

Supplementary Materials for

**Piecing the barcoding puzzle of Palearctic water frogs (*Pelophylax*) sheds  
light on amphibian biogeography and global invasions**

Dufresnes et al.

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**Table S1:** Taxonomic treatment of the recovered mtDNA and nuclear lineages with justifications in respect to recent taxonomic revisions

| mtDNA                   | nuclear                   | taxon                               | justification   |
|-------------------------|---------------------------|-------------------------------------|---|
| <i>perezi</i>           | <i>perezi</i>             | <i>P. perezi</i>                    |   |
| <i>saharicus</i> A      | <i>saharicus</i> A        | <i>P. saharicus</i>                 | The old split between the two main clades of <i>P. saharicus</i> (~7My), confirmed by phylogenomics (Doniol-Valcroze <i>et al.</i> , 2021) is consistent with two distinct species (A vs B/C). According to the lineage distributions (Figs. 1, 4), the name <i>saharicus</i> (described from In-Salah, Algeria) applies to lineage A and the younger name <i>riodeoroi</i> (described from Ad Dchira, Morocco) is a junior synonym. The name <i>zavataarii</i> (described from Gat, Libya) is potentially available for lineage C (and thus the species B/C), pending clarifications of the water frogs inhabiting Libya.  |
| <i>saharicus</i> B      | <i>saharicus</i> B/C      | <i>P. cf. saharicus</i>             |   |
| <i>saharicus</i> C      | <i>saharicus</i> B/C      | <i>P. cf. saharicus</i>             |   |
| <i>lessonae</i>         | <i>lessonae</i>           | <i>P. lessonae lessonae</i>         | The recent split (<3My) and widespread admixture between the <i>lessonae/bergeri</i> lineages is consistent with a single species (Speybroeck <i>et al.</i> , 2020). Further distinction of the two <i>bergeri</i> lineages may be warranted pending molecular divergence.  |
| <i>bergeri</i> A        | <i>bergeri</i> A/B        | <i>P. lessonae bergeri</i>          |   |
| <i>bergeri</i> B        | <i>bergeri</i> A/B        | <i>P. lessonae bergeri</i>          |   |
| <i>shqipericus</i>      | <i>shqipericus</i>        | <i>P. shqipericus</i>               |   |
| <i>epeiroticus</i>      | <i>epeiroticus</i>        | <i>P. epeiroticus</i>               |   |
| <i>cretensis</i>        | <i>cretensis</i>          | <i>P. cretensis</i>                 |   |
| <i>ridibundus</i>       | <i>ridibundus</i>         | <i>P. ridibundus ridibundus</i>     | The numerous lineages previously assigned or associated (“cf.”) to <i>P. ridibundus</i> , <i>P. kurtmuelleri</i> , <i>P. bedriagae</i> , <i>P. cypriensis</i> , <i>P. cerigensis</i> or <i>P. caralitanus</i> all belong to a fairly young diversification (<3.5My, Fig. 10) characterized by extensive admixture across the most divergent lineages in northern Greece (Hotz <i>et al.</i> , 2013), which altogether preliminary suggests a single species with high phylogeographic diversity (see also Speybroeck <i>et al.</i> , 2020). Lineages with available names are thus here treated as subspecies of <i>P. ridibundus</i> , and the remaining lineages are listed as unnamed subspecies ( <i>P. r. cf. ridibundus</i> ). If future investigations confirm their distinctiveness and leads to new taxonomic descriptions, the nominal taxa <i>P. ridibundus</i> may face a nomenclatural availability issue, as its type locality (N of the Caspian Sea in W-Kazakhstan) corresponds to hybrid populations (Plötner <i>et al.</i> , 2009, Fig. 4) with Central Asian lineages, and the name <i>fortis</i> (described from N-Germany) may be used instead (e.g., Dufresnes & Mazepa, 2020). |
| <i>kurtmuelleri</i>     | <i>kurtmuelleri</i>       | <i>P. ridibundus kurtmuelleri</i>   |   |
| <i>bedriagae</i>        | <i>bedriagae</i>          | <i>P. ridibundus bedriagae</i>      |   |
| <i>cypriensis</i>       | <i>cypriensis</i>         | <i>P. ridibundus cypriensis</i>     |   |
| cf. <i>ridibundus</i> A | cf. <i>ridibundus</i> A   | <i>P. ridibundus persicus</i>       |   |
| cf. <i>ridibundus</i> B | cf. <i>ridibundus</i> B   | <i>P. ridibundus caralitanus</i>    |   |
| cf. <i>ridibundus</i> C | -                         | <i>P. ridibundus cf. ridibundus</i> |   |
| cf. <i>ridibundus</i> D | cf. <i>ridibundus</i> D   | <i>P. ridibundus cf. ridibundus</i> |   |
| cf. <i>ridibundus</i> E | cf. <i>ridibundus</i> E   | <i>P. ridibundus cerigensis</i>     |   |
| cf. <i>ridibundus</i> F | cf. <i>ridibundus</i> F   | <i>P. ridibundus cf. ridibundus</i> |   |
| cf. <i>ridibundus</i> G | cf. <i>ridibundus</i> G   | <i>P. ridibundus cf. ridibundus</i> |   |
| cf. <i>ridibundus</i> H | cf. <i>ridibundus</i> H/I | <i>P. ridibundus cf. ridibundus</i> |   |
| cf. <i>ridibundus</i> I | cf. <i>ridibundus</i> H/I | <i>P. ridibundus cf. ridibundus</i> |   |
| cf. <i>ridibundus</i> J | cf. <i>ridibundus</i> J/K | <i>P. ridibundus cf. ridibundus</i> |   |
| cf. <i>ridibundus</i> K | cf. <i>ridibundus</i> J/K | <i>P. ridibundus cf. ridibundus</i> |   |
| cf. <i>ridibundus</i> L | -                         | <i>P. ridibundus cf. ridibundus</i> |   |
| cf. <i>ridibundus</i> M | -                         | <i>P. ridibundus terentievi</i>     |   |
| -                       | cf. <i>ridibundus</i> X   | <i>P. ridibundus cf. ridibundus</i> |   |

|                                     |                                  |   |   |
|-------------------------------------|----------------------------------|---|---|
| <i>mongolius</i>                    | -                                | <i>P. mongolius</i>                     |   |
| <i>fukienensis</i> A                | <i>fukienensis</i> A/B           | <i>P. fukienensis</i>                   | The shallow divergence between Taiwanese (A) and mainland (B) populations seems insufficient to warrant a taxonomic distinction based on the available data (see also Dufresnes & Litvinchuk, 2022)   |
| <i>fukienensis</i> B                | <i>fukienensis</i> A/B           | <i>P. fukienensis</i>                   |   |
| <i>pororus</i>                      | <i>pororus</i>                   | <i>P. pororus pororus</i>               | The recent split (<3My) of the <i>pororus/brevipodus</i> diversification and widespread admixture between the <i>brevipodus</i> lineage A ("Nagoya form") and B ("Okayama form") suggest a single species (Dufresnes & Litvinchuk, 2022). The name <i>brevipodus</i> applies to lineage A.  |
| <i>brevipodus</i> A                 | <i>brevipodus</i> A              | <i>P. pororus brevipodus</i>            |   |
| <i>brevipodus</i> B                 | <i>brevipodus</i> B              | <i>P. pororus</i> cf. <i>brevipodus</i> |   |
| cf. <i>nigromaculatus/plancyi</i> B | <i>nigromaculatus</i> - Japan    | <i>P. nigromaculatus nigromaculatus</i> | Populations related to <i>P. nigromaculatus</i> are composed of at least three nuclear lineages, all relatively young in the mitochondrial timetree (<4My, noting that the oldest mitogroup is a ghost mtDNA lineage), suggestive of a single species. The subspecies <i>nigromaculatus</i> (described from Japan) applies to the Japanese populations and preliminarily to the closely related Korean/Russian populations, while the name <i>reinhardtii</i> (described from China) is available for the Chinese populations. See also Dufresnes & Litvinchuk (2022).  |
| cf. <i>nigromaculatus/plancyi</i> F | <i>nigromaculatus</i> - Japan    | <i>P. nigromaculatus nigromaculatus</i> |   |
| cf. <i>nigromaculatus/plancyi</i> H | <i>nigromaculatus</i> - Japan    | <i>P. nigromaculatus nigromaculatus</i> |   |
| cf. <i>nigromaculatus/plancyi</i> A | <i>nigromaculatus</i> - mainland | <i>P. nigromaculatus nigromaculatus</i> |   |
| cf. <i>nigromaculatus/plancyi</i> C | <i>reinhardtii</i>               | <i>P. nigromaculatus reinhardtii</i>    |   |
| cf. <i>nigromaculatus/plancyi</i> G | <i>reinhardtii</i>               | <i>P. nigromaculatus reinhardtii</i>    |   |
| cf. <i>nigromaculatus/plancyi</i> E | <i>plancyi/chosenicus</i>        | <i>P. plancyi plancyi</i>               | Populations related to <i>P. plancyi</i> are composed of two shallow nuclear lineages corresponding to mitogroups of <2My of divergence (Figs. 3, 6; see also Komaki <i>et al.</i> , 2015) that argues for a single species with two subspecies (but noting that hybridization with <i>P. nigromaculatus</i> affected mtDNA evolution). One corresponds to <i>P. p. plancyi</i> (described from Jiangxi, China), with the name <i>chosenicus</i> (described from Korea) being a junior synonym, given its lack of mitochondrial (Fig. 3), nuclear (Fig. 6) and morphological differences (Zhou <i>et al.</i> , 2023). The other corresponds to <i>P. p. hubeiensis</i> (described from Hubei, China), which further shows morphological differences from <i>P. p. plancyi</i> (Zhou <i>et al.</i> , 2023). See also Dufresnes & Litvinchuk (2022) |
| cf. <i>nigromaculatus/plancyi</i> G | <i>plancyi/chosenicus</i>        | <i>P. plancyi plancyi</i>               |   |
| cf. <i>nigromaculatus/plancyi</i> D | <i>hubeiensis</i>                | <i>P. plancyi hubeiensis</i>            |   |

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# Bayesian

# Maximum-Likelihood

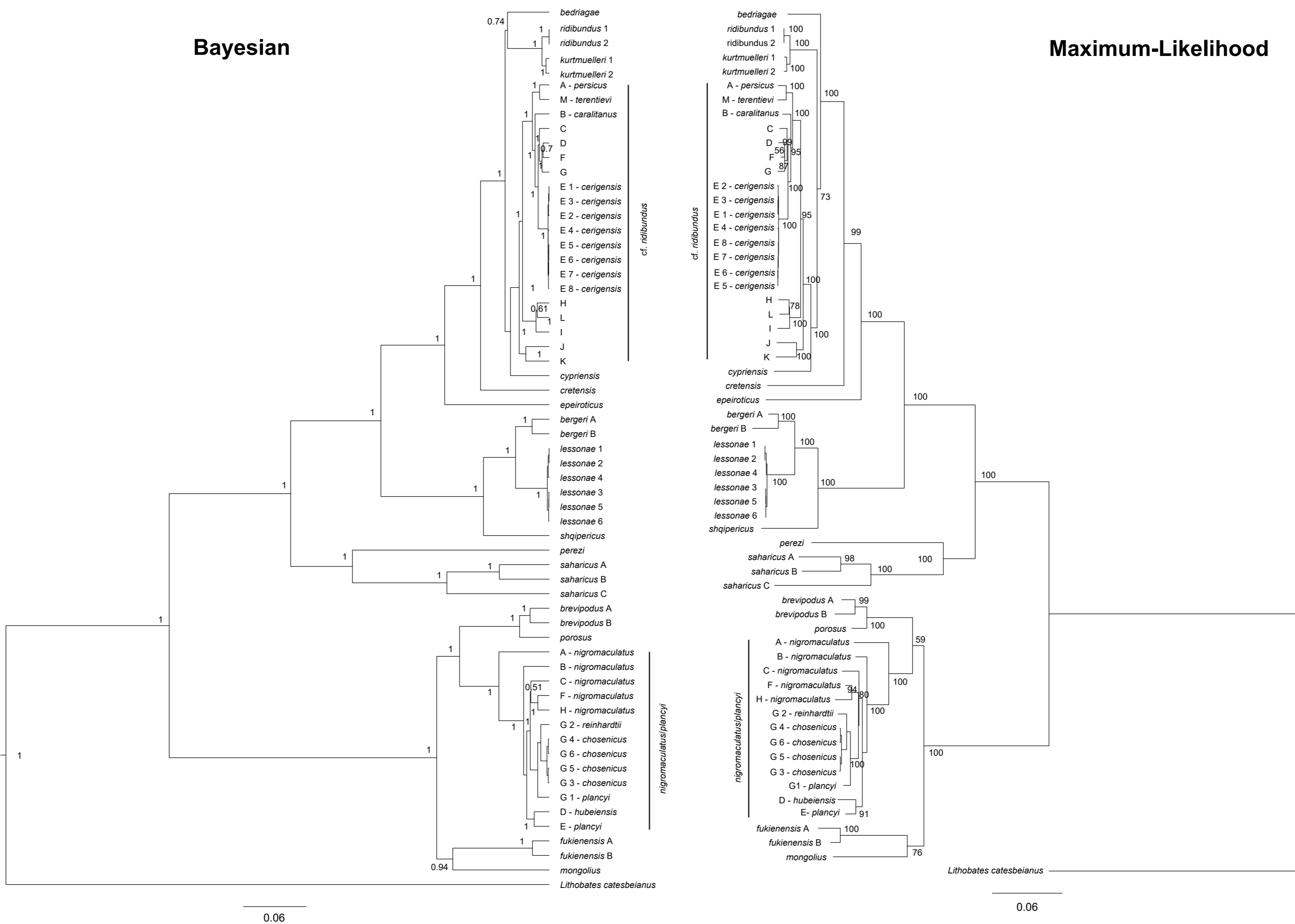


Fig. S1: Bayesian and maximum-likelihood phylogenies of the mitochondrial supermatrix, with terminal branches and support values.

# Bayesian

# Maximum-Likelihood

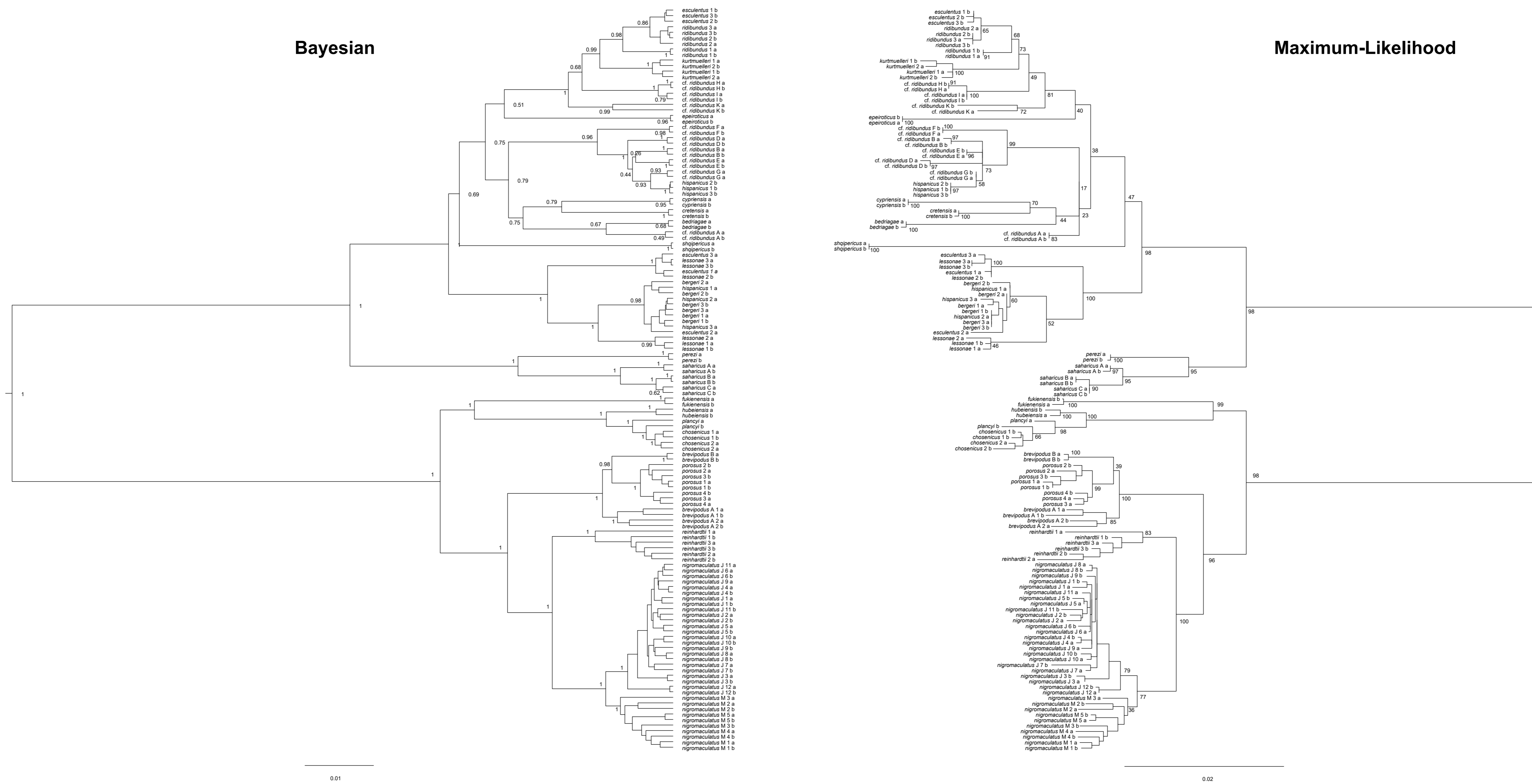
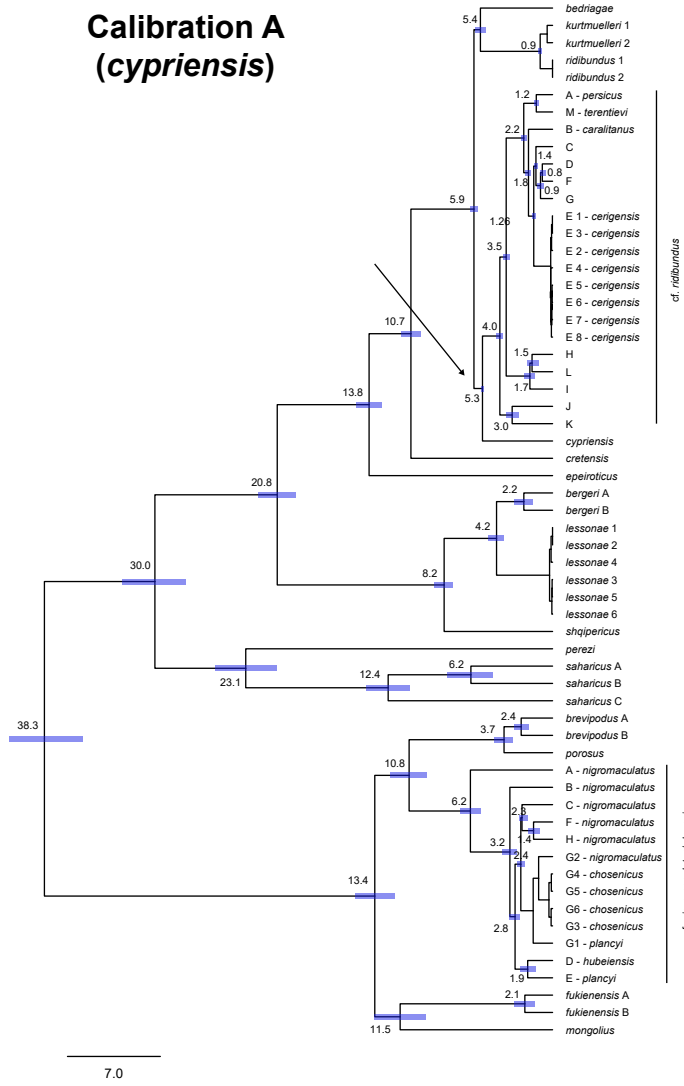
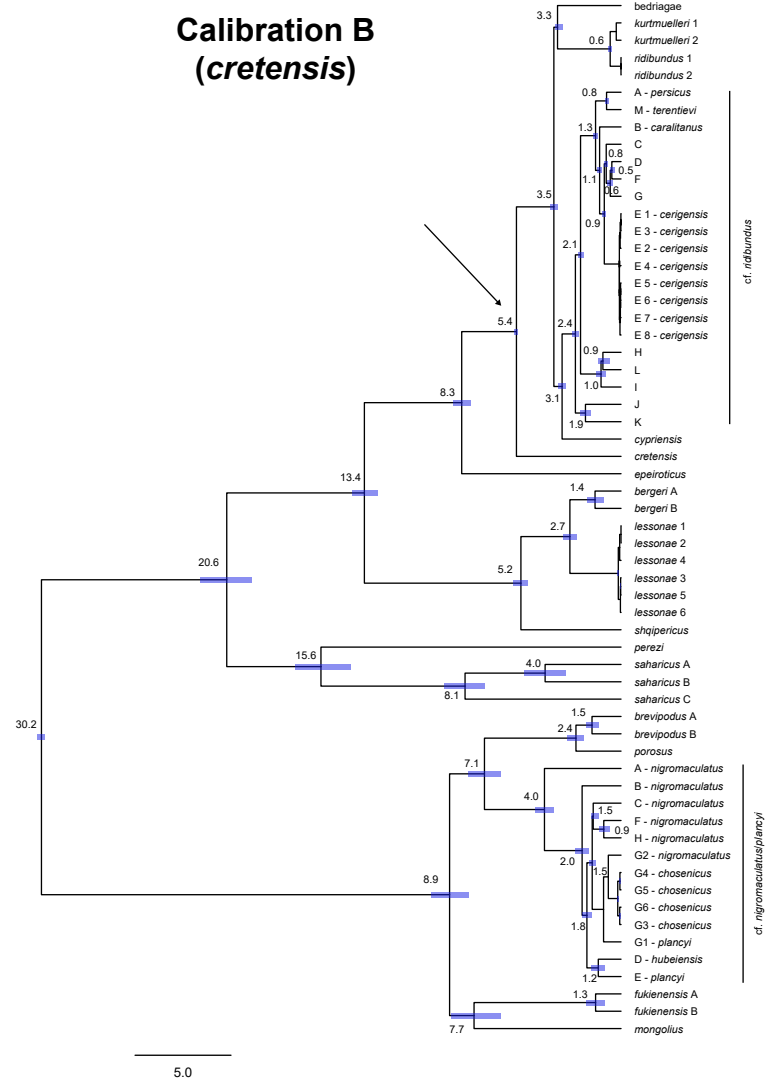


Fig. S2: Bayesian and maximum-likelihood phylogenies of the nuclear supermatrix, with details on terminal branches and support values.

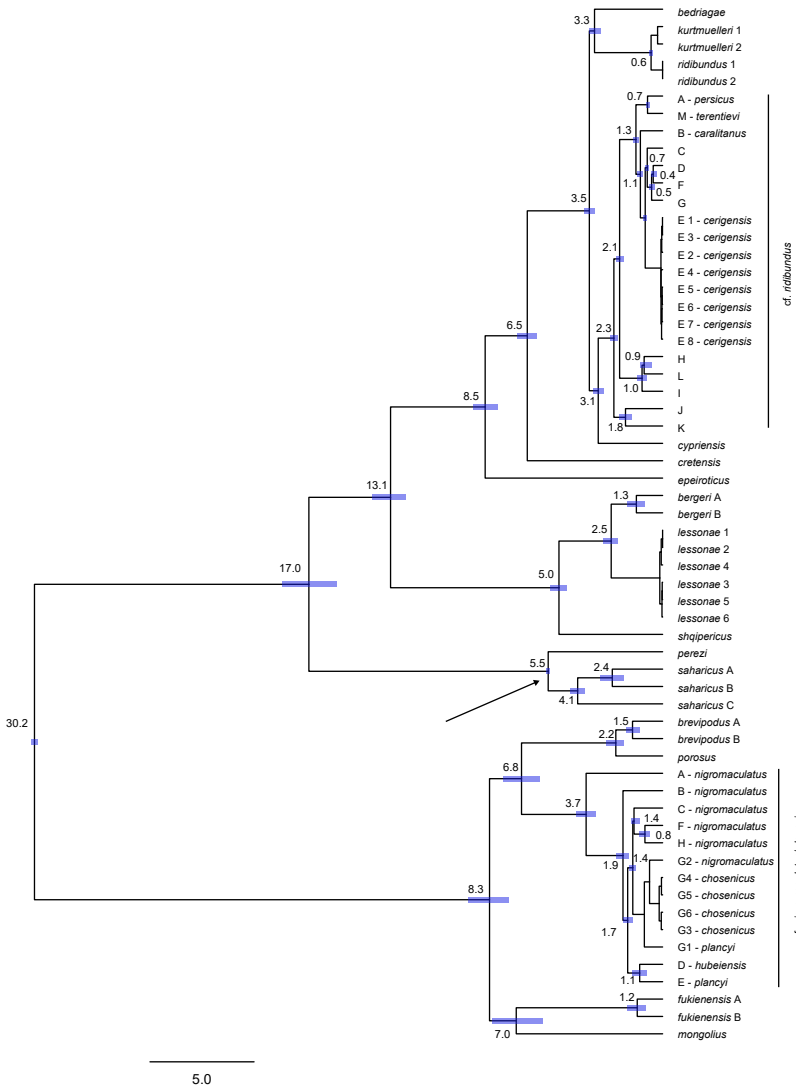
### Calibration A (*cypriensis*)



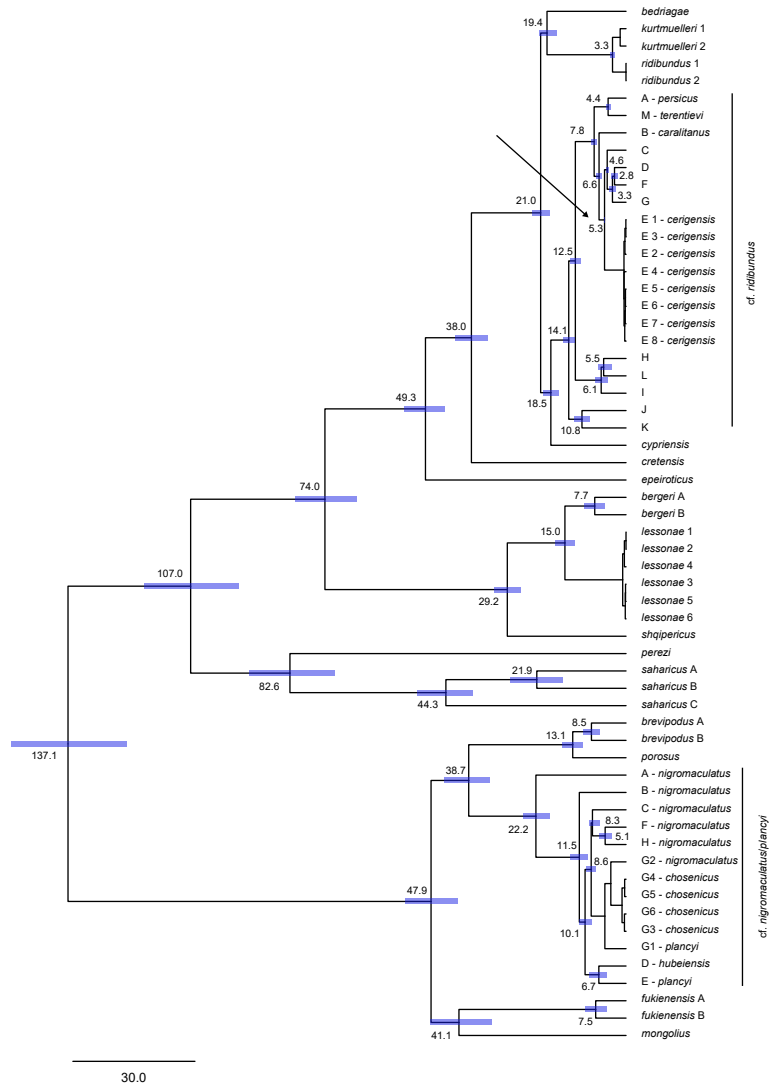
### Calibration B (*cretensis*)



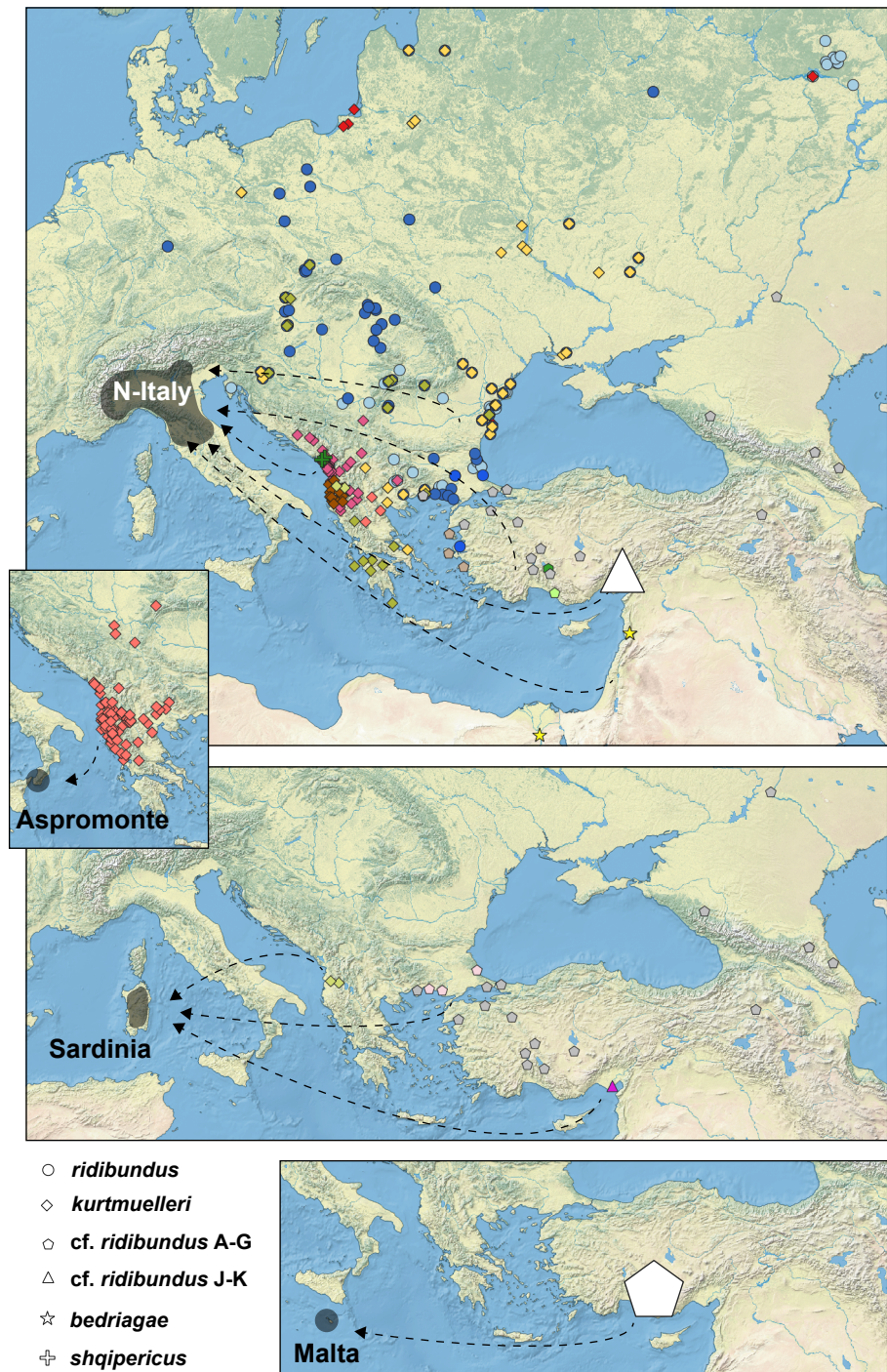
### Calibration C (*saharicus*)



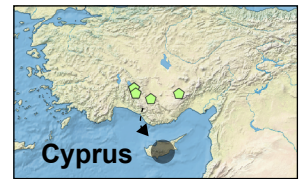
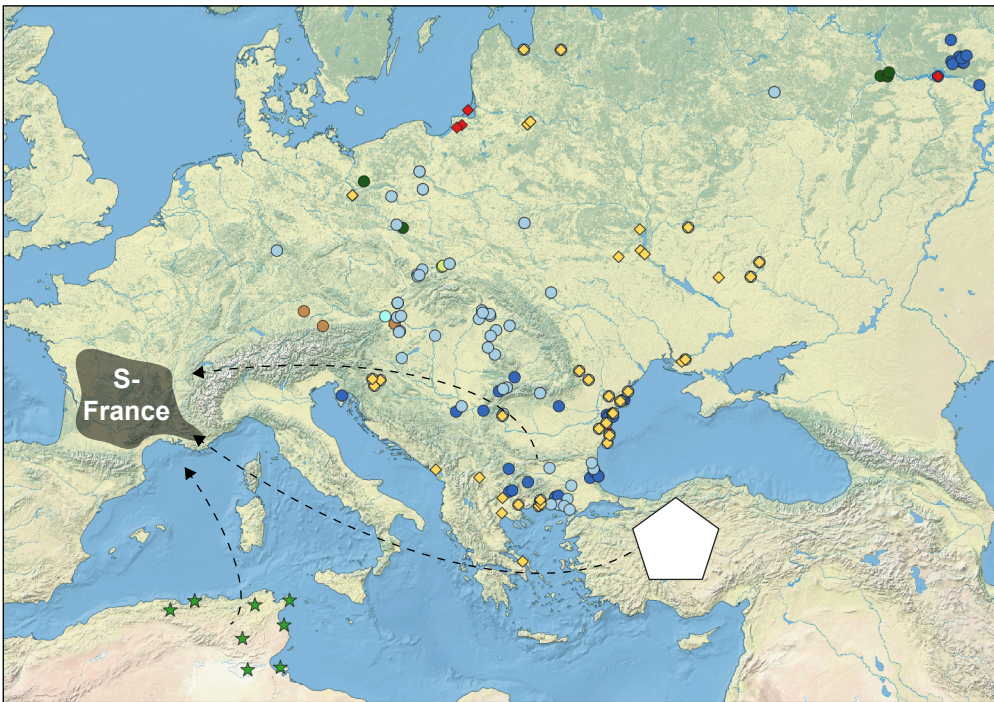
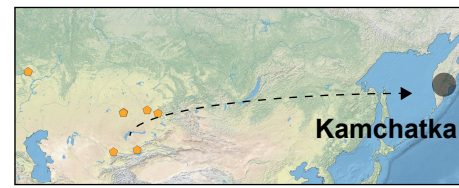
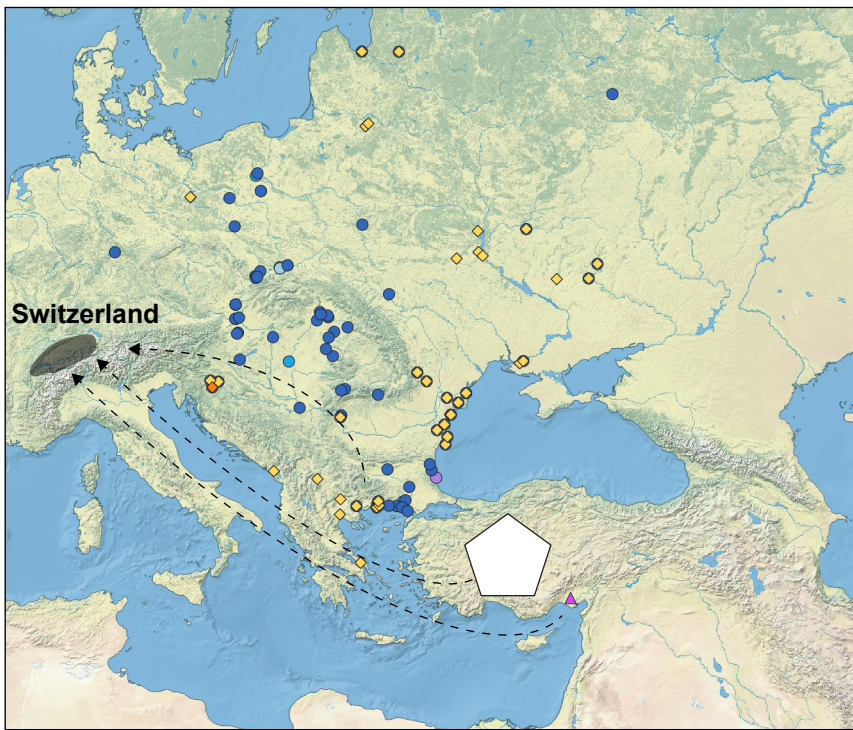
### Calibration D (*cerignensis*)



**Fig. S3:** Mitochondrial timetrees based on the four calibration schemes that differed in the taxon of Messinian origin (arrows).



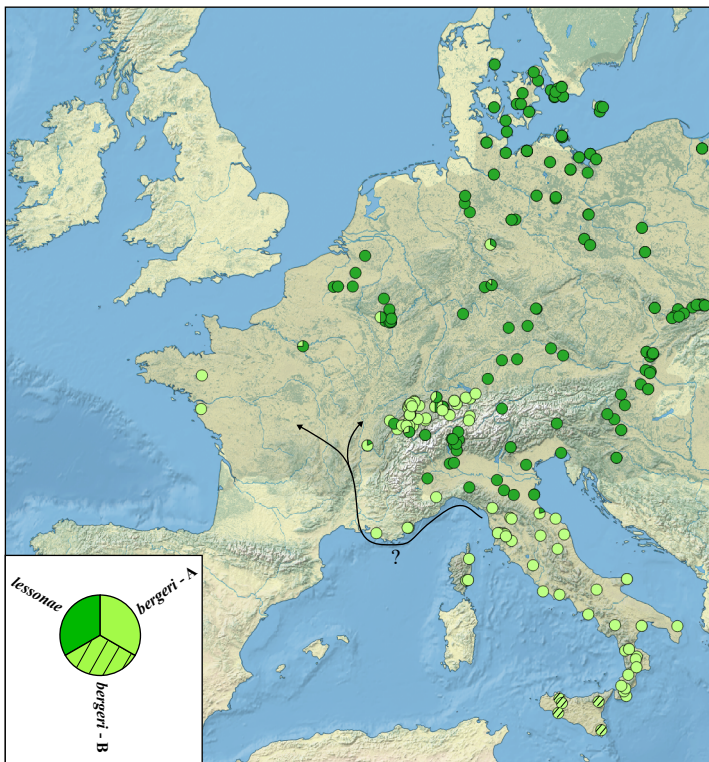
**Fig. S4:** Tracing Pelophylax invasions – part I (Italy and Malta). Symbols on the map show the natural occurrence of mtDNA haplotypes found in invasive ranges, when known. Shapes distinguish lineages, and colors distinguish haplotypes within lineages; large empty symbols: approximate ranges of introduced lineages when the corresponding haplotypes were not reported in natural ranges. Arrows point to potential common sources, i.e., ranges where a high diversity of introduced haplotypes/lineages are naturally found.



- *ridibundus*
- ◇ *kurtmuelleri*
- *cf. ridibundus* A-G
- △ *cf. ridibundus* J-K
- ☆ *saharicus*

**Fig. S5:** Tracing of Pelophylax invasions – part II (rest of the Western Palearctic + Kamchatka). Symbols on the map show the natural occurrence of mtDNA haplotypes found in invasive ranges, when known. Shapes distinguish lineages, and colors distinguish haplotypes within lineages; large empty symbols: approximate ranges of introduced lineages when the corresponding haplotypes were not reported in natural ranges. Arrows point to potential common sources, i.e., ranges where a high diversity of introduced haplotypes/lineages are naturally found.





**Fig. S6:** Tracing of *Pelophylax* invasions – part III (*P. i. bergeri*). Distributions of pool frog mitochondrial lineages in Western Europe. Rather than human-mediated introductions, the detection of *bergeri* mitotypes along the Mediterranean coastline suggests the possibility of a natural expansion outside the Apennine Peninsula. Shaded areas show pool frog distributions (see the legend of Fig. 1 for details).