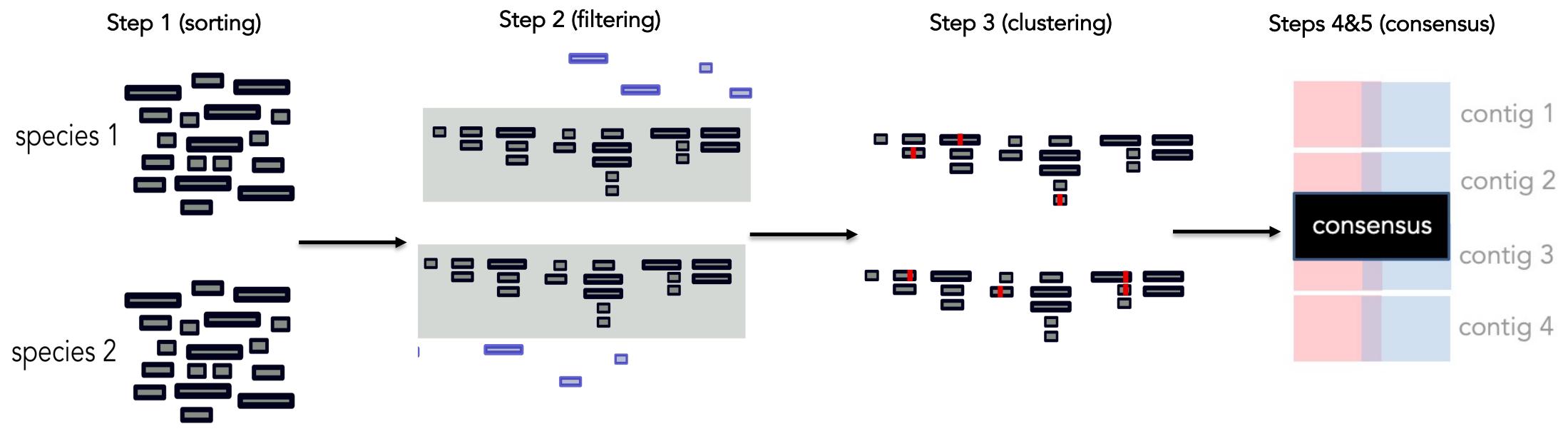
Step 3: clustering *within* inds (with clustering threshold)



Step 4&5: obtaining consensus reads (heterozygotes and error)



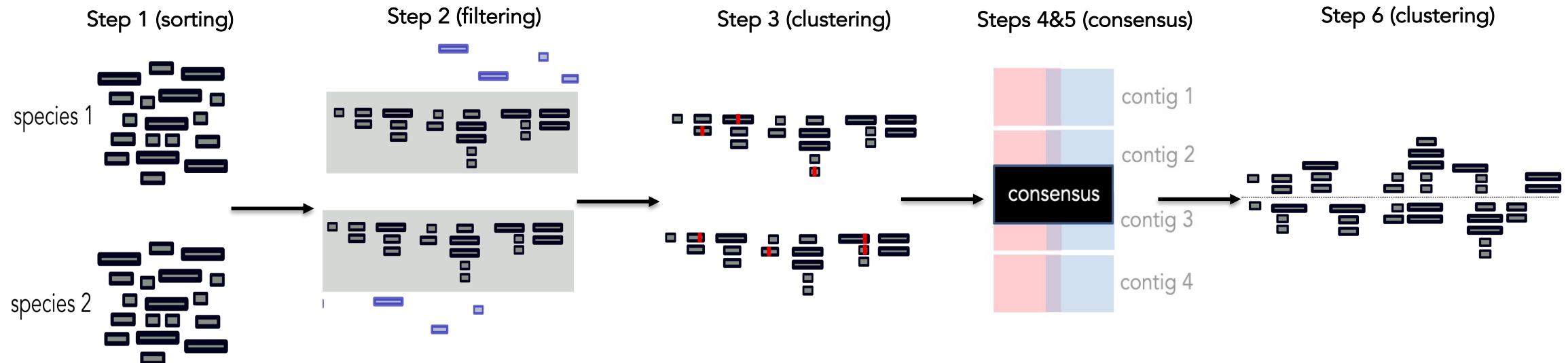
Step 6: Clustering among individuals



Step 6: Clustering among individuals

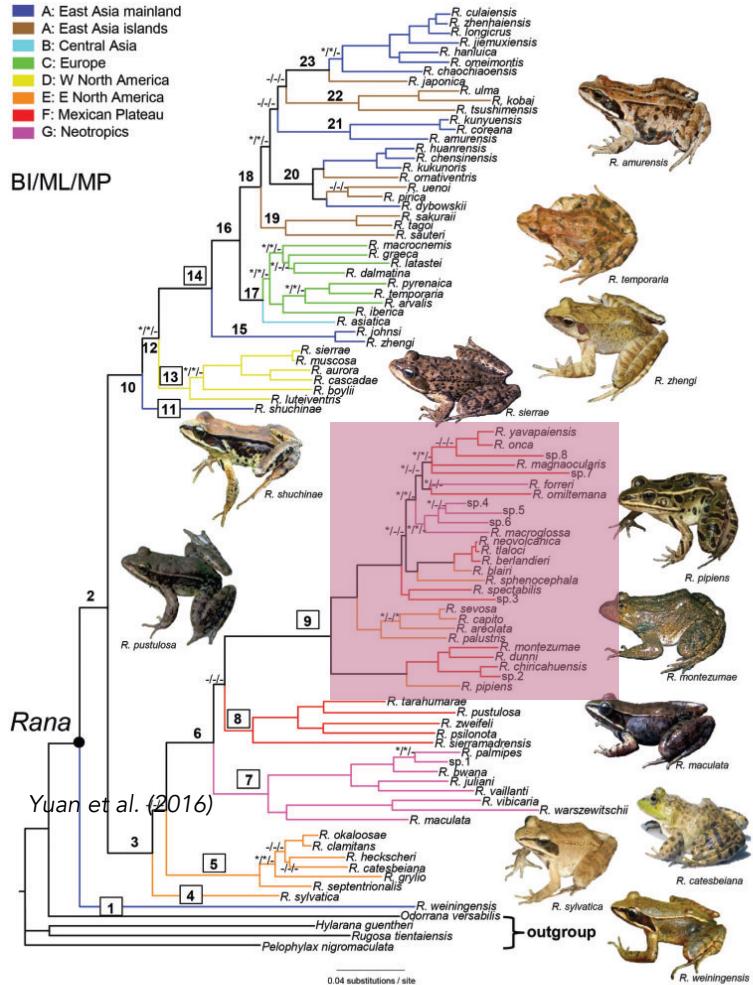
iPyrad relies on a number of programs

- iPyrad (along with other pipelines for NGS data) is just a string of executable programs that have different functions
- e.g., **vsearch** (de novo clustering), **muscle** (alignment), **samtools** (reference mapping)

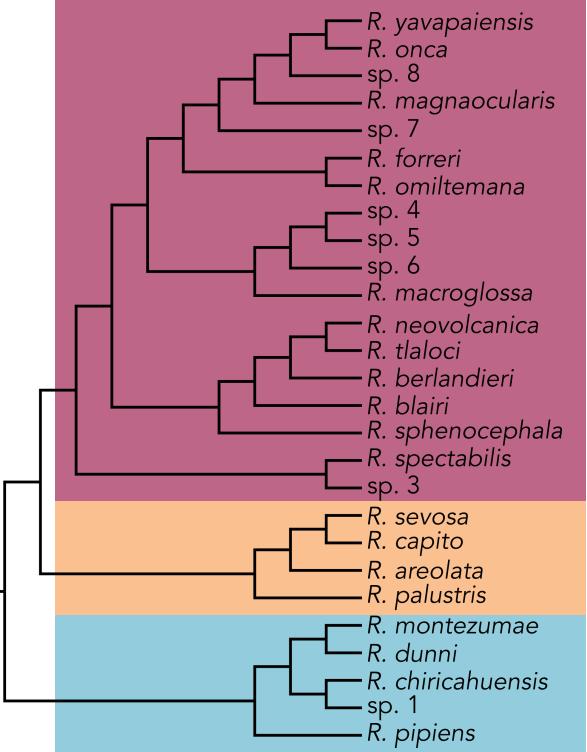


What will we be analyzing in this course?

Genus *Rana*



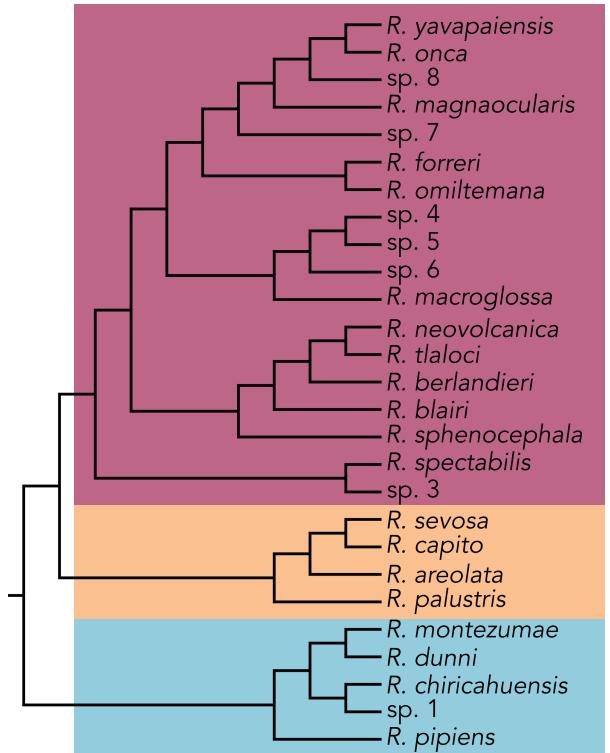
Pantherana subgenus



Adapted from Yuan et al. (2016)

Leopard frog complex (*Pantherana* subgenus)

Pantherana subgenus



Adapted from Yuan et al. (2016)

Scurrilirana



R. berlandieri

Nenirana

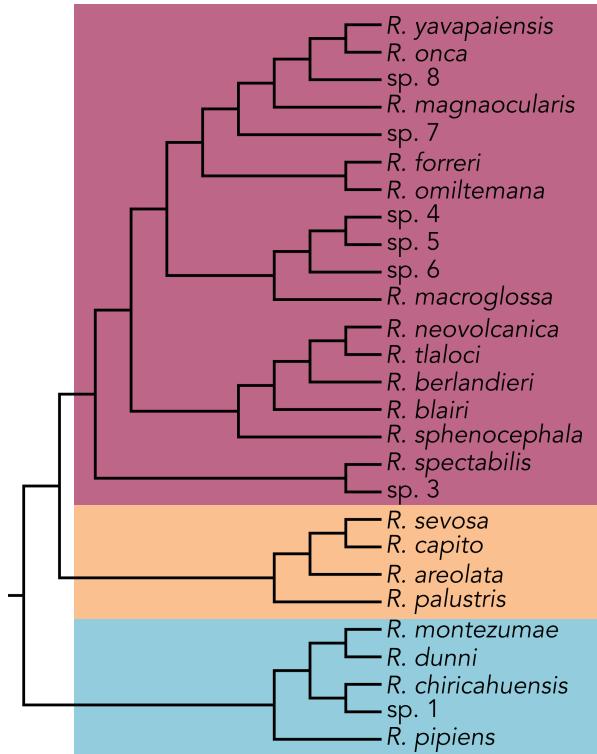


R. montezumae

Stertirana

Leopard frog complex (*Pantherana* subgenus)

Pantherana subgenus

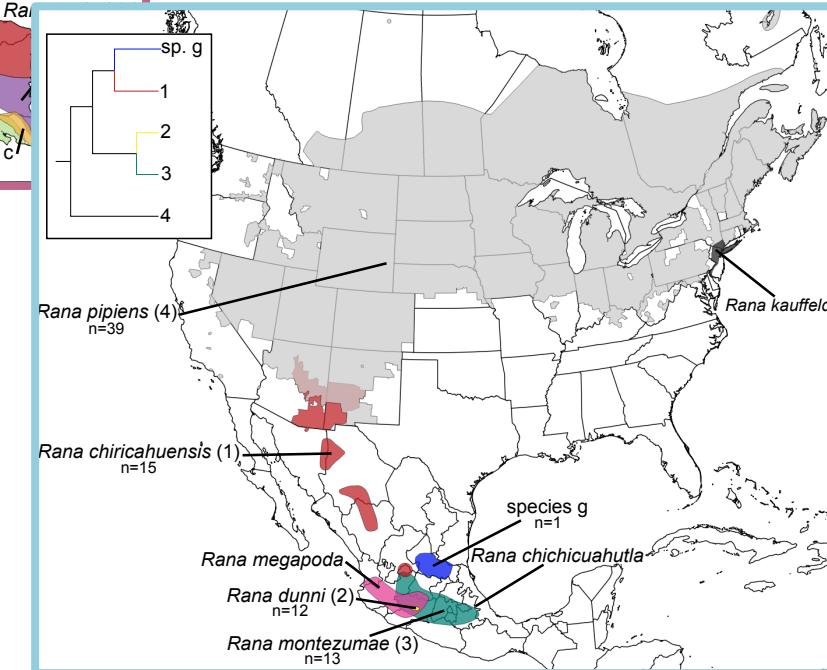
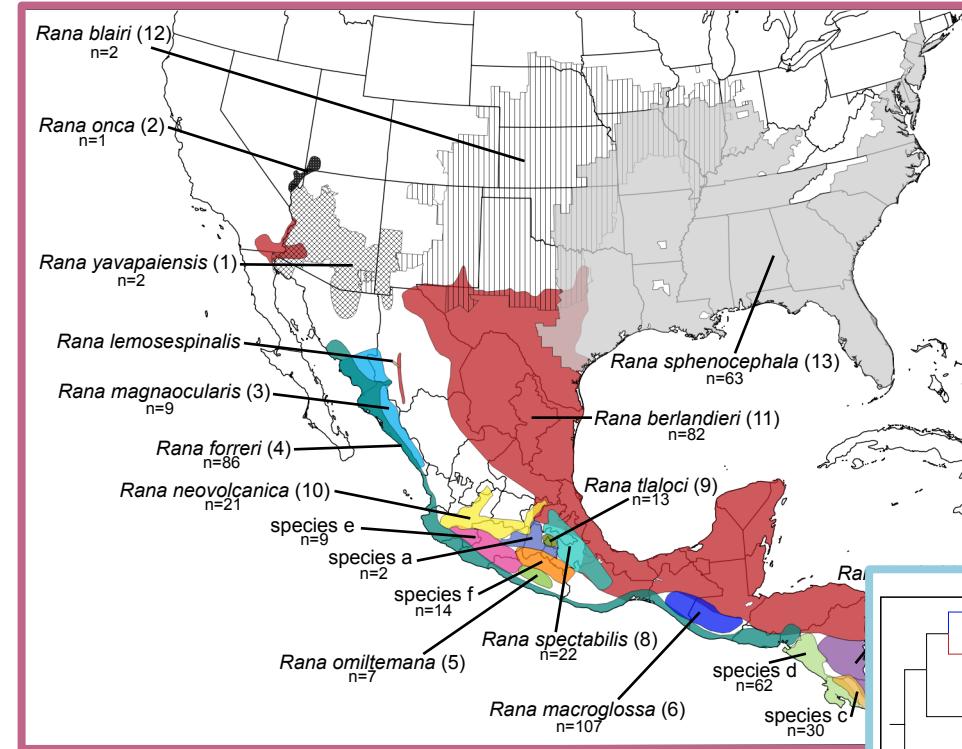


Adapted from Yuan et al. (2016)

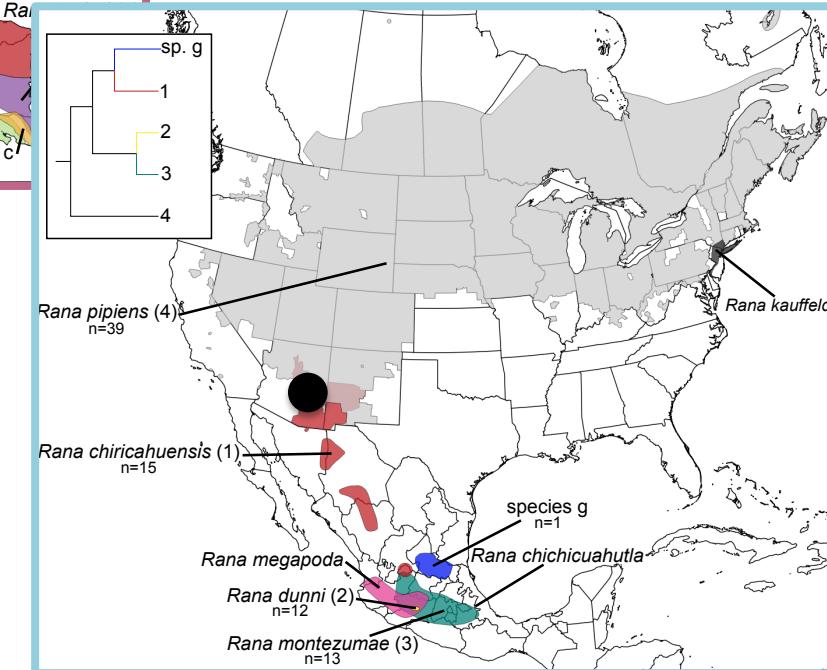
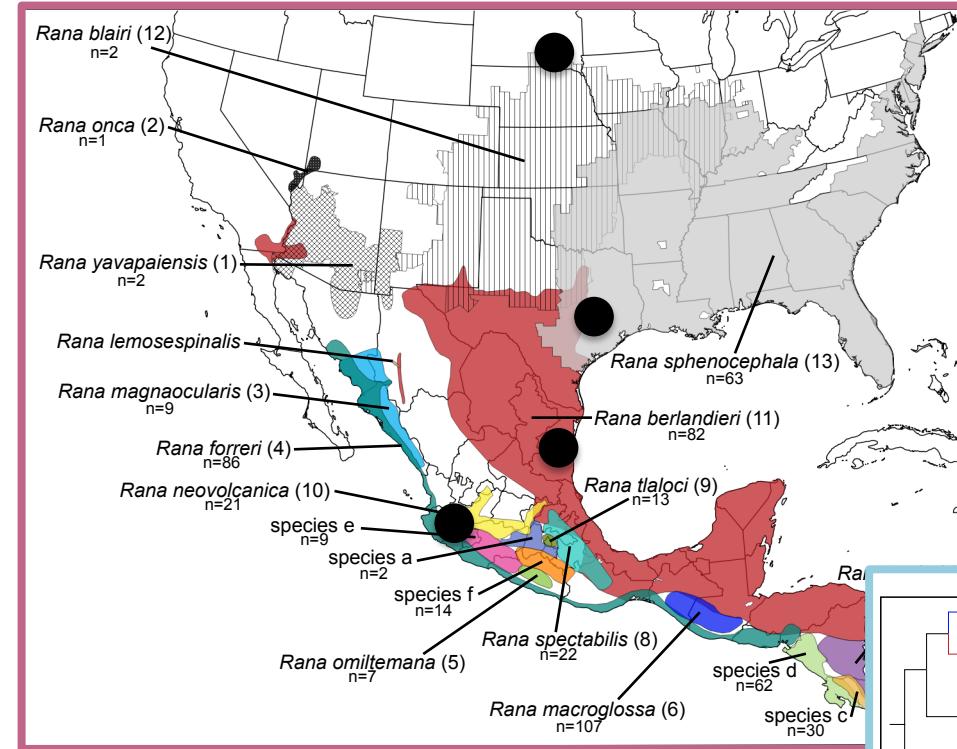
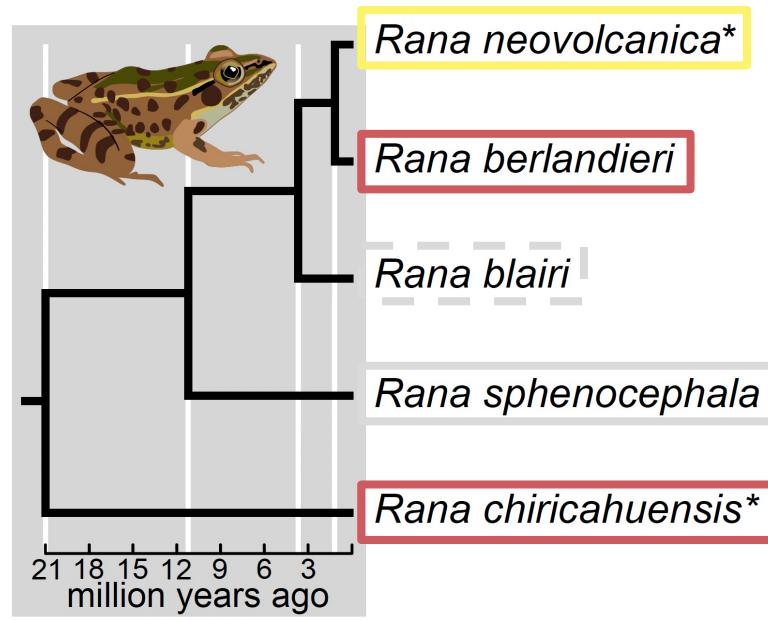
Scurrilirana

Nenirana

Stertirana



Leopard frog complex (*Pantherana* subgenus)



12 samples total, *replicate samples