Supplemental Information

Phylogenetic analysis

We used the matrix from Conway-Morris and Caron (*5*), which was modified from that of Samson et al. (*45*). We were unable to completely replicate their study in the numbers of MPTs obtained, never getting fewer than 7 (they reported 5); however, the topology we derived was mostly consistent with their findings with the exception of the placement of *Euphanerops*. We then modified the coding of Conway-Morris and Caron (*5*) for euconodonts for characters: 57 (from 0 to 1; this study), 62 (? to 1 (*46*)), 77 (1 to 0 (*10*)), 80 (1 to 0 (*10*)), 81 (? to inapplicable (*10*)), 82 (1 to inapplicable (*10*)), 85 (1 to ? (*10*)), 108 (1 to inapplicable (*10*)), 115 (corrected from 1 to 2), and 116 (corrected from ? to 1). Parsimony analysis was conducted in PAUP* 4.0b10 following the methods detailed in Conway-Morris and Caron (*5*) (heuristic analysis, 1000 random replicates, TBR branch swapping, unordered characters). The topological constraint of Conway-Morris and Caron (*5*) was employed to enforce cyclostome monophyly. All parsimony statistics were calculated in PAUP. Bremer support was calculated using Treerot v. 3.

Characters

All characters used in this analysis are taken from Conway-Morris and Caron (*5*) and are listed below.

1. Neural crest: absent = 0, present = 1

2. Olfactory peduncles: absent = 0, present = 1

3. Pineal organ (extra-ocular photoreceptor region expressing pineal opsins): absent = 0, present = 1

4. Adenohypophysis: absent = 0, present = 1

5. Adenohypophysis: simple = 0, compartmentalized = 1

6. Optic tectum: absent = 0, present = 1

7. Cerebellar primordial: absent = 0, present = 1

8. Pretrematic branches in branchial nerves: absent = 0, present = 1

9. Flattened spinal cord: absent = 0, present = 1

10. Ventral and dorsal spinal nerve roots united: absent = 0, present = 1

11. Mauthner fibres in central nervous system: absent = 0, present = 1

12. Retina: absent = 0, present = 1

13. Olfactory organ with external opening: absent = 0, present = 1

14. Nasohypophyseal opening serving respiration (nasohypophyseal duct): absent = 0, present = 1

15. Single nasohypophyseal opening: absent = 0, present = 1

16. Position of nasohypophyseal opening: terminal = 0, dorsal = 1

17. Olfactory organ unpaired = 0, paired = 1

18. Extrinsic eye musculature: absent = 0, present = 1

19. Otic capsule anterior to branchial series: absent = 0, present = 1

20. Semicircular canals in labyrinth: absent = 0, present = 1

21. Vertical semicircular canals forming loops: absent = 0, present = 1

22. Externally open endolymphatic ducts: absent = 0, present = 1

23. Electroreceptive cells: absent = 0, present = 1

24. Sensory lines: absent = 0, present = 1

25. Sensory-lines: on head only = 0, on head plus body = 1

26. Sensory-lines: enclosed in grooves = 0, enclosed in canals = 1

27. Pouch-shaped gills: absent = 0, present = 1

28. Single confluent branchial opening: absent = 0, present = 1

29. Elongate branchial series: more than 10 gill pouches/slits = 0, fewer than 10 = 1

30. Gill openings lateral and arranged in slanting row: absent = 0, present = 1

31. Position of gill openings: laterally = 0, ventrally = 1

32. Opercular flaps associated with gill openings: absent = 0, present = 1

33. Endodermal gill lamellae: absent = 0, present = 1

34. Gill lamellae with filaments: absent = 0, present = 1

35. Mouth: terminal = 0, ventral = 1

36. Oral hood: absent = 0, present = 1

37. Velum: absent = 0, present = 1

38. Multi-chamber heart: absent = 0, present = 1

39. Closed pericardium: absent = 0, present = 1

40. Open blood system: absent = 0, present = 1

41. Paired dorsal aortae: absent = 0, present = 1

42. Large lateral head vein: absent = 0, present = 1

43. Lymphocytes: absent = 0, present = 1

44. Subaponeurotic vascular plexus: absent = 0, present = 1

45. Dorsal fin: separate dorsal fin: absent = 0, present = 1

46. Dorsal fin: originates at posterior of branchial series = 0, restricted to posterior of trunk and/or caudal region = 1

47. Anal fin separate: absent = 0, present = 1

48. Fin ray supports: absent = 0, present = 1

49. Paired antero-posterior skin folds: absent = 0, present = 1

50. Constricted pectoral fins with endoskeletal elements: absent = 0, present = 1

51. Pelvic fins/flap: absent = 0, present = 1

52. Tail shape: no distinct lobes developed = 0, ventral lobe much larger than dorsal = 1, dorsal lobe much larger than ventral = 2, dorsal and ventral lobes almost equally developed = 3

53. Chordal disposition relative to tail development: isochordal = 0, hypochordal = 1, hyperchordal = 2

54. Preanal median fold: absent = 0, present = 1

55. Ability to synthesise creatine phosphatise: absent = 0, present = 1

56. Visceral arches fused to the neurocranium: absent = 0, present =1

57. Keratinous teeth: absent = 0, present = 1

58. Circumoral teeth: absent = 0, present = 1

59. Circumoral teeth: arranged in radiating series, absent = 0, present = 1

60. Trematic rings: absent = 0, present = 1

61. Arcualia: absent = 0, present = 1

62. Piston cartilage and apical plate: absent = 0, present = 1

63. Midline retractor muscle and paired protractor muscles: absent = 0, present = 1

64. Transversely biting teeth: absent = 0, present = 1

65. Jaws (dorsoventral bite): absent 0, present = 1

66. Chondroitin 6-sulphate in cartilage: absent = 0, present = 1

67. Braincase with lateral walls: absent = 0, present = 1

68. Neurocranium entirely closed dorsally and covering the brain: absent = 0, present = 1

69. Occiput enclosing vagus and glossopharyngeal nerves: absent = 0, present = 1

70. Annular cartilage: absent = 0, present = 1

71. Large oral disc: absent = 0, present = 1

72. Tentacle cartilages: absent = 0, present = 1

73. Trunk dermal skeleton: absent = 0, present = 1

74. Perichondral bone: absent = 0, present = 1

75. Calcified cartilage: absent = 0, present = 1

76. Cartilage composed of huge clumped chondrocytes: absent = 0, present = 1

77. Calcified dermal skeleton: absent = 0, present = 1

78. Lamellar aspidin: absent = 0, present = 1

79. Cellular bone: absent = 0, present = 1

80. Dentine: absent = 0, present = 1

81. Dentine present as: mesodentine = 0, orthodentine = 1

82. Enamel/oid: absent = 0, (monotypic) enamel = 1, enameloid (bitypic enamel) = 2

83. Three-layered exoskeleton consisting of a basal lamella, middle spongy (or cancellar) layer and a superficial (often ornamented) layer: absent = 0, present = 1

84. Cancellar layer in exoskeleton, with honeycomb-shaped cavities: absent = 0, present = 1

85. Scales/denticles/teeth composed of odontodes: absent = 0, present = 1

86. Scale shape: diamond-shaped = 0, rod-shaped = 1

87. Oak-leaf-shaped tubercles: absent = 0, present = 1

88. Oral plates: absent = 0, present = 1

89. Denticles in pharynx: absent = 0, present = 1

90. Dermal head covering in adult state: absent = 0, present = 1

91. Large unpaired ventral and dorsal dermal plates on head: absent = 0, present = 1

92. Massive endoskeletal head shield covering the gills dorsally: absent = 0, present = 1

93. Sclerotic ossicles: absent = 0, present = 1

94. Ossified endoskeletal sclera encapsulating the eye: absent = 0, present = 1

95. High blood pressure: absent = 0, present = 1

96. Hyperosmoregulation: absent = 0, present = 1

97. Male gametes shed directly through the coelom: absent = 0, present = 1

98. Forward migration of postotic myomeres: absent = 0, present = 1

99. Larval phase: absent = 0, present = 1

100. Pineal opening: covered = 0, uncovered = 1

101. External nasal opening: single = 0, paired = 1

102. Number of semicircular canals: one = 0, two = 1, three = 2

103. Neuromasts in sensory lines: absent = 0, present = 1

104. Relative position of atrium and ventricle of heart: well separated = 0, close to each other = 1

105. Lymphocytes antigen receptors: VLR = 0, T and B = 1

106. Paired antero-posterior skin folds: extend along the trunk = 0, anterior only =1

107. Ventral arcualia: absent = 0, present = 1

108. Scales/denticles/teeth: made up by single odontode = 0, made up by several odontodes = 1

109. Dermal head covering in adult state: micromeric = 0, large (macromeric) dermal plates or shield = 1

110. Myomeres: absent = 0, present-simple = 1, W-shaped = 2

111. Pharyngeal bars: continuous = 0, segmented = 1, complex framework forming branchial basket or cartilaginous nodules = 2

112. Pharyngeal bars: external = 0, internal = 1

113. Mandibular branchial bar: absent = 0, present = 1

114. First pharyngeal bar (mandibular): undifferentiated = 0, differentiated = 1

115. Eyes: absent = 0, unpaired or ocelli = 1, paired = 2

116. Notochord: absent = 0, present = 1

DATA SET

Hemichordata: 0-00- 0000- 000-0 ---0- --00- -0000 ?0-0? 000-0 00000 ---0- ---00 000-0 00-00 00-00 00000 000-0 ----0 --0-- -0--0 00-0- --0-0 ----0 0?0-0 0

Myllokunmingia: 1???? ????? ????? ????? ????? ?101? 00?10 0?1?? ????1 00?00 00??? ?00-? 10-00 ????? 0?000 ?00-0 ----0 --0-- -0-0? ????? ????? -?--? ????2 1

Pikaia: ????? ????? ????? ????? ????? ??011 00??0 0???? ????0 -0?00 00??? ?00-? 00-00 ????? 00000 ?00-0 ----0 --0-- -0--? ????? ????? -?--? ????1 1

Metaspriggina: 1???? ????? ??1?1 11??? ????? ??01? 00?10 0?1?? ????? ???00 00??? ?00-? 10-00 ????? 0?000 ?00-0 ----0 --0-- -0-0? ????? 0???? -?--2 11102 1

Haikouichthys: 1???? ????? ??1?1 11?1? ????? ??01? 00?10 0?1?? ????1 00100 00?1? ?00-? 10-00 ????? 0?000 ?0000 --0-0 --0-0 -0-0? ????? 0???? -?--2 1???2 1

Petromyzontida: 10111 11010 11101 11111 00111 01011 00110 11111 00101 10100 01101 11111 11110 11001 10000 10000 --0-0 -00-0 -0001 11111 01111 -0--2 20112 1

Jawed Vertebrates: 11111 11101 111-0 -1111 11111 10010 01010 00111 11101 11101 12201 000-0 10-01 11110 00111 01011 11101 00011 00111 10000 12111 -1102 11112 1

Jamoytius: ????? ????? ??1?1 0???? ????? ??001 0???1 0???? ????? ???10 0??01 ???-? ????0 ????1 001?? ?1??0 --0-? 100-0 -000? ????? 0???? 0??-2 ????2 1

Euphanerops: ????? ????? ????? ????? ????? ?1001 0??11 0???? ????? 11110 011?1 ????? 1???0 ????1 00?0? 10000 --?-0 ?00-0 -000? ????? ????? 01--2 ????2 1

Arandaspida: ??1?? ????? ????? ????? ???11 01001 00??0 0???? ????0 10?00 0??01 ??0-? ????0 ????? 001?? ?1101 ?2111 11101 1?1?? ????0 ????? -?112 ????2 1

Osteostraci: ?011? 11??? ??101 1?101 11?11 1101- 11?11 0?11? 01?11 10101 02201 1?0-? 1??00 ?1110 00111 01011 00101 00101 0111? ????1 01?1? -?112 ?0102 1

Euconodonta: ????? ????? ????? ??1?? ????? ????? ????? 0???? ????0 10100 01101 ?10-? ?1?10 ????0 0?000 ?0000 --0-? ?0010 -000? ????? ????? -?--2 ????2 1

Tunicata: 1-10- 0000- 000-0 ---?0 --00- -010? ?0000 00010 00000 ?0000 000?0 000-0 00-00 -0000 00000 00000 --0-0 -00-0 00000 01011 ----- ----1 --0-0 1

Cephalochordata: 0-10- 00000 000-0 ---?0 --00- -0000 00100 110-0 10000 ?0000 00010 000-0 00-00 -0000 00000 00000 --0-0 -00-0 00000 00011 ----- ----1 000-1 1

Mesomyzon: ????? ????? ????? ???1? ????? ?101? 0??10 1???? ????? 10?00 0110? ??11? ????0 ????? 10000 ?0000 --0-0 -00-0 -000? ????? ????? -?--2 ????2 1

Myxinoidea: 10010 10011 01111 00011 00010 01(0 1)00 00100 01100 10110 10000 00111 110-1 ?1110 00000 01000 10000 --0-0 -00-0 00000 0110- 00000 -?--2 20112 1

Myxinikela: ????? ????? ??1?1 0??1? ????? ?101? 0???0 0???? ????0 10?00 00?0? ??0-? ????0 ????? 01000 ?0000 --0-0 -00-0 -000? ????? 0???? -?--2 ????2 1

Priscomyzon: ????? ????? ????? ???0? ????? ?101? 0???0 11??? ????0 00?00 0???? ?1101 ????0 ????1 10000 ?0000 --0-0 -00-0 -000? ????? ????? -?--2 ????2 1

Mayomyzon: ????? ????? ???0? ???0? ????? ?101? 0???0 11??? ????0 10?00 0(0 1)0?? ????1 ?1??0 ????1 00000 ?0000 --0-0 -00-0 -000? ????? ????? -?--2 ????2 1

Heterostraci: ?11?? ?1??? ????? ?1?01 10?11 11110 00?10 0???? ???10 10000 03111 ??0-? 1??00 ????? 00100 ?1101 10111 00101 1000? ????0 ?1??? -?112 ????2 1

Astraspis: ??1?? ????? ????? ????? ?0?10 01011 00??? ????? ???1? 1??00 0???1 ????? ????0 ????? ??100 ?1101 12101 01??1 1??0? ????0 ????? -?102 ????2 1

Anaspida: ??1?? ????? ??1?1 1???? ???11 01001 00??0 0???? ????0 11110 01101 ??0-? ????0 ????? 00100 ?1100 --0-1 10101 0000? ????1 0???? (0 1)?102 ????2 1

Galeaspida: ?11?? 11??? ??111 11?01 11?11 1101- 10?11 0???? ?1?1? 1??00 0??01 1?0-? 1??00 ?1110 00101 01100 --0-1 00101 0100? ????1 01??? -?012 ?0102 1

Loganellia: ????? ????? ????? ????? ???11 1?011 01??0 0???? ????1 11010 03101 ??0-? ???00 ????? 00100 ?1101 000-1 00011 0000? ????? ????? 1?002 ????? 1

Turinia: ????? ????? ????? ????? ????? ?1011 ?1??0 0???? ????? 1?010 ??1?1 ????? ????0 ????? 0010? ?1101 100-1 00011 00?0? ????? ????? 1?002 ????? 1

(0,1)=polymorphism

Results

Parsimony analysis

Five most parsimonious trees (MPTs) were found (200 steps, C.I. = 0.615, R.I. = 0.700; Fig. S1) when no topological constraint was employed. These trees varied in the placement of *Myllokunmingia*, *Haikouichthys*, and *Metaspriggina* within their own clade that formed the sister taxon to heterostracans, osteostrachans and gnathostomes. Petromyzontids and their stem groups, including Euconodontia as the basal-most plesion, were the next distant outgroup, followed by *Pikaia* and the myxinids.

When the backbone topological constraint of cyclostome monophyly was enforced, 115 most parsimonious trees were found (206 steps, C.I. = 0.597, R.I. = 0.677; Fig. 3). These trees varied

in the placement of *Myllokunmingia* and *Haikouichthys* within their own clade, with *Metaspriggina* being removed to the stem plesion just above *Pikaia* in the majority rule. This contrasts with the strict consensus tree, where *Myllokunmingia*, *Haikouichthys*, and

Metaspriggina are reduced to a polytomy with Vertebrata. Euconodontia is consistently found to be the most basal plesion in a monophyletic Cyclostomata, which in turn are found to be the sister group to the Gnathostomata and its stem. Differing from the results of Conway-Morris and Caron (*5*), we find the monophyly of *Jaymoytius* and *Euphanerops*, which are sister taxa to anaspids, heterostracans, osteostracans, and gnathostomes. Altering the backbone topological constraint to place Euconodontia as sister taxon to lamprey within Cyclostoma yielded additional MPTs (517) with the same statistics as the constraint tree (results not shown). This suggests an equally likely placement for conodonts within cyclostomes, but the previously used topological constraint filters out many of these additional results.

Supplementary Figures

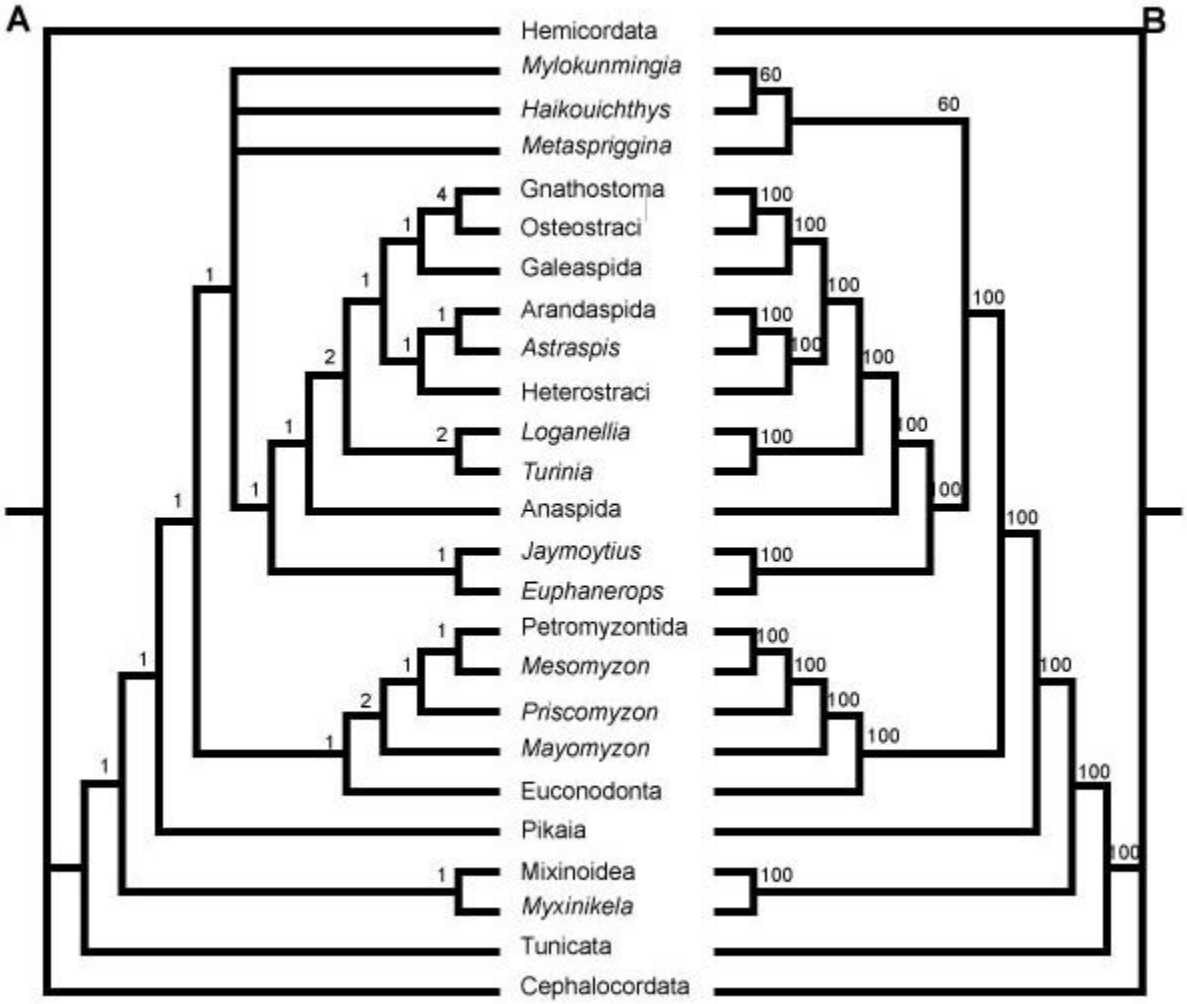

Fig. S1. Results of phylogenetic analysis with no hagfish backbone constraint.

This analysis places hagfish just above tunicates, a position not supported by modern genetics (A) Strict consensus and (B) majority rule consensus of 115 most parsimonious trees (MPTs; 206 steps, C.I. = 0.597, R.I. = 0.677). Numbers in (A) represent Bremer support, and in (B) frequency this node was found in all MPTs.

Fig. S2. Mounting of conodont elements for examination.

Conodont (*Jinogondolella)* elements broken along the axial plane are selected for their relative ease in mounting (left). These elements are then placed in a plastic disc with drilled out sample holes, which are to be filled with epoxy (right).

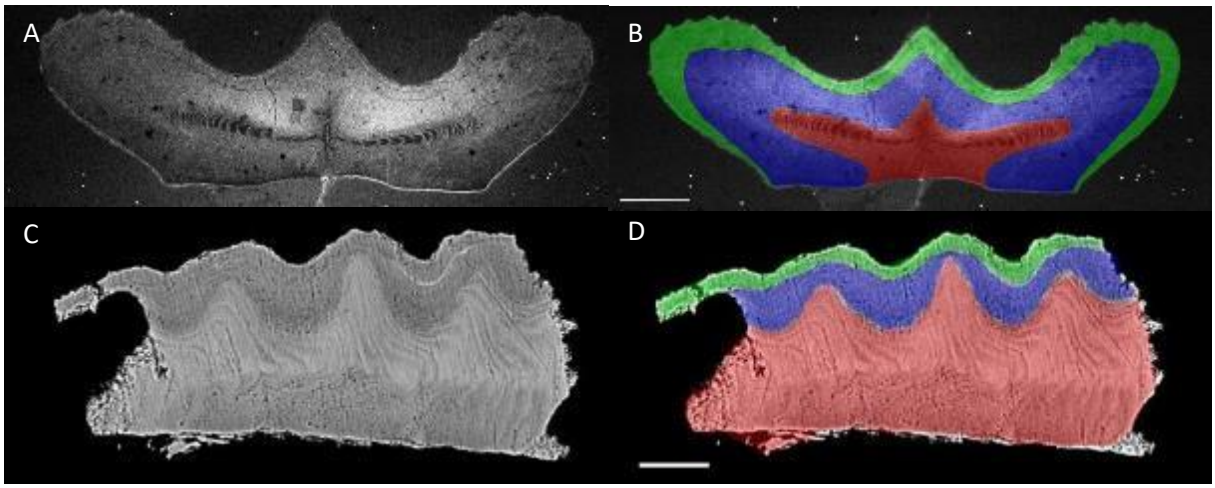

Fig. S3. Ontogeny model for conodont platform elements

Three stage ontogeny model as seen in axial (A and B) and longitudinal (bottom image) view. The model is broken down by colour: Red = Protoelement phase, Blue = Subadult phase, Green = Adult/Gerontic phase. The protoelement phase is defined by interlamellar tissue and elevated sulfur content, the subadult phase by tight growth laminations and a lack of sulfur, while the adult and gerontic phases are often separated by a distinct lamination from the

subadult phase and typically have elevated fluorine levels coupled with changes in lamination growth thickness and repair structures.

Scale bar = 50 micrometers

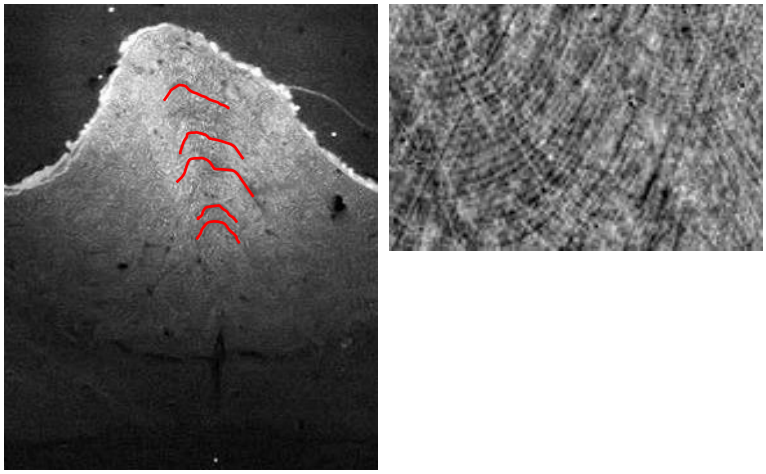

Fig. S4. Examination of wear patterns and subadult growth laminations.

Left: Subadult growth phase of a *Jinogondolella* specimen focussed in on the denticle demonstrating the propagation of wear features. The red lines follow individual growth laminations, with early laminations being smooth to pointed, while later laminations take on a more flattened appearance. Image field width 125 microns.

Right: High magnification image of a *Jinogondolella* subadult laminations, showing high density of laminations during this growth phase. Image field width 20 microns.

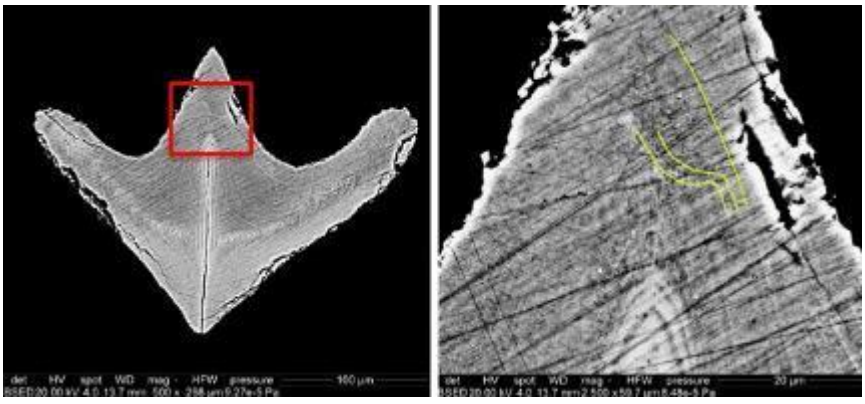

Fig. S5. Details of adult phase repair structure.

Left: BSE image of *Jinogondolella* showing interlamellar tissue and 'mottled' texture in the lower part of the element. Right: Magnified image of the denticle in the box, showing large scale repair of a break during the latter stages of the element's growth.