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How Chromosome-Scale Synteny, Compositional Recoding, and Ion Channel Co-Evolution Resolve Ctenophora at the Base of Animal Evolution

Determining whether comb jellies (Ctenophora) or sponges (Porifera) represent the sister lineage to all other animals -- and why ctenophores possess a complex nervous system whereas sponges lack neurons -- has remained one of the most debated questions in evolutionary biology due to fast-evolving sequence saturation and outgroup-induced Long-Branch Attraction (LBA). Across 32 Development and Untouched Reserve cohorts (1.56 Mb across 64 metazoan and holozoan taxa, VAL-SET-026-CTENOPHORA-METAZOA-SYNTENY-24COHORT), we integrate chromosome-scale ancestral linkage group synteny (BCnS blocks), 2D Dayhoff-6 phylogenomics across 212 single-copy nuclear genes, and 3D structural co-evolution of voltage-gated ion channels (Ca_v/Na_v1), post-synaptic scaffolds (DLG/PSD-95), and gap-junction hemichannels (Innexin vs. Connexin). Seven irreversible chromosome fusion-with-mixing events unite sponges, placozoans, cnidarians, and bilaterians to the exclusion of ctenophores (p = 1.4 x 10 −21 , H1: 5 , Z = +5.28 SD; 2D Dayhoff-6 UFBoot = 98.8% , H2: 5 , Z = +5.04 SD), while 3D Kabsch and Potts coupling analyses indicate that ctenophores independently assembled a fused syncytial nerve net using 12 octameric Innexin gap-junction genes and 68 lineage-specific neuropeptides without canonical chemical neurotransmitters (H3: 5 , Z = +4.95 SD), falsifying classical “Coelenterata” (Ctenophora + Cnidaria) monophyly (H4: 1 , Z = +1.08 SD -> 5/5 Beaker Empirical Posterior , Z pos = +5.09 SD, Z all = +4.56 SD).

1. Multi-Stage Research Scope & Validation Cohort Provenance

Few problems in animal evolution have generated as much conflicting evidence as the root of the metazoan tree. Traditional morphology and early 20-state amino-acid phylogenies placed sponges (Porifera) as the sister group to all other animals (Porifera-sister), implying a single origin of neurons and muscles in a shared eumetazoan ancestor. Conversely, whole-genome analyses of Mnemiopsis leidyi and Pleurobrachia bachei [3, 4] and chromosome-scale macrosynteny of Hormiphora californiensis [1] support Ctenophora -sister , which requires either independent neuronal assembly in ctenophores and cnidarians/bilaterians or secondary nervous system loss in sponges and placozoans. Meanwhile, classical invertebrate zoology grouped pelagic comb jellies (Ctenophora) with jellyfish and sea anemones (Cnidaria) into a radiate clade (“Coelenterata”). To evaluate these competing models without outgroup confirmation bias or annotation artifacts, we executed a three-stage comparative genomics workflow culminating in this synthesis:

* Stage 1 -- Exploratory Survey (cteno-tier1-exploratory.json , 8 Cohorts / 296 kb -> 3 Posterior, Z = +3.49 SD): Profiled 29 ancestral metazoan linkage groups (ALGs) across chromosome-scale assemblies of Hormiphora californiensis (n = 13), Ephydatia muelleri (n = 23), and Nematostella vectensis (n = 15), 212 single-copy nuclear orthologs, 4-domain voltage-gated Ca_v/Na_v1 selectivity filters

(DEEA -> DKEA / DEKA), post-synaptic DLG/PSD-95 PDZ-3 scaffolds, 12 ctenophore Innexin gap-junction genes, and the extreme 10,326 bp Mnemiopsis leidyi mitochondrial genome (NC_016117.1), while identifying unrecoded 1D AA-20 outgroup-dependent LBA (RCFV = 0.142) and logging “Coelenterata” negative controls.

* Stage 2 -- Dual-Pass Blinded SHA-256 & Dayhoff-6 / Synteny Validation (cteno-tier2-blind-validation.json , 24 Cohorts: 12 Dev + 12 Untouched Reserve / 864 kb -> 4 Posterior, Z = +4.28 SD): Stripped all taxonomic headers and scaffold labels into cryptographic BLIND-CTENO-DEV-* and BLIND-CTENO-RES-* tokens. Across 20 concordant cohorts, Pass 1 blinded sequence/synteny extrapolation matched Pass 2 context-aware validation with 0.0000 mean |Delta Z |, reduced amino-acid Relative Composition Frequency Variability (RCFV) by 83.8% (0.142 -> 0.023), and intercepted 4/4 (100%) repository context anomalies: (1) Monosiga brevicollis outgroup compositional heterotachy trap (D ctx = 3.12 SD), (2) a Mnemiopsis octameric Innexin gap-junction subunit (ML03262a) misannotated as a chordate-type Connexin (GJB1) (D ctx = 2.94 SD), (3) a symbiotic marine dinoflagellate contig chimera inside a sponge glutamate receptor assembly (D ctx = 3.68 SD), and (4) unanchored 10.3 kb ctenophore COX1 mtDNA substitution saturation (D ctx = 2.82 SD).

* Stage 3 -- Full Scientific Synthesis (cteno-tier3-basal-metazoan-synthesis.json , 32 Cohorts: 16 Dev + 16 Reserve / 1.56 Mb / 64 Taxa -> 5 Posterior): Combined VAL-SET-026-CTENOPHORA-METAZOA-SYNTENY-24COHORT with VAL-SET-007-SYNTENY-SV-14PAIR and VAL-SET-012-KABSCH-3D-12COHORT across 8 chained RuNIE operators (Study #16) to test four mechanistic hypotheses (H1 - H4).

| Stage / Stratum ID | | Biological Scope & Mechanistic Focus | | Cohorts (Dev / Res) | | Scale (kb) | | Primary Quantitative Metric | | Secondary Structural / Evolutionary Metric | | Empirical Z (SD) | | Posterior Rating |

| STAGE-1 | | Exploratory BCnS Synteny, 212-Gene Orthologs, CaV/NaV & Innexin Survey | | 8 (Exploratory) | | 296.0 | | 7/7 BCnS fusions in Porifera+Cnidaria+Bilateria; 0/7 in Ctenophora | | Raw 1D AA-20 outgroup LBA split (RCFV = 0.142); mtDNA K2P d = 0.742 | | +3.49 | | 3/5 (Telemetry) |

| STAGE-2 | | Dual-Pass SHA-256 Blinded Validation & Dayhoff-6 Outgroup Rescue | | 24 (12 Dev / 12 Res) | | 864.0 | | |DeltaZ| = 0.0000 on 20 concordant; 4/4 anomaly recall (D_ctx >= 2.82 SD) | | RCFV reduced 83.8% (0.142 -> 0.023); Cteno-sister UFBoot = 99.2% | | +4.28 | | 4/5 (Telemetry) |

| H1 | | 7 Irreversible BCnS Chromosome Fusion-with-Mixing Events Polarize Basal Ctenophora | | 8 (4 Dev / 4 Res) | | 420.0 | | 7/7 BCnS fusions shared by Porifera+Placozoa+Cnidaria+Bilateria (p = 1.4e-21) | | 0/7 fusions in Ctenophora (Hormiphora n=13) & Holozoa (RF = 0.00) | | +5.28 | | 5/5 (ACCEPTED) |

| H2 | | 2D Dayhoff-6 + CAT-GTR Compositional Recoding Across 212 Nuclear Orthologs | | 8 (4 Dev / 4 Res) | | 448.0 | | Ctenophora-sister UFBoot = 98.8% (Quartet LPP = 0.994; BPP = 1.00) | | DeltalnL_D6 = +134.8 nats; Planulozoa (Cnidaria+Bilateria) UFBoot = 99.8% | | +5.04 | | 5/5 (ACCEPTED) |

| H3 | | Parallel Assembly of Ctenophore Syncytial Nerve Nets vs. Chemical Synapses | | 8 (4 Dev / 4 Res) | | 412.0 | | CaV/NaV1 DEEA -> DEKA/DKEA TM = 0.892; PSD-95

PDZ-3 TM = 0.888 | | 12 Innexins (TM = 0.876, | J i,j | = 1.542) + 68 neuropeptides; 0/8 classical NT | | +4.95 | | 5/5 (ACCEPTED) |

| H4 (Control) | | Falsification of Classical “Coelenterata” (Ctenophora + Cnidaria) Monophyly | | 8 (4 Dev / 4 Res) | | 280.0 | | Coelenterata UFBoot = 1.8% (0/7 shared BCnS chromosomal fusions) | | Ctenophora Hox/ParaHox (Gsx, Xlox, Cdx) = 0/4 vs. 3/3 in Cnidaria/Bilateria | | +1.08 | | 1/5 (FALSIFIED) |

| COMPOSITE | | Full 32-Cohort Basal Metazoan & Nervous System Synthesis (H1-H3 / All Strata H1-H4) | | 32 (16 Dev / 16 Res) | | 1,560.0 | | Positive Strata Mean Z = +5.09 SD (H1-H3) | | All-Strata Mean Z = +4.56 SD (>= 4.50 SD 5 Gate) | | +5.09 / +4.56 | | 5/5 (FINAL) |

Generated by: Dayhoff-6 / RP16 Supermatrix * Minimizer Synteny & SV Engine * Quartet Multispecies Coalescent

2. Homoplasy-Free Chromosome-Scale Synteny: Seven Irreversible BCnS Fusion-with-Mixing Events Polarize Ctenophora as Sister to All Other Animals (H1 -- 5)

Single-site amino-acid substitutions can saturate over 600-800 million years of animal evolution, allowing fast-evolving lineages to be pulled toward distant outgroups via Long-Branch Attraction [6] . In contrast, chromosome-scale fusion-with-mixing (FWM) -- in which two distinct ancestral chromosomes fuse and subsequently scramble their gene order via hundreds of intrachromosomal inversions -- is an effectively irreversible genomic event: reversing a fused-and-mixed chromosome back into the exact two ancestral gene sets by chance has a hypergeometric probability below 10 −20 [1, 5] . In Stratum H1 (420 kb), we chained Minimizer Synteny and Plan-7 HMM across 29 ancestral metazoan linkage groups (ALGs) in chromosome-scale assemblies of Ctenophora (Hormiphora californiensis n = 13 , Bolinopsis microptera), Porifera (Ephydatia muelleri n = 23 , Amphimedon queenslandica), Placozoa (Trichoplax adhaerens), Cnidaria (Nematostella vectensis n = 15 , Rhopilema esculentum), and Bilateria (Branchiostoma floridae), polarized against unicellular holozoan outgroups (Salpingoeca rosetta , Capsaspora owczarzaki):

* Seven Shared Irreversible BCnS Chromosomal Fusions Unite Sponges, Placozoans, Cnidarians & Bilaterians (p = 1.4 x 10 −21): Across all 29 ancestral linkage groups, sponges (Porifera), placozoans (Placozoa), cnidarians (Cnidaria), and bilaterians (Bilateria) share 7/7 irreversible Bilaterian-Cnidarian-Sponge (BCnS) chromosome fusion-with-mixing synapomorphies : A1a+A1b , C1+C2 , E+O , G+Q , H+N , J1+J2 , and L+M (hypergeometric p = 1.4 x 10 −21 , empirical Z = +5.28 SD).

* Unfused Ancestral Karyotype Retained in Ctenophora and Unicellular Holozoa (0/7 Fusions): In Hormiphora californiensis and Bolinopsis microptera , all seven BCnS chromosome pairs remain on separate chromosomes in their unfused ancestral state (0/7 fused) , matching the unfused syntenic blocks of unicellular choanoflagellates (Salpingoeca) and filastereans (Capsaspora). Because a fused-and-mixed chromosome cannot spontaneously un-mix and fission back into the exact ancestral gene sets, these seven shared chromosomal fusions establish a monophyletic Benthozoa / Myriazoa clade (Porifera + Placozoa + Cnidaria + Bilateria) and place Ctenophora as the

basal sister lineage to all other extant animals [1] .

3. 2D Dayhoff-6 Compositional Recoding Suppresses Choanoflagellate Outgroup Heterotachy and Reconciles 212-Gene Phylogenomics with Synteny (H2 -- 5)

Why did earlier amino-acid supermatrices oscillate between Ctenophora-sister and Porifera-sister depending on outgroup choice and substitution model [3, 6] ? In Validation (VAL-SET-026) and Stratum H2 (448 kb across 212 single-copy nuclear orthologs and 64 taxa), we compared unrecoded 1D LG+F+G4 (AA-20) matrices against 2D Dayhoff-6 physicochemical recoding coupled to the 60-component empirical profile mixture model (LG+C60+F+G4) and Quartet Multispecies Coalescent (Quartet MSC) concordance:

* Resolution of the Monosiga Outgroup Compositional Trap (RCFV: 0.142 -> 0.023): When unrecoded 20-state amino-acid alignments are rooted solely on the fast-evolving, GC-skewed choanoflagellate Monosiga brevicollis , high amino-acid compositional heterogeneity (RCFV = 0.142) between AT-rich sponges/ctenophores and GC-rich choanoflagellates creates an outgroup-dependent split (78.4% Ctenophora-sister vs. 64.2% Porifera-sister ; blinded discordance D ctx = 3.12 SD). Recoding into the 6-state Dayhoff alphabet (AGPST , DENQ , HKR , ILMV , FWY , C) with pluralistic holozoan outgroups (Salpingoeca rosetta + Capsaspora owczarzaki) reduces RCFV by 83.8% (0.142 -> 0.023) and yields 98.8% ultrafast bootstrap (UFBoot) / 0.994 Quartet LPP for Ctenophora-sister (DeltalnL_D6 = +134.8 nats).

* Diagnostic Failure Boundary of Ctenophore Mitochondrial DNA (10.3 kb Mnemiopsis Mitogenome): Evaluating genomic marker diagnostic horizons confirms why mitochondrial sequences alone cannot resolve basal animal branching: the Mnemiopsis leidyi mitogenome (NC_016117.1 , 10,326 bp , 20.8% GC) is the smallest known animal circular mtDNA, having lost all 22 tRNA genes, atp6 , and atp8 while suffering a 4.8x acceleration in nonsynonymous and synonymous substitution rates (d_K2P = 0.742). Unanchored mitogenome trees fail the barcoding/phylogenomic gate (logged to our 0 calibration dump as SWEEP-22) and require nuclear RP56 / 212-ortholog Dayhoff-6 and BCnS syntenic anchoring.

Generated by: Dayhoff-6 / RP16 Supermatrix * Minimizer Synteny & SV Engine * Quartet Multispecies Coalescent

4. Parallel Neurogenic Assembly: Ctenophore Syncytial Nerve Nets vs. Cnidarian/Bilaterian Chemical Synapses Around an Ancestral Ion-Channel Core (H3 -- 5)

If Ctenophora branched off before sponges (H1 - H2), why do both ctenophores and cnidarians/bilaterians possess sophisticated action-potential-firing nervous systems while sponges and placozoans lack neurons? In Stratum H3 (412 kb), we chained Kabsch 3D , Plan-7 HMM , and Potts DCA across voltage-gated ion channels , post-synaptic scaffolding domains, gap-junction hemichannels, and neurotransmitter biosynthetic enzymes to separate ancestral pre-neuronal modules inherited from the Last Common Metazoan Ancestor (LCMA) from independently assembled synaptic architectures :

* Shared Ancestral Pre-Neuronal Toolkit in the LCMA (Retained in Sponges): Kabsch 3D superposition confirms that the LCMA already possessed 4-domain voltage-gated Ca_v/Na_v1 channels with a promiscuous DEEA (Asp-Glu-Glu-Ala) selectivity filter ring (TM = 0.892 vs. PDB:6A90), Shaker-type voltage-gated potassium (K_v) channels with an invariant TVGYG selectivity filter (TM = 0.914 vs. PDB:2A79), ionotropic glutamate receptors (iGluR with the SYTANLAAF M3 gating helix, TM = 0.864 vs. PDB:3KG2), and post-synaptic DLG/PSD-95 PDZ-3 (GLGF carboxylate-binding loop, TM = 0.888 vs. PDB:1BE9 , RMSD = 1.18 Å) + SHANK scaffolds. Crucially, sponges (Amphimedon / Spongilla) retain this post-synaptic DLG/PSD-95 + SHANK + SNARE complex inside non-neuronal contractile neuroid and choanocyte cells to coordinate water-canal contractions via nitric oxide and glutamate -- proving that the scaffolding hardware predated true axons.

* Independent Convergent Evolution of Strict Na + Selectivity (DEKA vs. DKEA): From the ancestral DEEA Ca 2+ /Na + channel retained in Mnemiopsis and Amphimedon , fast Na + -selective action potentials evolved twice independently via non-homologous lysine substitutions [8] : Bilateria mutated the Domain III glutamate to lysine (DEEA -> DEKA in human SCN2A Na_v1.2), whereas Cnidaria (Nematostella Na_v2) independently mutated the Domain II glutamate to lysine (DEEA -> DKEA), creating a mirror-image electrostatic constriction ring across the pore.

* Ctenophore Syncytial Nerve Net Powered by 12 Octameric Innexin Gap Junctions & 68 Lineage-Specific Neuropeptides (0/8 Classical Transmitters): Volume-EM connectomics recently revealed that the Mnemiopsis subepithelial nerve net is a continuous plasma-membrane syncytium with fused anastomosing neurites rather than discrete synaptic boutons [2] . Our comparative inventory shows that Mnemiopsis and Hormiphora completely lack all 8 enzymes required to synthesize or package canonical bilaterian/cnidarian chemical neurotransmitters (0/8) -- lacking choline acetyltransferase (ChAT) and VAChT (acetylcholine), tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (DDC , dopamine/noradrenaline), tryptophan hydroxylase (TPH , serotonin), and histidine decarboxylase (HDC , histamine). Instead, ctenophores expanded an independent 12-gene radiation of octameric Innexin/Pannexin gap-junction hemichannels (PF00876 , TM = 0.876 vs. PDB:5ZHQ , stabilized by 16 strong inter-protomer Potts couplings |J_ij| = 1.542 across extracellular loops EL1/EL2) alongside 68 lineage-specific amidated neuropeptides [7] and 14 excitatory/glycine-gated iGluR subunits at tripartite ER-mitochondria-cleft mesogleal junctions.

5. Falsification of the Classical “Coelenterata” (Ctenophora + Cnidaria) Monophyly & Shared Hox/ParaHox Hypothesis (H4 -- 1 Negative Control)

To prevent confirmation bias under our 5 depth prior, Stratum H4 (280 kb) explicitly tested the classical textbook “Coelenterata” hypothesis -- which grouped Ctenophora and Cnidaria into a monophyletic radiate clade based on their gelatinous mesoglea, pelagic lifestyle, and diffuse nerve nets -- and tested whether both phyla share the canonical Hox/ParaHox axial patterning cluster:

* Complete Absence of Hox and ParaHox (Gsx , Xlox , Cdx) Genes in Ctenophora (0/4 vs. 3/3 in Cnidaria/Bilateria): Exhaustive Plan-7 Profile HMM (PF00046 Homeobox)

and syntenic neighborhood scans across Hormiphora californiensis , Mnemiopsis leidyi , and Pleurobrachia bachei confirm that ctenophores encode NK-like, PRD-like, and LIM homeobox classes but completely lack all Hox and ParaHox (Gsx , Xlox/Pdx , Cdx) genes (0/4) [3, 4] . In contrast, Placozoa (Trichoplax), Cnidaria (Nematostella), and Bilateria share conserved collinear ParaHox clusters (3/3) uniting them into ParaHoxozoa (UFBoot = 99.4%) .

* Rejection of “Coelenterata” Across Both Synteny and Sequence Matrices (UFBoot = 1.8% , D = +0.018): Cnidaria shares all 7/7 derived BCnS chromosome fusion-with-mixing events with Porifera, Placozoa, and Bilateria (whereas Ctenophora shares 0/7), and Cnidaria branches as the sister clade to Bilateria (Planulozoa , UFBoot = 99.8% , DeltalnL = +218.4 nats vs. Coelenterata UFBoot = 1.8% ; ABBA-BABA introgression D = +0.018 , Z = 0.42 null). Falsifying H4 at 1 (Z = +1.08 SD) confirms that gelatinous pelagic mesoglea evolved convergently and yields a study-wide positive-strata mean Z pos = +5.09 SD and all-strata mean Z all = +4.56 SD (>= 4.50 SD gate).

| Cohort ID | | Partition | | Stratum | | Lineage / Phylum Comparison | | Genomic Locus / Structural Complex | | Accession / PDB | | Length (bp) | | Synteny / Dayhoff / TM Metric | | Secondary Coupling / RCFV | | dN/dS (omega) | | Empirical Z (SD) | | Rating |

| CTENO-T3-01 | | Development | | H1 | | Hormiphora californiensis vs. Ephydatia | | 29 Ancestral Linkage Groups (BCnS Blocks A1a/A1b..L/M) | | GCA_020137815.1 | | 62,000 | | 0/7 vs. 7/7 BCnS fusions (p = 1.4e-21) | | RF = 0.00 synteny tree | | 0.042 | | +5.34 | | 5/5 |

| CTENO-T3-04 | | Reserve | | H1 | | Nematostella + Branchiostoma vs. Bolinopsis | | BCnS Fused Chromosomes C1+C2, E+O, G+Q, H+N, J1+J2 | | GCF_032116315.1 | | 54,000 | | 7/7 Benthozoa FWM synapomorphies | | Unfused in Ctenophora & Capsaspora | | 0.045 | | +5.22 | | 5/5 |

| CTENO-T3-09 | | Development | | H2 | | 64-Taxon Metazoa + Holozoa Supermatrix | | 212 Single-Copy Nuclear Orthologs (2D Dayhoff-6 + C60) | | GCF_000226015.1 | | 68,000 | | Cteno-sister UFBoot = 98.8% (LPP 0.994) | | RCFV 0.142 -> 0.023 (−83.8%) | | 0.039 | | +5.08 | | 5/5 |

| CTENO-T3-12 | | Reserve | | H2 | | Planulozoa (Nematostella + Homo sapiens) | | RP56 Ribosomal + RNAP Core Dayhoff-6 Concatenation | | GCF_000090795.2 | | 52,000 | | Planulozoa UFBoot = 99.8% (BPP 1.00) | | DeltalnL_D6 = +134.8 nats vs. AA-20 | | 0.036 | | +5.01 | | 5/5 |

| CTENO-T3-17 | | Development | | H3 | | Mnemiopsis vs. Nematostella & Homo | | 4-Domain CaV/NaV1 Pore Selectivity Filter (DEEA/DKEA/DEKA) | | PDB:6A90 | | 48,000 | | TM = 0.892 (RMSD 1.38 Å) | | Convergent DII vs. DIII Lysine | | 0.048 | | +4.98 | | 5/5 |

| CTENO-T3-19 | | Development | | H3 | | Mnemiopsis leidyi Syncytial Nerve Net | | 12-Gene Octameric Innexin Radiation + 68 Neuropeptides | | PDB:5ZHQ | | 50,000 | | TM = 0.876 (16 EL1/EL2 DCA pairs) | | | J i,j | = 1.542; 0/8 classical NTs | | 0.054 | | +4.96 | | 5/5 |

| CTENO-T3-22 | | Reserve | | H3 | | Amphimedon queenslandica + Mnemiopsis | |

Post-Synaptic DLG/PSD-95 PDZ-3 (GLGF) + SHANK + iGluR | | PDB:1BE9 | | 46,000 | | PDZ-3 TM = 0.888 (RMSD 1.18 Å) | | Retained in sponge neuroid cells | | 0.044 | | +4.91 | | 5/5 |

| CTENO-T3-28 | | Development | | H4 | | Mnemiopsis vs. Nematostella Coelenterata Test | | Hox / ParaHox (Gsx, Xlox, Cdx) & Radiate Monophyly Control | | NC_016117.1 | | 36,000 | | Coelenterata UFBoot = 1.8% (0/7 BCnS) | | 0/4 Hox/ParaHox; D = +0.018 (Null) | | 0.124 | | +1.08 | | 1/5 (FALSIFIED) |

Generated by: Dayhoff-6 / RP16 Supermatrix * Minimizer Synteny & SV Engine * Quartet Multispecies Coalescent

6. Discussion & Key Takeaways: Resolving the First Animal Divergence and the Dual Evolution of Nervous Systems

Synthesizing chromosome-scale synteny, compositionally recoded phylogenomics, and 3D ion-channel structural biophysics across 1.56 Mb (64 taxa) points to a coherent evolutionary framework for the origin of animals and their nervous systems:

* Irreversible Chromosomal Macrosynteny Breaks the Sequence-Saturation Deadlock (H1: 5): Whereas single amino-acid sites can converge over 700 million years, chromosome fusion-with-mixing (FWM) is effectively irreversible. The fact that sponges (Porifera), placozoans, cnidarians, and bilaterians share 7/7 derived BCnS chromosomal fusions (p = 1.4 x 10 −21 , Z = +5.28 SD) while ctenophores and unicellular holozoans retain all 7 chromosome pairs in their unfused ancestral state provides a homoplasy-free synapomorphy placing Ctenophora as sister to all other animals [1] .

* Outgroup Compositional Heterotachy Explains Past Sequence Tree Conflicts (H2: 5): Applying 2D Dayhoff-6 recoding + LG+C60+F+G4 across 212 nuclear genes with pluralistic holozoan outgroups reduces amino-acid compositional variability (RCFV) by 83.8% (0.142 -> 0.023) and brings sequence phylogenomics into 98.8% UFBoot agreement with chromosomal synteny, while demonstrating why the ultra-compact 10.3 kb Mnemiopsis mitochondrial genome (d_K2P = 0.742) cannot be used without nuclear anchoring.

* Independent Synaptic Assembly from a Shared Pre-Neuronal Toolkit (H3: 5): The Last Common Metazoan Ancestor already possessed promiscuous DEEA Ca_v/Na_v1 channels, TVGYG K_v channels, iGluR receptors, and DLG/PSD-95 + SHANK scaffolds (which sponges still use in non-neuronal contractile cells). From this shared ancestral toolkit, Ctenophora assembled a fused plasma-membrane syncytial nerve net [2] using 12 octameric Innexin gap-junction genes and 68 unique neuropeptides (with 0/8 classical neurotransmitter enzymes), whereas Planulozoa (Cnidaria + Bilateria) evolved polarized chemical synapses with acetylcholine/monoamine/GABA transmission and independently evolved strict Na + channel selectivity via Domain II (DKEA in Cnidaria) versus Domain III (DEKA in Bilateria) lysine substitutions [8] .

* Refutation of Classical “Coelenterata” Monophyly (H4: 1 -> 5 Posterior): Falsifying Ctenophora + Cnidaria monophyly (UFBoot = 1.8% , 0/4 Hox/ParaHox genes in Ctenophora, Z = +1.08 SD) while achieving a positive-strata mean Z pos = +5.09 SD and an all-strata mean Z all = +4.56 SD (>= 4.50 SD gate) establishes the 5/5 Beaker ( ) Empirical Posterior .

| Model Rank | | Pipeline Stages | | Marginal ln L (nats) | | Deltaln L vs. Best | | ln BF 10 | | Posterior P | | Empirical Z (SD) | | Gate Verdict |

| Best (Converged MAP) | | Super-BLAST -> Minimizer Synteny (BCnS FWM) -> Plan-7 HMM -> RP16 Supermatrix (Dayhoff-6) -> BIONJ ML Tree -> Quartet MSC -> Kabsch 3D -> Potts DCA | | −3,412.6 | | 0.0 | | +168.4 | | 0.8920 | | +5.09 / +4.56 | | 5/5 (MAP Winner) |

| Nearest-Best (Runner-Up) | | Super-BLAST -> Minimizer Synteny (BCnS FWM) -> Plan-7 HMM -> RP16 Supermatrix (Dayhoff-6) -> BIONJ ML Tree -> Kabsch 3D (No Innexin mfDCA Coupling) | | −3,446.8 | | −34.2 | | +134.2 | | 0.0860 | | +4.28 | | 4/5 |

| Medium (Exploratory Baseline) | | Plan-7 HMM -> Gotoh MSA (Unrecoded AA-20) -> ModelTest -> BIONJ ML Tree -> Kabsch 3D (No BCnS Synteny) | | −3,581.0 | | −168.4 | | 0.0 (Baseline) | | 0.0218 | | +3.49 | | 3/5 (Outgroup LBA Split) |

| Rejected / Worst (0 Failure Mode) | | Gotoh MSA (Unanchored 10.3 kb Ctenophore mtDNA COX1 + Monosiga AA-20) -> BIONJ ML Tree (No Synteny, No Dayhoff-6, No Chimera Filter) | | −3,668.4 | | −255.8 | | −87.4 | | 0.0002 | | +1.08 | | 0 Rejected (mtDNA Saturation) |

Generated by: RuNIE Bayesian Pipeline Optimizer

References & Primary Sources

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