**Appendix S2**

We estimated rates of molecular evolution using Bayesian relaxed-clock dating with

PAML 4.1 [1] and the set of programs in Multidistribute [2-4]. Methods are presented in

the text, but here we expand upon the locus BFI, which had few sequences available

outside of *P. kentucki*. First, we estimated the rate of evolution using a three-taxon

statement, with *P. wehrlei* as the outgroup and *P. glutinous* + *P. kentucki* as the ingroup.

This is described in the main text. Because this estimate of molecular evolution was

derived from limited sampling, we also estimated the rate of evolution for BFI using a

phylogeny of the Salamandridae from Zhang et al.[5] in combination with sequence data

on salamandrids from Sequeira et al.[6]. The outgroup was designated as *Salamandrina*

*terdigitata*, and the ingroup included nine taxa from eight genera (Fig. B). The upper and

lower limits on node ages were set based on the BEAST 2 analyses of Zhang et al. [5],

including both their primary and additional calibration points (see [5] for details). For all

loci, MCMC analyses included 100,000 generations of burn-in. Thereafter, the Markov

chain was sampled every 100 generations until 10,000 samples were collected.

When BFI was calibrated using *Plethodon*, the estimated rate of evolution was

0.036%/myr, which is an intermediate rate among the nuclear loci. When BFI was dated

using salamandrids, the estimated rate of evolution given the two different calibrations

was 0.091%/myr (primary calibration) and 0.197%/myr (additional calibrations; see [7]).

These rates are higher than those estimated for our other nuclear loci. For our analyses,

we used the *Plethodon* calibration.

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**Table B.  Species and indentication numbers of taxa and loci used in the multidivtime analysis.** GenBank numbers are provided.

| Species | Cyt b | ND2 | tRNAs | MLC2A | GAPD | RAG1 | ILF3 | BFI |
|---|---|---|---|---|---|---|---|---|
| *albagula* | DQ994905.1 | DQ018689.1 | DQ018689 | JN798307.1 | JN798216.1 | DQ995008 | JN798264.1 | – |
| *amplus* | DQ994912.1 | AY874880.1 | AY874880.1 | JN798308.1 | JN798217.1 | DQ995010 | JN798265.1 | – |
| *angusticla* | DQ994913.1 | DQ018676.1 | DQ018676.1 | – | JN798218.1 | DQ995011 | – | – |
| *caddoensis* | DQ994916.1 | AY874875.1 | AY874875.1 | JN798311.1 | JN798221.1 | DQ995013 | JN798268.1 | – |
| *chattahooc* | DQ994919.1 | – | – | JN798312.1 | JN798222.1 | DQ995014 | JN798269.1 | – |
| *cheoah* | DQ994921.1 | – | – | JN798313.1 | JN798223.1 | DQ995015 | JN798270.1 | – |
| *chlorobryo* | DQ994923.1 | – | – | JN798314.1 | JN798224.1 | – | JN798271.1 | – |
| *cinereus* | AY378030 | AY874875.1 | AY874875.1 | JN798317.1 | JN798226.1 | DQ995017 | – | – |
| *cylindrace* | DQ994928.1 | – | – | JN798318.1 | JN798229.1 | DQ995022 | JN798272.1 | – |
| *dorsalis* | AY378077.1 | DQ018678.1 | DQ018678.2 | JN798319.1 | – | DQ995023 | JN798273.1 | – |
| *electromor* | DQ994932.1 | DQ018665.1 | DQ018665.2 | JN798320.1 | JN798230.1 | DQ995025 | – | – |
| *elongatus* | AY691746.1 | – | – | – | – | – | – | – |
| *fourchensi* | DQ994933.1 | AY874876.1 | AY874876.2 | JN798321.1 | JN798232.1 | DQ995026 | JN798274.1 | – |
| *glutinosus* | – | – | – | – | – | – | – | KU841065 |
| *grobmani* | DQ994941.1 | DQ018695.1 | DQ018695.2 | JN798325.1 | JN798236.1 | DQ995028 | JN798277.1 | – |
| *hoffmani* | AY378048.1 | DQ018666.1 | DQ018666.2 | JN798327.1 | JN798238.1 | DQ995029 | – | – |
| *hubrichti* | AY378058.1 | DQ018667.1 | DQ018667.2 | JN798328.1 | JN798239.1 | DQ995030 | – | – |
| *idahoensis* | DQ994945.1 | – | – | JN798329.1 | – | DQ995031 | – | – |
| *jordani* | DQ994947.1 | AY874887.1 | AY874887.2 | JN798330.1 | – | DQ995032 | JN798278.1 | – |
| *kentucki* | DQ994948.1 | KU841032 | KU841032 | JN798331.1 | JN798240.1 | DQ995033 | JN798279.1 | KU841122 |
| *kiamichi* | DQ994950.1 | DQ018696.1 | DQ018696.2 | JN798332.1 | JN798241.1 | DQ995034 | JN798280.1 | – |
| *kisatchie* | DQ994951.1 | DQ018698.1 | DQ018698.2 | JN798333.1 | JN798242.1 | DQ995035 | JN798281.1 | – |
| *meridianus* | DQ994954.1 | AY874898.1 | AY874898.2 | JN798335.1 | JN798244.1 | DQ995038 | JN798283.1 | – |
| *metcalfi* | DQ994956.1 | AY874903.1 | AY874903.2 | – | JN798245.1 | DQ995040 | JN798284.1 | – |
| *mississipp* | DQ994958.1 | DQ018700.1 | DQ018700.2 | – | JN798246.1 | – | JN798285.1 | – |
| *montanus* | DQ994961.1 | AY874960.1 | AY874960.2 | JN798336.1 | JN798247.1 | DQ995042 | JN798286.1 | – |
| *nettingi* | DQ994964.1 | – | – | – | JN798248.1 | DQ995045 | – | – |

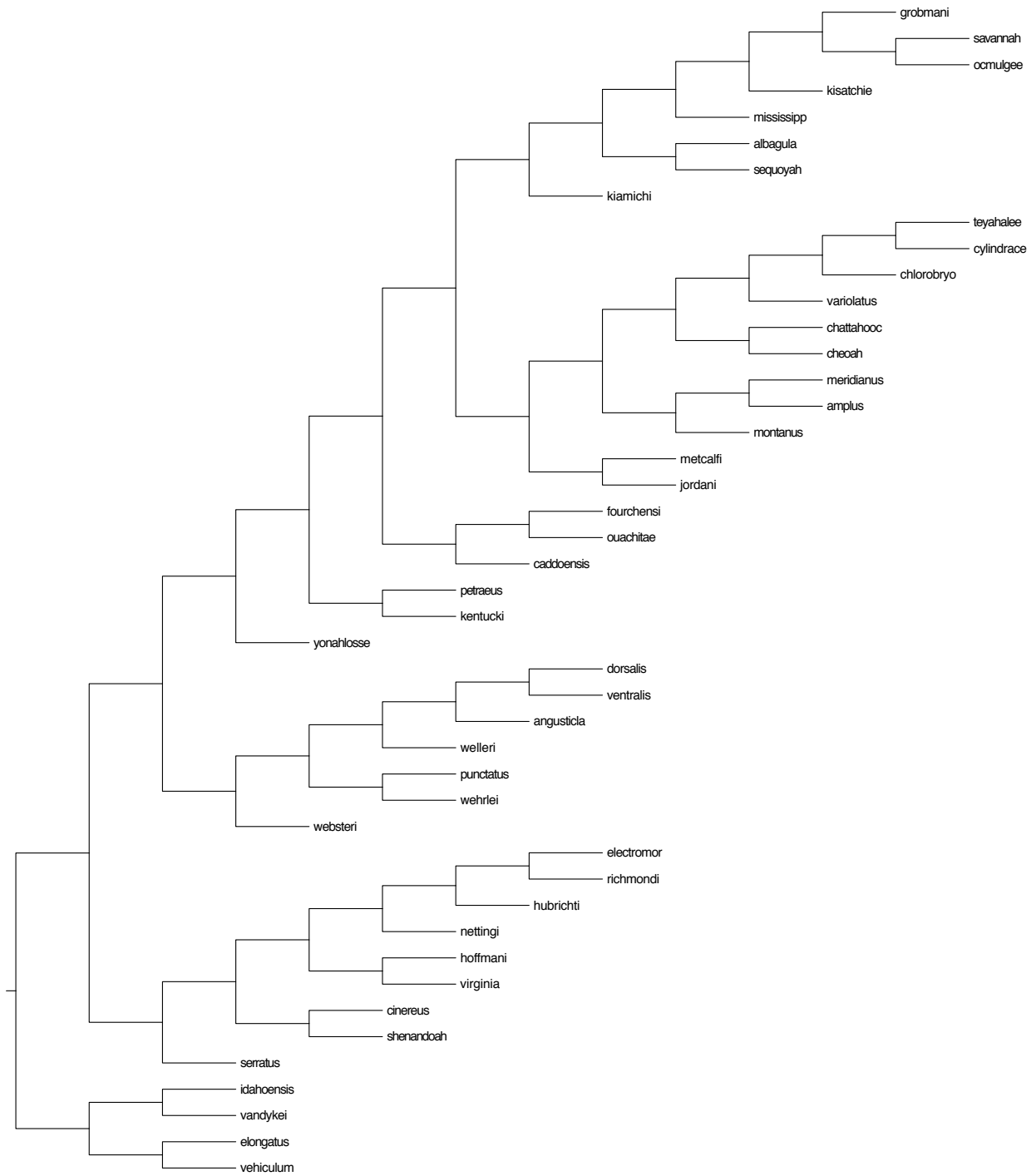| Species | Cyt b | ND2 | tRNAs | MLC2A | GAPD | RAG1 | ILF3 | BFI |
|---|---|---|---|---|---|---|---|---|
| *ocmulgee* | DQ994967.1 | DQ018702.1 | DQ018702.2 | JN798338.1 | JN798250.1 | DQ995048 | JN798288.1 | – |
| *ouachitae* | Y691747.1 | AY874877.1 | AY874877.2 | – | JN798251.1 | – | JN798289.1 | – |
| *petraeus* | DQ994973.1 | AY874878.1 | AY874878.2 | JN798339.1 | JN798252.1 | DQ995049 | JN798290.1 | – |
| *punctatus* | AY378078.1 | DQ018685.1 | DQ018685.2 | – | JN798253.1 | DQ995050 | JN798291.1 | – |
| *richmondi* | AY378070.1 | DQ018670.1 | DQ018670.2 | JN798340.1 | JN798254.1 | DQ995051 | – | – |
| *savannah* | DQ994978.1 | – | – | JN798341.1 | JN798255.1 | DQ995055 | JN798292.1 | – |
| *sequoyah* | DQ994979.1 | DQ018705.1 | – | JN798342.1 | – | DQ995056 | JN798293.1 | – |
| *serratus* | AY378073.1 | DQ018671.1 | DQ018671.2 | JN798343.1 | JN798256.1 | DQ995057 | – | – |
| *shenandoah* | AY378043.1 | DQ018674.1 | DQ018674.2 | JN798345.1 | JN798257.1 | DQ995062 | – | – |
| *teyahalee* | DQ994990.1 | – | – | JN798347.1 | – | DQ995068 | JN798295.1 | – |
| *vandykei* | AY691759.1 | – | – | – | JN798260.1 | – | – | – |
| *variolatus* | DQ994991.1 | – | – | JN798348.1 | – | DQ995069 | JN798296.1 | – |
| *vehiculum* | AY691760.1 | DQ018661.1 | DQ018661.2 | JN798349.1 | – | – | – | – |
| *ventralis* | DQ994993.1 | DQ018680.1 | DQ018680.2 | JN798350.1 | JN798261.1 | DQ995071 | JN798297.1 | – |
| *virginia* | AY378049.1 | DQ018675.1 | DQ018675.2 | JN798351.1 | – | DQ995072 | JN798298.1 | – |
| *websteri* | AY378076.1 | – | – | JN798352.1 | – | DQ995073 | JN798299.1 | – |
| *wehrlei* | AY378079.1 | DQ018686.1 | DQ018686.2 | JN798353.1 | – | DQ995075 | JN798300.1 | KU863657 |
| *welleri* | AY691761.1 | – | – | – | JN798262.1 | – | JN798301.1 | – |
| *yonahlosse* | AY378075.1 | AY874879.1 | AY874879.2 | JN798354.1 | JN798263.1 | DQ995078 | JN798302.1 | – |

**Figure A. Phylogeny of *Plethodon* used in the Multidivtime analyses**. See text for details.
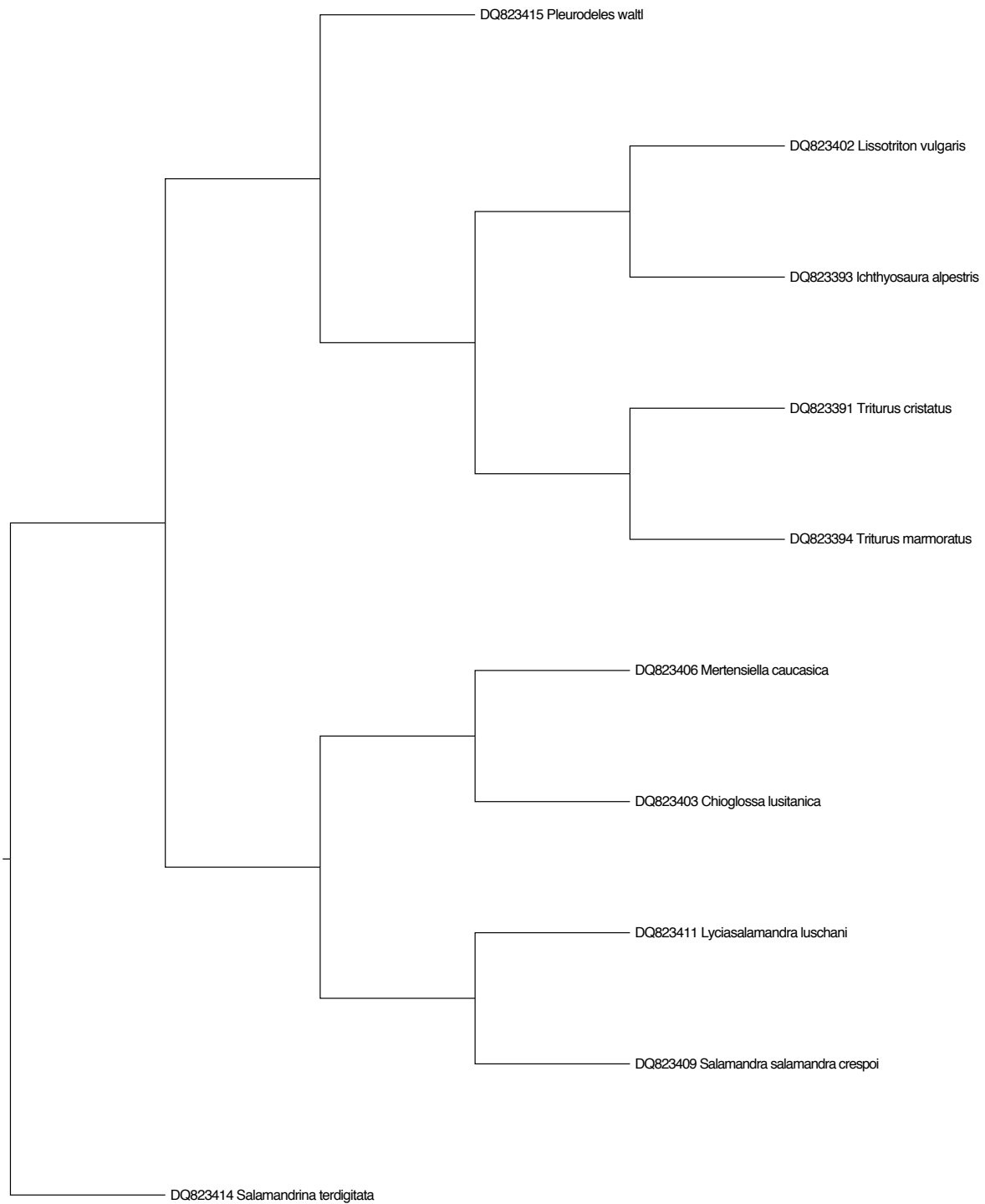
**Figure B. Phylogeny of** Salamandridae **used in the Multidivtime analyses**. See text for details.