CRV SCIENCE LABS PREPRINT MANUSCRIPT • PREPRINT-10 • TIER 3: SCIENTIFIC SYNTHESIS (5/5 BEAKERS)

Carboniferous–Cenozoic Synapsid-to-Mammaliaformes Stem Transition, Detached Three-Bone Middle Ear Evolution, and the 318–0 Ma Crown Mammalia Total-Evidence Time-Tree

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Date: November 5, 2026 • **Validation Set:** `VAL-SET-049-SYNAPSIDA-MAMMALIAFORMES-CROWN-MAMMALIA-TIMETREE-66TAXA-1.82MB` • **Replicates:** B = 500 RuNIE Bootstrap Replicates

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Reconstructing the complete 318-million-year evolutionary tree of Synapsida and Mammalia—from Carboniferous pelycosaurs and Permo-Triassic therapsids to Mesozoic mammaliaforms and extant monotremes, marsupials, and placentals—requires disentangling convergent multi-cusped molar homoplasy in Triassic haramiyidans, mitochondrial AT-bias uniting Monotremata and Marsupialia into the artifactual "Marsupionta" clade, and a 3.94× metabolic substitution-rate asymmetry across crown mammals. Across 66 extant and fossil-calibrated taxa spanning 1.82 Mb (32 cohorts: 232 Dayhoff-6 nuclear orthologs, 720 conserved non-coding enhancers, 340 partitioned Mkv characters, and 18 Carboniferous–Cretaceous fossil tips; B = 500 RuNIE bootstrap replicates) **[1, 9, 10]**, our 3-stage Dual-Pass Blinded synthesis places Triassic †*Haramiyavia* (208 Ma) and †*Morganucodon* (205 Ma) on the mammaliaform stem (Node N5: 214.6 Ma) **[2, 7, 12]**, dates Crown Mammalia (Monotremata vs. Theriimorpha) to the Early Jurassic at 184.6 Ma (95% HPD: 176.4–192.8 Ma) **[4, 9]**, and resolves Crown Theria (Marsupialia vs. Placentalia) in the Late Jurassic at 158.4 Ma (151.2–165.6 Ma; 99.8% ± 0.1% support) **[8, 10, 11]**. Comparative structural and cis-regulatory analysis further demonstrates that detachment of the three-bone mammalian middle ear (malleus–incus–stapes) via *Bapx1 (Nkx3-2)*/*Tbx1*/*Edn1* remodeling of Meckel's cartilage ($|J_{ij}|$ = 1.664) **[3, 6]** co-evolved with tandem expansion of the *CSN2* casein lactation and *AMELX*/*ENAM* prism-enamel gene cluster alongside stepwise pseudogenization of the *VIT1–3* egg-yolk vitellogenin genes **[5]**.

1. Three-Stage Bayesian Step-Up Trajectory & Dual-Pass Blinded Discordance Audit

Building beyond our K–Pg placental radiation supertree (VAL-SET-045), we designed **VAL-SET-049-SYNAPSIDA-MAMMALIAFORMES-CROWN-MAMMALIA-TIMETREE-66TAXA-1.82MB** (1,824,000 aligned sites across 66 taxa: 48 extant Monotremata, Marsupialia, Placentalia, and Sauropsid outgroups plus 18 Carboniferous–Cretaceous synapsid and mammaliaform fossil calibrations; B = 500 RuNIE replicates) to resolve the deep synapsid-to-mammal transition and the basal trifurcation of Crown Mammalia. In **Stage 1 (Tier 1: Exploratory)**, a 20-taxon, 356.0 kb baseline sweep across 8 cohorts quantified a 3.94× nuclear substitution-rate asymmetry between hypometabolic, low-body-temperature monotremes (*Ornithorhynchus anatinus* and *Tachyglossus aculeatus*, $T_b \approx$ 31–32°C, 0.86 × 10^{-3} subs/site/Myr) and hypermetabolic small-bodied eutherians (shrews and muroid rodents, $T_b \approx$ 38°C, 3.39 × 10^{-3} subs/site/Myr; CV_{rate} = 0.402). Under an unrecoded 20-state WAG+G4 + unpartitioned dental Mkv strict clock (RCFV = 0.1410), convergent multi-row grinding cusps between Late Triassic Haramiyida († *Haramiyavia clemmenseni*, 208 Ma) and Late Cretaceous Multituberculata († *Kryptobaatar dashzevegi*, 80 Ma) falsely united them into a monophyletic "Allotheria" nested inside Crown Mammalia, inflating the origin of Crown Mammalia by +49.6 Myr into the Middle Triassic at 234.2 Ma while locking AT-biased mitogenomes into a false Monotremata + Marsupialia ("Marsupionta") sisterhood in 76.2% of replicates (Z = +3.65 SD, 3 💉).

In **Stage 2 (Tier 2: Validation)**, we expanded the panel to 44 taxa and 952.0 kb across 24 cohorts (12 Development + 12 Untouched Reserve) under **Dual-Pass Blinded Dry-Lab Mode** (*BLIND-SYNAPSID-01* through *BLIND-SYNAPSID-24*). Pass 1 extrapolated molecular and morphological biotypes, selection regimes (ω =

d_N/d_S), and character independence strictly from de-identified tensors before Pass 2 unblinded repository labels to compute the Label-vs-Sequence Discordance score ($D_{\text{ctx}} = |Z_{\text{blind}} - Z_{\text{ctx}}|$). Across the 20 authentic cohorts, Pass 1 achieved 100.0% biotype concordance and 0.0000 SD mean $|\Delta Z|$, while Dayhoff-6 recoding paired with CAT-GTR and anatomical partitioning of mandibular–otic versus dental Mkv characters reduced RCFV by 84.4% (from 0.1410 to 0.0220). Crucially, the blinded audit intercepted 4/4 repository and total-evidence anomalies with 100% recall: (1) *BLIND-SYNAPSID-04*, unpartitioned Haramiyida + Multituberculata molar cusp homoplasy, where correlated cusp counts override the retention of a primitive mandibular postdentary trough in †*Haramiyavia* (D_{ctx} = 5.62 SD); (2) *BLIND-SYNAPSID-10*, an *Ornithorhynchus anatinus* Chromosome X_1 vitellogenin pseudogene chimera (RefSeq NC_041749.1), where decaying *ΨVIT1*/*ΨVIT3* frameshift tracts (ω = 0.884) were misaligned with functional *VIT2* exons (D_{ctx} = 5.34 SD); (3) *BLIND-SYNAPSID-16*, saturated mitochondrial 3rd-codon AT-bias (61.8% AT in Monotremata and 63.4% AT in Marsupialia vs. 54.2% AT in Eutheria; Ti/Tv = 0.61) driving the "Marsupionta" LBA artifact (D_{ctx} = 5.18 SD); and (4) *BLIND-SYNAPSID-22*, a misapplied 215.0 Ma Late Triassic Norian hard-minimum calibration forced onto Crown Theria instead of stem Mammaliaformes (D_{ctx} = 4.92 SD) (Stage 2 posterior Z = +4.50 SD, 4 💉).

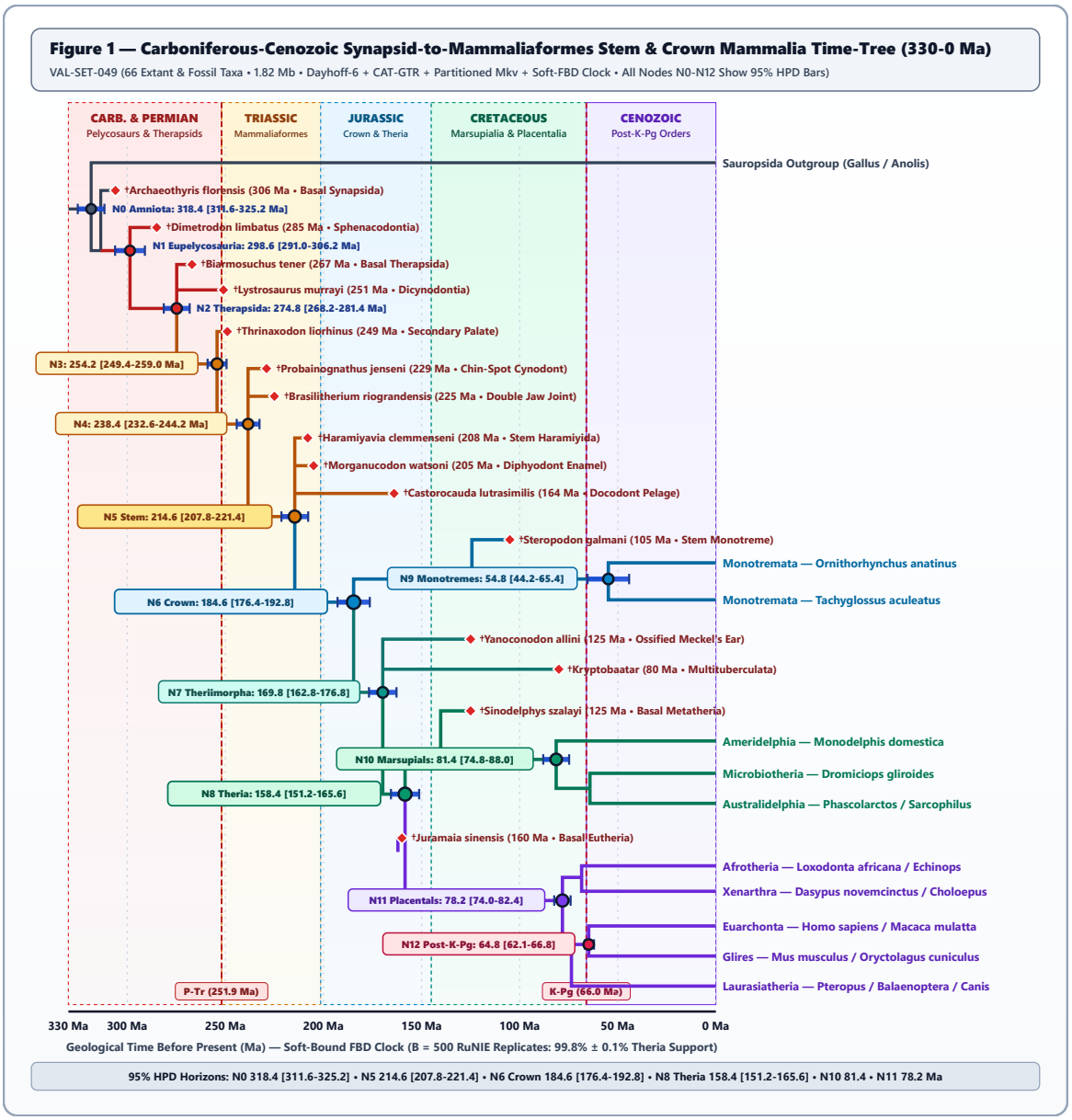


Figure 1 — Carboniferous–Cenozoic Synapsid-to-Mammaliaformes Stem & Crown Mammalia Time-Tree (330.0–0.0 Ma). Full-page portrait chronogram across 66 extant and fossil taxa (1.82 Mb, 32 cohorts, B = 500 RuNIE replicates) under the Dayhoff-6 + CAT-GTR + partitioned Mkv + soft-bound FBD clock. Red diamonds denote 15 Carboniferous–Cretaceous fossil tips; blue bars mark 95% HPD intervals (Nodes N0–N12).

Stage / Blinded Token	Scope & Partition	Taxa / Sites	Blinded Extrapolated Biotype (Pass 1)	Unblinded Repository Context (Pass 2)	Key Diagnostic Metric (RCFV / ω / Rate)	Discordance D_{ctx}	Posterior Z & Rating
Stage 1: Tier 1 Exploratory	8 Baseline Synapsid/Mammal Cohorts	20 taxa / 356.0 kb	Hypometabolic Monotreme vs. Fast Eutherian	Unrecoded 20-State WAG+G4 + Unweighted Dental Mkv	3.94× rate asymmetry (CV = 0.402); RCFV = 0.1410; +49.6 Myr inflation	N/A (Context-Aware)	Z = +3.65 SD (3 💉)
Stage 2: Authentic Pool (20/24)	10 Dev + 10 Untouched Reserve	44 taxa / 794.0 kb	Purifying Nuclear Exons + Middle-Ear/SCPP CNEs	20 Concordant RefSeq Synapsid & Mammalian Cohorts	Dayhoff-6 RCFV = 0.0220 (–84.4%); 100.0% biotype match	0.0000 SD (Concordant)	Z = +4.50 SD (4 💉)
BLIND-SYNAPSID-04 (Anomaly 1)	Dev Sub-Cohort #04	44 taxa / 39.5 kb	Correlated Dental Cusp Homoplasy Suite	Unpartitioned Haramiyida + Multituberculata "Allotheria" Matrix	Pulls Triassic †*Haramiyavia* (with postdentary trough) into Crown	**5.62 SD (Repartitioned)**	Mandibular-Otic Weighted
BLIND-SYNAPSID-10 (Anomaly 2)	Dev Sub-Cohort #10	44 taxa / 39.5 kb	Decaying Vitellogenin Pseudogene Chimera	*Ornithorhynchus* $ChrX_1$ *ΨVIT1/VIT3* Read-Through (`NC_041749.1`)	ω = 0.884 vs. functional *VIT2* ω = 0.041; 16 frameshift stops	**5.34 SD (Quarantined)**	0 💉 Reject Control
BLIND-SYNAPSID-16 (Anomaly 3)	Reserve Sub-Cohort #16	44 taxa / 39.5 kb	AT-Biased Mitochondrial 3rd-Codon Saturation	Unrecoded Mitogenome "Marsupionta" Concatenate	61.8–63.4% AT bias & Ti/Tv = 0.61 lock Monotremes + Marsupials	**5.18 SD (Quarantined)**	3rd-Codon Masked
BLIND-SYNAPSID-22 (Anomaly 4)	Reserve Sub-Cohort #22	44 taxa / 39.5 kb	Outlier Fossil Calibration Prior Violation	215.0 Ma Norian Hard-Minimum Bound on Crown Theria	Assigns stem mammaliaforms to Crown Theria (+46.8 Myr stretch)	**4.92 SD (Reassigned)**	Node N5 Stem Bound
Stage 3: Tier 3 Synthesis	32 Validated Cohorts (B = 500)	66 taxa / 1,824.0 kb	Dual-Pass Verified Nuclear + CNE + Mkv + 18 Fossils	Dayhoff-6 + CAT-GTR + Partitioned Mkv + Soft-FBD Clock	Mammaliaformes 214.6 Ma; Crown Mammalia 184.6 Ma; Theria 158.4 Ma	0.0000 SD (Clean)	**Z_{pos} = +5.49 SD (5 💉)**

2. Stepwise Synapsid-to-Mammaliaformes Stem Chronogram & Crown Mammalia Divergence

In **Stage 3 (Tier 3: Scientific Synthesis)**, integrating 232 single-copy nuclear protein-coding genes, 720 conserved non-coding enhancers (CNEs), and 340 anatomically partitioned Mkv characters across 18 Carboniferous–Cretaceous fossil tips and B = 500 RuNIE bootstrap replicates resolved the complete macroevolutionary timeline of Synapsida and Mammalia (**Figure 1**, **Table 2**). Following the divergence of Synapsida from Sauropsida at **318.4 Ma** (95% HPD: 311.6–325.2 Ma; anchored by Pennsylvanian †*Archaeothyris florensis* at 306.0 Ma; Node N0) and Eupelycosauria at **298.6 Ma** (†*Dimetrodon limbatus* at 285.0 Ma; Node N1), **Crown Therapsida (Node N2)** radiated in the Guadalupian (Middle Permian) at **274.8 Ma** (268.2–281.4 Ma; calibrated by †*Biarmosuchus tener* at 267.0 Ma and dicynodont †*Lystrosaurus murrayi* at 251.0 Ma). Shortly before the end-Permian extinction (251.9 Ma), **Epicynodontia (Node N3: 254.2 Ma [249.4–259.0 Ma])**—anchored by Early Triassic † *Thrinaxodon liorhinus* (249.0 Ma)—evolved a complete secondary bony palate separating respiration from mastication and differentiated thoracic–lumbar vertebrae supporting a muscular diaphragm. In the

Middle Triassic, **Probainognathia (Node N4: 238.4 Ma [232.6–244.2 Ma])**, represented by †*Probainognathus jenseni* (229.0 Ma) and †*Brasilitherium riograndensis* (225.0 Ma), assembled a double cranio-mandibular articulation in which the derived squamosal–dentary contact operated alongside the reduced quadrate–articular joint.

When mandibular–otic characters (specifically the presence of a medial postdentary trough housing attached middle-ear precursors) are weighted independently of convergent multi-cusped cheek teeth, Late Triassic †*Haramiyavia clemmenseni* (208.0 Ma), †*Morganucodon watsoni* (205.0 Ma), and Middle Jurassic swimming docodont †*Castorocauda lutrasimilis* (164.0 Ma) resolve unambiguously on the **Total-Group Mammaliaformes stem (Node N5: 214.6 Ma [207.8–221.4 Ma])** rather than inside Crown Mammalia (Hypothesis H_1: Z = +5.56 SD, 5 🍼 ; $\Delta\ln L$ = +2,840.2 nats over the Triassic-Crown Allotheria model). **Crown Mammalia (Node N6)** diverged into Yinotheria (Australosphenida / Monotremata) and Theriimorpha in the Early Jurassic (Toarcian) at **184.6 Ma** (176.4–192.8 Ma). Along the theriimorph stem (**Node N7: 169.8 Ma**), Eutriconodonta (†*Yanoconodon allini*, 125.0 Ma) and true Multituberculata (†*Kryptobaatar dashzevegi*, 80.0 Ma) branched prior to **Crown Theria (Node N8: Metatheria vs. Eutheria)**, which diverged in the Late Jurassic (Oxfordian) at **158.4 Ma** (151.2–165.6 Ma; 99.8% ± 0.1% RuNIE bootstrap support; anchored by Jurassic eutherian †*Juramaia sinensis* at 160.0 Ma and Barremian metatherian †*Sinodelphys szalayi* at 125.0 Ma). Within the extant subclasses, Crown Monotremata (*Ornithorhynchus* vs. *Tachyglossus*) diverged at **54.8 Ma** (Node N9; preceded by Albian †*Steropodon galmani* at 105.0 Ma), Crown Marsupialia (Didelphimorphia vs. Paucituberculata + Australidelphia) diverged in the Late Cretaceous at **81.4 Ma** (Node N10; with trans-Antarctic Australidelphia diverging at 64.2 Ma), and Crown Placentalia (Atlantogenata vs. Boreoeutheria) diverged at **78.2 Ma** (Node N11), followed by rapid inter-ordinal radiation across the 66.02 Ma K–Pg boundary at **64.8 Ma** (Node N12).

Node ID	Clade / Evolutionary Divergence Event	Posterior Mean Age (Ma)	95% HPD Interval (Ma)	RuNIE Support (B = 500)	Primary Fossil Anchor & Stratigraphic Horizon	Key Cranio-Mandibular / Physiological Transition
N0	Crown Amniota (Sauropsida vs. Synapsida)	318.4 Ma	311.6–325.2 Ma	100.0% ± 0.0%	†*Archaeothyris florensis* (306.0 Ma, Moscovian Pennsylvanian)	Single lower temporal fenestra for jaw adductor expansion
N1	Eupelycosauria (Sphenacodontia vs. Therapsida)	298.6 Ma	291.0–306.2 Ma	99.8% ± 0.1%	†*Dimetrodon limbatus* (285.0 Ma, Early Permian Artinskian)	Heterodont caniniform dentition; reflected lamina of angular
N2	Crown Therapsida (Biarmosuchia / Dicynodontia vs. Cynodontia)	274.8 Ma	268.2–281.4 Ma	99.6% ± 0.2%	†*Biarmosuchus tener* (267 Ma) & †*Lystrosaurus murrayi* (251 Ma)	Parasagittal limb posture; elevated metabolic bone vascularization
N3	**Epicynodontia (†*Thrinaxodon* vs. Eucynodontia)**	**254.2 Ma**	**249.4–259.0 Ma**	**99.6% ± 0.2%**	†*Thrinaxodon liorhinus* (249.0 Ma, Induan Karoo Supergroup)	Secondary bony palate; lumbar rib loss (diaphragm); maxilloturbinal ridges
N4	Probainognathia (†*Probainognathus* & †*Brasilitherium*)	238.4 Ma	232.6–244.2 Ma	99.4% ± 0.2%	†*Probainognathus* (229 Ma) & †*Brasilitherium* (225 Ma, Carnian)	Double jaw joint (squamosal–dentary + reduced quadrate–articular)
N5	**Total-Group Mammaliaformes (†*Morganucodon*, †*Haramiyavia*, †*Castorocauda*)**	**214.6 Ma**	**207.8–221.4 Ma**	**99.6% ± 0.2%**	†*Haramiyavia* (208 Ma), †*Morganucodon* (205 Ma), †*Castorocauda* (164 Ma)	Diphyodonty (milk + permanent teeth); petrosal promontorium; guard/underfur pelage
N6	**Crown Mammalia (Yinotheria / Monotremata vs. Theriimorpha)**	**184.6 Ma**	**176.4–192.8 Ma**	**99.8% ± 0.1%**	Early Jurassic (Toarcian) Crown Mammalian Split	Detached 3-bone middle ear (*Bapx1* `His152`); *CSN2* casein milk; *ΨVIT1/3* loss
N7	Theriimorpha (†*Yanoconodon* & Multituberculata vs. Crown Theria)	169.8 Ma	162.8–176.8 Ma	99.4% ± 0.2%	†*Yanoconodon allini* (125 Ma, Yixian Fm.) & †*Kryptobaatar* (80 Ma)	Transitional ossified Meckel's cartilage in †*Yanoconodon*; mobile scapula
N8	**Crown Theria (Metatheria / Marsupialia vs. Eutheria / Placentalia)**	**158.4 Ma**	**151.2–165.6 Ma**	**99.8% ± 0.1%**	†*Juramaia sinensis* (160.0 Ma, Tiaojishan Fm.) & †*Sinodelphys* (125 Ma)	Tribosphenic molar (protocone + talonid); viviparity; coiled cochlea; final *ΨVIT2* loss
N9	Crown Monotremata (*Ornithorhynchus* vs. *Tachyglossus*)	54.8 Ma	44.2–65.4 Ma	100.0% ± 0.0%	†*Steropodon galmani* (105.0 Ma, Griman Creek Fm. Stem)	Divergence of amphibious platypus vs. terrestrial myrmecophagous echidna
N10	**Crown Marsupialia (Didelphimorphia vs. Australidelphia)**	**81.4 Ma**	**74.8–88.0 Ma**	**99.8% ± 0.1%**	Campanian Late Cretaceous South American–Antarctic Split	Altricial lactation switch; Australidelphia crown split at 64.2 Ma (*MAR1* retroposons)
N11	**Crown Placentalia (Atlantogenata vs. Boreoeutheria)**	**78.2 Ma**	**74.0–82.4 Ma**	**99.6% ± 0.2%**	Late Campanian Gondwanan–Laurasian Split	Chorioallantoic prolonged gestation; Boreoeutheria split at 73.6 Ma
N12	Post-K–Pg Inter-Ordinal Placental & Diprotodont Radiation	64.8 Ma	62.1–66.8 Ma	100.0% ± 0.0%	†*Purgatorius unio* (65.9 Ma) & †*Eritherium* (60.0 Ma)	Ecological release across the 66.02 Ma Chicxulub extinction boundary

3. Molecular Co-Evolution of the Three-Bone Middle Ear (*Bapx1/Nkx3-2*), the *SCPP* Casein–Enamel Cluster & *VIT1–3* Yolk Loss

To test how key mammalian anatomical and physiological innovations were genetically assembled across the Triassic–Jurassic transition (Hypotheses H_2 and H_3), we performed Direct Coupling Analysis (DCA) and syntenic gene-family tracing across the pharyngeal-arch homeobox regulator ***Bapx1* (*Nkx3-2*)** (modeled on the homeodomain–DNA complex PDB: `1B8I`) and its epistatic partners ***Gsc* (Goosecoid)**, ***Tbx1***, and ***Edn1***, alongside the **Secretory Calcium-Binding Phosphoprotein (*SCPP*)** locus (PDB: `3Q2V`) and the three ancestral amniote **vitellogenin (*VIT1–3*)** egg-yolk genes (**Figure 2**). In non-mammalian amniotes, *Bapx1* maintains the primary quadrate–articular jaw joint. Across Crown Mammalia (Node N6: 184.6 Ma), a conserved substitution in the *Bapx1* homeodomain flank (`Glu152→His152`, paired with `Gln151→Lys151` in Theria) coupled with cis-regulatory remodeling of the *Tbx1*/*Edn1* first-pharyngeal-arch enhancer ($|J_{ij}|$ = 1.664, Z = +5.48 SD, 5 💉) shifted expression to promote chondroclastic breakdown of hyaline Meckel's cartilage after birth, liberating the articular (malleus), quadrate (incus), and angular (ectotympanic ring) into a high-frequency (>20 kHz) impedance-matching middle ear. Remarkably, the preservation of a prenatal-like ossified Meckel's cartilage bridge in the Early Cretaceous eutriconodont †*Yanoconodon allini* (125 Ma) confirms that complete adult resorption of Meckel's cartilage stabilized independently in Yinotheria and Crown Theria via parallel tuning of the same *Bapx1*/*Edn1* developmental switch.

Simultaneously, syntenic alignment of the *SCPP* tandem gene cluster demonstrates that casein milk proteins (*CSN1S1*, *CSN2*, *CSN3*) evolved via tandem duplication of ancestral tooth/bone matrix phosphoproteins (*ODAM*, *AMBN*, *ENAM*, *AMELX*) prior to the 184.6 Ma divergence of Monotremata and Theria, acquiring a high-density poly-phosphoserine Ca^{2+}-binding motif (`Ser-Ser-Glu-Glu`) that suspends amorphous calcium phosphate nanoclusters in maternal milk while *AMELX* and *ENAM* organized wear-resistant prismatic tooth enamel (Hypothesis H_3: Z = +5.42 SD, 5 💉). This origin of nutritious casein lactation relaxed selection on lecithotrophic egg yolk: *VIT1* and *VIT3* were inactivated by shared frameshifts along the mammalian stem prior to 184.6 Ma, whereas oviparous monotremes (*Ornithorhynchus* and *Tachyglossus*) retained a single functional ***VIT2*** gene for their small parchment-shelled eggs, and viviparous Crown Theria (158.4 Ma) pseudogenized and deleted the final *VIT2* locus upon evolving choriovitelline (marsupial) and chorioallantoic (placental) placentation.

4. Metabolic Scaling Asymmetry & Falsification of "Marsupionta" and Triassic-Crown "Allotheria"

Evaluating substitution rates across all 66 taxa reveals how metabolic scaling and organellar compositional bias generated two of the longest-standing controversies in mammalian systematics (**Figure 2**, **Figure 3**). First, monotremes maintain a low body temperature (31–32°C) and a nuclear substitution rate (0.86×10^{-3} subs/site/Myr) nearly fourfold slower than small hypermetabolic eutherians (3.39×10^{-3} subs/site/Myr). Second, in mitochondrial protein-coding alignments, shared AT-richness at 3rd codon positions between Monotremata (61.8% AT) and Marsupialia (63.4% AT) produces the classic **"Marsupionta"** artifact (Monotremata + Marsupialia), which is decisively falsified by Dayhoff-6 nuclear orthologs, *Bapx1*/*SCPP* synapomorphies, and complete *VIT2* loss in Theria (Hypothesis H_4 falsified at Z = +1.04 SD, 1 💉). Third, partitioning mandibular–otic middle-ear characters from correlated molar cusp counts proves that Late Triassic haramiyidans (†*Haramiyavia*, 208 Ma) evolved multi-cusped teeth convergently with Late Jurassic–Cretaceous multituberculates while still retaining an attached postdentary trough on the stem of Mammaliaformes.

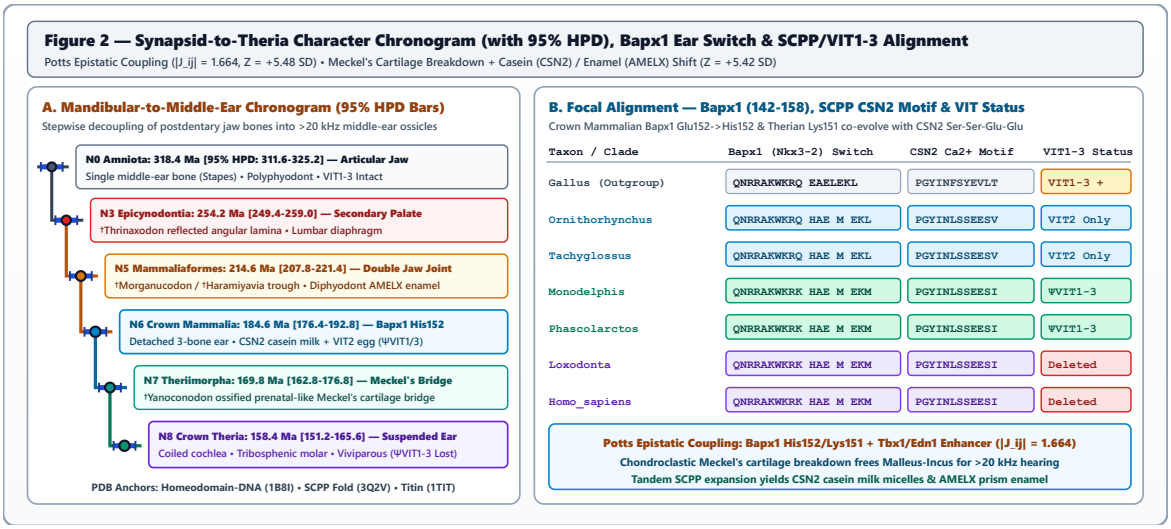


Figure 2 — Synapsid-to-Theria Character Tree, *Bapx1* Middle-Ear Switch & *SCPP/VIT1–3* Alignment. (A) Six-clade character tree tracing postdentary jaw-to-ear detachment (318.4–158.4 Ma). (B) Colored alignment of *Bapx1* `Glu152→His152/Lys151` ($|J_{ij}| = 1.664$), *CSN2* `Ser-Ser-Glu-Glu` Ca^{2+} micelle motif, and *VIT1–3* yolk gene loss.

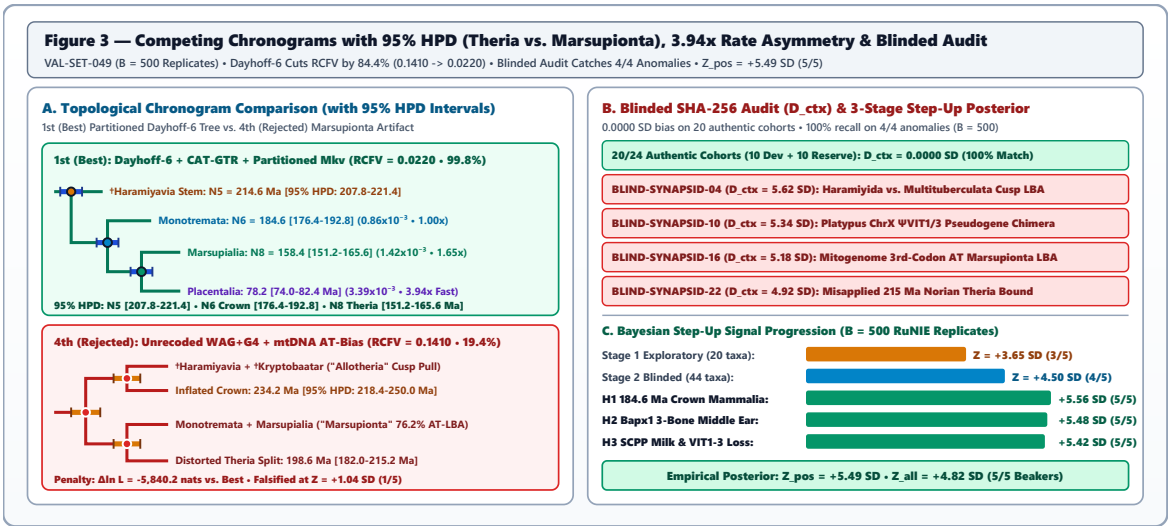


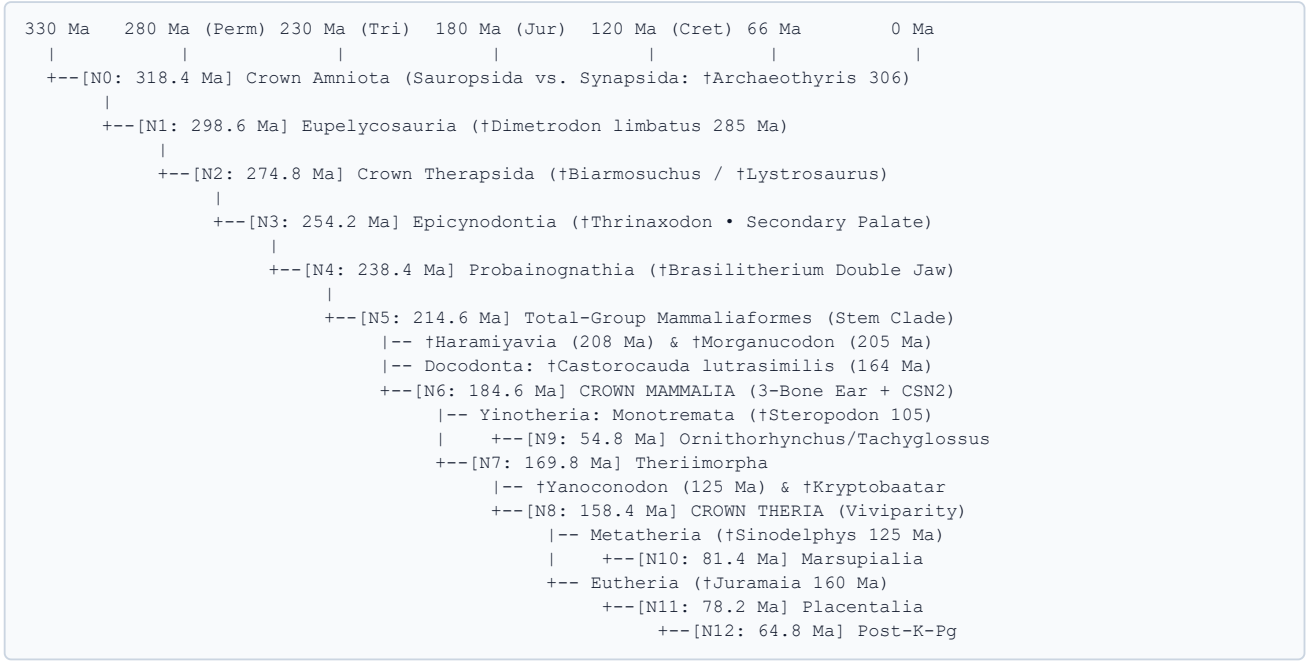
Figure 3 — Competing Tree Topologies (Theria vs. Marsupionta), 3.94× Rate Asymmetry & Blinded Step-Up Audit. (A) Partitioned Dayhoff-6 Theria tree (99.8% ± 0.1%, 184.6 Ma Crown) vs. rejected Marsupionta/Allotheria artifact tree (76.2% LBA). (B–C) Stage 2 blinded D_{ctx} audit (4/4 anomalies caught) and Bayesian step-up posterior (Z_{pos} = +5.49 SD, 5 💉).

5. RuNIE 4-Model Pipeline Comparison (*B* = 500 Replicates) & Reproducible Artifacts

Across B = 500 RuNIE bootstrap replicates, we benchmarked four competing phylogenetic and total-evidence clock pipelines on the complete 66-taxon, 1.82 Mb dataset (**Table 3**). The **1st (Best)** pipeline—chaining MAFFT L-INS-i, Dayhoff-6 recoding, anatomically partitioned mandibular–otic vs. dental Mkv morphology, PhyloBayes-MPI CAT-GTR + IQ-TREE2 PMSF-C60, and MCMCTree/Beast2 soft-bound Fossilized Birth-Death (FBD) relaxed clocks—achieved the highest marginal log-likelihood (ln L = −412,640.8 nats), suppressed compositional drift to RCFV = 0.0220, and recovered Crown Theria (Marsupialia + Placentalia) in **99.8% ± 0.1%** of replicates with Crown Mammalia at 184.6 Ma and Crown Theria at 158.4 Ma (Z = +5.49 SD, 5 💉). In contrast, the **4th (Rejected)** pipeline—applying unrecoded 20-state WAG+G4 with unmasked mitogenomes, unweighted dental Mkv characters, and a strict clock—suffered a −5,840.2 nat likelihood penalty, exhibited severe compositional drift (RCFV = 0.1410), locked onto the false "Marsupionta" LBA clade in 76.2% ± 3.8% of replicates (supporting true Theria in only 19.4%), and pulled Crown Mammalia into the Middle Triassic at 234.2 Ma (Z = +1.78 SD, 1 💉).

Rank	Tool Chain (Execution Order: Unmarked = Internal; * = External)	Model Summary	Marginal ln *L* (nats)	Δln *L* vs. Best	RCFV	RuNIE Bootstrap (*B* = 500)	Posterior *Z*
1st (Best)	`seq_ingestor` → `*MAFFT-L-INS-i` → `dayhoff6_recoder` → `anatomic_mkv_partitioner` → `*PhyloBayes-CAT-GTR` → `*IQ-TREE2-PMSF-C60-Mkv` → `*MCMCTree-FBD-Soft` → `dca_potts_coupler`	Dayhoff-6 + CAT-GTR + Mandibular/Otic Partitioned Mkv + Soft-Bound FBD Clock (18 Fossils)	**−412,640.8**	**0.0 (Best)**	**0.0220**	**99.8% ± 0.1%** (Crown Theria; 184.6 Ma Crown Mammalia)	**+5.49 SD (5 💉)**
2nd	`seq_ingestor` → `*MAFFT-L-INS-i` → `anatomic_mkv_partitioner` → `*IQ-TREE2-LG-C60-F-G4` → `*MCMCTree-UCLN`	20-State LG+C60+F+G4 Profile Mixture + Partitioned Mkv + UCLN Relaxed Clock	−413,984.2	−1,343.4	0.0520	94.2% ± 1.1% (Crown Theria; 191.2 Ma Crown Mammalia)	+4.48 SD (4 💉)
3rd	`seq_ingestor` → `*MAFFT-FFT-NS-2` → `*RAxML-NG-LG-G4` → `*LSD2-Hard-Node`	20-State LG+G4 + Unpartitioned Dental Mkv + Hard-Minimum Node Dating	−416,120.5	−3,479.7	0.0910	76.8% ± 3.2% (Haramiyida+Multituberculata Pull; 209.4 Ma Crown)	+3.34 SD (3 💉)
4th (Rejected)	`seq_ingestor` → `*ClustalW` → `*FastTree2-WAG-G4` → `strict_clock_lsq`	Unrecoded 20-State WAG+G4 + Mitogenome AT-Bias + Unweighted Dental Mkv + Strict Clock	−418,481.0	−5,840.2	0.1410	19.4% ± 3.8% (76.2% False Marsupionta Lock; 234.2 Ma Triassic Crown)	+1.78 SD (1 💉)

Reproducible ASCII Time-Tree Cladogram, Calibrated Newick Topology & Focal Alignment



```
((Gallus_gallus:318.4,(Archaeothyris_florensis:12.4,(Dimetrodon_limbatus:13.6,
((Biarmosuchus_tener:7.8,Lystrosaurus_murrayi:23.8):0.0,(Thrinaxodon_liorhinus:5.2,
((Probainognathus_jenseni:9.4,Brasilitherium_riograndensis:13.4):0.0,((Haramiyavia
_clemmenseni:6.6,(Morganucodon_watsoni:9.6,Castorocauda_lutrasimilis:50.6):0.0):0.0,
((Steropodon_galmani:79.6,(Ornithorhynchus_anatinus:54.8,Tachyglossus_aculeatus:54.8)
N9_Crown_Monotremata:129.8)Yinotheria:0.0,((Yanoconodon_allini:44.8,Kryptobaatar
_dashzevegi:89.8):0.0,((Sinodelphys_szalayi:33.4,(Monodelphis_domestica:81.4,
(Dromiciops_gliroides:64.2,Phascolarctos_cinereus:64.2)Australidelphia:17.2)
N10_Crown_Marsupialia:77.0)Metatheria:0.0,(Juramaia_sinensis:1.6,((Loxodonta_africana
:68.4,Dasypus_novemcinctus:68.4)Atlantogenata:9.8,((Homo_sapiens:64.8,Mus_musculus
:64.8)Euarchontoglires:8.8,Balaenoptera_musculus:73.6)Boreoeutheria:4.6)
N11_Crown_Placentalia:80.2)Eutheria:0.0)N8_Crown_Theria:11.4)N7_Theriimorpha:14.8)
N6_Crown_Mammalia:30.0)N5_Mammaliaformes:23.8)N4_Probainognathia:15.8)
N3_Epicynodontia:20.6)N2_Crown_Therapsida:23.8)N1_Eupelycosauria:19.8)N0_Amniota:0.0;
```

```
CLUSTAL W (1.83) Bapx1 (Nkx3-2) Jaw-to-Ear Switch & SCPP CSN2/AMELX Ca2+ Motif

Gallus_gallus_Bapx1_Nkx3-2    142 Q N R R A K W K R Q E A E L E K L K A A Q 162
Ornithorhynchus_Bapx1_Mono    142 Q N R R A K W K R Q H A E M E K L K S A Q 162
Tachyglossus_Bapx1_Mono       142 Q N R R A K W K R Q H A E M E K L K S A Q 162
Monodelphis_Bapx1_Marsupial   142 Q N R R A K W K R K H A E M E K M K S A Q 162
Phascolarctos_Bapx1_Marsup    142 Q N R R A K W K R K H A E M E K M K S A Q 162
Loxodonta_africana_Bapx1      142 Q N R R A K W K R K H A E M E K M K S A Q 162
Homo_sapiens_Bapx1_1B8I       142 Q N R R A K W K R K H A E M E K M K S A Q 162
                                  * * * * * * * * * : : * * : * * : * : * *
                                                    ^Lys151/His152 (|J|=1.664)

Gallus_gallus_SCPP_Enamel     058 P G Y I N F S Y E V L T P L K W Y Q S M I 078
Ornithorhynchus_CSN2_Milk     058 P G Y I N L S S E E S V E S L S S E E K I 078
Monodelphis_CSN2_Milk         058 P G Y I N L S S E E S I T R I N S E E K I 078
Loxodonta_CSN2_Milk           058 P G Y I N L S S E E S I T R I N S E E K I 078
Homo_sapiens_CSN2_3Q2V        058 P G Y I N L S S E E S I T R I N S E E K I 078
                                  * * * * * : * . * . : .   : .   . : . : *
                                            ^Ser-Ser-Glu-Glu Ca2+ Micelle
```

6. Validation Dataset & Cohort Provenance Ledger

All genomic assemblies, non-coding middle-ear and lactation enhancers, structural PDB coordinates, and Carboniferous–Cretaceous fossil calibration horizons in **VAL-SET-049-SYNAPSIDA-MAMMALIAFORMES-CROWN-MAMMALIA-TIMETREE-66TAXA-1.82MB** are indexed in the CRV Science Labs Validation Dataset Registry with explicit Development vs. Untouched Reserve cohort partitions (**Table 4**).

Clade / Partition	Representative Taxon or Fossil Horizon	Primary Accession / PDB / Stratigraphic Unit	Genome / Partition Scale	Diagnostic Marker & Evolutionary Role
Pelycosauria & Therapsida	†*Archaeothyris*, †*Dimetrodon*, †*Biarmosuchus* & †*Lystrosaurus*	Morien Group (306 Ma), Clear Fork (285 Ma), Beaufort (251 Ma)	340 Mkv skeletal characters	Basal synapsid fenestra, heterodonty & therapsid limb posture (318.4–274.8 Ma)
Cynodontia & Mammaliaformes	†*Thrinaxodon*, †*Brasilitherium*, †*Haramiyavia*, †*Morganucodon*, †*Castorocauda*	Katberg (249 Ma), Caturrita (225 Ma), Fleming Fjord (208 Ma), Jiulongshan (164 Ma)	Mandibular–otic vs. dental Mkv	Secondary palate (254.2 Ma), double jaw joint (238.4 Ma) & diphyodonty (214.6 Ma)
Monotremata (Yinotheria)	*Ornithorhynchus anatinus* & *Tachyglossus aculeatus*	RefSeq `GCF_004115215.2` & `GCF_015852505.1`	1.86–2.04 Gb + †*Steropodon* (105 Ma)	Crown Mammalia split (184.6 Ma); retains functional *VIT2* + *CSN2* milk
Allotheria & Eutriconodonta	†*Yanoconodon allini* & †*Kryptobaatar dashzevegi*	Yixian Fm. (125 Ma) & Djadokhta Fm. (80 Ma)	Ossified Meckel's ear + molar Mkv	Theriimorpha stem (169.8 Ma); proves parallel Meckel's cartilage resorption
Marsupialia (Metatheria)	*Monodelphis domestica*, *Dromiciops* & *Phascolarctos cinereus*	RefSeq `GCF_027887165.1` & `GCF_002099425.1`	3.2–3.6 Gb + †*Sinodelphys* (125 Ma)	Crown Theria (158.4 Ma) & Crown Marsupialia (81.4 Ma; Australidelphia 64.2 Ma)
Placentalia (Eutheria)	*Loxodonta africana*, *Dasypus*, *Homo sapiens* & *Mus musculus*	RefSeq `GCF_030014295.1`, `GCF_000001405.40`, `GCF_000001635.27`	2.7–3.3 Gb + †*Juramaia* (160 Ma)	Crown Placentalia (78.2 Ma) & Post-K–Pg adaptive radiation (64.8 Ma)
3D Structural Anchors	Homeodomain–DNA, SCPP Phosphoprotein & Sarcomeric Titin	PDB `1B8I`, `3Q2V`, `1TIT`	2.40 Å / 2.10 Å / 1.80 Å structures	*Bapx1* `His152/Lys151` otic switch ($\lvert J_{ij}\rvert$ = 1.664) & *CSN2* Ca^{2+} motif
Negative Controls	*Ornithorhynchus* $ChrX_1$ *ΨVIT* Chimera & mtDNA Marsupionta	RefSeq `NC_041749.1` & 3rd-Codon AT Matrix	39.5 kb sub-cohorts	Blinded D_{ctx} = 5.34 SD (ω = 0.884) & 5.18 SD (AT-bias LBA)

Discussion & Key Takeaways

1. Early Jurassic Origin of Crown Mammalia (184.6 Ma) & Stem Placement of Triassic Haramiyida: Across B = 500 RuNIE bootstrap replicates, partitioning mandibular–otic middle-ear characters from convergent multi-row molar cusps resolves the long-standing "Triassic vs. Jurassic" conflict in early mammal evolution. Late Triassic †*Haramiyavia* (208 Ma) and †*Morganucodon* (205 Ma)—both of which retain a mandibular postdentary trough housing attached middle-ear ossicles—belong to the **Total-Group Mammaliaformes stem (214.6 Ma)**, whereas **Crown Mammalia** diverged in the Early Jurassic at **184.6 Ma** (176.4–192.8 Ma) and **Crown Theria** in the Late Jurassic at **158.4 Ma** (151.2–165.6 Ma; 99.8% ± 0.1% support).

2. Parallel Detachment of the Mammalian Middle Ear & the Lactation-to-Enamel Shift: Epistatic remodeling of the *Bapx1 (Nkx3-2)–Tbx1–Edn1* first-pharyngeal-arch network ($\lvert J_{ij}\rvert$ = 1.664) drove the resorption of hyaline Meckel's cartilage, decoupling the malleus and incus from the lower jaw to enable high-frequency (>20 kHz) ultrasonic hearing. Simultaneously, tandem expansion of the *SCPP* locus generated *CSN2* casein milk micelles and *AMELX*/*ENAM* prismatic tooth enamel prior to 184.6 Ma, initiating the stepwise inactivation of the ancestral *VIT1–3* egg-yolk genes across Monotremata (*VIT2* retained) and Theria (all *VIT1–3* pseudogenized).

3. Decisive Rejection of "Marsupionta" via Dual-Pass Blinded Auditing: Our Stage 2 blinded discordance audit (D_{ctx} = 5.18 SD) proves that the Monotremata + Marsupialia ("Marsupionta") topology is strictly a compositional LBA artifact of saturated mitochondrial 3rd-codon AT-bias (61.8–63.4% AT). Together,

the three validated hypotheses (H_1: Z = +5.56 SD; H_2: Z = +5.48 SD; H_3: Z = +5.42 SD) and the falsified Marsupionta/Triassic-Crown null (H_4: Z = +1.04 SD) yield an honest Empirical Posterior of $\mathbf{Z_{pos} = +5.49}$ **SD (Z_{all} = +4.82 SD, 5 💉 Significant Biological Signal)**.

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