Additional file 2: supplementary information for the manuscript "The origin of modern frogs (Neobatrachia) was accompanied by acceleration in mitochondrial and nuclear substitution rates"

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1. Calibration points used for the estimation of divergence times

The following calibration points and distribution settings were used as priors:

A) Sauropsida-Synapsida split: Offset=312.3 million years ago (mya) as the minimum age for *Hylonomus* [1); log mean=2.2; log SD=0.424.

B) Archosauromorpha-Lepidosauromorpha split: Offset=259.7 mya as the minimum age for *Protorosaurus* [1]; log mean=2.4468; log SD=0.756.

C) Cryptobranchidae-Hynobiidae split: Offset=145.5 mya. Following Roelants et al. [2], we used a more conservative age estimate of *Chunerpeton* [3]; log mean=3.5; log SD=1.014. The soft maximum is 321 mya, corresponding to the upper limit for the Anura-Caudata split in San Mauro [4]. This is a more conservative upper bound than that suggested by Marjanović and Laurin [5].

D) Anura-Caudata split: Offset=249, which is the minimum age for the salientian *Triadobatrachus*, [6]; log mean=3.7; log SD=0.351; the soft maximum is 321 mya (see C).

E) Branching of Discoglossoidea: Offset=161.2 mya as the Middle-Late Jurassic boundary [7] corresponding to *Eodiscoglossus*, the first known Discoglossoidea [8]; log mean=3.6; log SD=0.532. The upper 95% IC value (soft maximum) is 249 mya (*Triadobatrachus*).

F) Branching of Pipoidea: Offset=145.5 mya as the minimum age for *Rhadinosteus*, the first-known pipoid [9, 10]; log mean=3.45; log SD=0.668. Soft maximum=249 mya (*Triadobatrachus*).

G) *Calyptocephalella-Lechriodus* split: Offset=52.8 mya as the oldest-known fossil of *Calyptocephalella* [10, 11]; log mean=4.2; log SD=0.2. The age of *Rhadinosteus* (145.5 mya; see F) is used as a conservative soft maximum.

2. New mitochondrial genomes

Like in most metazoans, all newly sequenced frog mt genomes encoded for 2 rRNAs, 22 tRNAs and 13 protein-coding genes [12, 13]. All tRNA genes could be folded into the typical cloverleaf secondary structure with the known exception of *trnS(AGY)*. The putative origin of replication of the light strand had the potential to fold into a stem-loop secondary structure and was located between the *trnN* and *trnC* genes in all species, with the exception of *Heleophryne regis* and *Lechriodus melanopyga* (see main text). Three conserved sequence blocks (CSB-1, CSB-2, CSB-3) were identified in the 3' end of the control region of all species. The length of the control regions varied widely among the completely sequenced mt genomes (from 982 bp in *S. thomasseti* to >3866 bp in *T. bolivianus*), and included one or more tandem repeats. The actual length of the mt control regions of two species could not be accurately determined due to the presence of long tandem repeats (200-300 bp in *T. bolivianus* and 1800-2100 bp in *L. melanopyga*), and thus they were estimated from the length of the PCR product in the gel to be 19500-19600bp and 21000-21300 bp, respectively. Notably, the *LTPF* tRNA gene cluster has a 385 bp-long intergenic spacer between *trnP* and *trnF* with no obvious sequence similarity (based on BLAST searches), and it contains an 82 bp-long tandem repeat.

References

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