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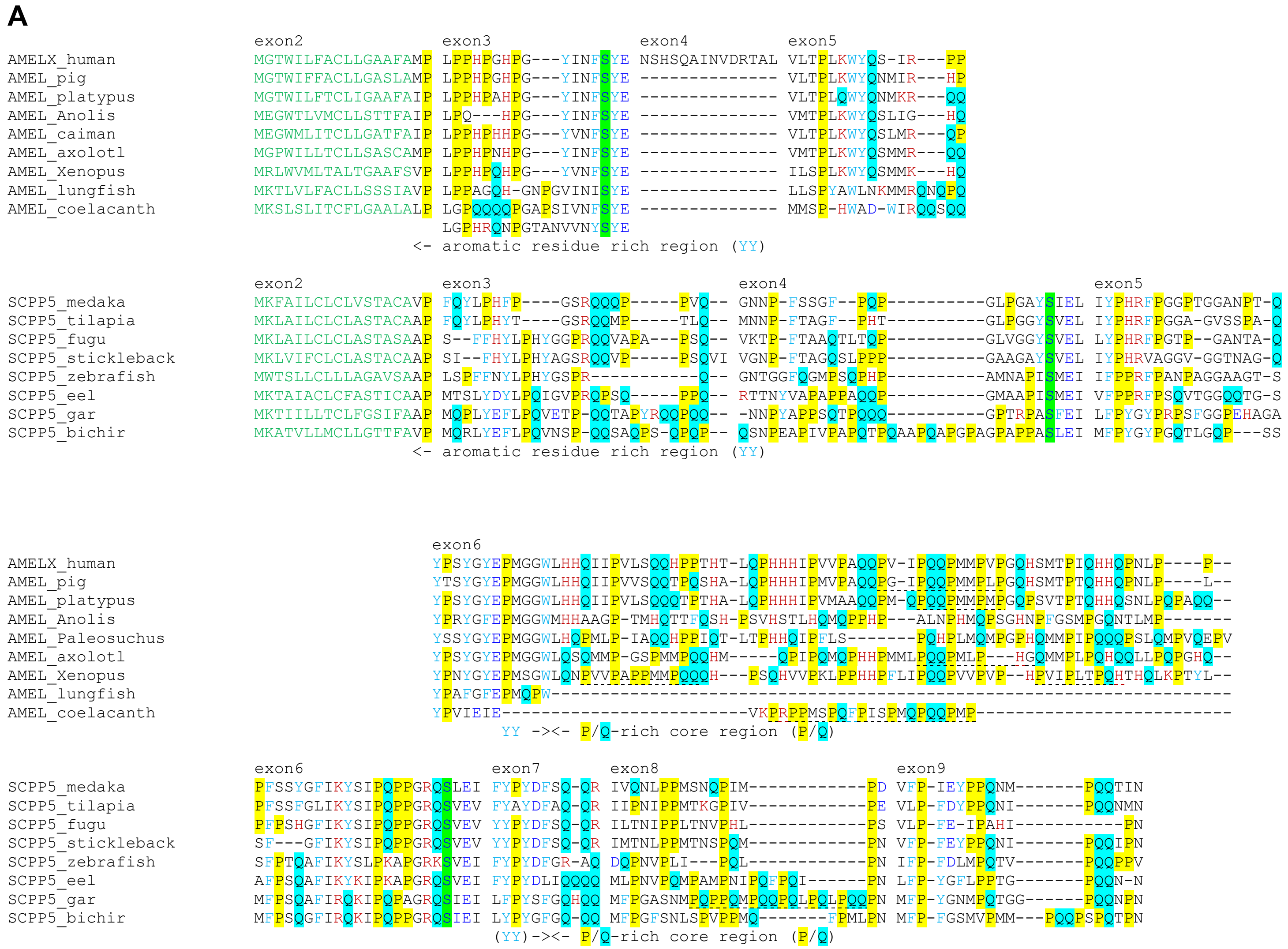
Supplemental Information

Coevolution of enamel, ganoin, enameloid, and their matrix SCPP genes in osteichthyans

Kazuhiko Kawasaki, Joseph N. Keating, Mitsushiro Nakatomi, Monique Welten, Masato Mikami, Ichiro Sasagawa, Mark N. Puttick, Philip C.J. Donoghue, and Mikio Ishiyama



A

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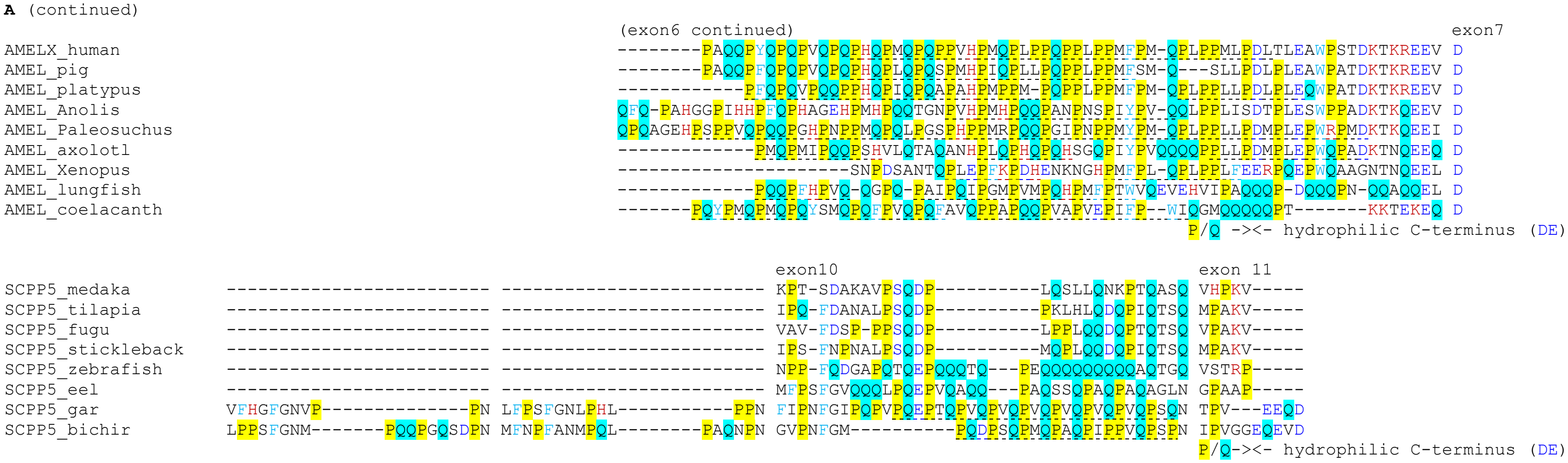
                         exon2              exon3               exon4          exon5
AMELX_human              MGTWILFACLLGAAFAMP LPPHPGHPG---YINFSYE NSHSQAINVDRTAL VLTPLKWYQS-IR---PP
AMEL_pig                 MGTWIFFACLLGASLAMP LPPHPGHPG---YINFSYE -------------- VLTPLKWYQNMIR---HP
AMEL_platypus            MGTWILFTCLIGAAFAIP LPPHPAHPG---YINFSYE -------------- VLTPLQWYQNMKR---QQ
AMEL_Anolis              MEGWTLVMCLLSTTFAIP LPQ---HPG---YINFSYE -------------- VMTPLKWYQSLIG---HQ
AMEL_caiman              MEGWMLITCLLGATFAIP LPPHPHHPG---YVNFSYE -------------- VLTPLKWYQSLMR---QP
AMEL_axolotl             MGPWILLTCLLSASCAMP LPPHPNHPG---YINFSYE -------------- VMTPLKWYQSMMR---QQ
AMEL_Xenopus             MRLWVMLTALTGAAFSVP LPPHPQHPG---YVNFSYE -------------- ILSPLKWYQSMMK---HQ
AMEL_lungfish            MKTLVLFACLLSSSIAVP LPPAGQH-GNPGVINISYE -------------- LLSPYAWLNKMMRQNQPQ
AMEL_coelacanth          MKSLSLITCFLGAALALP LGPQQQQPGAPSIVNFSYE -------------- MMSP-HWAD-WIRQQSQQ
                                            LGPHRQNPGTANVVNYSYE
                                         <- aromatic residue rich region (YY)

                         exon2              exon3                         exon4                               exon5
SCPP5_medaka             MKFAILCLCLVSTACAVP FQYLPHFP----GSRQQQP-----PVQ-- GNNP-FSSGF--PQP----------GLPGAYSIEL IYPHRFPGGPTGGANPT-Q
SCPP5_tilapia            MKLAILCLCLASTACAAP FQYLPHYT----GSRQQMP-----TLQ-- MNNP-FTAGF--PHT----------GLPGGYSVEL IYPHRFPGGA-GVSSPA-Q
SCPP5_fugu               MKLAILCLCLASTASAAP S--FFHYLPHYGGPRQQVAPA---PSQ-- VKTP-FTAAQTLTQP----------GLVGGYSVEL LYPHRFPGTP--GANTA-Q
SCPP5_stickleback        MKLVIFCLCLASTACAAP SI--FHYLPHYAGSRQQVP-----PSQVI VGNP-FTAGQSLPPP----------GAAGAYSVEL IYPHRVAGGV-GGTNAG-Q
SCPP5_zebrafish          MWTSLLCLLLAGAVSAAP LSPFFNYLPHYGSPR-----------Q-- GNTGGFQGMPSQPHP----------AMNAPISMEI IFPPRFPANPAGGAAGT-S
SCPP5_eel                MKTAIACLCFASTICAAP MTSLYDYLPQIGVPRQPSQ-----PPQ-- RTTNYVAPAPPAQQP----------GMAAPISMEI VFPPRFPSQVTGGQQTG-S
SCPP5_gar                MKTIILLTCLFGSIFAAP MQPLYEFLPQVETP-QQTAPYRQQPQQ-- -NNPYAPPSQTPQQQ----------GPTRPASFEI LFPYGYPRPSFGGPEHAGA
SCPP5_bichir             MKATVLLMCLLGTTFAVP MQRLYEFLPQVNSP-QQSAQPS-QPQP-- QSNPEAPIVPAPQTPQAAPQAPGPAGPAPPASLEI MFPYGYPGQTLGQP---SS
                                         <- aromatic residue rich region (YY)

                                           exon6
AMELX_human                                YPSYGYEPMGGWLHHQIIPVLSQQHPPTHT-LQPHHHIPVVPAQQPV-IPQQPMMPVPGQHSMTPIQHHQPNLP----P--
AMEL_pig                                   YTSYGYEPMGGWLHHQIIPVVSQQTPQSHA-LQPHHHIPMVPAQQPG-IPQQPMMPLPGQHSMTPTQHHQPNLP----L--
AMEL_platypus                              YPSYGYEPMGGWLHHQIIPVLSQQQTPTHA-LQPHHHIPVMAAQQPM-QPQQPMMPMPGQPSVTPTQHHQSNLPQPAQQ--
AMEL_Anolis                                YPRYGFEPMGGWMHHAAGP-TMHQTTFQSH-PSVHSTLHQMQPPHP---ALNPHMQPSGHNPFGSMPGQNTLMP-------
AMEL_Paleosuchus                           YSSYGYEPMGGWLHQPMLP-IAQQHPPIQT-LTPHHQIPFLS-------PQHPLMQMPGPHQMMPIPQQQPSLQMPVQEPV
AMEL_axolotl                               YPSYGYEPMGGWLQSQMMP-GSPMMPQQHM-----QPIPQMQPHHPMMLPQQPMLP---HGQMMPLPQHQQLLPQPGHQ--
AMEL_Xenopus                               YPNYGYEPMSGWLQNPVVPAPPMMPQQQH---PSQHVVPKLPPHHPFLIPQQPVVPVP--HPVIPLTPQHTHQLKPTYL--
AMEL_lungfish                              YPAFGFEPMQPW---------------------------------------------------------------------
AMEL_coelacanth                            YPVIEIE-------------------------VKPRPPMSPQFPISPMQPQQPMP--------------------------
                                                  YY -><- P/Q-rich core region (P/Q)

                         exon6                   exon7       exon8                        exon9
SCPP5_medaka             PFSSYGFIKYSIPQPPGRQSLEI FYPYDFSQ-QR IVQNLPPMSNQPIM------------PD VFP-IEYPPQNM-------PQQTIN
SCPP5_tilapia            PFSSFGLIKYSIPQPPGRQSVEV FYAYDFAQ-QR IIPNIPPMTKGPIV------------PE VLP-FDYPPQNI-------PQQNMN
SCPP5_fugu               PFPSHGFIKYSIPQPPGRQSVEV YYPYDFSQ-QR ILTNIPPLTNVPHL------------PS VLP-FE-IPAHI-----------PN
SCPP5_stickleback        SF---GFIKYSIPQPPGRQSVEV YYPYDFSQ-QR IMTNLPPMTNSPQM------------PN VFP-FEYPPQNI-------PQQIPN
SCPP5_zebrafish          SFPTQAFIKYSLPKAPGRKSVEI FYPYDFGR-AQ DQPNVPLI---PQL------------PN IFP-FDLMPQTV-------PQQPPV
SCPP5_eel                AFPSQAFIKYKIPKAPGRQSVEI FYPYDLIQQQQ MLPNVPQMPAMPNIPQFPQI------PN LFP-YGFLPPTG-------PQQN-N
SCPP5_gar                MFPSQAFIRQKIPQPAGRQSIEI LFPYSFGQHQQ MFPGASNMPQPPQMPQQPQLPQLPQQPN MFP-YGNMPQTGG------PQQNPN
SCPP5_bichir             MFPSQGFIRQKIPQPPGRQSIEI LYPYGFGQ-QQ MFPGFSNLSPVPPMQ-------FPMLPN MFP-FGSMVPMM---PQQPSPQTPN
                                                 (YY)-><- P/Q-rich core region (P/Q)
```



A (continued)

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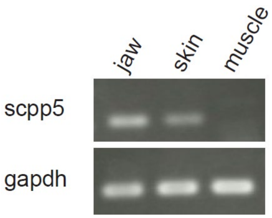
                                                          (exon6 continued)                                              exon7
AMELX_human                                               --------PAQQPYQPQPVQPQPHQPMQPQPPVHPMQPLPPQPPLPPMFPM-QPLPPMLPDLTLEAWPSTDKTKREEV D
AMEL_pig                                                  --------PAQQPFQPQPVQPQPHQPLQPQSPMHPIQPLLPQPPLPPMFSM-Q---SLLPDLPLEAWPATDKTKREEV D
AMEL_platypus                                             ------------PFQPQVPQQPPHQPIQPQAPAHPMPPM-PQPPLPPMFPM-QPLPPLLPDLPLEQWPATDKTKREEV D
AMEL_Anolis                                               QFQ-PAHGGPIHHPFQPHAGEHPMHPQQTGNPVHPMHPQQPANPNSPIYPV-QQLPPLISDTPLESWPPADKTKQEEV D
AMEL_Paleosuchus                                          QPQAGEHPSPPVQPQQPGHPNPPMQPQLPGSPHPPMRPQQPGIPNPPMYPM-QPLPPLLPDMPLEPWRPMDKTKQEEI D
AMEL_axolotl                                              -------------PMQPMIPQQPSHVLQTAQANHPLQPHQPQHSGQPIYPVQQQQPPLLPDMPLEPWQPADKTNQEEQ D
AMEL_Xenopus                                              ----------------------SNPDSANTQPLEPFKPDHENKNGHPMFPL-QPLPPLFEERPQEPWQAAGNTNQEEL D
AMEL_lungfish                                             -------------PQQPFHPVQ-QGPQ-PAIPQIPGMPVMPQHPMFPTWVQEVEHVIPAQQQP-DQQQPN-QQAQQEL D
AMEL_coelacanth                                           -------PQYPMQPMQPQYSMQPQFPVQPQFAVQPPAPQQPVAPVEPIFP--WIQGMQQQQQPT-------KKTEKEQ D
                                                                                                               P/Q -><- hydrophilic C-terminus (DE)

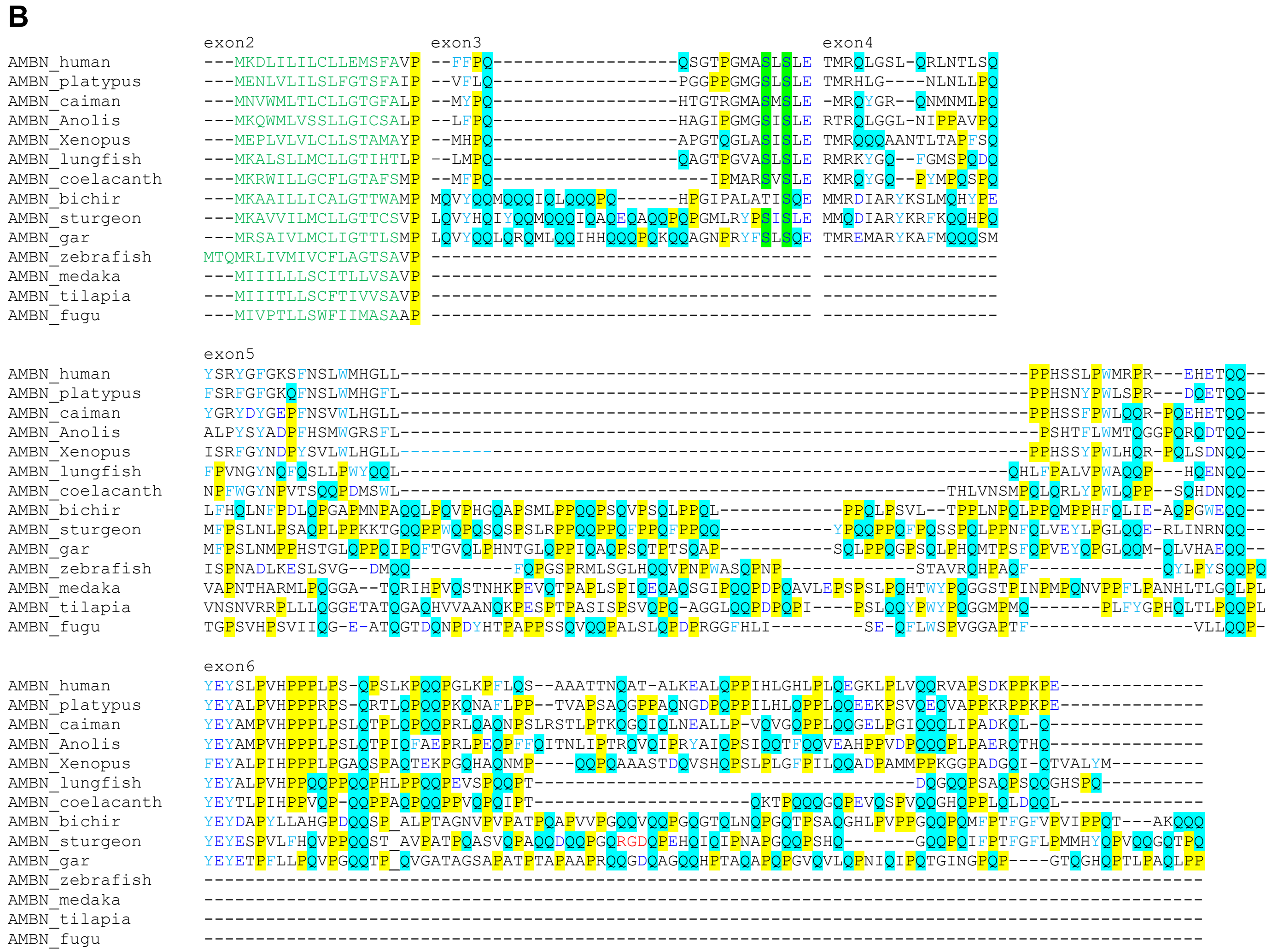
                                                                         exon10                                  exon 11
SCPP5_medaka         ------------------------- ------------------------- KPT-SDAKAVPSQDP----------LQSLLQNKPTQASQ VHPKV-----
SCPP5_tilapia        ------------------------- ------------------------- IPQ-FDANALPSQDP----------PKLHLQDQPIQTSQ MPAKV-----
SCPP5_fugu           ------------------------- ------------------------- VAV-FDSP-PPSQDP----------LPPLQQDQPTQTSQ VPAKV-----
SCPP5_stickleback    ------------------------- ------------------------- IPS-FNPNALPSQDP----------MQPLQQDQPIQTSQ MPAKV-----
SCPP5_zebrafish      ------------------------- ------------------------- NPP-FQDGAPQTQEPQQQTQ---PEQQQQQQQQQAQTGQ VSTRP-----
SCPP5_eel            ------------------------- ------------------------- MFPSFGVQQQLPQEPVQAQQ---PAQSSQPAQPAQAGLN GPAAP-----
SCPP5_gar            VFHGFGNVP--------------PN LFPSFGNLPHL-----------PPN FIPNFGIPQPVPQEPTQPVQPVQPVQPVQPVQPVQPSQN TPV---EEQD
SCPP5_bichir         LPPSFGNM-------PQQPGQSDPN MFNPFANMPQL--------PAQNPN GVPNFGM----------PQDPSQPMQPAQPIPPVQPSPN IPVGGEQEVD
                                                                                                                 P/Q-><- hydrophilic C-terminus (DE)
```

Chromosomal locations of *AMEL* and *SCPP5*.

	ARHGAP6	*AMEL*	P/Q-rich SCPP genes	*SCPP5*
Homo sapiens	X	X, Y	4	ND
Anolis carolinensis	3	3	2, 5	ND
Xenopus tropicalis	2	2	1	ND
Latimeria chalumnae	JH126997	JH126997	JH128739, JH128762, JH129486	ND
Lepisosteus oculatus	17	ND	2, 4, 22	4
Danio rerio	7	ND	1, 5, 10, 23	1
Fugu rubripes	1	ND	17	17

Expression of bichir *scpp5* in the jaw and skin, detected by RT-PCR.





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                   exon2                 exon3                                 exon4
AMBN_human         ---MKDLILILCLLEMSFAVP --FFPQ------------------QSGTPGMASLSLE TMRQLGSL-QRLNTLSQ
AMBN_platypus      ---MENLVLILSLFGTSFAIP --VFLQ------------------PGGPPGMGSLSLE TMRHLG----NLNLLPQ
AMBN_caiman        ---MNVWMLTLCLLGTGFALP --MYPQ------------------HTGTRGMASMSLE -MRQYGR--QNMNMLPQ
AMBN_Anolis        ---MKQWMLVSSLLGICSALP --LFPQ------------------HAGIPGMGSISLE RTRQLGGL-NIPPAVPQ
AMBN_Xenopus       ---MEPLVLVLCLLSTAMAYP --MHPQ------------------APGTQGLASISLE TMRQQQAANTLTAPFSQ
AMBN_lungfish      ---MKALSLLMCLLGTIHTLP --LMPQ------------------QAGTPGVASLSLE RMRKYGQ--FGMSPQDQ
AMBN_coelacanth    ---MKRWILLGCFLGTAFSMP --MFPQ---------------------IPMARSVSLE KMRQYGQ--PYMPQSPQ
AMBN_bichir        ---MKAAILLICALGTTWAMP MQVYQQMQQQIQLQQQPQ------HPGIPALATISQE MMRDIARYKSLMQHYPE
AMBN_sturgeon      ---MKAVVILMCLLGTTCSVP LQVYHQIYQQMQQQIQAQEQAQQPQPGMLRYPSISLE MMQDIARYKRFKQQHPQ
AMBN_gar           ---MRSAIVLMCLIGTTLSMP LQVYQQLQRQMLQQIHHQQQPQKQQAGNPRYFSLSQE TMREMARYKAFMQQQSM
AMBN_zebrafish     MTQMRLIVMIVCFLAGTSAVP ------------------------------------- -----------------
AMBN_medaka        ---MIIILLLSCITLLVSAVP ------------------------------------- -----------------
AMBN_tilapia       ---MIIITLLSCFTIVVSAVP ------------------------------------- -----------------
AMBN_fugu          ---MIVPTLLSWFIIMASAAP ------------------------------------- -----------------

                   exon5
AMBN_human         YSRYGFGKSFNSLWMHGLL-------------------------------------------------------------PPHSSLPWMRPR---EHETQQ--
AMBN_platypus      FSRFGFGKQFNSLWMHGFL-------------------------------------------------------------PPHSNYPWLSPR---DQETQQ--
AMBN_caiman        YGRYDYGEPFNSVWLHGLL-------------------------------------------------------------PPHSSFPWLQQR-PQEHETQQ--
AMBN_Anolis        ALPYSYADPFHSMWGRSFL--------------------------------------------------------------PSHTFLWMTQGGPQRQDTQQ--
AMBN_Xenopus       ISRFGYNDPYSVLWLHGLL-------------------------------------------------------------PPHSSYPWLHQR-PQLSDNQQ--
AMBN_lungfish      FPVNGYNQFQSLLPWYQQL-----------------------------------------------------------QHLFPALVPWAQQP---HQENQQ--
AMBN_coelacanth    NPFWGYNPVTSQQPDMSWL-----------------------------------------------------THLVNSMPQLQRLYPWLQPP--SQHDNQQ--
AMBN_bichir        LFHQLNFPDLQPGAPMNPAQQLPQVPHGQAPSMLPPQQPSQVPSQLPPQL------------PPQLPSVL--TPPLNPQLPPQMPPHFQLIE-AQPGWEQQ--
AMBN_sturgeon      MFPSLNLPSAQPLPPKKTGQQPPWQPQSQSPSLRPPQQPPQFPPQFPPQQ-----------YPQQPPQFPQSSPQLPPNFQLVEYLPGLQQE-RLINRNQQ--
AMBN_gar           MFPSLNMPPHSTGLQPPQIPQFTGVQLPHNTGLQPPIQAQPSQTPTSQAP-----------SQLPPQGPSQLPHQMTPSFQPVEYQPGLQQM-QLVHAEQQ--
AMBN_zebrafish     ISPNADLKESLSVG--DMQQ----------FQPGSPRMLSGLHQQVPNPWASQPNP-------------STAVRQHPAQF-------------QYLPYSQQPQ
AMBN_medaka        VAPNTHARMLPQGGA--TQRIHPVQSTNHKPEVQTPAPLSPIQEQAQSGIPQQPDPQAVLEPSPSLPQHTWYPQGGSTPINPMPQNVPPFLPANHLTLGQLPL
AMBN_tilapia       VNSNVRRPLLLQGGETATQGAQHVVAANQKPESPTPASISPSVQPQ-AGGLQQPDPQPI----PSLQQYPWYPQGGMPMQ-------PLFYGPHQLTLPQQPL
AMBN_fugu          TGPSVHPSVIIQG-E-ATQGTDQNPDYHTPAPPSSQVQQPALSLQPDPRGGFHLI---------SE-QFLWSPVGGAPTF----------------VLLQQP-

                   exon6
AMBN_human         YEYSLPVHPPPLPS-QPSLKPQQPGLKPFLQS--AAATTNQAT-ALKEALQPPIHLGHLPLQEGKLPLVQQRVAPSDKPPKPE--------------
AMBN_platypus      YEYALPVHPPPRPS-QRTLQPQQPKQNAFLPP--TVAPSAQGPPAQNGDPQPPILHLQPPLQQEEKPSVQEQVAPPKRPPKPE--------------
AMBN_caiman        YEYAMPVHPPPLPSLQTPLQPQQPRLQAQNPSLRSTLPTKQGQIQLNEALLP-VQVGQPPLQQGELPGIQQQLIPADKQL-Q---------------
AMBN_Anolis        YEYAMPVHPPPLPSLQTPIQFAEPRLPEQPFFQITNLIPTRQVQIPRYAIQPSIQQTFQQVEAHPPVDPQQQPLPAERQTHQ---------------
AMBN_Xenopus       FEYALPIHPPPLPGAQSPAQTEKPGQHAQNMP----QQPQAAASTDQVSHQPSLPLGFPILQQADPAMMPPKGGPADGQI-QTVALYM---------
AMBN_lungfish      YEYALPVHPPQQPPQQPHLPPQQPEVSPQQPT-------------------------------------DQGQQPSAQPSQQGHSPQ----------
AMBN_coelacanth    YEYTLPIHPPVQP-QQPPAQPQQPPVQPQIPT---------------------QKTPQQQGQPEVQSPVQQGHQPPLQLDQQL--------------
AMBN_bichir        YEYDAPYLLAHGPDQQSP_ALPTAGNVPVPATPQAPVVPGQQVQQPGQGTQLNQPGQTPSAQGHLPVPPGQQPQMFPTFGFVPVIPPQT---AKQQQ
AMBN_sturgeon      YEYESPVLFHQVPPQQST_AVPATPQASVQPAQQDQQPGQRGDQPEHQIQIPNAPGQQPSHQ-------GQQPQIFPTFGFLPMMHYQPVQQGQTPQ
AMBN_gar           YEYETPFLLPQVPGQQTP_QVGATAGSAPATPTAPAAPRQQGDQAGQQHPTAQAPQPGVQVLQPNIQIPQTGINGPQP----GTQGHQPTLPAQLPP
AMBN_zebrafish     -------------------------------------------------------------------------------------------------
AMBN_medaka        -------------------------------------------------------------------------------------------------
AMBN_tilapia       -------------------------------------------------------------------------------------------------
AMBN_fugu          -------------------------------------------------------------------------------------------------
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B (continued)

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                   exon7         exon8         exon9         exon10
AMBN_human         LPRVDFADPQGPS LPGMDFPDPQGPS LPGLDFADPQGST IFQIAR-LISHG--------PMPQN--KQSP
AMBN_platypus      LPALDFTVQLRQS ------------- ------------- IFPIAQKLISQG--------PRQKA--EQSP
AMBN_caiman        LPALEYSGHLGQV ------------- ------------- MYPIVHQLVHQG--------PMQPQ--QQPA
AMBN_Anolis        ------------- ------------- ------------- IYPALH-GTQQD--------PSQQL--QQQS
AMBN_Xenopus       ------------- ------------- ------------- YQTIMNKLLQQGAGETIPD-PAGTLPVNQQH
AMBN_lungfish      ------------- ------------- ------------- IEVPIF---QTPQVAQQP-QPPQQM--IFPA
AMBN_coelacanth    ------------- ------------- ------------- VSPSNGNAVQTPQVTQQPLQPGQQG--QQPQ
AMBN_bichir        ------------- ------------- ------------- -------------------------------
AMBN_sturgeon      ------------- ------------- ------------- -------------------------------
AMBN_gar           ------------- ------------- ------------- -------------------------------
AMBN_zebrafish     ------------- ------------- ------------- -------------------------------
AMBN_medaka        ------------- ------------- ------------- -------------------------------
AMBN_tilapia       ------------- ------------- ------------- -------------------------------
AMBN_fugu          ------------- ------------- ------------- -------------------------------

                   exon11
AMBN_human         L--YPGMLYVPF--------GANQL ---------------------------------------------------------------------------
AMBN_platypus      L--YPELVYVPF--------GANQQ ---------------------------------------------------------------------------
AMBN_caiman        L--HPALFYMSY--------AANQG ---------------------------------------------------------------------------
AMBN_Anolis        I--YPSLYYMPY--------VANQG ---------------------------------------------------------------------------
AMBN_Xenopus       P--YPGLFFMQY--------GGGPG ---------------------------------------------------------------------------
AMBN_lungfish      F--E---YFYQF----PVQQGQQPM MFPSFENSP-QFPGQQGQYPVMLPTSDILPPVIQQPDIQQPQLPQVPPTNSFLPSIVQQGQGQQQQQQQQQQPQQ
AMBN_coelacanth    IQFFPTYEYLQMLPQVPGQQGQQPG GYPPYSFFP--ALAQQPGLDP------------------------------QQQQQQQQQQQQQQQQQQQPQQQP
AMBN_bichir        ------------------------- FSGYGLLL-PASFPPQPV-----------------------------------------QPGLEQQPPQQQPSQS
AMBN_sturgeon      ------------------------- FPGYSFLI-PAAFPQQ------------------------------------------------QAQPGQEQQQQ
AMBN_gar           V--FNTF---GILPIMPPQAQDEQQ FPGYGVFF-NTAFPQQPV------------------------------------------QPPQAAQPGAPPQQQ
AMBN_zebrafish     F--F----YYPILGQQS----RNPT FS-YGVSAFQHAASQQYPANAAPALRPARP-------------------------QQGQNAHQMRQPQQQQQQQQ
AMBN_medaka        M--FPPYGFYPLFAPPY----GNQM FSPYGFPSIRESPFLQAPTQQLQNNQAPPAENVV------------------------------PAGAAPQQTQQ
AMBN_tilapia       I--FPPYGYLPLFSSPY----GNQL FSPYGYPMILESGLPQIPANQLPNSPVLPAENAAGVAV-----------------DPSIDATQGIQQPQQQLQQQ
AMBN_fugu          I-SSPR-SFIPLISPAF----INQL ---------------------------------------------------------------------------

                                        exon12
AMBN_human         -------------------- N-APARLGIMSSEEVA
AMBN_platypus      -------------------- NAAPARLGKVSSEEMP
AMBN_caiman        -------------------- G-APARLGIVSSEEML
AMBN_Anolis        -------------------- A-APARQGIVSSEEMQ
AMBN_Xenopus       -------------------- G-PPARLGAMSSEEIT
AMBN_lungfish      GFPIQFYLQALGHQ-NLPQN V-----QGAISSEEIQ
AMBN_coelacanth    PFPSFVYVPLPARQEQLPQG G-FPNTFGSMSSEEMQ
AMBN_bichir        MFPQLVYFPLQT----GVMA G----AIGSVSSEEMQ
AMBN_sturgeon      MYPQLVYIQLQP----GMAG G----ASGSVSSEELQ
AMBN_gar           MIPQILYLQIQPM--RSAMG G---GGFGSASSEEMQ
AMBN_zebrafish     FQPIF-YMVPQMP----QRM A---GSYGGLSSEELQ
AMBN_medaka        QNPPIVYMLQ-------QPM N---PAIGGLSSEELE
AMBN_tilapia       N-PQILYMLQ-------QPM S---SPLGSLSSEELQ
AMBN_fugu          -------------------- D---STLSSFSSEEFE
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B (continued)

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                   exon13
AMBN_human         GGRE-DPMAYGAMFPGFGGMRP---------------------------------GFEGMPHNPAMGGDFTLEFDSPVAATKGPEN-------EEGGAQ----GSPMPEANPDNLENPAFLTELEPAPHAG-LLV
AMBN_platypus      GGGG-EPLAYGSIFPGFGSMRP---------------------------------GFGAMQINPALGKDFTLEDDSPTVAGK-PPG-------QGGEVL----GSPRDGAIPAGHPNPGLLPERISRLRSGAALT
AMBN_caiman        GGRG-GVPAYGAMIP----------------------------------------GFRGMPQDPALQGDFTTEDDNPATAHN-PAI-------QGGANQGFSRGSRFPAVNRAGHGSAILLPDGTPAGHEG----
AMBN_Anolis        GGGF-GAPAYRAPGPDLFAMDT---------------------------------RFGNSPLN--QPGDYTVEDDTLGITEQ-PDV-------KGGANA---------GANPSGKGNNAVLLDGTQGGALP----
AMBN_Xenopus       GGRTGAAHAFSSLYPGLLGMGP---------------------------------RLESHPQDPALQGDFTIEDDSPVAGQK-PTG-------QEVSQG--------PIESPQGVGSSIMIPGLEGSPTGQ-GET
AMBN_lungfish      GFPIQFYLQALGHQNLPQNVQGAISSEEIQANRAAGAAAAAVFNSMFPAFAANYPGFGSPVARPISPVDATIEDDHPVVGQGEQAGQGTGTPGVQGNLPNAVGT--------------PSSPAVNIPIPEGNPTG
AMBN_coelacanth    AAGGGLS-PALGGIIPGYVGNIPG-FGAGVPSFGGSVPGAAANIPMF--------GFPNTAVNPAGQGGVTVEDDPPPAAEK-PASPGN-SPGT-GANPSGQGGSAPNPEGNPTGQVGSIPNPQANPPPSEGTPS
AMBN_bichir        QSAGDMS-PPMAGFIPGFATPT---FGGGFPNFVGSGPAVGGTVPV---AGGI--PTAQGTQPGVLPEV------------------------------------------------------------KLPIPV
AMBN_sturgeon      AAGG-IS-PGFAGFIPGFAGQA---FGGSIPSFGGSVPGAEGSIPF---AGGV--PTGQGTNP-VVPEA--------------------------------------------------------GLPTVEILPA
AMBN_gar           AAGG-VA--GFGGFVPGFAGSG---YGGFGGTVPGTE--GVFPTGQGILPVAPDAGLF-PVGQGG------------------------------------------------------------SPTAPAVFPA
AMBN_zebrafish     NMGR-VNMHLPAIRGNVFPVSGPQTVVPIGPVRPPNPFPATGMTYPGITELQQPSIIAVPPSRDAIPATGGSH----------PNSAVLPSRISGTQDRDRAPCVSAEIPLTNSFAPLD--SGHGAFISADVP--
AMBN_medaka        MQAK-LNQMSV--YSVLTNLPAGAGPVQPENQAAGLTNPGEQGATPHVGGSA-----------------------------------------AGVRSLQGPACSGSLLNSNSVPAGVKTAVPEANAVQTQNHAK
AMBN_tilapia       MAAT-MGQLGV--YMPNVLTNQPAGAVQPESQAAGLTHPEQQGVQPAVGTSA-----------------------------------------AGAQPIKALPC-SGSQPNASGFSAG-LAGPDAATAQTPAEPQ
AMBN_fugu          --------VNI--YLSTALTDPSAATVQPVKQAAGL-----QNLVSTVGTPS-----------------------------------------TGAPQNQ-VPTSSGPPMDTNGVPVG-SEKPAEEMATVQHPVE

                   exon13 (continued)
AMBN_human         ---------------------FLKDDIPGLPRDPSGKMKGLPSVTPAAVDPLMTPELADVYRTYDADMTTSVDFQEEATMDTTMAPNSLQTSMPGNKAQ----------------------EPEMMHDAWHFQEP
AMBN_platypus      ---------------------FPSDFIPHRGQGPAGQSKLPPGVTPPPADPLMTPGPSDPLEFFEGDGTTPLGLQREALLDTTMTPDTQSTYMPGNEAQ----------------------QMQVTQDQWQFQEP
AMBN_caiman        -------------------FLPNINDMPGQGVNPVGQ-RGTPEVTPTATAPELTQGIPDSFMTFGAEGTVPLGIQKEVTADPTMFPEAQHTLMAGNGAE----------------------QPQVMQDVWHFQEP
AMBN_Anolis        -------------------------NANSHLFSPAGQSQGLSGLAQATAAPHATQG---PFLPLDAADPS-------MPLDATMFPD-LYTSNVGNEAG----------------------LAQVGQDAWHFQEP
AMBN_Xenopus       ---------------------VPFPNINSPNMGFNPQSKIPPGVTP-ANAPRLTHDTGAGYVPFALDDTMPFGIQRENVISGDITRANNAKGIESPIMQH---------------------EVHLQNHNNYFQEP
AMBN_lungfish      QIGNPQIP----------------------------QSNPSSPGQGGIMQNPDINPSNQGLPATPADIPAASAGNPSAATASPNEGGL----------------------------PVIYYEGLNHPAARGPWFP
AMBN_coelacanth    VQGVPQPAPEGNLPNLEGN------PTGQVGNQPAPQSKGPMGGATTPASTKNLTPANVPGGPEGGIVPFGAGDTASAAARKVVPTIPAFFNPSLGGTAPTENNNAVRSNAAGVLPPIVVNEGLN-PLGGGWIYP
AMBN_bichir        GQG------GSPTIPEVIPIGQGNMPPKTEGAVPAQGGQQPSPQMKLPNMTPDGVLLDGPVPPEGTVQSFAPGSTQDTIGKKSPTAAPDPATVP---------------KAQAPTPPEVEMPDLDDYTANFYLHP
AMBN_sturgeon      GQG------GGPTIPEVIPTGQGSLP-----VMSKTGNQPAPQSKLPTVGLTTPAWVRMLTPGNSPAAA.
AMBN_gar           GHGNLPPTPGS-TVPV-----QGS------------NNQPAPQSRDPAFSMTTPAGVRAATVGNPPAAAAGTAQPNAAAAAATAAAE-----------------RVHVNHAERNIPADDEMPEADDYRAP-FIYP
AMBN_zebrafish     ------------------------------------------------------------------------------------TVHPDPQSSGPNLVPDPLPTAEYKEDLFPAAPP-LLSDTVDTNTAN-EMYP
AMBN_medaka        SLPTNRNLVRSKAN.
AMBN_tilapia       LQPMQETLV.
AMBN_fugu          AELLPLTQTRSV.
```

C

```
                       exon3                  exon4             exon5
ENAM_human             ---MK-ILLVFLGLLGNSVAMP MHMPRMPGFSSKSEE-- --------MMR-YNQFN--FMNGPH-
ENAM_pig               ---MK-ILLVFLGLLCYSAAMP MQMPRMPGFSSKSEE-- --------MMR-YGHFN--FMNAPH-
ENAM_platypus          ---MK-SFLLFLGLFGASTAMP MHMPRMPGFGSKSEE-- --------MMP-YGQYN--FMNSPH-
ENAM_Anolis            MQRMK-QLLLYLCLIGTSWAVR LRKPRKAGFGSKSEE-- --------MMQ-YGPFG--YLNSPQ-
ENAM_Xenopus           --MMV-PIILLLCFLISSNSVP MHF---SHMSSNSEE-- --------GMP-YGPYG--YQN-PQ-
ENAM_coelacanth        ---MK-TLLLLVCLLGTSLAGL ARL--VRKARSNSEE-- --------MVQKFTSYNRHMNPQQRL
ENAM_bichir            ---MK-RGLLLLCFLGTALAFP V----GPVALSASQE-- --------ALMQQ-RWNALLGAFQQQ
ENAM_gar               ---MK-IALLVLCFLGTSLAFP I----VPGAFSDSHE-- --------AKMQQYRWYG-APQQQRQ
ENAM_zebrafish         ---MK-AVALLMCLLGSSLAAP -------APDSGSNE-- QAIASHANTALQLMELYRMLGQLRQQ
ENAM_trout             ---MI-SVVLLMCLLGFSLAAP -------TPDSGSDE-- Q-VAAHANEALRWMELYRMYGSL---
ENAM_fugu              ---MVTILVALMVLLGTAWSAP -------ALGSESAEEN --VAAHANQALRWMHMYRLY---QQQ
ENAM_medaka            ---MK--LFVFLYLIISASAAP -------APDSDSSE-- --VAAHANAALRLMELFRMY---QQQ
ENAM_tilapia           ---MK-QLVFFICLLVSTLAAP -------APASESSE-- --IAAHANEALRWMEMYRLY---QQQ

                       exon6
ENAM_human             M-AHL-GPFFGNG--LPQQFPQYQM--------------------------------PMWPQPPPNT-WHPRKSS-------------APKRHNKTD-
ENAM_pig               M-AHL-GTLYGNGMQLPQFFPQYQM--------------------------------PMWPQPPPNK-KHPQKPS-------------ASKQQSKTD-
ENAM_platypus          M-AQL-GPLYGYGLQLPQFFQPPQM--------------------------------PPWPQPPPNG-WPAQNPGPPQ----------GGKPQAKPG-
ENAM_Anolis            L-TQLAASLYGYRSGFPQMVPQQ----------------------------------PTFPLQRFYL-WPQQMPVHQS----------ARPPQQKAH-
ENAM_Xenopus           M-PGI-GSMYGLG--FPHYSQMIQL--------------------------------QQAFGRQPSS-WLPQNIARTQQIHKETIPLNLPRFHLQPV-
ENAM_coelacanth        QVMQL-RSLYGNAPQRL-MYPQQQLQQQQQRKLLYARLLAQQALKQTQQSRAQQNAAPQLQVPKPQLQWPLQEPQVQQ----------KQKQQQQLLQ
ENAM_bichir            --------------------------------------------------------------------------------------------------
ENAM_gar               --------------------------------------------------------------------------------------------------
ENAM_zebrafish         --------------------------------------------------------------------------------------------------
ENAM_trout             --------------------------------------------------------------------------------------------------
ENAM_fugu              --------------------------------------------------------------------------------------------------
ENAM_medaka            --------------------------------------------------------------------------------------------------
ENAM_tilapia           --------------------------------------------------------------------------------------------------

                       exon6 (continued)
ENAM_human             QTQETQKPNQ-----------------TQSKKPP-QKRPL------------KQPSHNQPQPE--------EEAQPPQ-------
ENAM_pig               PAPESQKPNQ-----------------PQPKTPT-PKQPL------------NEPSPTPTQPE--------EETQTPQ-------
ENAM_platypus          QSQNTKQPNP-----------------SQPKKPP-QQQPP--------KQAAKQPAPGPSKPT--------EETRQPQ-------
ENAM_Anolis            QTPVPKKPNSRPRLKPRLVPQQPRYQQPQPKIHLPAKQPP------------VATHPFQPQPQTPIQPPKGEKPQQPQ-------
ENAM_Xenopus           QSQLSQLPAP--------PVQQPQLNPPQSKSNYVTKQP-------------HQPLQLPPAPD--------QQAQAPM-------
ENAM_coelacanth        QAQNLKNVLP---------------QQPQRLQQKPSQQPQGLQHQKPPQQAKKQPQSRQR-----------FSRKQPQQKDLQQL
ENAM_bichir            -------------------------------------------------------------------------------------
ENAM_gar               -------------------------------------------------------------------------------------
ENAM_zebrafish         -------------------------------------------------------------------------------------
ENAM_trout             -------------------------------------------------------------------------------------
ENAM_fugu              -------------------------------------------------------------------------------------
ENAM_medaka            -------------------------------------------------------------------------------------
ENAM_tilapia           -------------------------------------------------------------------------------------

                       exon7                                                        exon8
ENAM_human             AFPPFGNGLFPYQQPPWQI--PQ ----------------- ------------ RLPPPGY-------------------GRPPISNEE--GG
ENAM_pig               AFPPFGNGLFPYQQPLWHV--PH ----------------- ------------ RIPP-GY-------------------GRPPTSNEE--GG
ENAM_platypus          MYPPFGNGFFPFQLQPWQI--PP ----------------- ------------ QMPP-GY-------------------GRPPGSNEE--GG
ENAM_Anolis            AFPP-------HPQQPWQF--PQ IFGHGGFQPQLFNPYQG ------------ RMPF----------------------GRPPNSEE----G
ENAM_Xenopus           MFPPFSNIMNPY-QPYW----QQ ----------------- ------------ PFMH-GF-------------------GRPPNSNE---GN
ENAM_coelacanth        VYPSQGYIRMLP-QMLWPQRQQQ ----------------- ------------ PV----------------------GRRKQSGSYEEQGMG
ENAM_bichir            GNYP----KVVYIQLPSPP-MAG ----------------- ------------ MGPNR---------------------AAPAESEE---GG
ENAM_gar               QDFP----KVVYVHLPP---MAG ----------------- ------------ MGPNT---------------------AAPAESEEA--GA
ENAM_zebrafish         GY-GA-----VAG--PQL----P ----AAPAAPVDPAPVD TQPQVSPQVPQQ GFFFNPAAFA----------------PTGDGSDEE--VA
ENAM_trout             GQ-LA-----APA---QP-PMLN ----APAAAPATPAQDP ------------ KFFYPPSPMN---------------------SDEE---A
ENAM_fugu              AMLQN-----PFV---FPAGRAQ ----------------- ------------ VNAAPVQPLAVAPQAAVAHPAAVAHPAAVGDSDEE--AQ
ENAM_medaka            GLA-N-----PFL----PAAGAH ----AGPAAAAD----- ------------ INV-PVQPAA-------------AAIPAGDASDEE--AE
ENAM_tilapia           GIVQN-----PFV----PSANSP ----AVSAGAT------ ------------ VDAAPAQPADVPP---------PAHVPVGDASEEE--TE
```

C (continued)

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                        exon9
ENAM_human              -NPYFGY---------FGYHGFGG-RPPYYSEEMFEQDFEKPKEEDPPKAESPGTEPTANSTVTETNSTQ-PNPK----GSQGGNDTSPTGNSTPG---L
ENAM_pig                -NPYFGF---------FGYHGFGG-RPPYYSEEMFEQDFEKPKEKDPPKTETPATEPSVNTTVPETNSTQ-PNAP----N-PRGNDTSPTGTSGQG---P
ENAM_platypus           -NPYFGY---------FGHQGFGG-RPPYYSEEMKEEEKE------SPKEDSPSPEPTANSTVAETNSTQ-PSGP-SQAGSQGGNATDPGQSAGNGSPAQ
ENAM_Anolis             -NPYFGP----------GYQGMGG-RPPYYSEEMFEQEDK-----EAPK-ESPATDPVTNST--DTNSTV-SNP-----ASQGGNGTMSGLSEKATGANS
ENAM_Xenopus            -GNYFGF------------FGMGG-RAPYNSEEINEDEEPAKAEKESPKTESPVAE-TSNTTIADNNSTT-ISQ-------SQGNATTNNESMAGDITQS
ENAM_coelacanth         -ISYFGA-----------YYPEI-RRRPLESEEMYPESEG-----KSKKTED-------NSPVPEVSSPTSASPP----AGQDGNSTNAGGNHTVHGENL
ENAM_bichir             QPQYAGYMPPAYGYGPYGGYGHFGKRMPL-SEEINEEGPAKAEGGKEPTKPPKAEEATPTPGGTATKPDGAAPNTEGGAPAAGGSPPAAEGSPNGQGGTP
ENAM_gar                -PQYGGYVPSFYGYGPFG-FGNFGRRAPL-SEEINEVNEKESESPGKPEAPAATEAPKAEDLPPTPGGV--PTNTEGAGAAASANGTAANGAGNQPATEG
ENAM_zebrafish          -PQYRGF------YPHYGYHQPAQPAAPLNSDEAEGAEAEGAEAEAGAEAGAEAGAEAGAEVGAEAE----PAGTAATVDAAAANEI---LPVDVAEPAA
ENAM_trout              -PVPRYGGYGRY-YPYPGLAAPAAPAVPLNSDEVGEDEAAT-EAEAE----------PAVDHAAEEPAVVDTAAPVDPAD-AAPVDPAMDVPIDVVIPAA
ENAM_fugu               -MQ---------------AAGPGAPAAPLNSDEAEEASEAEPAADPEAAGAAADATPAPAGA--EVAATAAADAPADAAAASGPEAAAADVPAAA-DVPA
ENAM_medaka             -DGNP---------------GPGVGVAPLNSDEEEEVEEVEATEVE-----------PAVVETAAEATAAPPAAAAEPAVVDVAVDAAPVDDVV-VDVPP
ENAM_tilapia            -DGNP--------------ATKAAGPAPLNSDEVEEAEEVEAAEAD-----------PAMVEAEPSANPAAVVEPAVVDVPIDV---APVDDAAAVDVPP

                        exon9 (continued)
ENAM_human              NTGNNPSARNGI---GPLPAVNASGQGGPGSQIPW--RPSQPNIRENHPYPNI-RNFPSGRQWYF------------TGTVMGHRQNRPFYRNQQVQRG
ENAM_pig                NPRSNPTGQNG-------PAVNVSGQGVPRSQSPW--GPRQTIIHENYPNPNI-RGFPARRQWRP------------PGPAMGHRRNGPFYRNQQIQRG
ENAM_platypus           TPGHGTVPDNGE---PPGPLLNASDHGGARGPKPTGPGPGQPPFRGRPANPNAAPGFPAGRQRRPFVHPFGRPFDRPVGRPVGRRDHGPTYGNTPKARG
ENAM_Anolis             PGLQ-NNMFSGNAVSTPLPTTQTSEGNGVA---QN--VLEQIRHRSKLPNGDVIQSFPSGRQHST------------MQVYNI---HKPHEHMEN----
ENAM_Xenopus            STIA-LNAQNGH---IEGPPVIAVVHNKHKNTLHG--ASRQAHYVVRAPNPDTILSN-----------------------------HQPHQKYHI----
ENAM_coelacanth         ---------PNTDINPSGQGDNTSNTGNVHFGRGDNP-QNPLSNPTGQEGSFPNPEFNPLGQGNMPPNTEVNPLGQGSTNPDPGYNSVGQGSIPPNTRY
ENAM_bichir             ---------TNPEANPPASGGNSPANGQSQPDRGDSP-NQGVNQPN------PAQGINPMYPGNVNPFSG-----------------------------
ENAM_gar                GPNTPGSSPPKPEGAPAGPGGNTPAVGPNLPGRGDSGPNAGPSSP-GVAGGFPVPGTNPLYP-NLDPFAG-----------------------------
ENAM_zebrafish          DVIAAAV-----AAV---------DPALSPE--------------APAVAMEIDTTV-----------------------V---GPDVAAGEQVL----
ENAM_trout              VDIPATV-----ATD---------IIVVDPA--------------LIAPI---DTIV-----------------------AADVPAEVDAGATDV----
ENAM_fugu               D-AAAADVPVDAAAADVPVDAAGSGVPAAAAVDVPADAAAADVPVAAACS-DV--PAGAEAAAAEVPVDAAAADVPV---------RGDATAADVPTGV
ENAM_medaka             VDVAAVD-----IAP---------EVAVDVA--------------GPAAA-DI--PA-----------------------AADVPAAGDTVATDA----
ENAM_tilapia            VDVVPVDS--APAAP---------EVAVDPA--------------APATA-DI--LA-----------------------V------------------

                        exon9 (continued)
ENAM_human              P-RWNFFAWERKQVARPGNPVYHKAYPPTS-RGN--Y-PN-YAGNPANLRR-KPQGP-NKHPVGTTVAPLGPK-PGPVVRNEKIQNPKEKPLGPKEQI-
ENAM_pig                P-RWNSFTLEGKQAVRPGYPTYRRVYGSTA-RSN--P-PN-YAGNSANLRR-KPEGP-NKNPMVTNVAPPGPK-HGTVDQNENIQNPREKQVSQKERT-
ENAM_platypus           PLRWNAVTSEGKRVARPGYPMYRRPFPVAA-PGRG-F-PN-RAGNPLNLRK-NPQAP-AKHPGEANSGPVYSQRGGLMAPREPVASPQENPPSPREKP-
ENAM_Anolis             --------I-KQNLISRGNPSIQSEDPTYP-MGNG-WNSN-HRGNIQNTNVNNPSTN-T-------------R-HIPYGQQEQPHYPERNEFSQIDRV-
ENAM_Xenopus            --------VDKILVGRGGFPSQNTIYHQEQITGKVPL-TNIHTEKSSYIRH-VPQTNSDKIPLRTVATKDGDS-F-----------------VREARGM
ENAM_coelacanth         NPSGQGRIPPHTRANPPSQRSMSYPNTQRVGQKGIQPNPDYYPSGQESIPSNTRYHTFSQGSMTPNHIYKPSGEENIPPSPEYDPSDHGSIPPNHRYNP
ENAM_bichir             ------------------------------------------------------------------VFLPAGIRKVTPSPDTG----------------
ENAM_gar                ------------------------------------------------------------------VMWPAGVRKVTPSPSAG----------------
ENAM_zebrafish          -------M------------AEAPDAALSV---------------------------------------------------------------------
ENAM_trout              -------GA-APLDAEANMTGGPADPTATLLA-------------------------------------------------------------------
ENAM_fugu               DAAAADTAAHADLAAEGPAAAGSSCPDATLLA-------------------------------------------------------------------
ENAM_medaka             --------------AENEVTGGIADPALPV-M-------------------------------------------------------------------
ENAM_tilapia            --------------------AGPLDPTAL----------------------------------------------------------------------

                        exon9 (continued)
ENAM_human              -IVPTKNPTSPWRNSQQYEVNKSNYKLPHSEGY---MPVPNFNSVDQHENSYYPRGDS--RKVPNS--DGQTQSQNLPKGIVLGSRRMPYESETNQSEL
ENAM_pig                -VVPTRDPSGPWRNSQDYGINKSNYKLPQPEDN---MLVPNFNSIDQRENSYYPRGES--KRAPNS--DGQTQTQIIPKGIVLEPRRIPYESETNQPEL
ENAM_platypus           -SFPTRGPAVPWRKPQNYGPGKPVYRPPLSEGR---PADPGPSPFDPRENPYYPRGDATDRRFLSS--GGSAPGRHFPEGIVLKP-------EDDQPPS
ENAM_Anolis             -PFPSSDPVGQWNKDPIYRDNDPKNSPP--EGH---PLDPQFKTM-RIDNIYNARQDA--ERFQPS--GMHQSNALSHQGVNLATRRTPFEIETHQYDW
ENAM_Xenopus            HFDPTMNTIGNDAIQQIYRNNVPDNASPKVDNEHLSFLVN-KNKTPNRKIILVQHQGR--EHFPGHIMESVIESEYAPRGIFSSHRNTPSYTQINKEDQ
ENAM_coelacanth         SGQVNMPLNPNHKSFDKGGSSHIPQYSHLGHESVPPNPKYNPSGQESILPEQVYNPSGQE-RLTPYSKYNPPGQERIMPNSRYDLSGPENIQPNYRYSP
ENAM_bichir             --------------------------------------------STQILPGGFV-PSADGGATIP----------------------------------
ENAM_gar                --------------------------------------------ATQ----GFAEPGGED----A----------------------------------
ENAM_zebrafish          ---------------------------------------------------------------------------------------------------
ENAM_trout              ---------------------------------------------------------------------------------------------------
ENAM_fugu               ---------------------------------------------------------------------------------------------------
ENAM_medaka             ---------------------------------------------------------------------------------------------------
ENAM_tilapia            ---------------------------------------------------------------------------------------------------
```

C (continued)

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                        exon9 (continued)
ENAM_human              KHSSYQPAVYPEEIPSP-A----KEHFPAGRNTW-------------------------DHQEISPP---FKEDPGRQEEHLPHPSHGSRGSV-FYPEY
ENAM_pig                KHSAYQPV-YTEGIPSP-A----KEHFPAGRNTW-------------------------NQQEISPP---FKEDPGRQEEHLPHLSHGSRVHV-YYPDY
ENAM_platypus           KQKPFHPV-TSERTPR---------EPSPTKPQR-------------------------TSPEPPPP---LEEGPGRQDKSLTYPPISPRSGI-PYPDH
ENAM_Anolis             REQPFNHI-DQKR----------GQFLSAQTPTW-------------------------NNREASQL---FQEAPPRQSSMYSSGSFNQREMP-TYSGK
ENAM_Xenopus            KLSVPNNG-DNSGN-INTAHIKSEDLFRGGEFFQHNGENAALYQKEGTASDIGTASDIGTAYDIGTAYDIGTATTGQQLESIYLVKHFPTNHFQVYGDN
ENAM_coelacanth         SGQESISPNSRYNPSGQEIHPPNSRYNPSGQENIPSNPNYNPSDQGRMLPHHEVNLPNKKSIQPNSGYHPSGQKNLSPRHMYNPSDHGSNPQNHEIDPL
ENAM_bichir             -----------------------VTSSPSGNNNAVLNIPYYPSNGG------------------------------------------------NINPV
ENAM_gar                -----------------------TTPAPMGKGSPALNIPFSPSEGA------------------------------------------------KRNPS
ENAM_zebrafish          ---------------------------------------------------------------------------------------------------
ENAM_trout              ---------------------------------------------------------------------------------------------------
ENAM_fugu               ---------------------------------------------------------------------------------------------------
ENAM_medaka             ---------------------------------------------------------------------------------------------------
ENAM_tilapia            ---------------------------------------------------------------------------------------------------

                        exon9 (continued)
ENAM_human              N-------PYDPRENSPYLRGNTWDERDDSPNTM--GQKESPL---YPI-NTP-DQKEIVPYNEEDPVDPTGDEVFPGQNRWG-EELSFKG--------
ENAM_pig                N-------PYDPRENSPYLRSNTWYERDDSPNTM--GQPENPH---YPM-NTP-DPKETIPYNEEDPIDPTGDEHFPGQSRWDMEELSFKE--------
ENAM_platypus           H-------PYDPK--------GTWDEGNEPTPSS--GPAEQPGRQPSPCSPRM-GQRGEVPYNEEDPVDPTGDEPNLGPGPWE-ETANGKE--------
ENAM_Anolis             KSYNQYTHPFPP---------RAWEDREHSPTISPSDQRESSP---YPP-VSPSDQIQRNTYY--RRIQPE-YEPYSRQDPWT-REQHLHD--------
ENAM_Xenopus            N-------PKNPS----HLLSNTWQKKPDQ--------LESPR---N---NLL-GQKEIYLYPDTELYHQTNNELPLGKQHWD-NEIDFSAVQFENPGS
ENAM_coelacanth         GQRHQPSSPVTNTLVQEDMSHYPRSNPLRQGGNSPNHETFSHGQKKYSHYDRSNHVRGKGNSPCHGSHPSGQVIHHKPQFKGNSQIHDENPWGQEGQMS
ENAM_bichir             GMRKTP---------------------VKNGC-------------------------------------------------------------------
ENAM_gar                GIRKPP---------------------VQVPC-------------------------------------------------------------------
ENAM_zebrafish          ---------------------------------------------------------------------------------------------------
ENAM_trout              ---------------------------------------------------------------------------------------------------
ENAM_fugu               ---------------------------------------------------------------------------------------------------
ENAM_medaka             ---------------------------------------------------------------------------------------------------
ENAM_tilapia            ---------------------------------------------------------------------------------------------------

                        exon9 (continued)
ENAM_human              -------------GPTVR--HYEGEQYT---SNQPKEYLPYSLDNPSKPREDFYYSEFYPWSPDENFPSYNTASTMPPPIESRGYYVNNAAGPEESTLF
ENAM_pig                -------------DPTVR--HYEGEQYT---SNQPKEYLPYSLDNPSKPREDFLYGEFYPWNPEENFPSYNTAPTVSSPVESRGYYANNAVGQEESTMF
ENAM_platypus           -------------DPS-R--YHKSPQYPQSRATEPQDYPPYPEGGLEK-HRDFPYGDFYPRGPEDNLQPYNGADQ--LPVEGRGYYTHDAVGQGGPLQF
ENAM_Anolis             --------------PD-N--QYSNSPYN---PTHHQTYQKYTTENPPSEISNLPYRKINQWTQEEHSPVH-TAGHL-RQMENVPYRTNSKFEQGE-RKF
ENAM_Xenopus            STYYMKDSQRQKQWPTASNNRVELDRQP---TDQPKYLLISKLD------------------PAKNTDVS--TFTTNEPMAQNGYITNQEAY----LQS
ENAM_coelacanth         NAGRIPSNRGDSSPYPGLFLSENRESSPYLASKPVNQGQISPNHEVYSSAQGGNRPYTGMIPTDKIISPNHGPSSSIQGDLSQNYRGYQSGKERNQPYT
ENAM_bichir             -------NMG-----------------------------------------------------------NHGPTTV-----------------------
ENAM_gar                -------NKGP-----------------------------------------------------------HGPY-------------------------
ENAM_zebrafish          ---------------------------------------------------------------------------------------------------
ENAM_trout              ---------------------------------------------------------------------------------------------------
ENAM_fugu               ---------------------------------------------------------------------------------------------------
ENAM_medaka             ---------------------------------------------------------------------------------------------------
ENAM_tilapia            ---------------------------------------------------------------------------------------------------

                        exon9 (continued)
ENAM_human              PSRNSWDHRIQAQGQRERRPYFNRNIWDQATHLQKAPARPPDQKGNQPYYSNTPAGLQKNPIWHEG-E-NLNYGMQITRMNSPEREH------SSFPNF
ENAM_pig                PSWSSWDPRIQAQGQKEGRPYLNRNFWDQSTNLYKTPTSSPHQKENQPYSNNSPAGLQKNPTWHEG-E-NLNYGMQITRLNSPERDH------LAFPDL
ENAM_platypus           PSRDSWDWGLRSAAQERRAPYDERDSWDHAINS---PKSTAGATETLSPSHSVPAGIRGKPHEESV-D-WSYRGGQVSDLGVPEAEG------PALMEP
ENAM_Anolis             QNVGSGSPQQRNTFQEE-AQYAERNTWVPQRV------DPSAQKETAPYYNIYSTDFRGNPTHVEDRERIIHMNAPISTENAPRRRH------YLDRMG
ENAM_Xenopus            PNN--KSPFIFNKPSTDGILYIPIDELHDS--------------------QNNPVHLKLTP------------------YSKAHSENHSPGVFRSLQEV
ENAM_coelacanth         EVIPRGQKSIYSAPSGQRGNSENLRINPSGNSENLFYPERKPSGKESDLPSYRVYVKGQGENSPYSQPV-PSSQRIMTSDPLLNQSTQKGTMPYFTLNS
ENAM_bichir             -------------------------------------EPVAPSAGNSEIPSFGE--------------------GILMA--------------------
ENAM_gar                --------------------------------------------GNGE-------------QPGFVDPMNPDAEGIILA--------------------
ENAM_zebrafish          ---------------------------------------------------------------------------------------------------
ENAM_trout              ---------------------------------------------------------------------------------------------------
ENAM_fugu               ---------------------------------------------------------------------------------------------------
ENAM_medaka             ---------------------------------------------------------------------------------------------------
ENAM_tilapia            ---------------------------------------------------------------------------------------------------
```

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C (continued)
                        exon9 (continued)
ENAM_human              IPPSYPSGQKEAHL---------------------FHLS-QRG-SCCAGSSTGPKDNPLAL--------------------------------------
ENAM_pig                IPPDYPGGQKESHV---------------------FHLS-QRG-PCCAGGSMWPKNNPLAL--------------------------------------
ENAM_platypus           VARSDPADPREASS---------------------YPVAVARS-SCCAGGSPGPRDVPLAPPDYPPLALPGYLPLDPLDYPPLDRPDYLPPAPRDYPPP
ENAM_Anolis             YSDDYPKERRMIT----------------------SQST-ANQ-LCCADDSPMPRENRLAP--------------------------------------
ENAM_Xenopus            NPNIYPSSLRRQSLNPRKTGHYSCTIPLTGVDPTLEHSN-ALILVCCKLNDM-GQSHPSAN--------------------------------------
ENAM_coelacanth         PGQKTNSPYISENPSDQRGTSPKNRIYPAGQRARLPYPASSVFSQRDNHPIHGTNPSGQEDIPPNQDTNLLSKIGFTPYTLENPSEQRQSGPCPRNNQL
ENAM_bichir             ---------AAEAGSDGEGRT------------------------------------------------------------------------------
ENAM_gar                ---------AADA--------------------------------------------------------------------------------------
ENAM_zebrafish          ---------------------------------------------------------------------------------------------------
ENAM_trout              ---------------------------------------------------------------------------------------------------
ENAM_fugu               ---------------------------------------------------------------------------------------------------
ENAM_medaka             ---------------------------------------------------------------------------------------------------
ENAM_tilapia            ---------------------------------------------------------------------------------------------------

                        exon9 (continued)
ENAM_human              ----------------------QDYTPSYGLAPGENQDTSPLYTDGSHTKQTRDII-SPTSILPGQRNSSE-----KRESQNPFRDGVSTLRRNTPCSI
ENAM_pig                ----------------------QDYTQSFGLAPGENPDTSIGYAEDSHIKYARQTV-SPTSIVPGQRNSSEKILPGESQNPSPFKDDVSTLRRSTPCSV
ENAM_platypus           DTPNYSPLDTPDYPPHYPPLAPQDYSSPLGLSLWELQVGGPRSPGGSRSRHVQQAA-SPVGYPAIRRNGTEEAPPTRREEPAPSRDGLA-PGRSPPCSL
ENAM_Anolis             ----------------------LRSAPQFRHALWEAKESST-YPDGSHANYVRHAH-SPAGIQA---NNPLKNGGREQEELGAFRVENAGSEKSLPCSN
ENAM_Xenopus            ----------------------EDNVHHLKVVLNEYKESVP-HQDQTHRQHTRDVIESPKFSI--Q-NNVMTVRPNSSNNHDNIQSSRGKSSDSVPCTS
ENAM_coelacanth         CQRSNSPDFKGNSPITERKSLGWRERTHSVGATPIHGNDHPDSFSPRKSQFPADNFPYQAGNPETPRRNQFVDRENMPITRDSPLGSEGNSMKPSLSEP
ENAM_bichir             ---------------------------------------------------------------------------------------------------
ENAM_gar                ----------------------------------IHGDS------------------------------------------------------------
ENAM_zebrafish          ---------------------------------------------------------------------------------------------------
ENAM_trout              ---------------------------------------------------------------------------------------------------
ENAM_fugu               ---------------------------------------------------------------------------------------------------
ENAM_medaka             ---------------------------------------------------------------------------------------------------
ENAM_tilapia            ---------------------------------------------------------------------------------------------------

                        exon9 (continued)
ENAM_human              KNQLGQKEI-MPFPEASSLQSKNTPCLKNDLGGDGNNILEQVFEDN-QLNERTVDLTPEQLVIGTPDEGSNPEGIQSQVQENESERQQQRPSN--ILHL
ENAM_pig                KSQLSQRGI-MPLPEANSLQSKNTPCLTSDLGGDGNNVLEQIFEGN-QLNERTVDLTPEQLVFGTPDKEPRPEGIPNEMQGNESERQQQRQSS--ILQL
ENAM_platypus           RPRASPRAG-LAFPEAGLPPAKPAPCFKSRLRGDVDSVPEQILDPD-PPVAGNDGPAPGDLAVVVPERDPGPVGIRGDP----EPGPEAGAPG--FQWV
ENAM_Anolis             SH-LSQDSKQEANLQRGLFKFRNMPCHGSNIRGDRHNPLAHLVGTGQSFGRGTLNFLPEQ--------FPQPHGIQSEALVSEDDGKEYAALG--AKRI
ENAM_Xenopus            KPSYEQNSE-YPY------AFPKKQCS---YRGDADSYLPPSQDESISLKK--RFLASENVYMPL-ANTPEPDL-PDRRI--KDDGKVKKGIGKLLEIL
ENAM_coelacanth         ---------------------------------RVNLFDRDNPALFSHTTNANNFEEGISTTSPKKEMPPPGVDLTRHATKSWIPAAAGDMLPAPIKKA
ENAM_bichir             ---------------------------------------------------------------------------------------------------
ENAM_gar                -------------------------------------------------------------------------------------------------RV
ENAM_zebrafish          ---------------------------------------------------------------------------------------------------
ENAM_trout              ---------------------------------------------------------------------------------------------------
ENAM_fugu               ---------------------------------------------------------------------------------------------------
ENAM_medaka             ---------------------------------------------------------------------------------------------------
ENAM_tilapia            ---------------------------------------------------------------------------------------------------

                        exon9 (continued)
ENAM_human              PCFGSKLAKHHSSTT-----GTPS---------------SDGRQSP-----------FDGDSI--TPTE-------NPNTLVELA--TEEQFKS-----INVDP-
ENAM_pig                PCFGSKLANYHTSSI-----GTPS---------------SLGRQDS-----------FDGDPI--MPTE-------TPNSLAGLA--TGAQFQN-----INVDP-
ENAM_platypus           PCFGSETRDSK-SLL-----GMDGRSPWQREAGPIRAGVHRTRRDPGRGLWASDRGAFRGPQP--SPFIRTFTALQDPPVGSGRR--SGTTGSR-----LLVAP-
ENAM_Anolis             PCFGSWLKQYL-SNT-----EVPS---------------DDQQRDP-----------FYGENP--VPTE-------RPNSIPPKPEPISSSFLS-----NGVEE-
ENAM_Xenopus            TCPQDQEIHTNKKAINIAKTRPTA---------------SLLNHDK-----------LSADAIP-----------------------------------VLNTEQ
ENAM_coelacanth         PCFGDRTSPTSRDKIMSFGGSATLAGVRKAPPGNGDSMVLAVKPNLVPPASKTTVATDPKNDMALTSTLNKFPGTEKDALSAPPIAVNEDPRENRAPDCVLLRD-
ENAM_bichir             PCRVHDTATAGKESNFYY---------------------------------------------------------------------------------------
ENAM_gar                PCVVHDSPAQAEENHYYY---------------------------------------------------------------------------------------
ENAM_zebrafish          ---------------------------------------------------------------------------------------------------------
ENAM_trout              ---------------------------------------------------------------------------------------------------------
ENAM_fugu               ---------------------------------------------------------------------------------------------------------
ENAM_medaka             ---------------------------------------------------------------------------------------------------------
ENAM_tilapia            ---------------------------------------------------------------------------------------------------------
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Figure S1. Multiply aligned amino acid sequences encoded by matrix SCPP genes, Related to Figure 1.

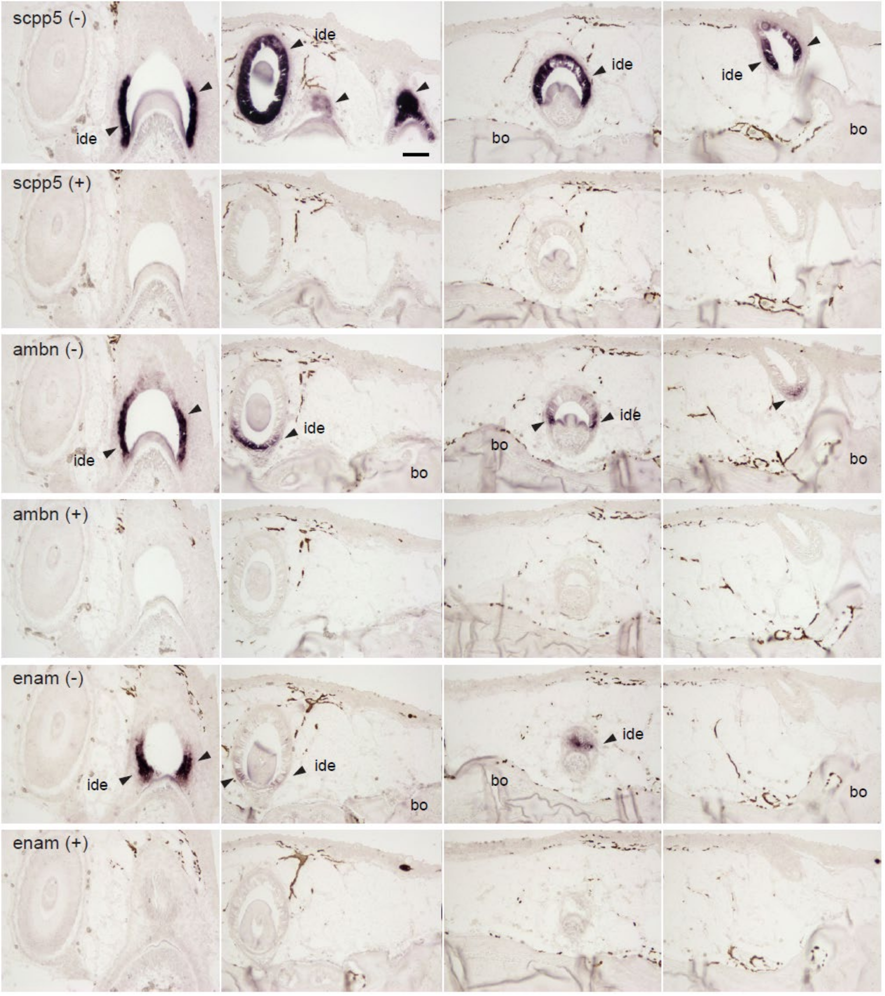
(A) Multiply aligned amino acid sequences encoded by *AMEL* and *SCPP5*, their chromosomal locations, and expression of bichir *scpp5* in the jaw and skin. Aromatic residues, negatively charged residues, and positively charged residues are shown in sky blue, blue, and brown, respectively, while Pro, Gln, and potentially phosphorylated Ser residues (potential pSer residues: Ser residues in Ser-Xaa-Glu or Ser-Xaa-pSer sequences, where Xaa represents any amino acids, are known to be phosphorylated in SCPPs.) (Kawasaki and Amemiya, 2014) are highlighted in yellow, blue, and green, respectively. Uninterrupted Pro-Xaa-Yaa (Yaa also represents any amino acid; PXY) repeats are shown with dashed underlines. The three conserved modules, the aromatic residue-rich region (YY), the Pro/Gln-rich region containing uninterrupted PXY repeats (P/Q), and the hydrophilic C-terminus (DE) are shown at the bottom. Unaligned sequence gaps are shown by dashes. Amino acid sequences were retrieved from GenBank (accession numbers for *AMEL*: NP_873632.1 for humans, NP_998965.1 for pig, reconstructed from GCA_000002275 for platypus, XP_003228746.1 for *Anolis*, AAC78133.1 for caiman, AAZ23149.1 for axolotl, NP_001107153.1 for *Xenopus*, AYU58914.1 for lungfish, and reconstructed from GCA_000225785.1 for coelacanth; accession numbers for *SCPP5*: AYU58917.1 for bichir, XP_015200896.1 for gar, XP_035273951.1 for eel, NP_001138708.1 for zebrafish, reconstructed from GCA_000180675.1 for stickleback, NP_001032946.1 for fugu, reconstructed from GCA_000188235.2 for tilapia, and reconstructed from GCA_002234675.1 for medaka). Note that all teleost species used for multiple amino acid sequence alignments (A, B, and C) belong to different orders. In sarcopterygians, *AMEL* is located within intron 1 of *ARHGAP6*, while in actinopterygians *SCPP5* is located within a P/Q-rich SCPP gene cluster (Kawasaki et al., 2017). In *L. chalumnae*, locations of genes are shown as contig names (JHxxxxxx). Expression of bichir *scpp5* was detected in the jaw and skin that secretes the ganoin matrix on scales, but not in the skeletal muscle (negative control). Bichir *gapdh* (glyceraldehyde 3-phosphate dehydrogenase) was used as the positive control.

(B) Multiply aligned amino acid sequences encoded by *AMBN*. Cys residues is shown in red. See the legend above. *AMBN* in sturgeon, medaka, and tilapia has a small last exon, and the C-terminus is shown by a period. The amino acid sequences shown under exon 6 of human *AMBN* are encoded by two different exons in bichir, sturgeon, and gar (separated by an underscore). In a previous study, *ambn* was named *scpp6* in teleosts (Kawasaki, 2009). However, conservation of the amino acid sequences encoded by exons 11 and 12 (corresponds to exon 12 of human *AMBN*) between *AMBN* and *SCPP6*, the absence of the entirely untranslated last exon in both *ANBN* and *SCPP6*, the presence and locations of four potential pSer residues, and an equivalent chromosomal location of gar *ambn* and zebrafish *scpp6* (between *scpp3* genes and *scpp7*) implies the orthology of *AMBN* and *SCPP6* (Qu et al., 2015; Braasch et al., 2016; Kawasaki et al., 2017). In the present study, the orthology of *AMBN* and *SCPP6* was confirmed by a similar expression pattern during tooth development. Amino acid sequences were retrieved from GenBank (AAG35772.1 for humans, XP_007667389.2 for platypus, AAK92227.1 for caiman, XP_016846623.1 for *Anolis*, XP_002938667.2 for *Xenopus*, AYU58913.1 for lungfish, XP_006011890.1 for coelacanth, AYU58915.1 for bichir, reconstructed from SRX424534 for sturgeon, AMD08894.1 for gar, NP_001138709.1 for zebrafish, reconstructed from GCA_002234675.1 for medaka, reconstructed from GCA_000188235.2 for tilapia, and NP_001032945.1 for fugu).

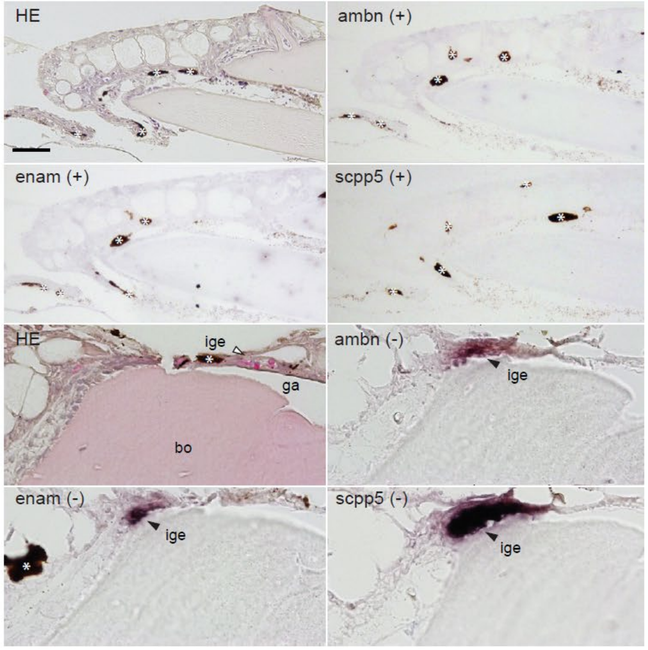
(C) Multiply aligned amino acid sequences encoded by exons 3, 4, 5, 6, 7, 8, and a 5' half of exon 9 of *ENAM*. The amino acid sequence of porcine 32-kDa enamelin (Tanabe et al., 1990; Yamakoshi et al., 1998) was underlined. Small amino acids (Ala and Gly) in the 32-kDa enamelin and corresponding regions are highlighted in grey. In the region corresponding to exon 6 of human *ENAM*, many small duplicate exons were identified in gar and bichir *enam* genes but are not shown in the figure. Amino acid sequence similarities in the portion located C-terminal to the 32-kDa enamelin (not highlighted or not shown in color) are low or undetectable between phylogenetically distant vertebrates (e.g., tetrapods and teleosts). In teleosts, *enam* was originally referred to as *fa93e10* (Goldsmith et al., 2003). However, sequence similarities in the 32-kDa enamelin and its N-terminal region encoded by *ENAM* and *FA93E10*, the absence of the entirely untranslated last exon in both *ENAM* and *FA93E10*, the presence and locations of four pSer residues, and an equivalent chromosomal location of far *enam* and zebrafish *enam* (immediately downstream of *scpp5*) imply the orthology of *ENAM* and *FA93E10* (Qu et al., 2015; Braasch et al., 2016; Kawasaki et al., 2017). In the present study, the orthology of *ENAM* and *FA93E10* was confirmed by a similar expression pattern during tooth development. Amino acid sequences were retrieved from GenBank (AAG43242.1 for humans, NP_999406.1 for pig, reconstructed from GCA_000002275.2 for platypus, ADJ67842.1 for *Anolis*, NP_001139215.1 for *Xenopus*, reconstructed from GCA_000225785.1 for coelacanth, AYU58916.1 for bichir, AMD08897.1 for gar, NP_001139028.1 for zebrafish, CDQ64219.1 for trout, reconstructed from GCA_000180735.1 for fugu, reconstructed from GCA_002234675.1 for medaka, and XP_003454248.1 for tilapia).

A

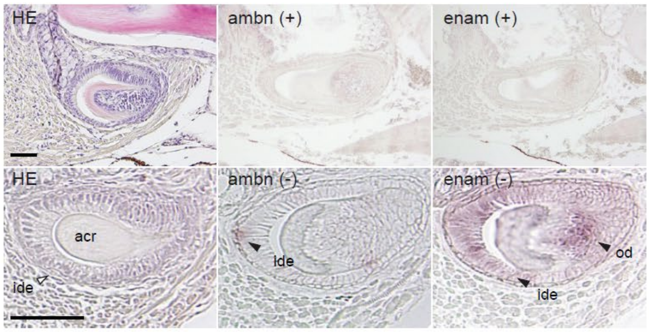
ISH: gar teeth



ISH: gar scales

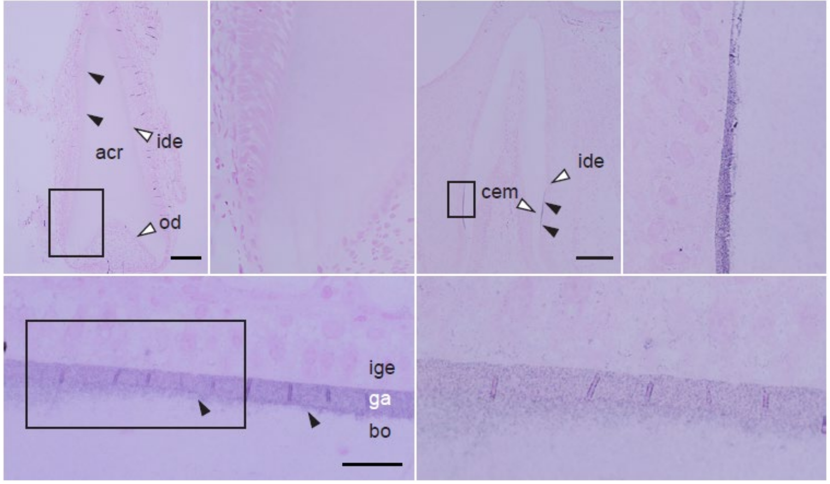


ISH: zebrafish teeth



B

Optical IHC: gar acrodin, collar enamel, and ganoin



C

TEM IHC: gar acrodin, collar enamel, and ganoin

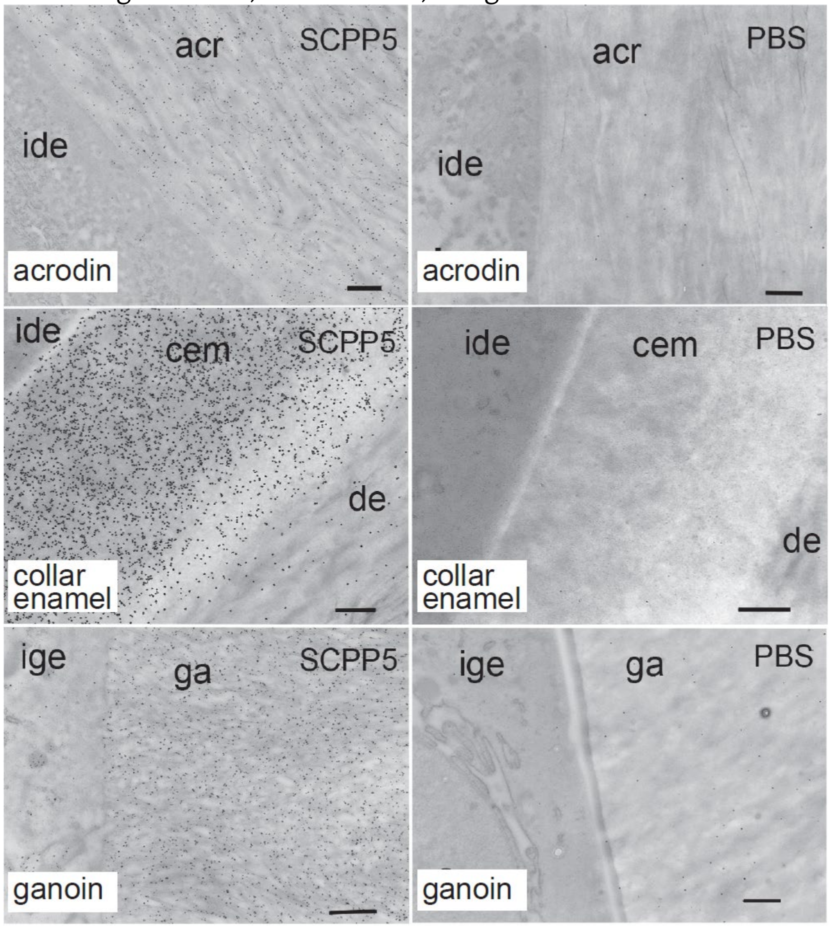
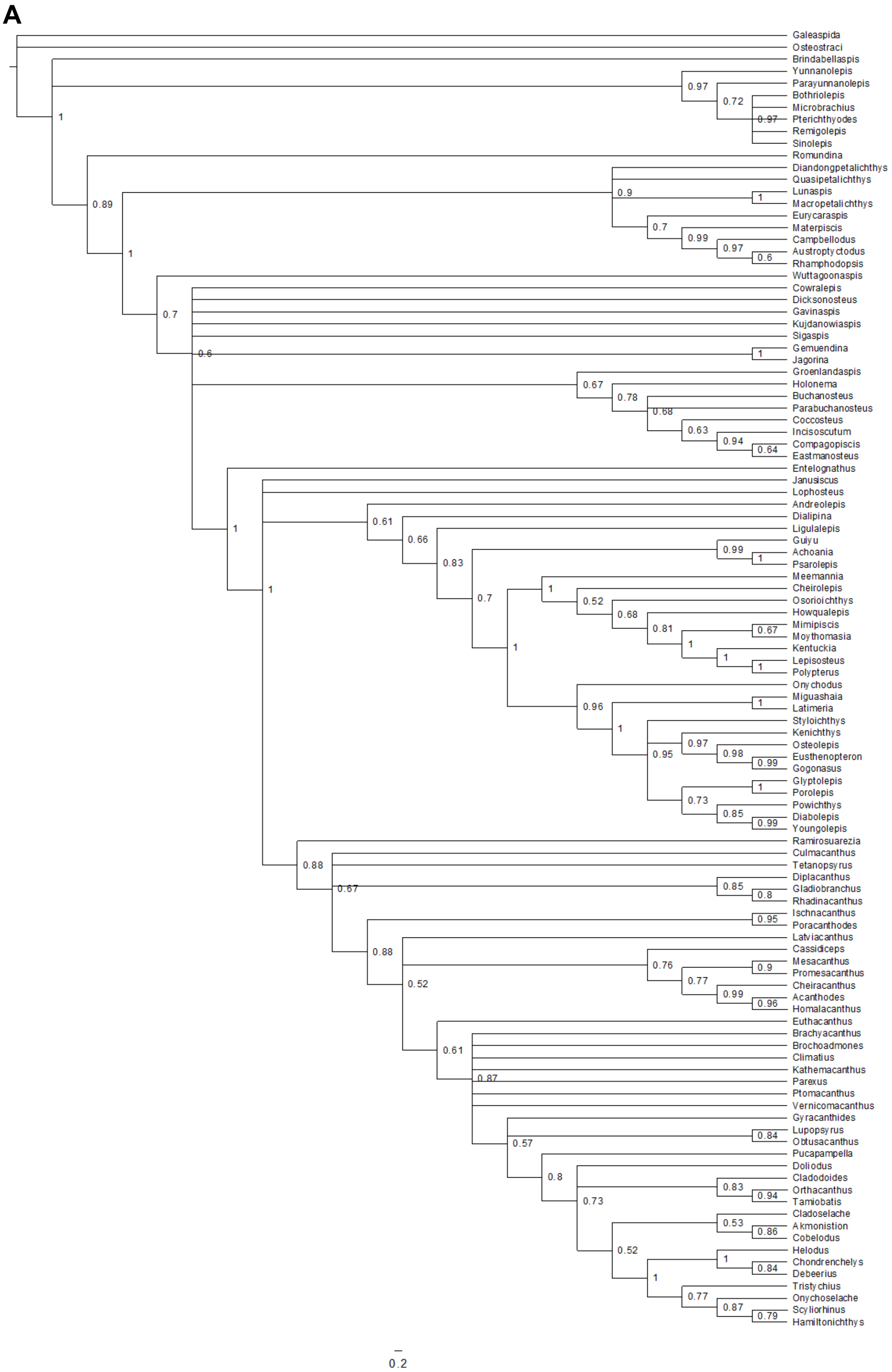


Figure S2. Expression of gar and zebrafish *scpp5*, *ambn*, and *enam* genes in teeth and/or scales, and distribution of gar Scpp5 in teeth and scales, Related to Figures 2-4.

(A) ISH analysis of gar teeth, gar scales, and zebrafish teeth. Expression of *scpp5*, *ambn*, and *enam* genes were detected using antisense (-) probes, but not using sense (+) probes, which serve as negative controls. Images of HE staining are shown for gar scales and zebrafish teeth. All our ISH results are summarized in Figure 2P. Abbreviations: acr, acrodin; bo, bone; ga, ganoin; ide, inner dental epithelial cells; ige, inner ganoin epithelial cells; od, odontoblasts. Scale bar, 50 μm.

(B) Original images for optical IHC analysis (Figures 3A-3F) without enhancing image contrast. See the legend of Figure 3. Scale bar, 100μm (upper row) or 20 μm (lower row).

(C) TEM IHC analysis of gar Scpp5 in acrodin, collar enamel, and ganoin. PBS was used as negative controls. Abbreviations: acr, acrodin, cem, collar enamel; de, dentine; ga, ganoin; ide, inner dental epithelial cells; ige, inner ganoin epithelial cells. Scale bar, 500 nm.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Sinolepis
Romundina
Diandongpetalichthys
Quasipetalichthys
Lunaspis
Macropetalichthys
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Eusthenopteron
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Doliodus
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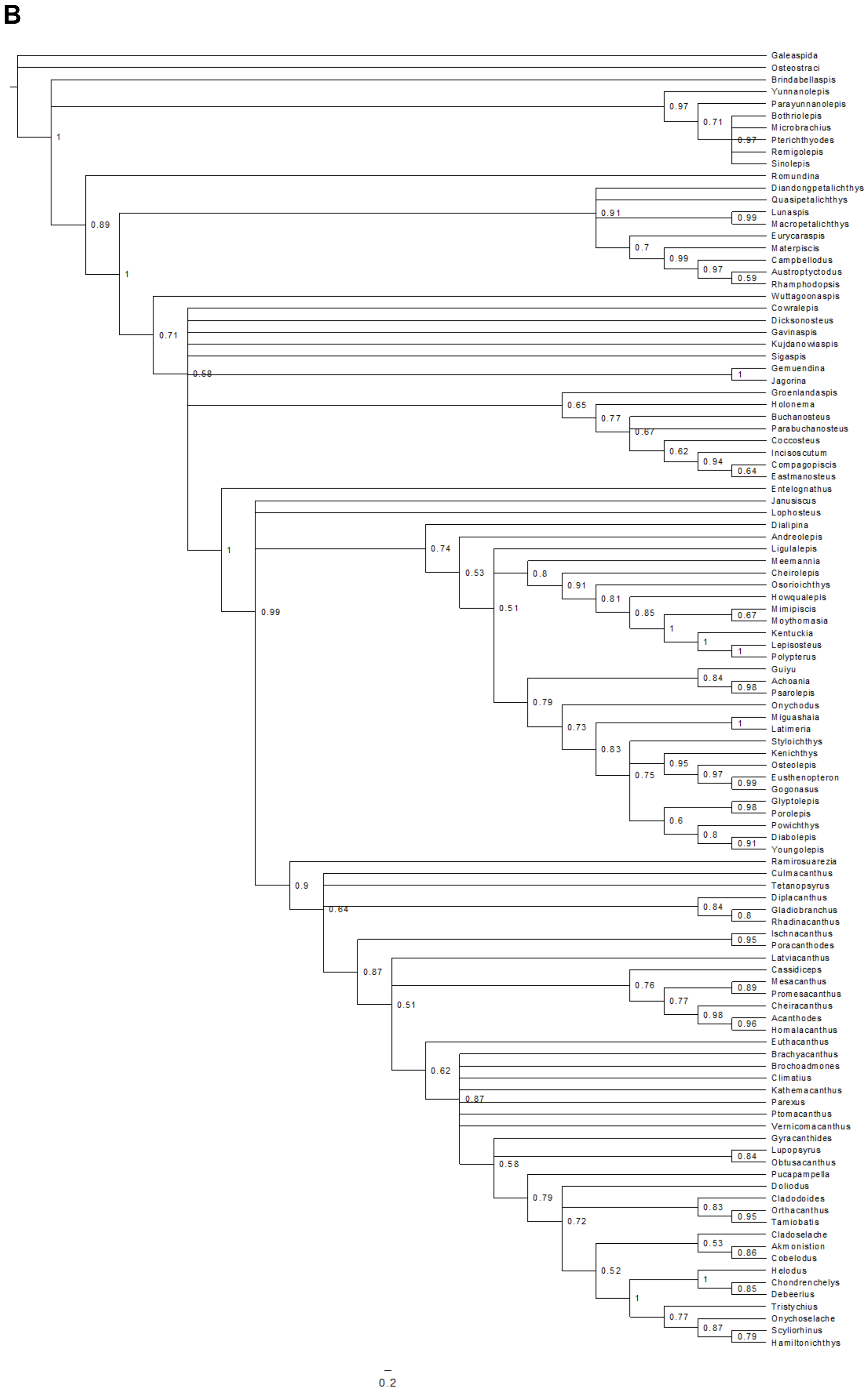
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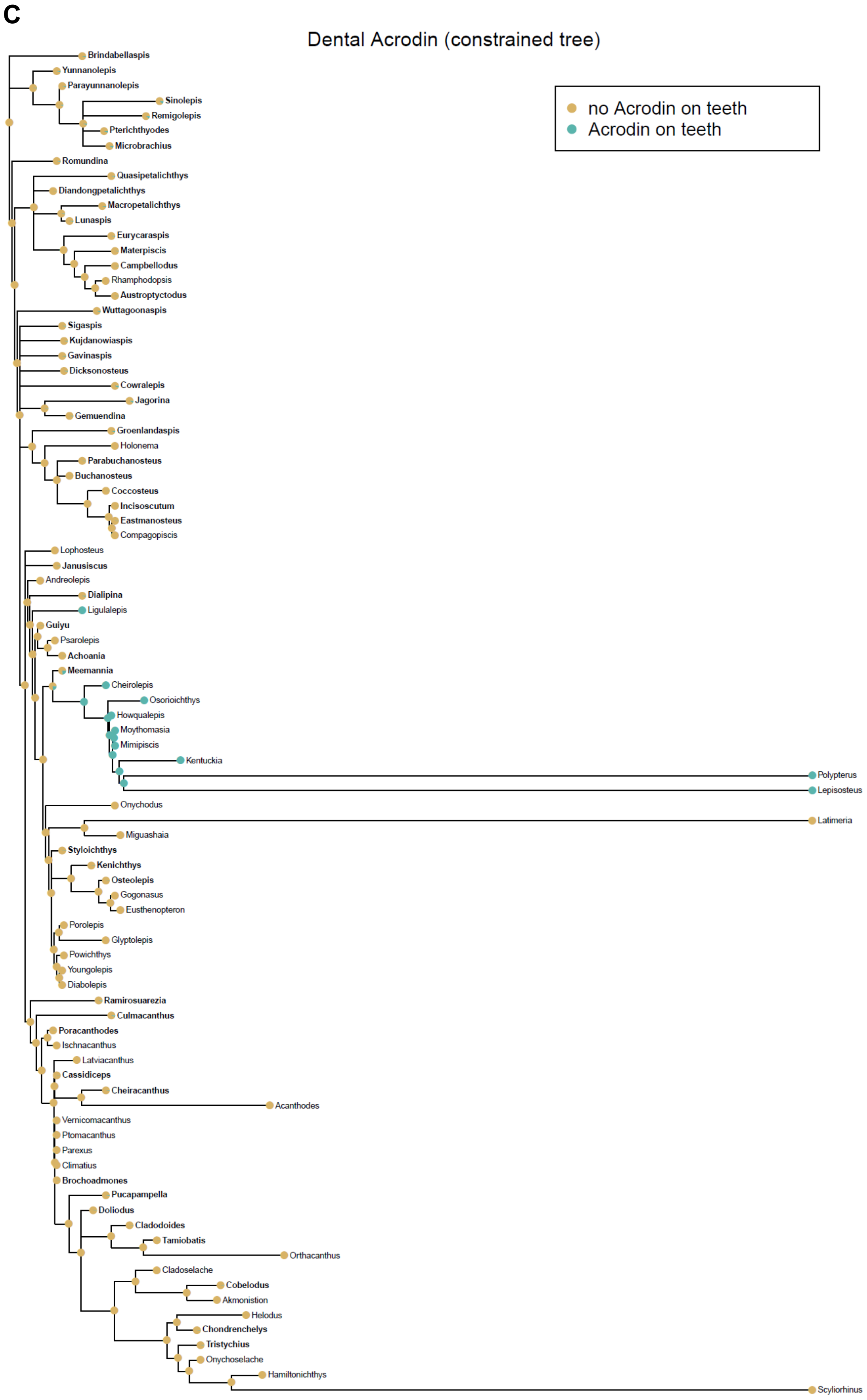
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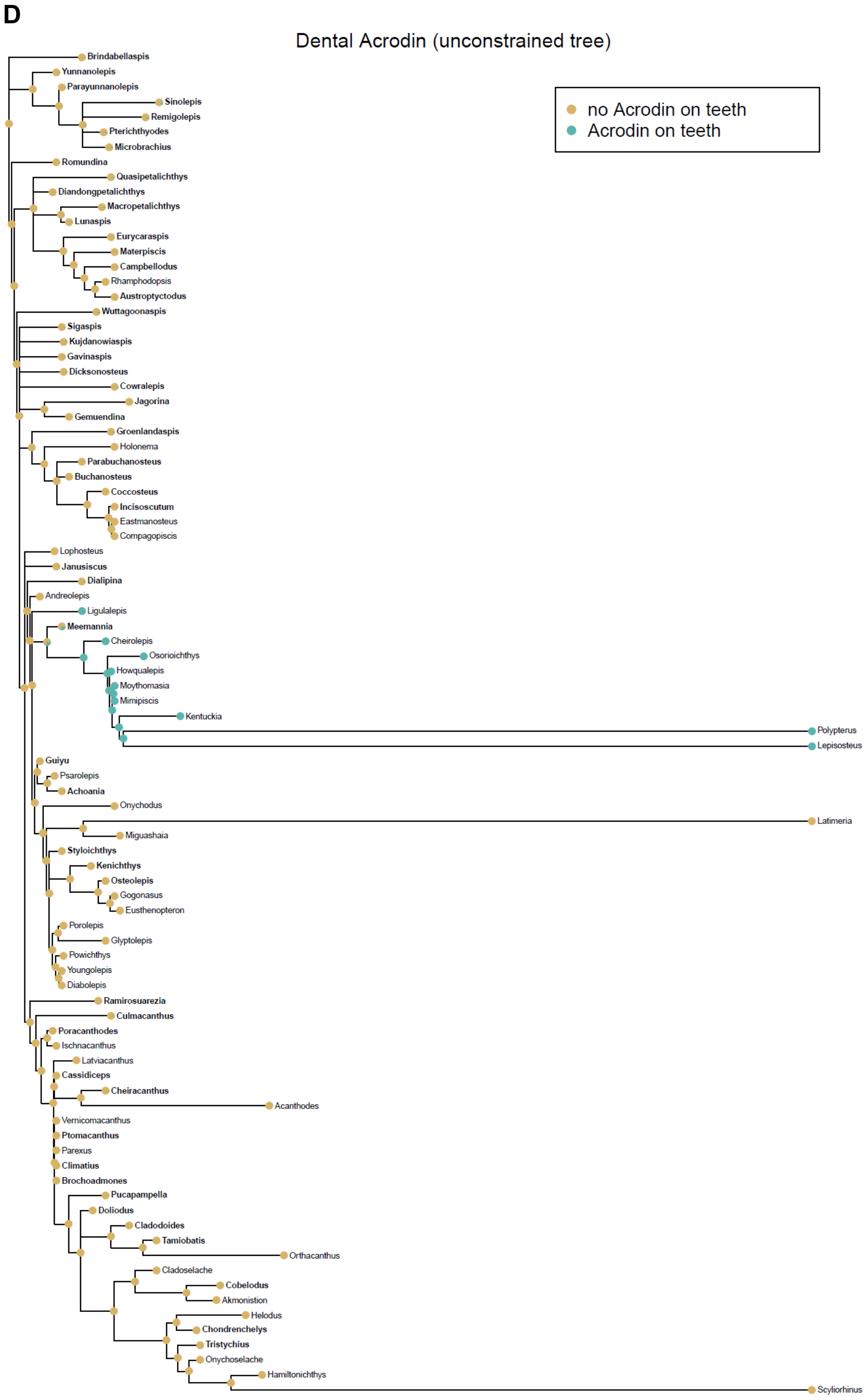
Dental Acrodin (unconstrained tree)

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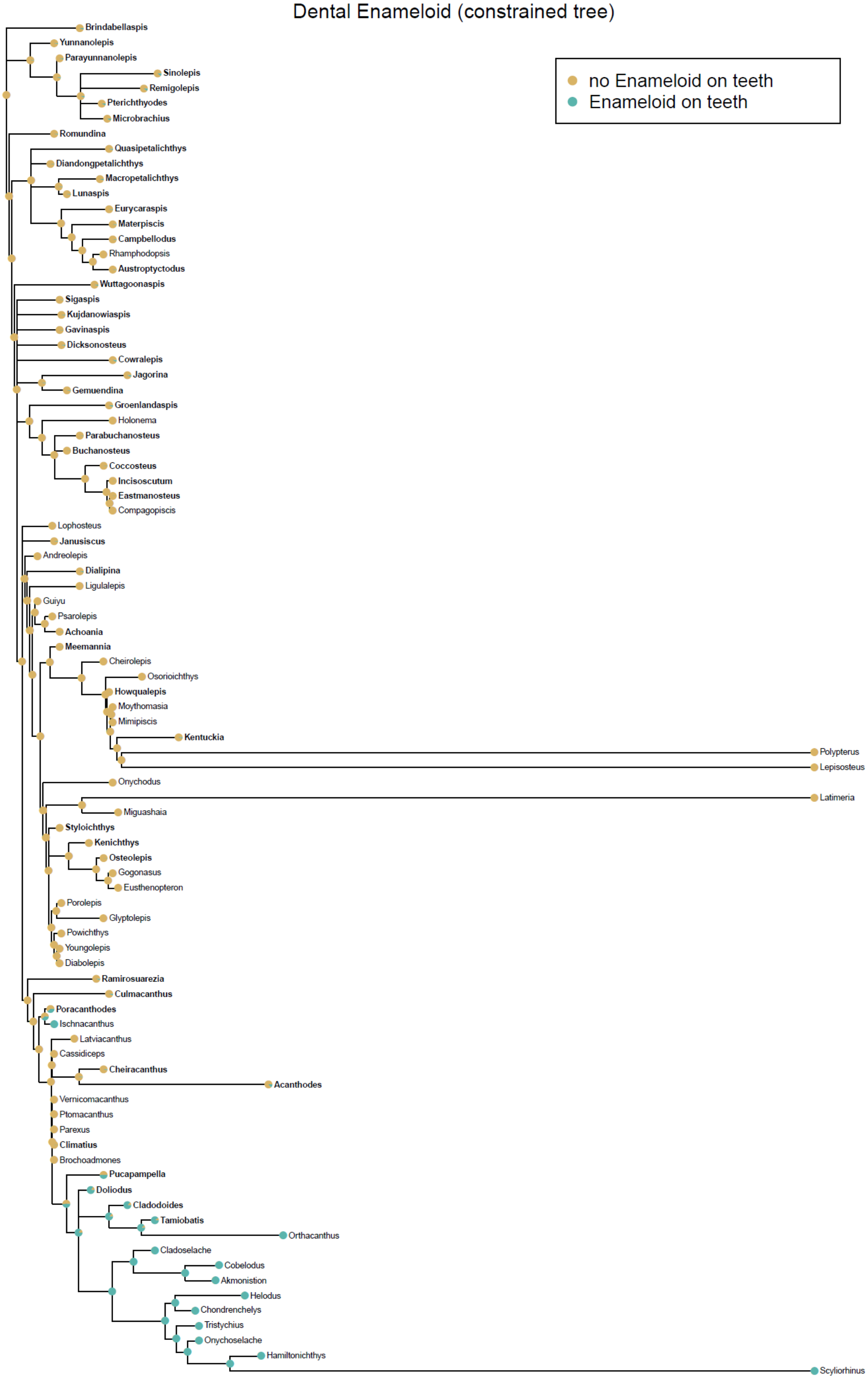

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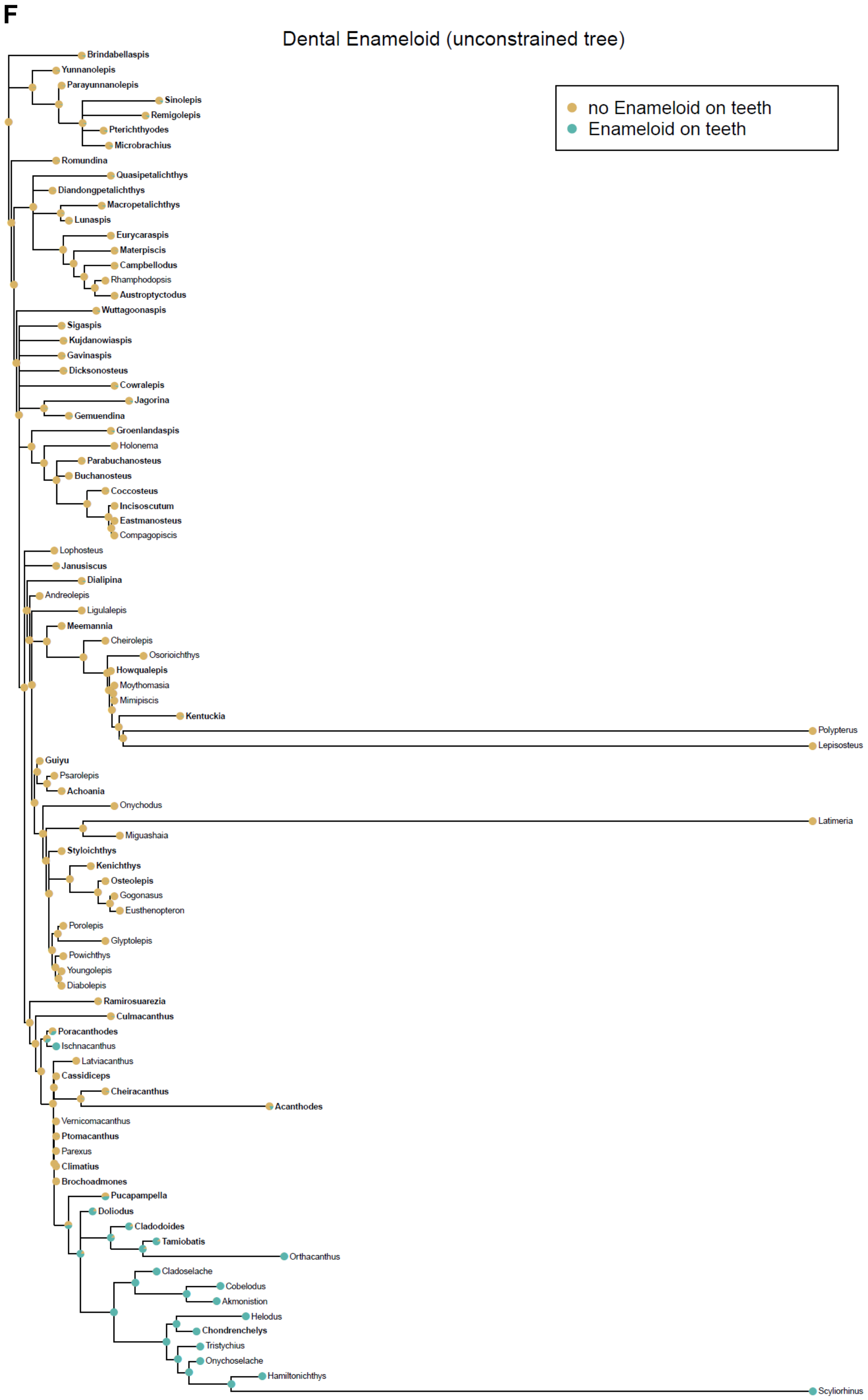
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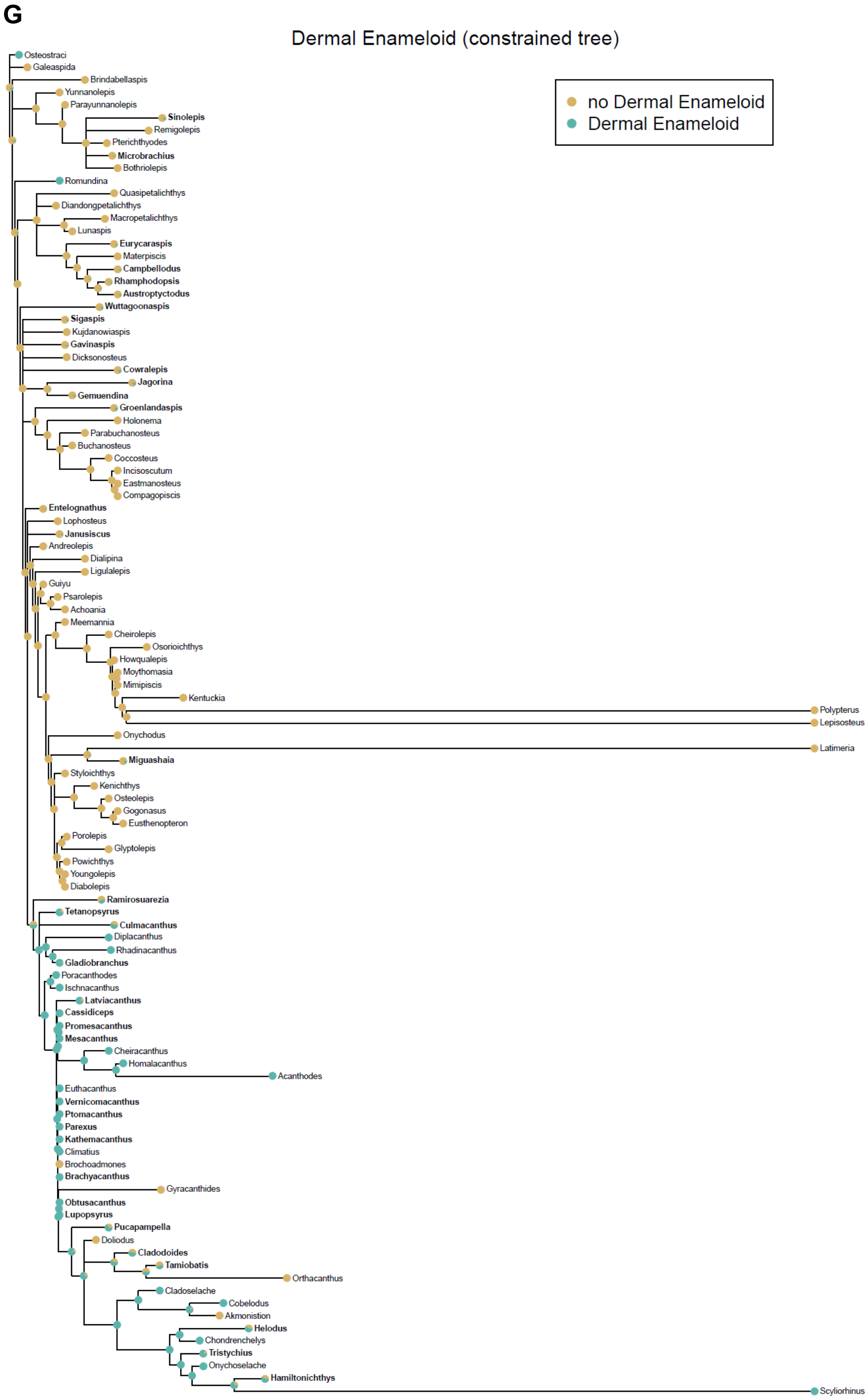
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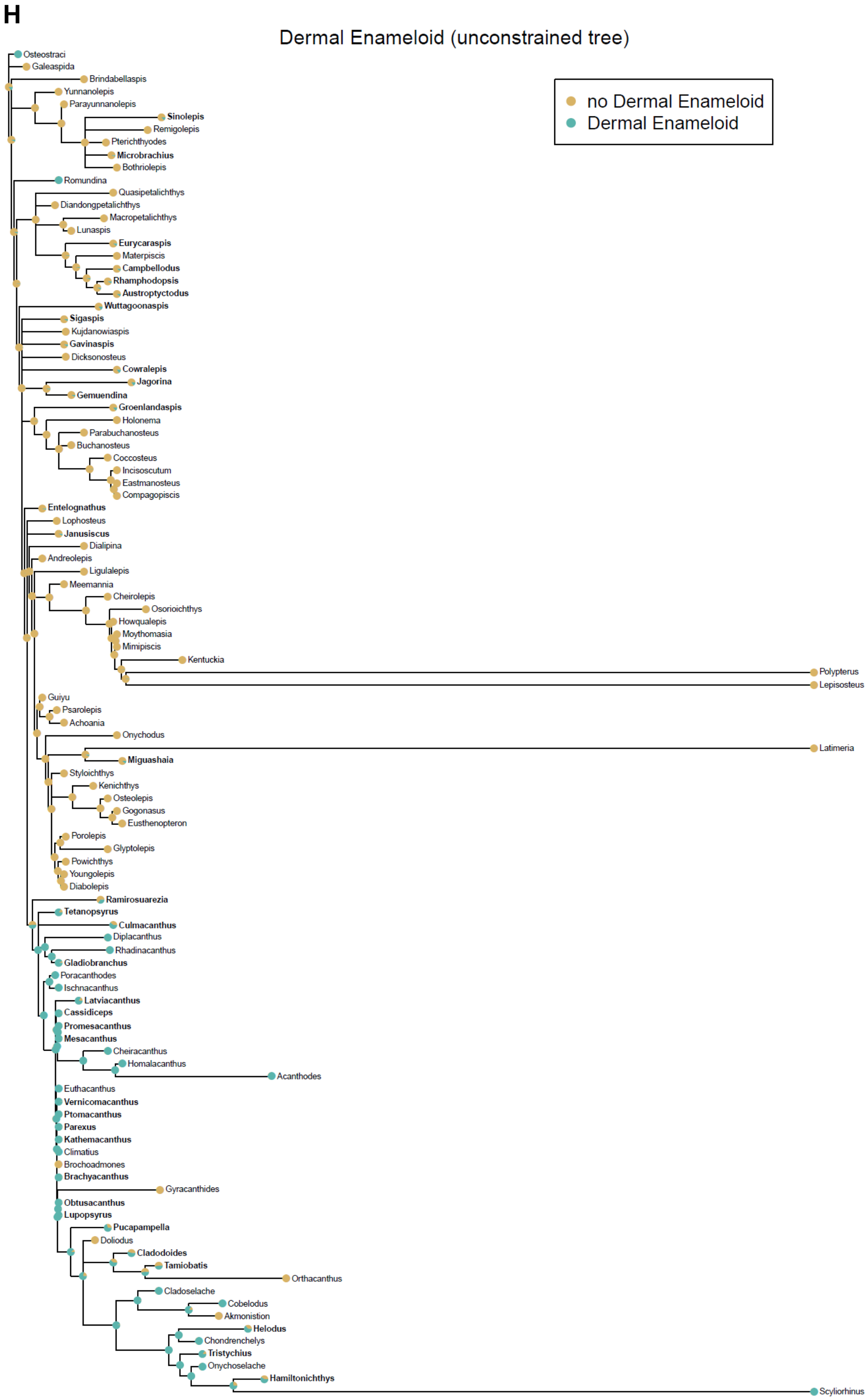
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Ligulalepis
Meemannia
Cheirolepis
Osorioichthys
Howqualepis
Moythomasia
Mimipiscis
Kentuckia
Polypterus
Lepisosteus
Guiyu
Psarolepis
Achoania
Onychodus
Latimeria
Miguashaia
Styloichthys
Kenichthys
Osteolepis
Gogonasus
Eusthenopteron
Porolepis
Glyptolepis
Powichthys
Youngolepis
Diabolepis
Ramirosuarezia
Tetanopsyrus
Culmacanthus
Diplacanthus
Rhadinacanthus
Gladiobranchus
Poracanthodes
Ischnacanthus
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Promesacanthus
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Cheiracanthus
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Euthacanthus
Vernicomacanthus
Ptomacanthus
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Kathemacanthus
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Doliodus
Cladodoides
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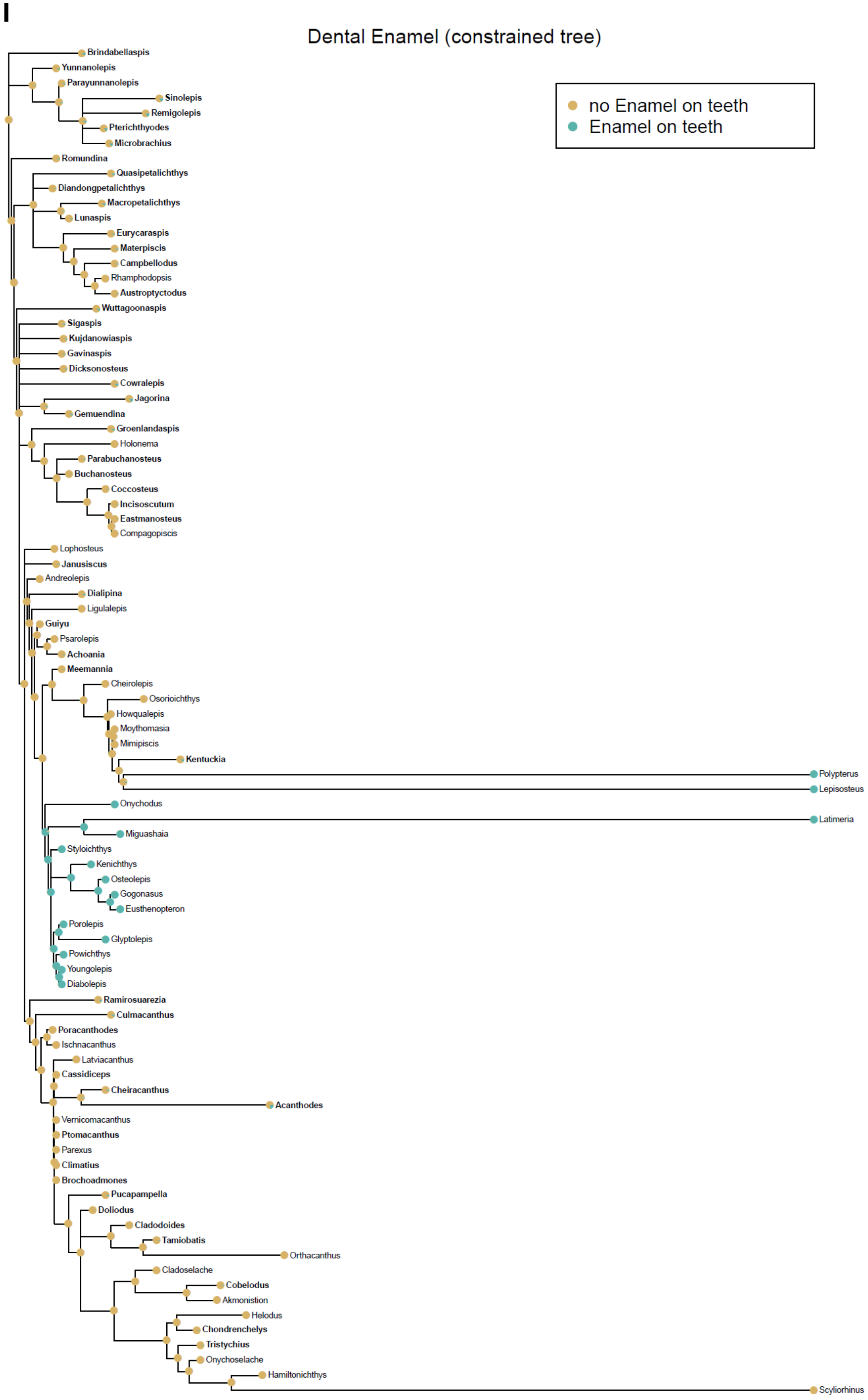I
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Coccosteus
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Eastmanosteus
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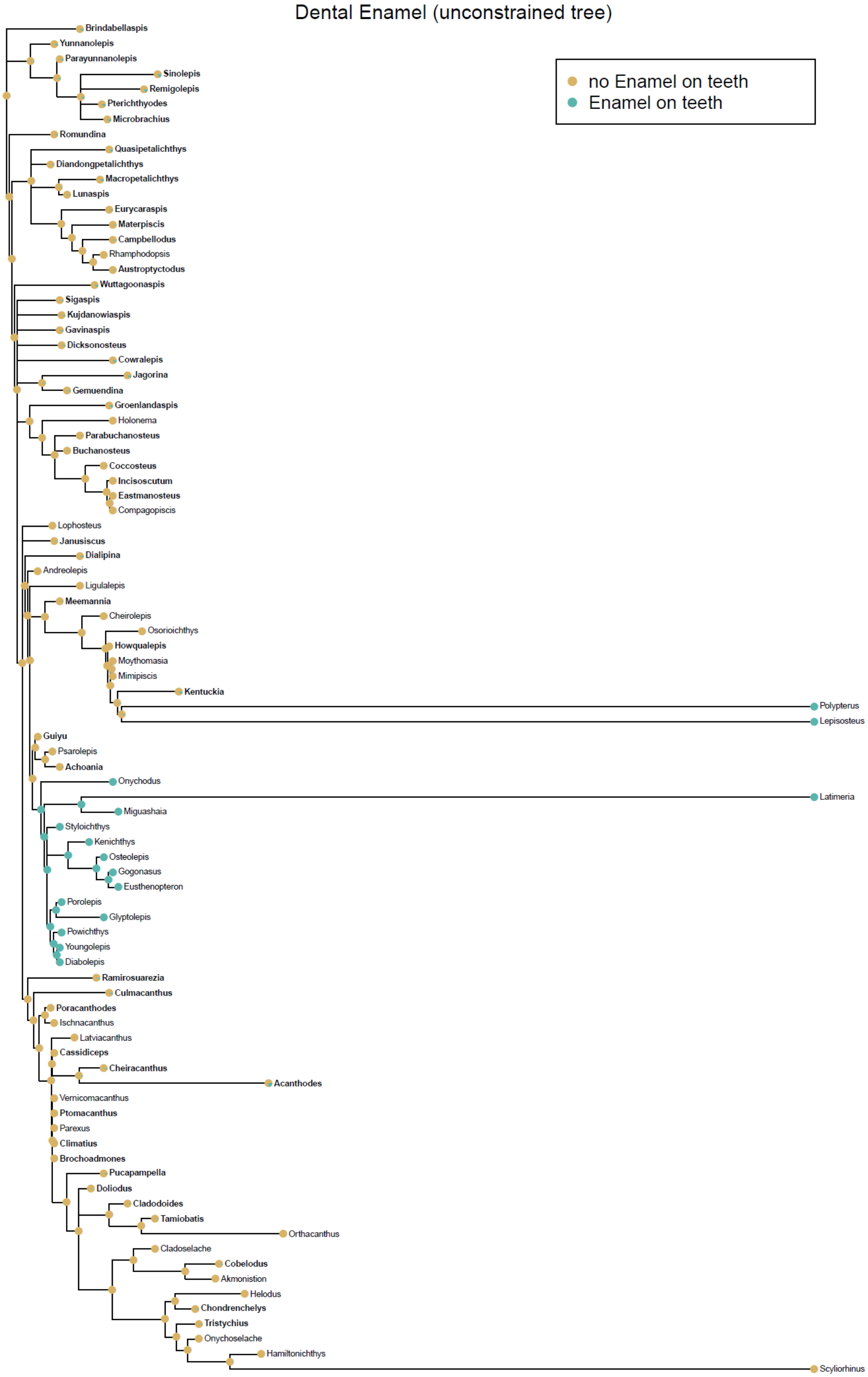
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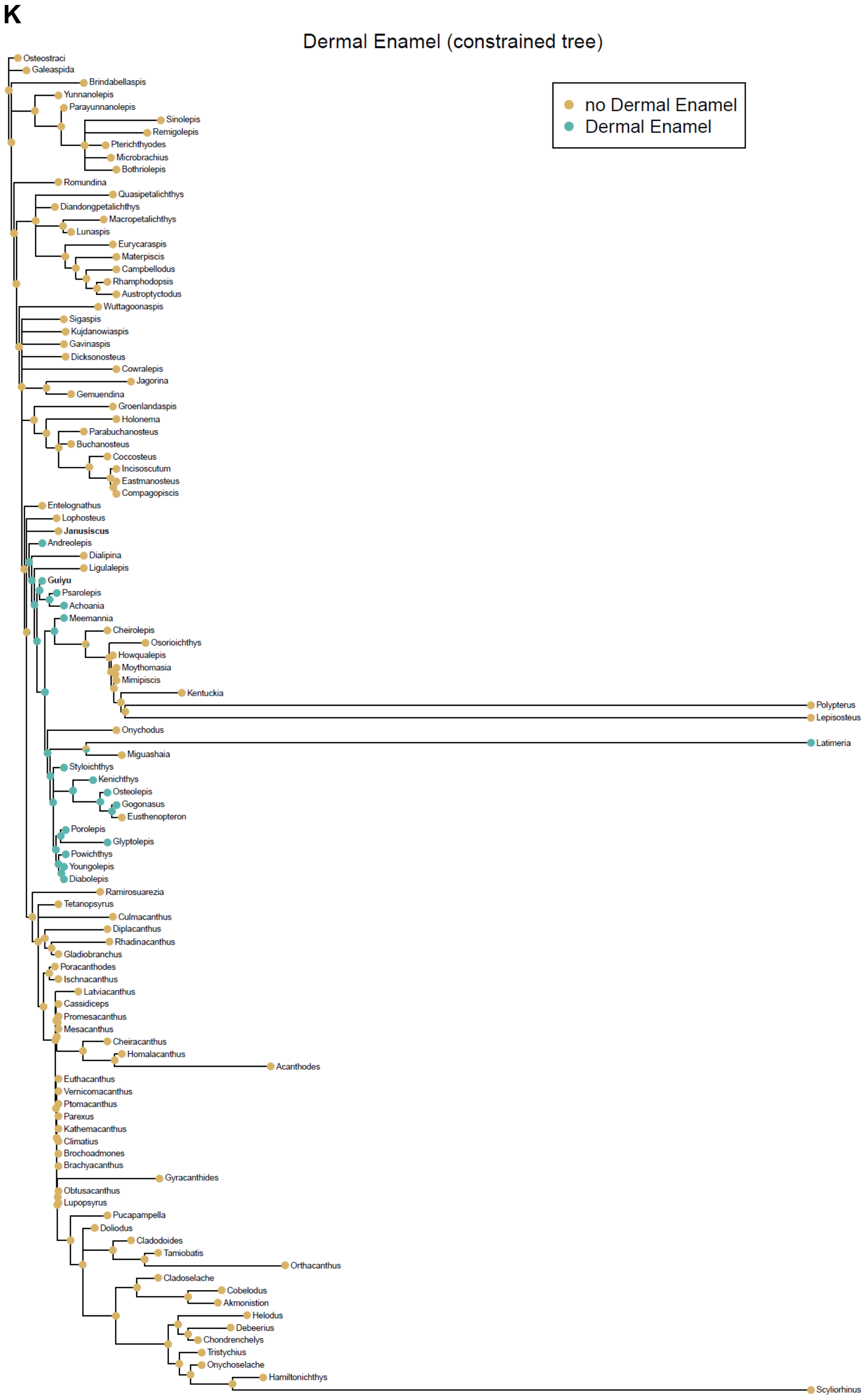
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Diandongpetalichthys
Macropetalichthys
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Rhamphodopsis
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Sigaspis
Kujdanowiaspis
Gavinaspis
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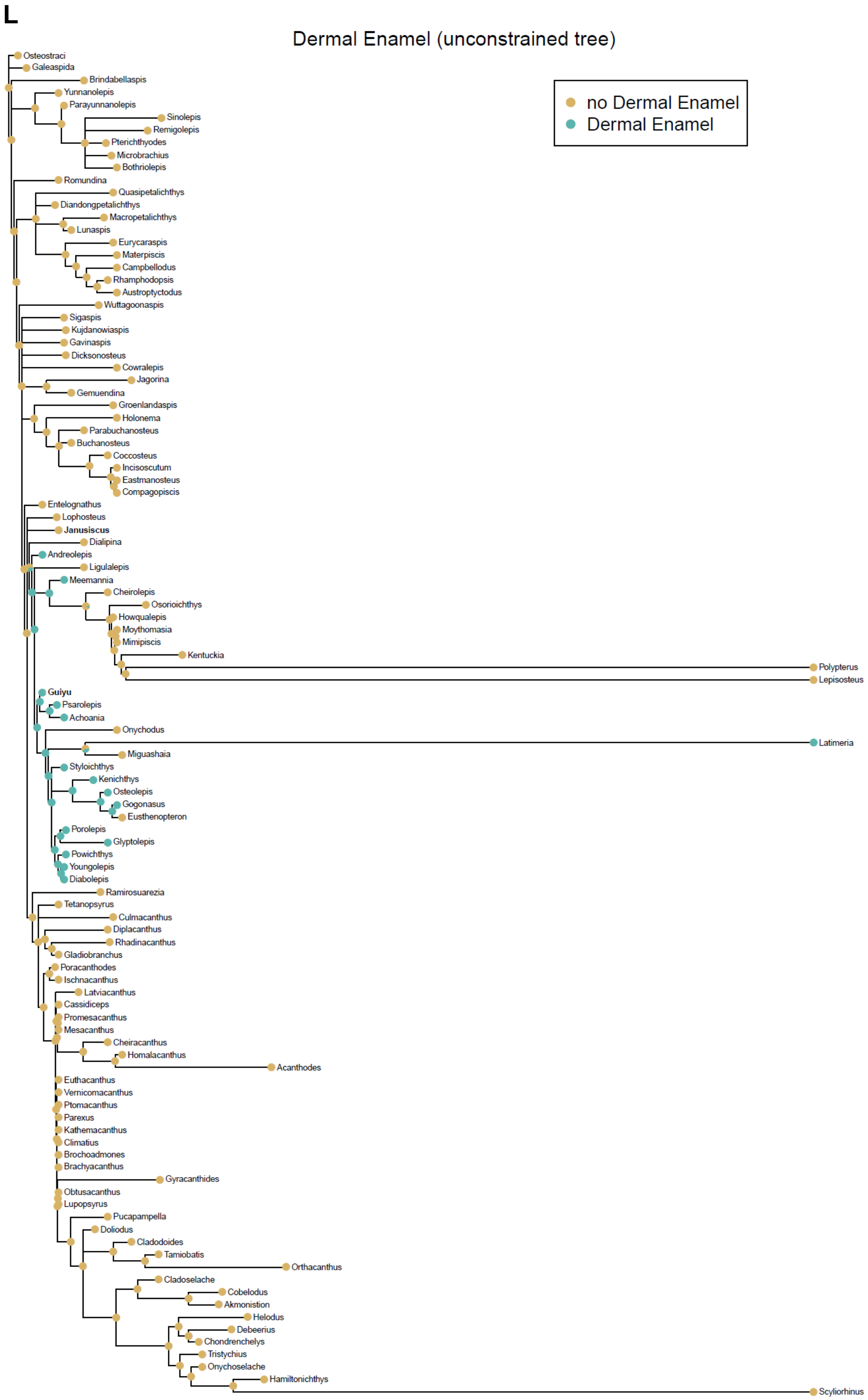
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Macropetalichthys
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Austroptyctodus
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Gavinaspis
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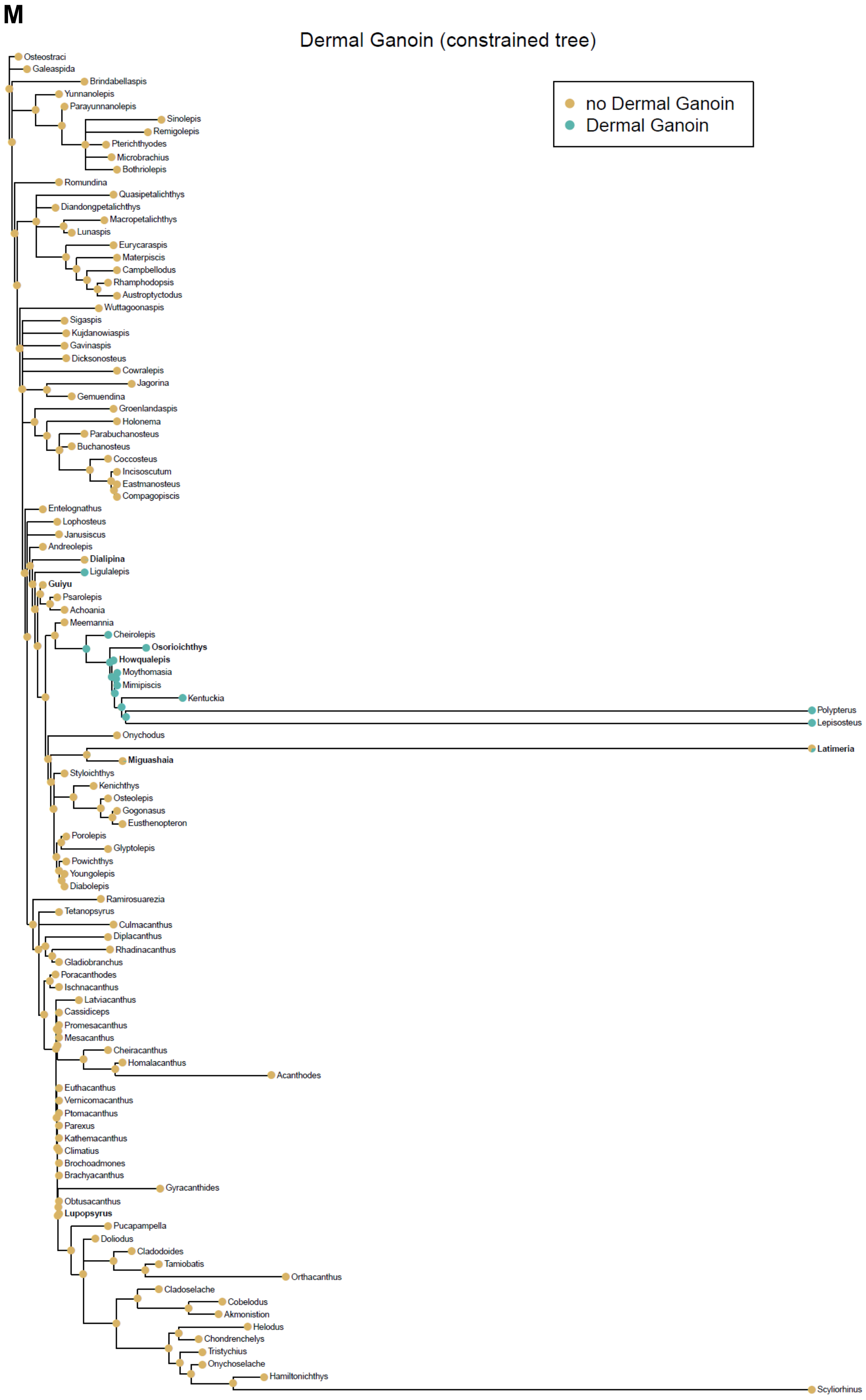
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Sinolepis
Remigolepis
Pterichthyodes
Microbrachius
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Macropetalichthys
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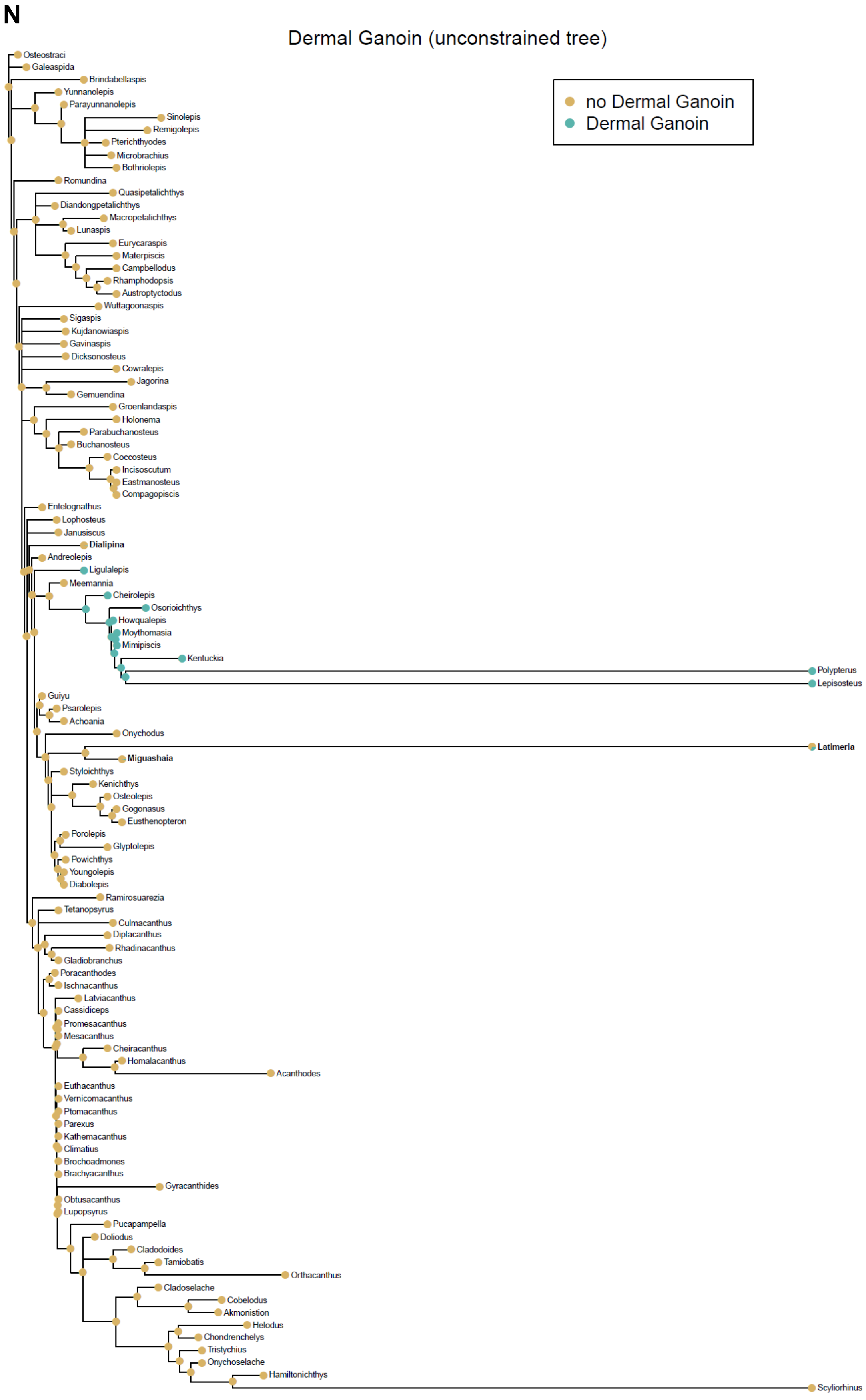
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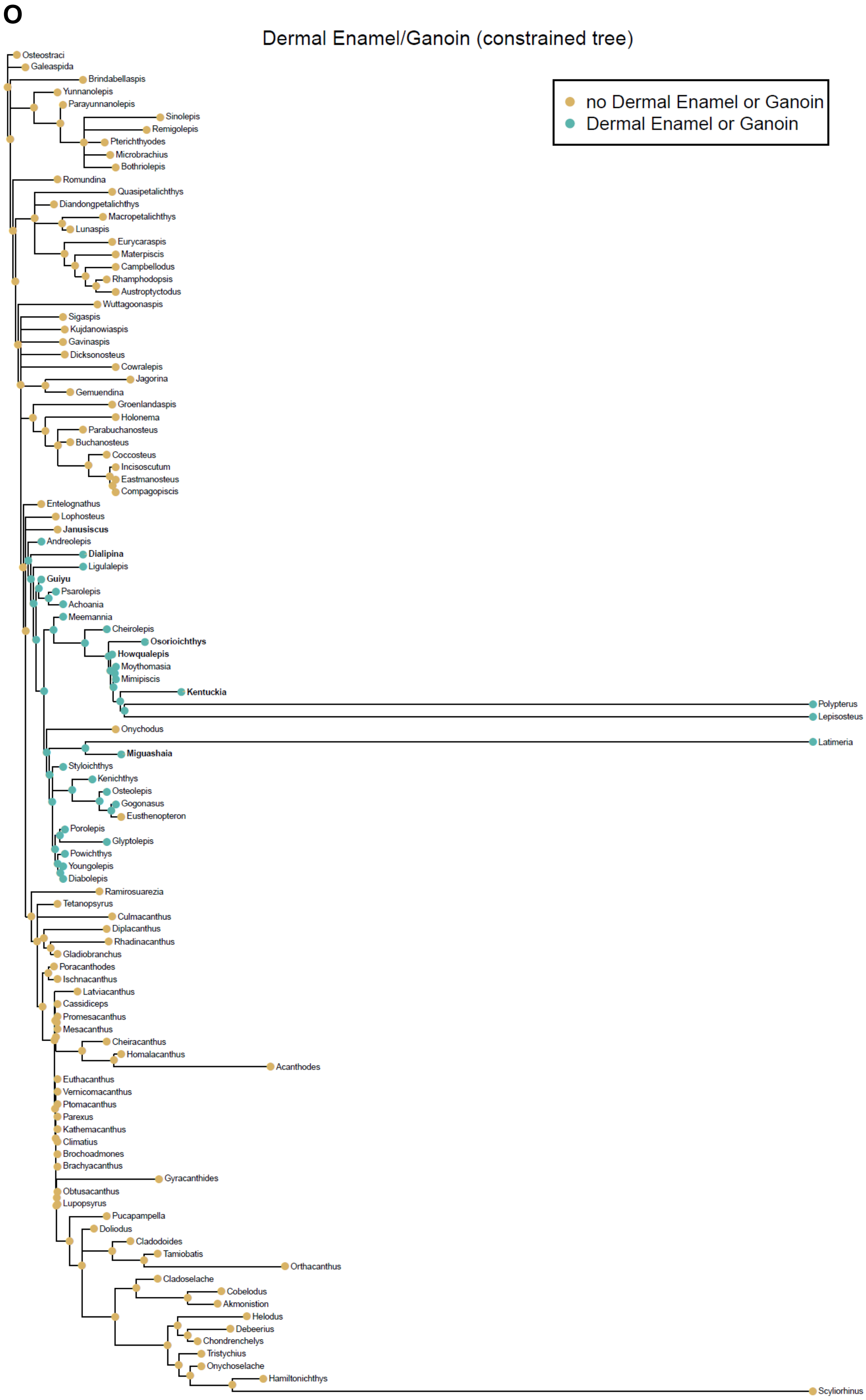
Dermal Ganoin (unconstrained tree)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● Dermal Enamel or Ganoin
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Galeaspida
Brindabellaspis
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Parayunnanolepis
Sinolepis
Remigolepis
Pterichthyodes
Microbrachius
Bothriolepis
Romundina
Quasipetalichthys
Diandongpetalichthys
Macropetalichthys
Lunaspis
Eurycaraspis
Materpiscis
Campbellodus
Rhamphodopsis
Austroptyctodus
Wuttagoonaspis
Sigaspis
Kujdanowiaspis
Gavinaspis
Dicksonosteus
Cowralepis
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Groenlandaspis
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Orthacanthus
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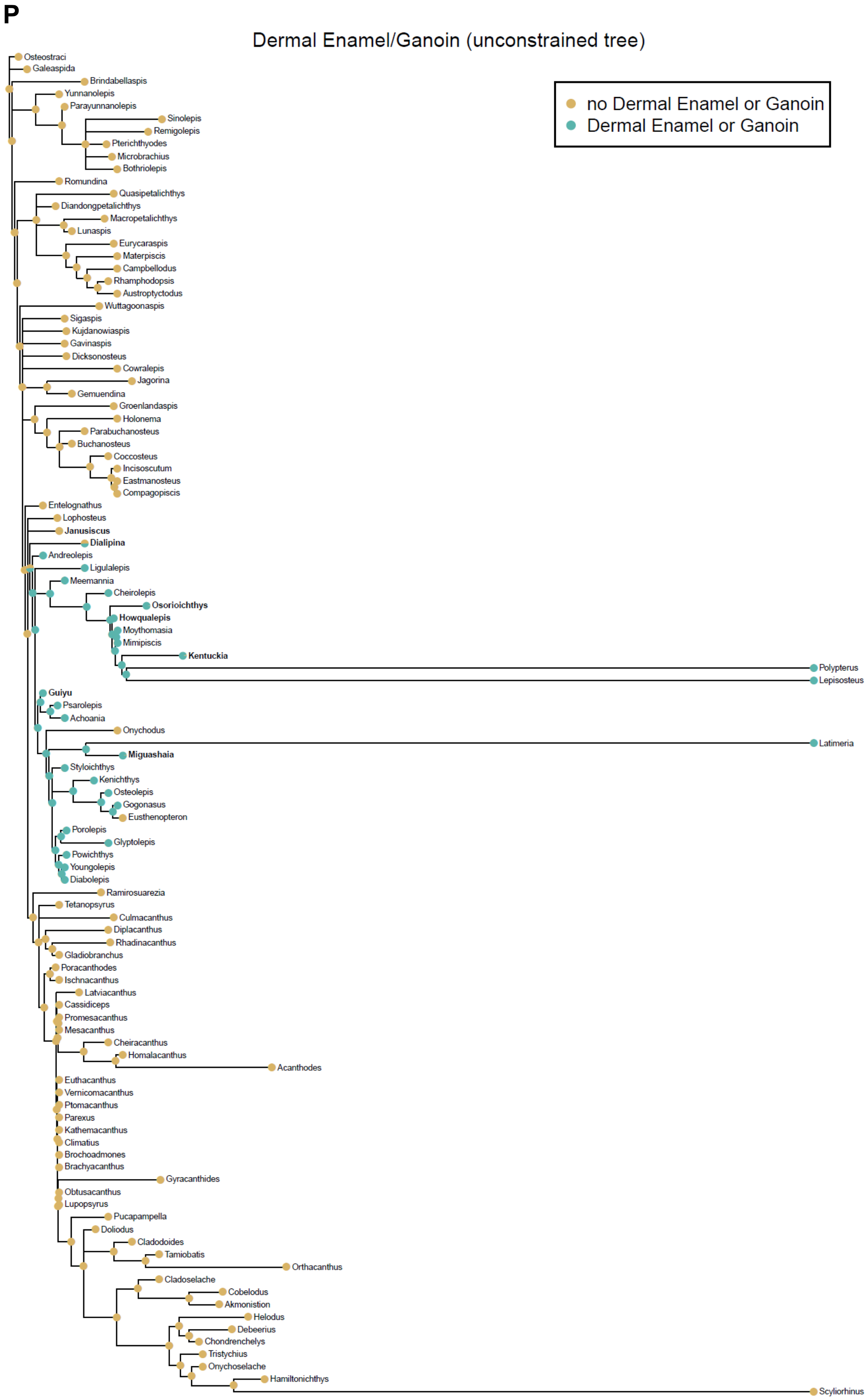
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Galeaspida
Brindabellaspis
Yunnanolepis
Parayunnanolepis
Sinolepis
Remigolepis
Pterichthyodes
Microbrachius
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Romundina
Quasipetalichthys
Diandongpetalichthys
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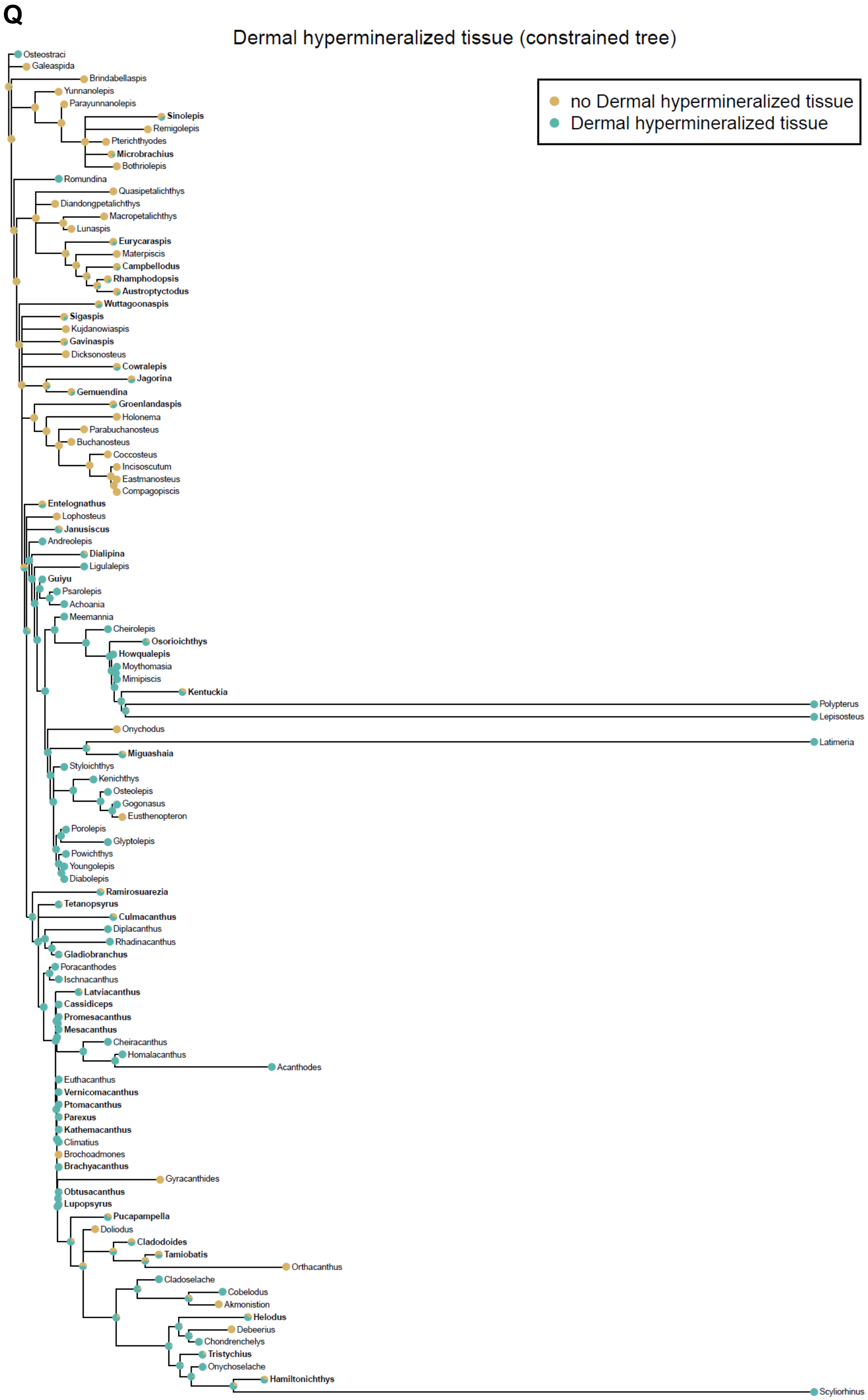
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Dermal hypermineralized tissue
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Sinolepis
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Microbrachius
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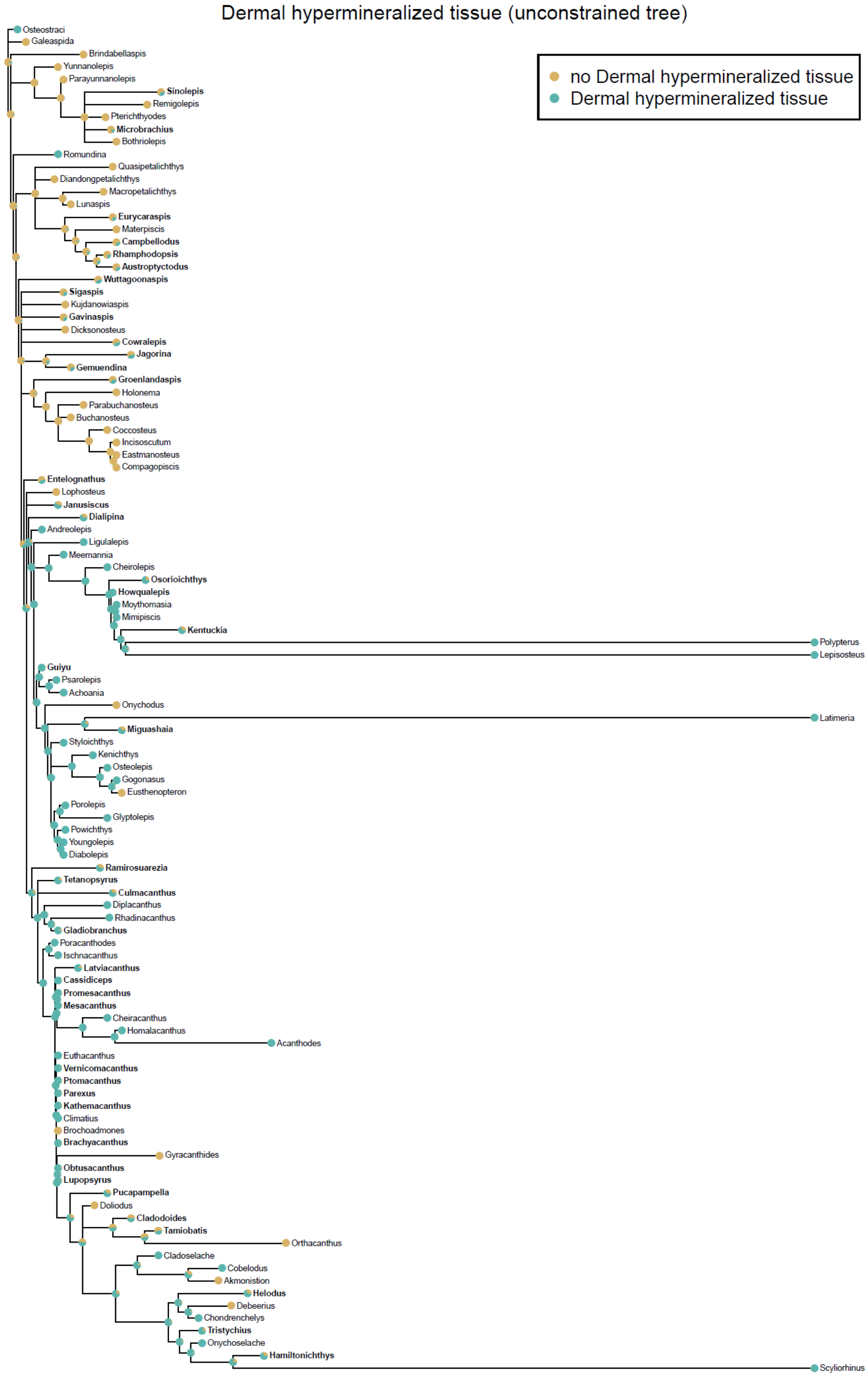
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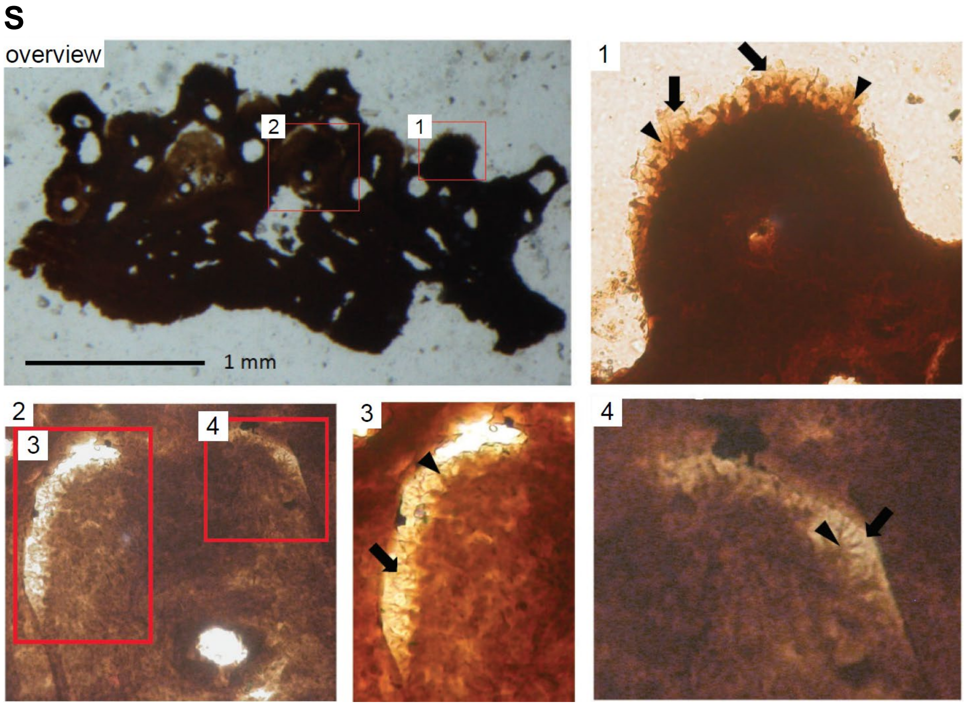

Figure S3. Reconstructing ancestral states of hypermineralized tissues, Related to Figure 5.

(A) Constrained tree used in ancestral state estimation. The GAP clade is constrained to a stem-osteichthyan affinity. Reliabilities of branches are shown at nodes as posterior probabilities.

(B) Unconstrained tree used in ancestral state estimation. The affinity of the GAP clade was unconstrained in the analysis and is resolved to a stem-sarcopterygian affinity. Reliabilities of branches are shown at nodes as posterior probabilities.

(C) Prediction of ancestral states of dental acrodin - GAP clade stem-osteichthyans, shown as pie charts.

(D) Prediction of ancestral states of dental acrodin - GAP clade unconstrained, shown as pie charts.

(E) Prediction of ancestral states of dental enameloid - GAP clade stem-osteichthyans, shown as pie charts.

(F) Prediction of ancestral states of dental enameloid - GAP clade unconstrained, shown as pie charts.

(G) Prediction of ancestral states of dermal enameloid - GAP clade stem-osteichthyans, shown as pie charts.

(H) Prediction of ancestral states of dermal enameloid - GAP clade unconstrained, shown as pie charts.

(I) Prediction of ancestral states of dental enamel - GAP clade stem-osteichthyans, shown as pie charts.

(J) Prediction of ancestral states of dental enamel - GAP clade unconstrained, shown as pie charts.

(K) Prediction of ancestral states of dermal enamel - GAP clade stem-osteichthyans, shown as pie charts.

(L) Prediction of ancestral states of dermal enamel - GAP clade unconstrained, shown as pie charts.

(M) Prediction of ancestral states of dermal ganoin - GAP clade stem-osteichthyans, shown as pie charts.

(N) Prediction of ancestral states of dermal ganoin - GAP clade unconstrained, shown as pie charts.

(O) Prediction of ancestral states of dermal enamel+ganoin - GAP clade stem-osteichthyans, shown as pie charts.

(P) Prediction of ancestral states of dermal enamel or ganoin - GAP clade unconstrained, shown as pie charts.
(Q) Prediction of ancestral states of dermal enamel, ganoin, or enameloid - GAP clade stem-osteichthyans, shown as pie charts.
(R) Prediction of ancestral states of dermal enamel, ganoin, or enameloid - GAP clade unconstrained, shown as pie charts.
(S) Cladoselache skin denticles [specimen P.9294 (NHMUK)]. Detail of boxed areas (1-4) in overview is enlarged. The dentin tubules (arrowheads), protruding through the enameloid layer (arrow), are clearly visible.

	lpq20	lpq8	lpq7	enam	scpp5	scpp7	lpq6	ambn	lpq5	lpq4	scpp3dl	scpp3cl	scpp3bl	scpp3al	odam	lpq3	scpp9	lpq2	lpq1
Coverage[1]	173	16	5	1761	6385	1768	3689	825	12	4	0	0	189	237	209	1119	69	336	2755
FPKM	54	6	2	548	2204	547	1118	262	4	2	0	0	101	106	66	338	21	102	1181
Conf[2]	4	3	1	8	35	31	45	7	1	1	0	0	45	22	5	12	4	9	62

(continued)

	dmp1	scpp1	dsppl1	ibsp	mepe1	mepe2	Spp1	lpq17	lpq16b	lpq16a	lpq15	lpq14	lpq13	lpq12	lpq19	lpq11	lpq18	lpq1	lpq9
Coverage[1]	33	237	33	632	513	991	1089	66	305	264	1010	79	126	951	386	1043	1201	3180	108
FPKM	12	72	11	211	193	313	326	20	125	101	341	23	38	317	117	331	383	1037	35
Conf[2]	1	5	1	7	22	35	11	2	21	16	8	9	5	6	4	9	7	13	7

Table S1. Expression levels gar SCPP genes in the skin, Related to Figure 2. Relative expression levels of SCPP genes in the skin were estimated as fragments per kilobase of transcript per million mapped reads (FPKM values) (Trapnell et al., 2010). [1]Coverage shows the estimate for the absolute depth of read coverage across the whole transcript. [2]Conf represents the 95% confidence interval.

	Dermal characters					**Dental characters**		
Taxa	**Enameloid**	**Ganoin**	**Enamel**	**Enamel+Ganoin**	**Enamel+Ganoin+Enameloid**	**Acrodin**	**Enameloid**	**Enamel**
Galeaspida	0	0	0	0	0	–	–	–
Osteostraci	1	0	0	0	1	–	–	–
Acanthodes	1	0	0	0	1	0	0/1	0/1
Achoania	0	0	1	1	1	0/1	0/1	0/1
Akmonistion	0	0	0	0	0	0	1	0
Austroptyctodus	0/1	0	0	0	0/1	0/1	0/1	0/1
Bothriolepis	0	0	0	0	0	–	–	–
Brachyacanthus	0/1	0	0	0	0/1	–	–	–
Brindabellaspis	0	0	0	0	0	0/1	0/1	0/1
Brochoadmones	0	0	0	0	0	0/1	0/1	0/1
Buchanosteus	0	0	0	0	0	0/1	0/1	0/1
Campbellodus	0/1	0	0	0	0/1	0/1	0/1	0/1
Cassidiceps	0/1	0	0	0	0/1	0/1	0/1	0/1
Cheiracanthus	1	0	0	0	1	0/1	0/1	0/1
Cheirolepis	0	1	0	1	1	1	0	0
Chondrenchelys	1	0	0	0	1	0/1	0/1	0/1
Cladodoides	0/1	0	0	0	0/1	0/1	0/1	0/1
Cladoselache	1	0	0	0	1	0	1	0
Climatius	1	0	0	0	1	0/1	0/1	0/1
Cobelodus	1	0	0	0	1	0/1	0/1	0/1
Coccosteus	0	0	0	0	0	0/1	0/1	0/1
Compagopiscis	0	0	0	0	0	0	0	0
Cowralepis	0/1	0	0	0	0/1	0/1	0/1	0/1
Culmacanthus	0/1	0	0	0	0/1	0/1	0/1	0/1
Debeerius	–	–	0	0	0	–	–	–
Diabolepis	0	0	1	1	1	0	0	1
Dialipina	0	0/1	0	0/1	0/1	0/1	0/1	0/1
Diandongpetalichthys	0	0	0	0	0	0/1	0/1	0/1
Dicksonosteus	0	0	0	0	0	0/1	0/1	0/1
Diplacanthus	1	0	0	0	1	–	–	–
Doliodus	0	0	0	0	0	0/1	0/1	0/1
Eastmanosteus	0	0	0	0	0	0/1	0/1	0/1
Entelognathus	0/1	0	0	0	0/1	–	–	–
Eurycaraspis	0/1	0	0	0	0/1	0/1	0/1	0/1
Eusthenopteron	0	0	0	0	0	0	0	1
Euthacanthus	1	0	0	0	1	–	–	–
Gavinaspis	0/1	0	0	0	0/1	0/1	0/1	0/1
Gemuendina	0/1	0	0	0	0/1	0/1	0/1	0/1
Gladiobranchus,	0/1	0	0	0	0/1	–	–	–
Glyptolepis	0	0	1	1	1	0	0	1
Gogonasus	0	0	1	1	1	0	0	1
Groenlandaspis	0/1	0	0	0	0/1	0/1	0/1	0/1
Guiyu	0	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Gyracanthides	0	0	0	0	0	–	–	–
Scyliorhinus	1	0	0	0	1	0	1	0
Hamiltonichthys	0/1	0	0	0	0/1	0	1	0
Helodus	0/1	0	0	0	0/1	0	1	0
Holonema	0	0	0	0	0	0	0	0
Homalacanthus	1	0	0	0	1	–	–	–
Howqualepis	0	0/1	0	0/1	0/1	0/1	0/1	0/1
Incisoscutum	0	0	0	0	0	0/1	0/1	0/1
Ischnacanthus	1	0	0	0	1	0	1	0
Jagorina	0/1	0	0	0	0/1	0/1	0/1	0/1
Janusiscus	0/1	0	0/1	0/1	0/1	0/1	0/1	0/1

Kathemacanthus	0/1	0	0	0	0/1	–	–	–
Kenichthys	0	0	1	1	1	0/1	0/1	0/1
Kentuckia	0	0/1	0	0/1	0/1	0/1	0/1	0/1
Kujdanowiaspis	0	0	0	0	0	0/1	0/1	0/1
Latviacanthus	0/1	0	0	0	0/1	0	0	0
Ligulalepis	0	1	0	1	1	1	0	0
Lophosteus	0	0	0	0	0	0	0	0
Lunaspis	0	0	0	0	0	0/1	0/1	0/1
Lupopsyrus	0/1	0/1	0	0/1	0/1	–	–	–
Macropetalichthys	0	0	0	0	0	0/1	0/1	0/1
Materpiscis	0	0	0	0	0	0/1	0/1	0/1
Meemannia	0	0	1	1	1	0/1	0/1	0/1
Mesacanthus	0/1	0	0	0	0/1	–	–	–
Microbrachius	0/1	0	0	0	0/1	0/1	0/1	0/1
Miguashaia	0/1	0/1	0	0/1	0/1	0	0	1
Lepisosteus	0	1	0	1	1	1	0	1
Polypterus	0	1	0	1	1	1	0	1
Mimipiscis	0	1	0	1	1	1	0	0
Moythomasia	0	1	0	1	1	1	0	0
Obtusacanthus	0/1	0	0	0	0/1	–	–	–
Onychodus	0	0	0	0	0	0	0	1
Onychoselache	1	0	0	0	1	0	1	0
Orthacanthus	0	0	0	0	0	0	1	0
Osorioichthys	0	0/1	0	0/1	0/1	1	0	0
Osteolepis	0	0	1	1	1	0/1	0/1	0/1
Parabuchanosteus	0	0	0	0	0	0/1	0/1	0/1
Parayunnanolepis	0	0	0	0	0	0/1	0/1	0/1
Parexus	0/1	0	0	0	0/1	0	0	0
Poracanthodes	1	0	0	0	1	0/1	0/1	0/1
Porolepis	0	0	1	1	1	0	0	1
Powichthys	0	0	1	1	1	0	0	1
Promesacanthus	0/1	0	0	0	0/1	–	–	–
Psarolepis	0	0	1	1	1	0	0	0
Pterichthyodes	0	0	0	0	0	0/1	0/1	0/1
Ptomacanthus	0/1	0	0	0	0/1	0/1	0/1	0/1
Pucapampella	0/1	0	0	0	0/1	0/1	0/1	0/1
Quasipetalichthys	0	0	0	0	0	0/1	0/1	0/1
Ramirosuarezia	0/1	0	0	0	0/1	0/1	0/1	0/1
Remigolepis	0	0	0	0	0	0/1	0/1	0/1
Rhadinacanthus	1	0	0	0	1	–	–	–
Rhamphodopsis	0/1	0	0	0	0/1	0	0	0
Romundina	1	0	0	0	1	0/1	0/1	0/1
Sigaspis	0/1	0	0	0	0/1	0/1	0/1	0/1
Sinolepis	0/1	0	0	0	0/1	0/1	0/1	0/1
Styloichthys	0	0	1	1	1	0/1	0/1	0/1
Latimeria	0	0/1	1	1	1	0	0	1
Tamiobatis	0/1	0	0	0	0/1	0/1	0/1	0/1
Tetanopsyrus	0/1	0	0	0	0/1	–	–	–
Tristychius	0/1	0	0	0	0/1	0/1	0/1	0/1
Vernicomacanthus	0/1	0	0	0	0/1	0	0	0
Wuttagoonaspis	0/1	0	0	0	0/1	0/1	0/1	0/1
Youngolepis	0	0	1	1	1	0	0	1
Yunnanolepis	0	0	0	0	0	0/1	0/1	0/1
Andreolepis	0	0	1	1	1	0	0	0

Table S2. Presence or absence of enameloid, ganoin, enamel, enamel or ganoin, enamel, ganoin, or enameloid in the dermal skeleton, and acrodin, enameloid, and enamel on teeth, Related to Figure 5. 0, 1, and 0/1 represent the absence, presence, and unknown status of the tissue, respectively. “-“ in dental characters represents the absence of teeth.

Taxa	Minimum age (Mya)	Reference
Galeaspida	432.6	
Osteostraci	437.4	
Acanthodes	298	King et al. 2016
Achoania	412	King et al. 2016
Akmonistion	327	King et al. 2016
Austroptyctodus	383	King et al. 2016
Bothriolepis	383	King et al. 2016
Brachyacanthus	415	King et al. 2016
Brindabellaspis	401	King et al. 2016
Brochoadmones	415	King et al. 2016
Buchanosteus	408	King et al. 2016
Campbellodus	383	King et al. 2016
Cassidiceps	415	King et al. 2016
Cheiracanthus	388	King et al. 2016
Cheirolepis	388	King et al. 2016
Chondrenchelys	338	King et al. 2016
Cladodoides	375	King et al. 2016
Cladoselache	360	King et al. 2016
Climatius	415	King et al. 2016
Cobelodus	325	King et al. 2016
Coccosteus	388	King et al. 2016
Compagopiscis	383	King et al. 2016
Cowralepis	383	King et al. 2016
Culmacanthus	385	King et al. 2016
Debeerius	320	King et al. 2016
Diabolepis	412	King et al. 2016
Dialipina	401	King et al. 2016
Diandongpetalichthys	417	King et al. 2016
Dicksonosteus	411	King et al. 2016
Diplacanthus	388	King et al. 2016
Doliodus	395	King et al. 2016
Eastmanosteus	383	King et al. 2016
Entelognathus	424	King et al. 2016
Eurycaraspis	385	King et al. 2016
Eusthenopteron	380	King et al. 2016
Euthacanthus	415	King et al. 2016
Gavinaspis	412	King et al. 2016
Gemuendina	408	King et al. 2016
Gladiobranchus	415	King et al. 2016
Glyptolepis	388	King et al. 2016
Gogonasus	383	King et al. 2016
Groenlandaspis	385	King et al. 2016
Guiyu	424	King et al. 2016
Gyracanthides	359.3	Warren et al. 2000
Scyliorhinus	0	
Hamiltonichthys	302	King et al. 2016
Helodus	311	King et al. 2016
Holonema	383	King et al. 2016
Homalacanthus	380	King et al. 2016
Howqualepis	385	King et al. 2016
Incisoscutum	383	King et al. 2016
Ischnacanthus	415	King et al. 2016
Jagorina	375	King et al. 2016
Janusiscus	415	King et al. 2016
Kathemacanthus	415	King et al. 2016
Kenichthys	396	King et al. 2016
Kentuckia	347	King et al. 2016
Kujdanowiaspis	411	King et al. 2016
Latviacanthus	404	King et al. 2016
Ligulalepis	401	King et al. 2016
Lophosteus	416	Cunningham et al. 2012
Lunaspis	408	King et al. 2016
Lupopsyrus	415	King et al. 2016
Macropetalichthys	390	King et al. 2016
Materpiscis	383	King et al. 2016
Meemannia	412	King et al. 2016
Mesacanthus	415	King et al. 2016
Microbrachius	386	King et al. 2016
Miguashaia	380	King et al. 2016
Lepisosteus	0	
Polypterus	0	
Mimipiscis	383	King et al. 2016
Moythomasia	383	King et al. 2016

Obtusacanthus	415	King et al. 2016
Onychodus	383	King et al. 2016
Onychoselache	336	King et al. 2016
Orthacanthus	290	King et al. 2016
Osorioichthys	367	King et al. 2016
Osteolepis	388	King et al. 2016
Parabuchanosteus	401	King et al. 2016
Parayunnanolepis	412	King et al. 2016
Parexus	415	King et al. 2016
Poracanthodes	417	King et al. 2016
Porolepis	411	King et al. 2016
Powichthys	411	King et al. 2016
Promesacanthus	415	King et al. 2016
Psarolepis	416	King et al. 2016
Andreolepis	424	Chen et al. 2016
Pterichthyodes	389	King et al. 2016
Ptomacanthus	415	King et al. 2016
Pucapampella	388	King et al. 2016
Quasipetalichthys	385	King et al. 2016
Ramirosuarezia	392	King et al. 2016
Remigolepis	366	King et al. 2016
Rhadinacanthus	386.9	Lukševičs et al. 2010
Rhamphodopsis	388	King et al. 2016
Romundina	415	King et al. 2016
Sigaspis	412	King et al. 2016
Sinolepis	358.5	Zhu et al. 2000
Styloichthys	412	King et al. 2016
Latimeria	0	
Tamiobatis	360	King et al. 2016
Tetanopsyrus	415	King et al. 2016
Tristychius	336	King et al. 2016
Vernicomacanthus	415	King et al. 2016
Wuttagoonaspis	393	King et al. 2016
Youngolepis	412	King et al. 2016
Yunnanolepis	415	King et al. 2016

Table S3. Tip ages used for the ancestral state estimation, Related to Figure 5.

Transparent Methods

Bioinformatic analyses

We searched for SCPP genes in RNA-seq datasets (GenBank accession numbers: SRX796494 and SRX1016233-SRX1016241 for lungfish, SRX796491 and SRX1386644- SRX1386647 for bichir, and SRX424533 and SRX424534 for sturgeon) by tblstn (http://www.ncbi.nlm.nih.gov/) (Altschul et al., 1990) using amino acid sequences of gar orthologs as queries (Kawasaki et al., 2017).

Relative expression levels of gar SCPP genes were estimated as FPKM values (Trapnell et al., 2010), calculated for a dataset of gar skin (GenBank accession number, SRP042013) (Braasch et al., 2016) using Galaxy (https://usegalaxy.org/) (Afgan et al., 2016). The dataset was retrieved from the EBI SRA database, trimmed using Trimmatic (Bolger et al., 2014), aligned with the gar genome sequence (LepOcu1) using TopHat (Kim et al., 2013). FPKM values and confidence intervals were calculated using Cufflinks (Trapnell et al., 2010) based on BAM files obtained by the TopHat analysis. All default conditions were used in Galaxy analysis, except additional options in Trimmomatic (ILLUMINACLIP and MINLEN=50) and Cufflinks (multi-read correction). Genomic coordinates of SCPP genes were determined using Splign (http://www.ncbi.nlm.gov./) (Kapustin et al., 2008), and used for the Cufflinks analysis.

Molecular analysis

All animals used in our study (gar, 16-55 cm in total length; zebrafish, 3.0 cm in total length; bichir, 20 cm in total length; and lungfish, 20 cm in total length; sex not determined for these animals) were sacrificed according to the guidelines issued by the Ministry of Justice in Japan. SCPP genes identified in bichir and lungfish RNA-seq datasets were confirmed by PCR using cDNA libraries made from a tooth plate (lungfish) and tooth germs (bichirs), as described (Braasch et al., 2016). These libraries were made using SMART cDNA library construction kit (Takara Bio), and PCR products were size fractionated by agarose gel electrophoresis, purified from the agarose gel using the FastGene Gel/PCR Extraction Kit (Nippon Genetics), and ligated into T-vector pMD20 (Takara Bio). The ligation mix was then used to transform *E.*

coli HST08 Premium Competent Cells (Takara Bio). Exon-intron borders were also determined by PCR using genomic DNA as the substrate. For expression analysis of bichir *scpp5*, total RNA molecules were isolated using RNAiso Plus (Takara Bio) and cDNA was synthesized using the PrimerScript II 1st strand cDNA synthesis kit (Takara bio). Primer sequences used for RT-PCR are as follows: 5’-GAGACTTGCGATCTTCTCTTCTG-3’ and 5’-GTGAATTGACCTGAGGCAGGA-3’ for *scpp5*; and 5’-CACAGTTTGCCAGATGGTCC-3’ and 5’-CACCACCAATTGCCTTGCTC-3’ for *gapdh*.

ISH analysis

For ISH analysis, jaws and skin of gar and zebrafish were fixed with neutralized 4% paraformaldehyde, decalcified with Morse’s solution (10% w/v sodium citrate and 22.5% v/v formic acid), dehydrated through a graded ethanol series and xylene, embedded in paraffin, sectioned in the coronal plane at 4 μm in thickness, and mounted on glass slides. These glass slides were deparaffined, treated with Proteinase K (20 mg/ml) for 10 min, and used for hybridization (50% formamide, 10 mM Tris pH7.6, 1xDenhart’s solution, 5% dextran sulfate, 600 mM NaCl, 0.25% SDS, 1 mM EDTA, and 100μg/ml *Escherichia coli* tRNA) at 70C (Nakatomi et al., 2006). A specific portion of *scpp5*, *ambn*, and *enam* were amplified from the cDNA libraries (primer sequences: 5'-GTTGGTGCTACAGCAGGAAGT-3' and 5'-GTTGTTGCTTCCCTGAACTG-3' for gar *ambn*; 5'-AAGGCCTCAGCTTCGTCCAG-3' and 5'-ACTCCTTCTCGTTGACTTCGT-3' for gar *enam*; 5'-TATTCTGAGGAGTCACCAA-3' and 5'-ATTCTGACTTGGCTGGACG-3' for gar *scpp5*; 5'-ACTTCATCAACAGGTGCCCAATC-3' and 5'-TGAAAGCTCCGTGACCTGAATCT-3' for zebrafish *ambn*; and 5'-CTGCCCCTGACAGTGGCAGTAATG-3' and 5'-CATCAGGCCCAACAACAGTGGTGT-3' for zebrafish *enam*), cloned using the pGEM-T Easy Vector (Promega), and labeled with digoxigenin using the DIG RNA Labeling Kit (Roche), and used for hybridization. After hybridization, glass slides were washed with 2xSSC. Hybridization signals were detected using Anti-Digoxigenin-AP, Fab fragments (Roche) and NBT/BCIP Solution (Roche), as specified.

IHC analysis

For IHC analysis, jaws and scales were fixed (4% paraformaldehyde-0.2% glutaraldehyde, 0.05M HEPES buffer pH7.4), dehydrated, and embedded in LR-White resin (LR-W, London Resin), and processed for the Protein A-gold (PAG) method (Sasagawa et al., 2012). For light microscopic analysis, semi-thin sections were made from LR-W resin block, mounted on glass slides, masked with 5% goat serum in phosphate buffered saline (PBS), and reacted with the antibody diluted 1:100 or 1:200 in PBS containing 0.5% bovine serum albumin (PBS-BSA) at 4C. These sections were washed with PBS, incubated with the PAG conjugate (gold nanoparticles 5nm, BBI) diluted 1:100 with PBS-BSA, washed with PBS, treated with the silver enhancer solution (50 mM citrate buffer, 0.85% hydroquinone, 0.1% maleic acid, 0.11% silver lactate, and 1% acacia powder) (Uchida et al., 1991) for 10 min in a dark box, and rinsed with water. These sections were then immersed in photographic fixatives, and stained with fast red or toluidine blue (Sasagawa et al., 2012).

For TEM analysis, ultrathin sections obtained from LR-W resin block were mounted on nickel grids, floated on a drop of 1% goat serum and then the antibody diluted 1:200-1:400 in PBS-BSA. These sections were subsequently washed with PBS-BSA, treated with 1% goat serum and then with the PAG conjugate diluted 1:10 in PBS-BSA. These sections were stained with platinum blue and lead citrate (additionally stained with phosphotungstic acid for some samples), and examined using TEM (JEM-1010, JEOL) (Sasagawa et al., 2016). In some sections, immunoreactions were enhanced using the silver enhancer solution (Uchida et al., 1991) or the Silver Enhancer Kit (Kirkegaard & Perry Laboratories). Polyclonal antibodies to gar Scpp5 were raised against YRQQPQQQN (SIGMA-ALDRICH).

Phylogenetic analysis

We augmented an existing phylogenetic dataset (Qiao et al., 2016) with codings for *Scyliorhinus*, *Lepisosteus*, *Polypterus*, and *Latimeria,* representing the three major living clades of jawed vertebrates. We also coded *Andreolepis,* a putative stem-osteichthyan (Qu et al., 2015), and coded *Ligulalepis* as present for dental acrodin (Schultze, 2016; Schultze, 2018). Morphological data were analyzed using the Mkv+G model in MrBayes 3.2.7 (Ronquist et al., 2012). We constrained the positions of the extant taxa to mitigate long branch effects: *Scyliorhinus* with *Helodus, Chondrenchelys, Debeerius, Tristychius, Hamiltonichthys, Onychoselache* (Qiao et al., 2016); *Latimeria* with *Miguashaia* (Arratia and Schultze, 2015); and *Polypterus, Lepisosteus, Kentuckia* with *Moythomasia, Mimipiscis* (Giles et al., 2017). We conducted two phylogenetic analyses of this dataset, one in which the GAP clade were constrained to be stem-osteichthyans (King et al., 2017; Lu et al., 2017), and the other in which these taxa were topologically unconstrained, yielding a stem-sarcopterygian affinity (Lu et al., 2016; Qiao et al., 2016; Choo et al., 2017). Each analysis used four independent runs of four chains over 10,000,000 generations, sampling every 10,000 generations, with 25% of burnin. Convergence was assessed using Tracer 1.7 (Rambaut et al., 2018). Results were summarized using majority rule consensus trees (Figures S3A and S3B).

Ancestral state estimation

We compiled data on the distribution of five dermal (enameloid, ganoin, enamel, ganoin/enamel, hypermineralized tissue) and three dental (acrodin, enameloid, enamel) skeletal characters (Table S2); we confirmed the presence of dental enameloid in *Cladoselache* (Figure S3S).

Branch lengths were estimated using the function *timePaleoPhy* in the R package (https://www.R-project.org/) *paleotree* (Bapst, 2012) using the 'equal' method (Brusatte et al., 2008), with the root age increased by 5 million years. Tip ages are from King *et al*. (King et al., 2017) (Table S3) but the root (Tinn and Märss, 2018), and the crown clades of chondrichthyans, sarcopterygians, actinopterygians, osteichthyans, and gnathostomes (Benton et al., 2015) were calibrated to be minimally as old as oldest fossil representative. Using these time-scaled topologies, we estimated ancestral states using Stochastic Character Mapping (Huelsenbeck et al., 2003) in the R package *phytools* (Revell, 2012). Each character was coded as a binary presence or absence state for all taxa; uncertain tip states were assigned an equal prior probability of 0.5. Taxa were excluded from an analysis when the character was inapplicable. By running all separate analyses for each character, there is zero modelled co-variance between each character. Stochastic character mapping was run using the *make.simmap* function (Bollback, 2006) in *phytools* but with a single *Q* matrix for all simulations under the ‘all rates different’ and a naïve equal prior probability for absence or presence at the root. Each character model ran for 1000 simulations and each iteration used the same ‘empirical’ *Q* matrix with the highest likelihood transition probabilities. Results were summarized using the *describe.simmap* function and the posterior probabilities of node and tip states were plotting on each tree.

Supplemental References

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