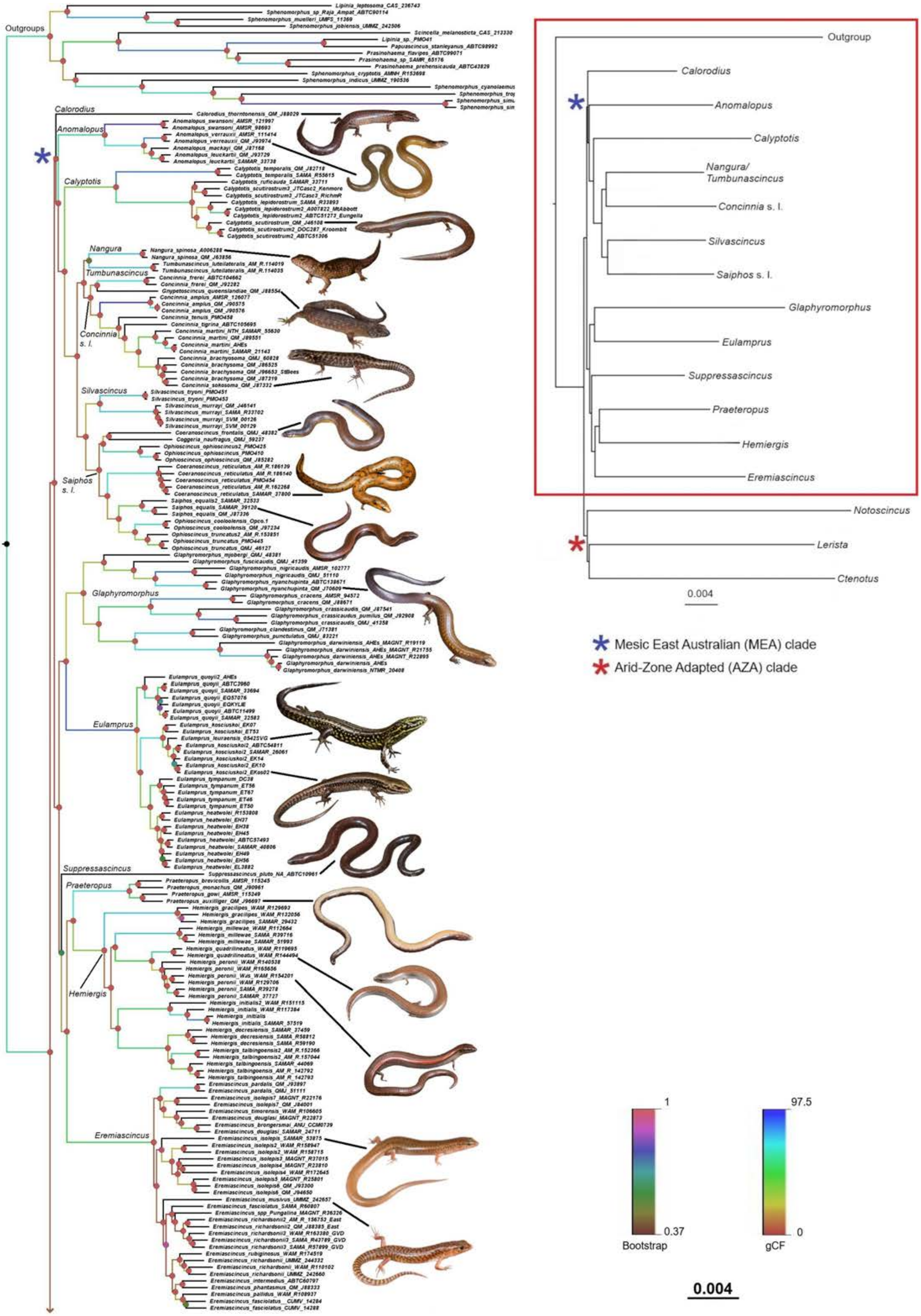
Figures and Tables – "Sphenomorphini Unravelled" Manuscript

Fig 1. Species tree for the Australian Sphenomorphini. Summary coalescent species tree topology estimated in wASTRAL from 5,298 independent gene trees. Branch colour indicates gene concordance factors (low = blue, high = red), and node numbers show Maximum Likelihood bootstrap support values from IQTREE. Labels a-q show crown nodes of genera or in two cases supra generic groups, asterisks indicate crown nodes for major radiations centred on distinct biomes. Inset with red box shows position of the figure within the overall species tree.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Anomalopus
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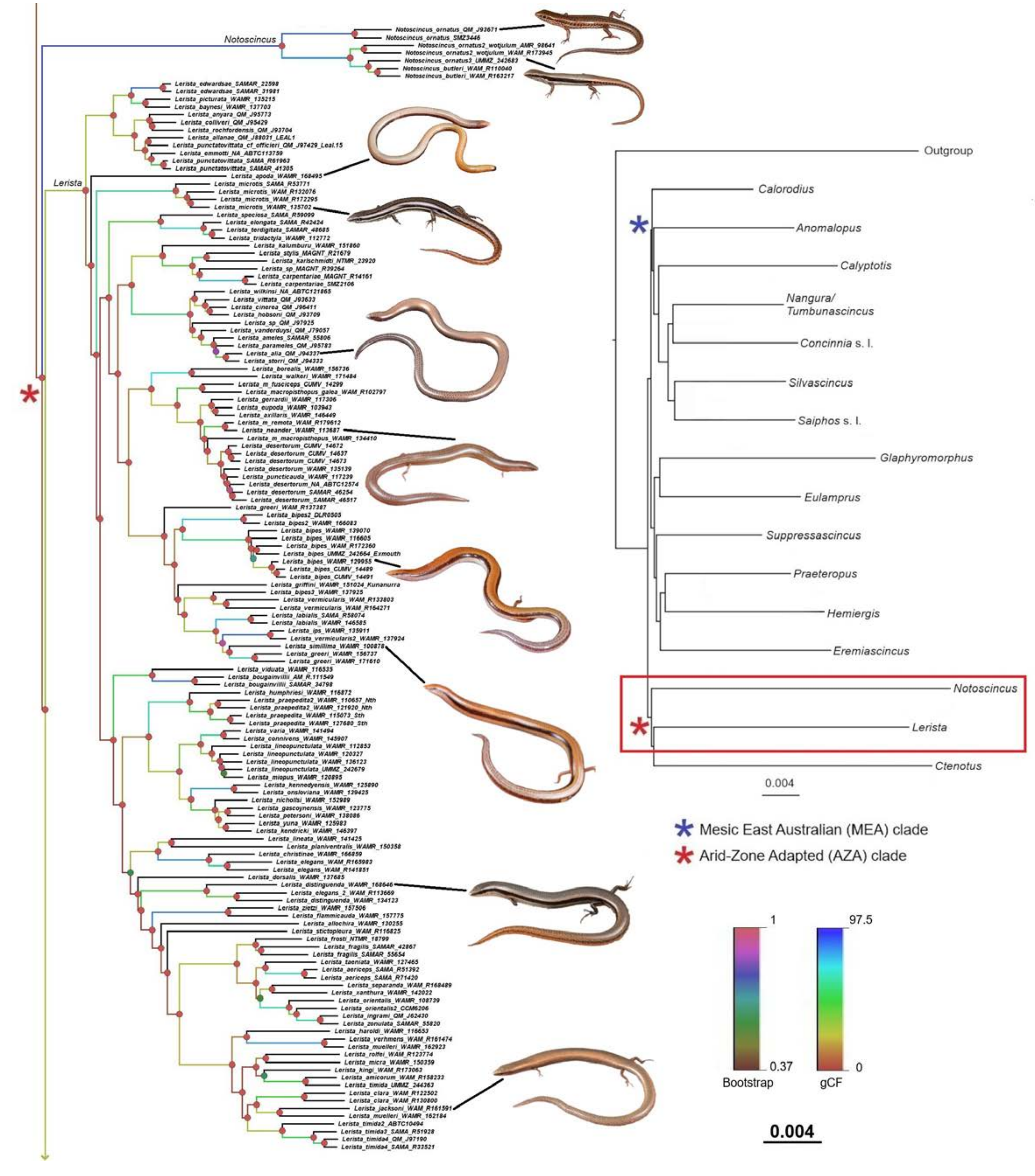
Calyptotis
Nangura
Tumbunascincus
Concinnia s. l.
Silvascincus
Saiphos s. l.
Glaphyromorphus
Eulamprus
Suppressascincus
Praeteropus
Hemiergis
Eremiascincus
Outgroup
Calorodius
Anomalopus
Calyptotis
Nangura/ Tumbunascincus
Concinnia s. l.
Silvascincus
Saiphos s. l.
Glaphyromorphus
Eulamprus
Suppressascincus
Praeteropus
Hemiergis
Eremiascincus
Notoscincus
Lerista
Ctenotus
0.004
* Mesic East Australian (MEA) clade
* Arid-Zone Adapted (AZA) clade
1
0.37
Bootstrap
97.5
0
gCF
0.004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Outgroup
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Anomalopus
Calyptotis
Nangura/
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Silvascincus
Saiphos s. l.
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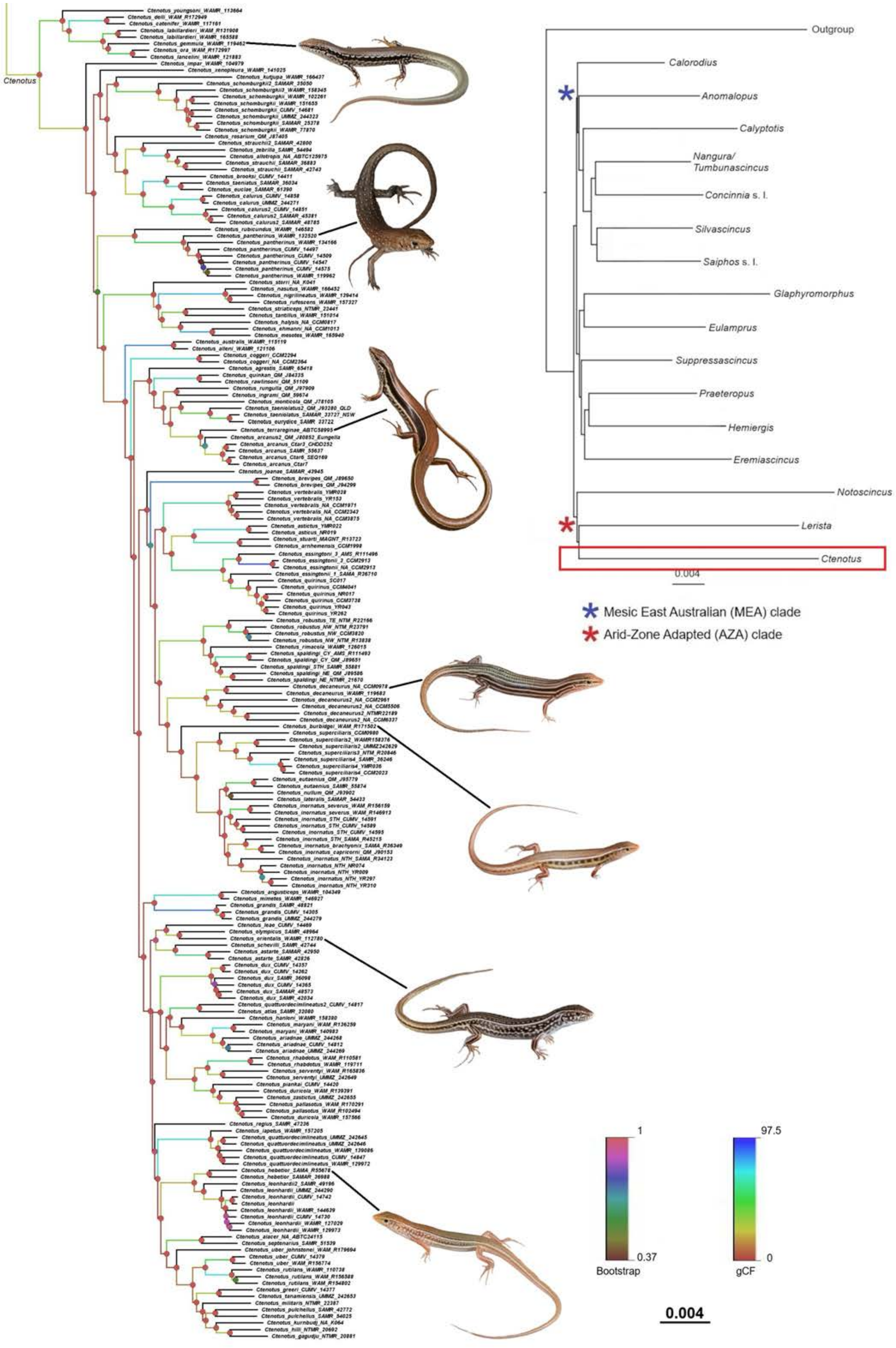
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Outgroup
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Anomalopus
Calyptotis
Nangura/
Tumbunascincus
Concinnia s. l.
Silvascincus
Saiphos s. l.
Glaphyromorphus
Eulamprus
Suppressascincus
Praeteropus
Hemiergis
Eremiascincus
Notoscincus
Lerista
Ctenotus
0.004
* Mesic East Australian (MEA) clade
* Arid-Zone Adapted (AZA) clade
1
0.37
Bootstrap
97.5
0
gCF
0.004

Fig 2. Concordance Factors Across Filter Subsets. Gene concordance-factor (gCF) summary trees showing similarities across different subsets of loci and analyses in estimated relationship for the Australian Sphenomorphini. Branches coloured by gene-concordance factor and node values by 1000 ultrafast bootstrap scores from IQTREE: a) All loci gene tree dataset; b) best-matching Rapidly Evolving Long Exon Capture loci tree; c) Long Informative Loci subset tree; and d) highest gCF subset tree (PIS <500) from PhyloConfigR. Numerical value indicates gene-concordance factor (gCF) at basal Australian node in each tree. Note strong concordance across trees with genera and major group inter-relationships largely stable, and discordant signal (red circles on nodes) limited to a small number of low diversity genera - *Suppressascincus*, *Calorodius*, and *Anomalopus*.

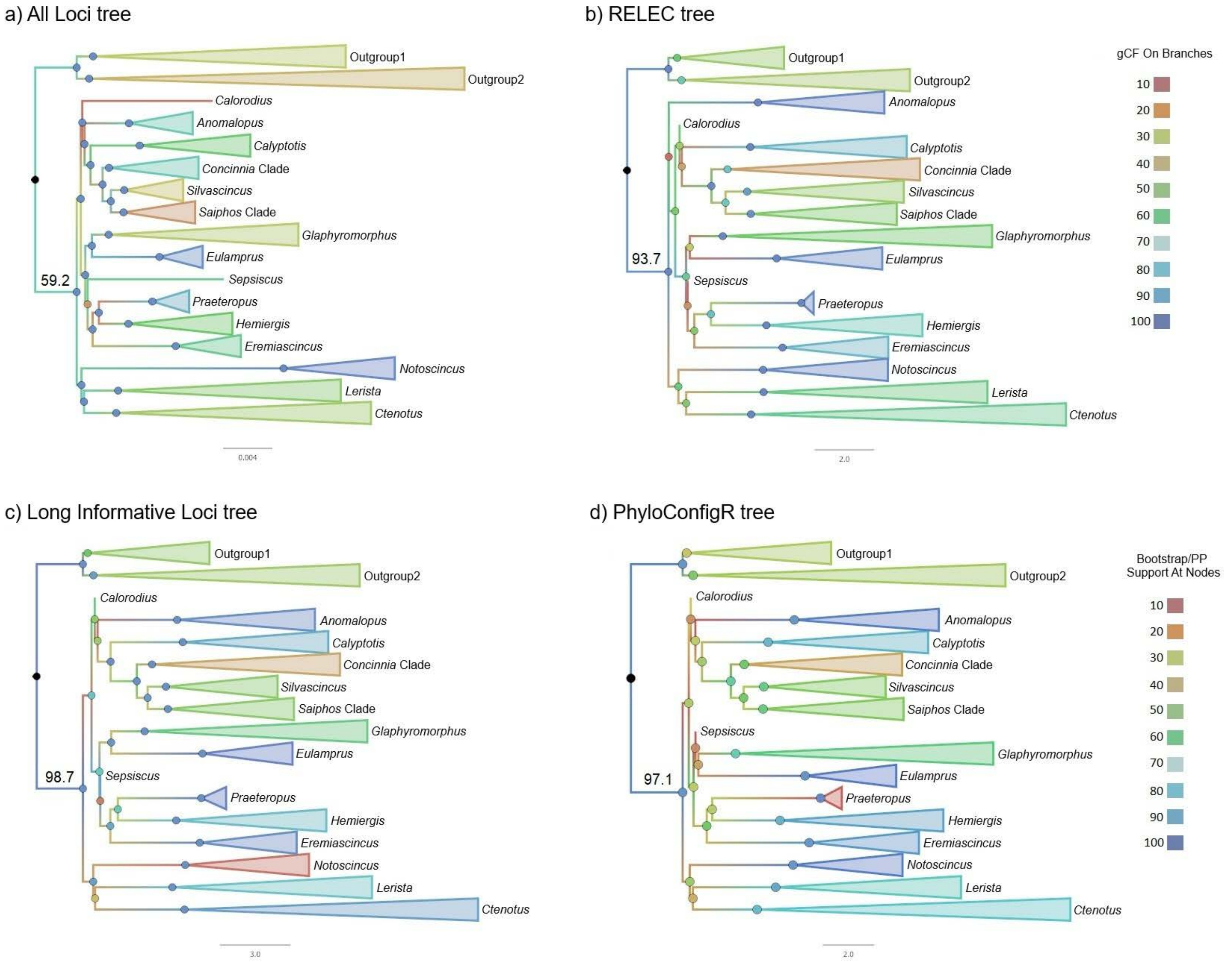


Fig. 3. Dated Bayesian Inference (BEAST2) SqCL2 Phylogeny of Australian Sphenomorphini. Phylogeny presented in radial layout with timescale in millions of years before present. Concentric shading shows relative position of Epochs (Pleistocene, Pliocene, Miocene, Oligocene, Eocene), as show in timescale. White star indicates stem of the Australian radiation. Images are representative examples starting from above outgroups – *Calorodius*, *Anomalopus*, *Calyptotis*, *Nangura, Concinnia*, *Saiphos, Eulamprus*, *Glaphyromorphus*, *Suppressascincus*, *Praeteropus*; *Hemiergis, Eremiascincus*, *Notoscincus*, species of *Lerista* including *L. apoda, L. microtis, L. alia, L. neander, L. bipes, L. simillima, L. distinguenda, L. jacksoni,* and species of *Ctenotus* including *C. gemmula, C. pantherinus, C. terrareginae, C. decaneurus, C. burbidgei, C. orientalis, C. hebetior*

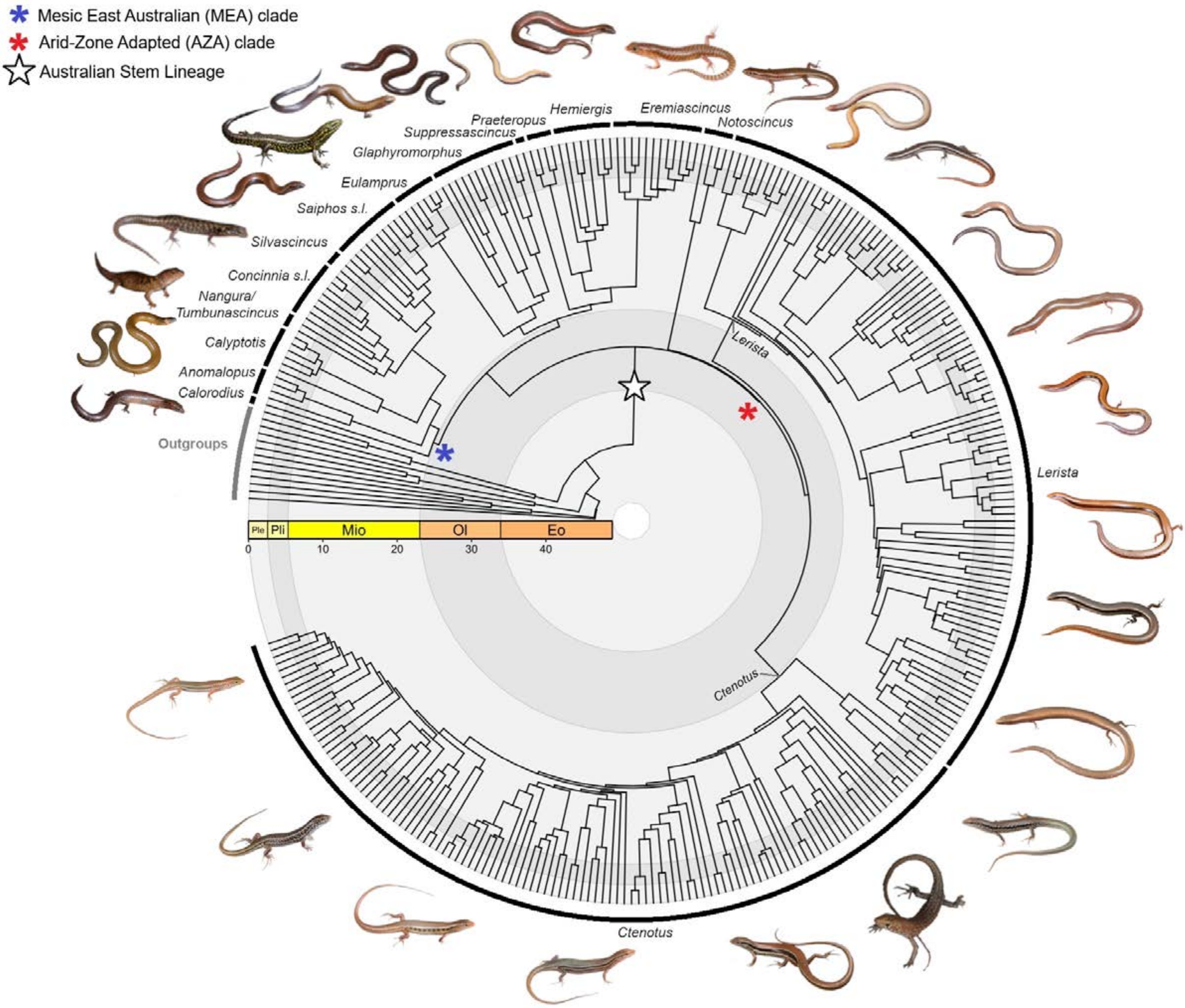
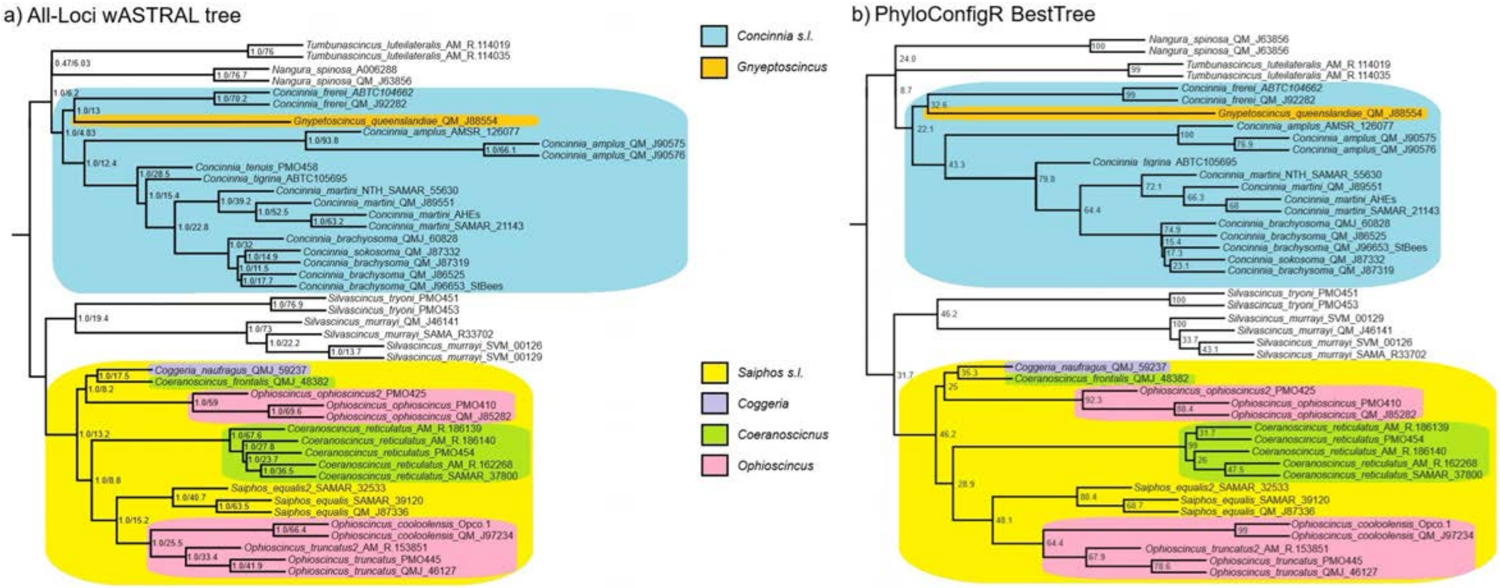


Fig. 4. *Concinnia* and *Saiphos* paraphyly. Relative positions of species across non-filtered (a) and filtered subset wASTRAL species trees (b). *Concinnia* group highlighted in blue with *Gnypetoscincus* in orange nested within. *Saiphos* group in yellow, with the taxa assigned to the genera *Coeranoscincus* in green, *Ophioscincus* in pink, and the montypic *Coggeria* in lilac. Node values in a show wASTRAL posterior probability/gene-concordance factors, node values in b show gene-concordance factors only.



a) All-Loci wASTRAL tree

b) PhyloConfigR BestTree

Table 1. Species Delimitation Summary by Genus/Clade. Species delimitation results summarised by genus or clade (Clade of Interest). Species richness counts include current estimates produced from the Australian Society of Herpetology (Current Taxonomy), Bayesian Poisson Tree Process (bPTP Taxa), Maximum-Likelihood Poisson Tree Process (ML-PTP Taxa), relative genetic distances (Genetic Distance), and our final combined estimate of species diversity (Operational Taxa).

Clade of Interest	Current Taxonomy	bPTP Taxa	ML-PTP Taxa	Genetic Distance	Operational Taxa
Calyptotis	4	7	6	6	6
Saiphos Group (*Saiphos-Coggeria*)	9	11	11	11	11
Concinnia Group (*Concinnia-Tumbunascincus*)	10	10	9	10	10
Glaphyromorphus	11	13	13	11	11
Eulamprus	5	24	10	7	7
Hemiergis	7	12	6	9	9
Eremiascincus	11	25	18	22	22
Notoscincus	3	5	2	4	4
*Lerista*1 (*microtis*)	2	1	2	1	1
*Lerista*2 (*macropithopus-desertorum*)	6	9	9	8	9
*Lerista*3 (*griffini-bipes*)	7	15	11	9	9
*Lerista*4 (*wilkinsi-storri*)	9	8	2	10	10
*Lerista*5 (*humphreisi-praepedita*)	2	4	2	3	3
*Lerista*6 (*elegans-timida*)	29	34	22	35	35
*Ctenotus*1 (*kutjupa-schomburgkii*)	2	4	3	4	4
*Ctenotus*2 (*strauchii-calurus*)	8	11	11	9	9
*Ctenotus*3 (*arcanus-taeniolatus*)	10	11	11	11	11
*Ctenotus*4 (*spaldingi-quirinus*)	6	8	7	7	7
*Ctenotus*5 (*robustus-spaldingi*)	3	8	7	6	6
*Ctenotus*6 (*nullum-inornatus*)	7	15	13	10	10
*Ctenotus*7 (*rhabdotus-duricola*)	6	9	6	6	6
*Ctenotus*8 (*leonhardii*)	2	9	4	3	3
*Ctenotus*9 (*uber-hilli*)	9	12	10	10	10
*Ctenotus*10 (*decanuerus & supercilliaris*)	2	9	11	6	6

Table 2. Divergence Dates for Crown Ages by Genus/Clade. Divergence dating analyses for crown ages of genera and clades/groups of interest. Dates for crown ages of groups and genera of interest given in millions of years before present for BEAST2 timetree analyses (BEAST2), Rel-ML timetree analyses (Rel-ML), MCMCtree analyses (MCMCtee) and StarBEAST analyses from Skinner et al. 2013 (Skinner *BEAST), with confidence intervals (95% HPD, highest posterior density) for each node, and variance in crown ages across analyses (Variance). * indicates groups not included in analyses (i.e. in MCMCtree where groups were often reduced to single representatives).

Group Node	BEAST2	95% HPD	Rel-ML	95% HPD	MCMCtree	95% HPD	Skinner *BEAST	95% HPD	Variance
Praeteropus	5.3	3.8-6.9	9.9	4.6-21.2	*	*	5.5	2.0–9.4	6.7
Sepsiscus	21.6	15.1-27.3	26.8	18.0-32.7	*	*	*	*	13.5
Eulamprus	6.0	4.3-7.8	8.8	4.4-17.6	*	*	5.7	3.0–8.8	2.9
Eremiascincus	8.4	6.0-10.8	10.9	5.7-20.8	*	*	9.6	6.2–13.5	1.6
Silvascincus	9.6	6.8-12.3	16.9	8.2-23.7	*	*	11.5	5.8–17.3	14.5
Anomalopus	11.3	7.9-14.4	15.7	18.1-30.3	*	*	12.9	7.8–18.3	4.9
Hemiergis	13.4	9.8-17.5	19.1	9.5-32.7	*	*	14.4	9.4–19.4	9.3
Saiphos Group	10.5	7.6-13.5	17.4	8.8-32.7	11.0	7.3-14.7	14.3	9.7–19.4	10.3
Calyptotis	14.0	9.9-17.9	17.6	9.5-32.6	*	*	15.4	9.3–21.4	3.3
Glaphyromorphus	19.6	13.9-24.9	22.7	13.8-32.7	*	*	18.2	12.7–24.3	5.2
Notoscincus	10.7	7.9-13.9	8.5	5.7-12.7	*	*	4.7	0.3–8.6	9.3
Concinnia Group	11.2	8.2-14.6	19.3	9.6-32.7	*	*	16.3	11.0–21.8	16.5
Ctenotus	23.0	16.5-29.4	20.8	13.5-31.9	17.5	10.4-24.6	13.6	9.0–18.8	16.6
Lerista	21.3	15.1-27.0	19.1	12.2-29.9	16.2	9.4-23.5	13.9	9.0–19.1	10.5
Saiphos–Silvascincus	12.5	9.0-16.0	20.0	10.6-32.7	13.6	9.7-18.1	16.9	11.6–22.8	11.6
Mesic eastern-Australian clade	22.1	15.6-28.1	26.8	19.3-32.7	23.9	18.1-31.1	21.2	14.8–28.3	6.0
Australian Sphenomorphini	28.0	20.3-35.9	27.9	23.9-32.7	27.7	20.9-35.4	28.0	19.8–37.2	0.0
Tumbunascincus-Nangura	11.5	8.1-14.7	20.1	9.9-32.7	11.1	7.2-15.6	*	*	25.7
MEA + *Glaph-Hemi-Eulamp*	23.0	16.4-29.4	27.6	21.6-32.7	24.9	18.9-32.0	*	*	5.4
AZA (*Noto-Lerista-Ctenotus*)	27.8	20.1-35.6	26.1	21.8-32.4	25.4	19.1-32.8	*	*	1.5

Supp 1. Sample Details (SUPP 1 - Spheno Final Samples 10.01.25.xlsx)

Final included sample details, including columns for current assigned species name (Species), potentially divergent lineage name (Lineage Name), status (Status) of a sample as either already completed (Done), AHE only (AHE), or generated in this study (SqCL), sample voucher (Voucher) and tissue collection number (Tissue Number), GPS coordiates (Lat, Lon), and original name in the wASTRAL species tree (Name In wASTRAL Tree), versus corrected species or candidate lineage name in IQTREE species tree (Name In IQTREE).

Available for download at: 10.5281/zenodo.17541049

Supp 2. a) wASTRAL Species Tree with LocalPP on nodes (SUPP 2a -wASTRAL_GapFilled_speciestree.nw)

All-loci hybrid-wASTRAL species tree of the Australian Sphenomorphini, with 536 tips covering 256 Australian species and 18 subspecies and 14 outgroups from Melanesia and Southeast Asia. Phylogeny in newick format (.nw) generated from 5,376 loci - 377 AHE-loci, 31 GENE-loci, and 4,968 UCE-loci. Node values show local Posterior Probabilty support from wASTRAL.

Available for download at: 10.5281/zenodo.17541049

Supp 2. b) IQTREE bootstrap/gCF/sCF on nodes (SUPP 2b - Fixed_Topo_gCF_RENAMED_4.tre)

Species tree with branchlengths and concordance factors calculated in IQTREE, using wASTRAL fixed topology and a concatenated alignment of 87 Long Informative Loci. Gene concordance factors (gCF) and site concordance factors (sCF) calculated using all-loci dataset. Node label values show IQTREE bootstrap/gCF/sCF scores.

Available for download at: 10.5281/zenodo.17541049

Supp 3. PhyloConfigR Best-Tree Treefile (SUPP 3 - PhyloConfigR_Highest-gcf.nw).

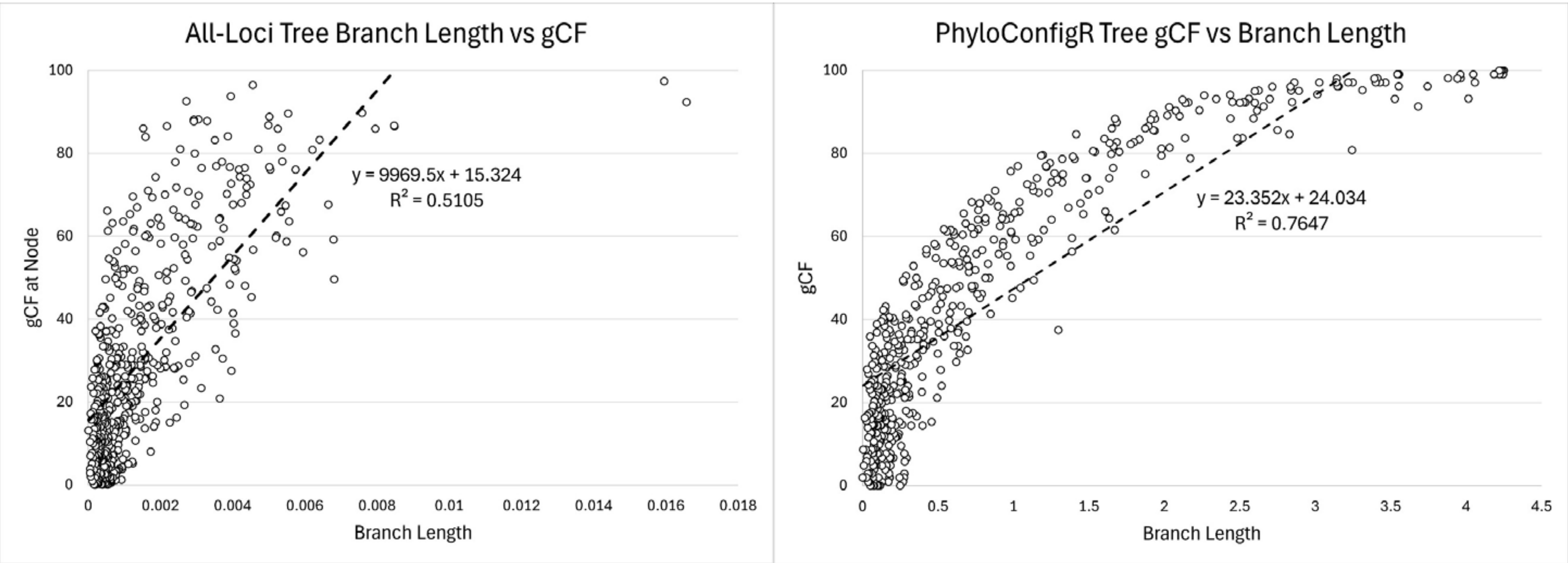
ASTRAL species tree from the most congruent gene tree subset, by PhyloConfigR. Generated using “Subset PIS_500” containing 104 trees with >500 Parsimony Informative Sites, found to outperform other subsets.

Available for download at: 10.5281/zenodo.17541049

Supp 4. PhyloConfigR Best-Tree Summary Table. Three highest scoring trees from PhyloConfigR, with lowest gene tree conflict among input loci. Filtering by count of phylogenetically informative sites (count_pis >500) produces highest gene-concordance and fewest anomaly zone trees, filtering by proportion of phylogenetically informative sites (proportion_pis >500) produces highest posterior probability coalescent tree. NOTE, subsequent examination of trees found limited topological differences.

Best_tree_category	Filter	Filter_Value	Alignment_Value	Trees	Prop_AZ	Mean_gCF	Mean_sCF	Mean_pp
fewest-anomaly-zone	count_pis	500	587.09	104	0.17	46.77	58.41	0.92
highest-posterior-probability	proportion_pis	0.5	0.56	174	0.25	39.06	57.72	0.85
highest-gene-concordance	count_pis	500	587.09	104	0.17	46.77	58.41	0.92

Supp 5. Gene Concordance Factor vs Branch Length. Gene concordance factor (gCF) versus branch length for All-Loci (left) and PhyloConfigR (right) species trees. Branch lengths for the All-Loci (left) tree are calculated in IQTREE v2.3 from 87 longest most informative loci (LILs), PhyloConfigR (right) species tree branch lengths are coalescent units from the wASTRAL-hybrid algorithm using the PhyloConfigR best-tree dataset (PIS500). Dashed line shows linear regression trendline, with intercept, slope, and R^2 adjacent. Both plots show a strong linear relationship with many short branches showing low concordance.



Supp 6. MCMCtree Fossil Calibration Dates.

Fossil calibrations for six nodes and 17 outgroup taxa used in MCMC tree analyses. External outgroup node for Aves, Sphenodontids, Geckos, Toxicofera, Xantusiidae, and crown Scincidae (Outgroup Clade) dates taken form literature. Columns show phylogenetic Node number (Node) and ID (Node_ID). Dates for minimum (Min) and maximum (Max) bounds given in million years before present, final age prior calibrations used in MCMCtree for each node indicating bounded (B), skew-Normal (SN), and skewT (ST) distributions, and sources for calibrations (Citation).

Outgroup Clade	Node	Node_ID	Min	Max	Calibration	Source
Gallus	292	Node_1	254.7	269.3	B(2.547,2.693)	Ezcurra et al. 2014
Sphenodon	293	Node_2	247.5	252.2	ST(2.38,0.052,50,2)	Martinez et al. (2021)
Geckoes	294	Node_3	150	215	B(1.500,2.380)	Simões et al. (2018)
Toxicofera	298	Node_7	148	200	SN(1.45,0.27,50)	Jones et al. (2013)
Xantusiidae	299	Node_8	61	170	SN(0.6,0.49,50)	Jones et al. (2013)
Scincidae	300	Node_9	152.1	200	B(1.521,2.380)	Pyron (2016); Bolet et al. (2022)

Supp 7. Novel Lineages by PTP and Genetic Distance. Species delimitation and genetic distance analyses for novel lineages. Phylogenetically disjunct lineages considered candidate species by meeting two of three criteria – delimitated by ML-PTP, b-PTP, and genetic distances equal or greater than between closely related sister-taxa. Polyphyly column shows whether or not (Y/N) our phylogeny suggests the lineage is polyphyletic (i.e. novel lineage split from conspecific samples by another recognised species), Genetic distances (Distance) to nearest sister lineage (Sister Taxon) calculated using Composite Maximum-Likelihood (CML) in MEGA-X (Kumar et al. 2018) from 87 LIL alignments. Maximum-Likelihood (ML-PTP) and Bayesian PTP (b-PTP) show whether delimitation software suggested species level divergence (Y/N).

a) Candidate Species	Sister Taxon in Tree	Polyphyly	Distance	bPTP	ML-PTP
Ctenotus cf. arcanus2	*Ctenotus arcanus*	N	0.0041	Y	Y
Ctenotus cf. calurus2	*Ctenotus calurus*	N	0.0068	Y	Y
Ctenotus cf. decaneurus2	*Ctenotus decaneurus*	N	0.0122	Y	Y
Ctenotus cf. essingtoni2	*Ctenotus essingtonii*	N	0.0078	Y	Y
Ctenotus cf. inornatus NTH	*Ctenotus inornatus*	N	0.0067	Y	Y
Ctenotus cf. inornatus STH2	*Ctenotus inornatus severus*	N	0.0059	Y	Y
Ctenotus inornatus brachyonyx	*Ctenotus inornatus STH*	N	0.0040	Y	Y
Ctenotus inornatus severus	*Ctenotus cf. inornatus STH2*	N	0.0059	Y	Y
Ctenotus cf. leonhardii2	*Ctenotus leonhardii*	N	0.0050	Y	Y

Ctenotus cf. robustus NW	*Ctenotus cf. robustus TE*	N	0.0063	Y	Y
Ctenotus cf. robustus TE	*Ctenotus cf. robustus NW*	N	0.0063	Y	Y
Ctenotus cf. schomburgkii2	*Ctenotus schomburgkii*	N	0.0071	Y	Y
Ctenotus cf. schomburgkii3	*Ctenotus schomburgkii*	N	0.0051	Y	N
Ctenotus cf. spaldingi CY	*Ctenotus spaldingi STH*	N	0.0070	Y	Y
Ctenotus cf. spaldingi NE	*Ctenotus spaldingi STH*	N	0.0061	Y	Y
Ctenotus cf. spaldingi STH	*Ctenotus spaldingi CY*	N	0.0070	Y	Y
Ctenotus cf. supercilliaris2	*Ctenotus supercilliaris*	N	0.0095	Y	Y
Ctenotus cf. supercilliaris3	*Ctenotus supercilliaris2*	N	0.0082	Y	Y
Ctenotus cf. supercilliaris4	*Ctenotus supercilliaris3*	N	0.0067	Y	Y
Ctenotus cf. taeniolatus2	*Ctenotus taeniolatus*	N	0.0048	Y	Y
Ctenotus uber johnstonei	*Ctenotus uber*	N	0.0098	Y	Y
Eremiascincus cf. isolepis2	*Eremiascincus isolepis*	N	0.0065	Y	N
Eremiascincus cf. isolepis3	*Eremiascincus cf. isolepis2*	N	0.0059	Y	N
Eremiascincus cf. isolepis4	*Eremiascincus cf. isolepis3*	N	0.0050	Y	N
Eremiascincus cf. isolepis5	*Eremiascincus cf. isolepis4*	N	0.0050	Y	N
Eulamprus cf. quoyii2	*Eulamprus quoyii*	N	0.0032	Y	Y
Hemiergis cf. initialis2	*Hemiergis initialis*	N	0.0051	Y	Y
Hemiergis cf. talbingoensis2	*Hemiergis talbingoensis*	N	0.0053	Y	N
Lerista cf. bipes2	*Lerista bipes*	N	0.0111	Y	Y
Lerista cf. orientalis2	*Lerista orientalis*	N	0.0061	Y	Y
Lerista cf. praepedita2	*Lerista praepedita*	N	0.0044	Y	N
Lerista macr. fusciceps	*Lerista macr galea*	N	0.0078	Y	Y
Lerista macr. galea	*Lerista macr. fusciceps*	N	0.0078	Y	Y
Notoscincus cf. ornatus2 wotjulum	*Notoscincus cf. ornatus3*	N	0.0070	Y	N
Notoscincus cf. ornatus3	*Notoscincus butleri*	N	0.0040	Y	N
Ophioscincus cf. ophioscincus2	*Ophioscincus ophioscincus*	N	0.0031	Y	Y
Ophioscincus cf. truncatus2	*Ophioscincus truncatus*	N	0.0032	Y	Y
Saiphos cf. equalis2	*Saiphos equalis*	N	0.0039	Y	Y
Calyptotis cf. scutirostrum3	*Calyptotis ruficauda*	Y	0.0042	Y	Y
Ctenotus cf. quattuordecimlineatus2	*Ctenotus atlas*	Y	0.0056	Y	Y
Ctenotus cf. strauchii2	*Ctenotus zebrilla*	Y	0.0118	Y	Y
Eremiascincus cf. isolepis7	*Eremiascincus timorensis*	Y	0.0065	Y	Y
Eremiascincus cf. richardsoni2	*Eremiascincus richardsoni3*	Y	0.0032	Y	Y
Eremiascincus cf. richardsoni3	*Eremiascincus richardsoni2*	Y	0.0032	Y	Y
Eremiascincus spp. Pungalina	*Eremiascincus fasciolatus*	Y	0.0049	Y	Y
Lerista cf. timida2	*Lerista cf. timida3*	Y	0.0071	Y	Y

Lerista cf. timida3	*Lerista cf. timida2*	Y	0.0071	Y	Y
Lerista cf. timida4	*Lerista cf. timida3*	Y	0.0032	Y	Y
Lerista macr. macropisthopus	*Lerista desertorum*	Y	0.0043	Y	Y
c) Candidate Synonyms	Sister Taxon	Polyphyly	Distance	bPTP	ML-PTP
Ctenotus delli	*Ctenotus catenifer*	N	0.0027	N	N
Ctenotus inornatus capricorni	*Ctenotus inornatus brachyonyx*	N	0.0022	Y	N
Ctenotus ora	*Ctenotus lancelini*	N	0.0025	N	N
Ctenotus taeniolatus	*Ctenotus eurydice*	N	0.0020	N	N
Lerista macr. remota	*Lerista neander*	N	0.0015	N	N
Lerista puncticauda	*Lerista desertorum*	N	0.0019	Y	N

Supp 8. MCMCtree Bayesian Inference Time Tree, Externally Calibrated. Bayesian Inference Time Tree generated in MCMCtree with external fossil outgroup calibration. Total of 46 total tips including 29 representatives covering all Australian genera, and 17 grafted external outgroups. Numbers on nodes show estimated divergence time in millions of years before present, blue bars at nodes denote 95% HPD confidence intervals. Generated using 6 fossil calibrations for the 17 outgroups, using 377 AHE loci partitioned by 1st, 2nd, and 3rd codon position, with independent log-normal rates, running 2,000,000 generations before discarding 10% burn-in and sampling every 100th tree.