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**COMPARATIVE DEVELOPMENT AND EVOLUTION OF TWO LATERAL LINE
PHENOTYPES IN LAKE MALAWI CICHLIDS**

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Short Title: Development and Evolution of Cichlid Lateral Line

17 **ABSTRACT** A comparison of the pattern and timing of development of cranial lateral line
18 (LL) canals and canal neuromasts in three species of Lake Malawi cichlids, *Labeotropheus*
19 *fuelleborni* and *Metriaclima zebra* (narrow LL canals), and *Aulonocara baenschi* (widened LL
20 canals) were used to test the hypothesis that the evolution of widened canals (an adaptive
21 phenotype in the lateral line system) from narrow canals is the result of heterochrony. Using
22 histological analysis and SEM, this study has provided the first detailed and quantitative
23 description of the development of widened lateral line canals in a teleost, and demonstrated that:
24 1) canal neuromast number and the pattern of canal morphogenesis are conserved among species
25 with different adult canal morphologies, 2) heterochrony (“dissociated heterochrony” in
26 particular) can explain the evolution of widened canals and variation in morphology between
27 canals in a species with respect to canal diameter and neuromast size, and 3) the morphology of
28 the lateral line canals and the dermal bones in which they are found (e.g., the mandibular canal
29 contained within the dentary and anguloarticular bones of the mandible) can evolve
30 independently of each other, thus requiring the addition of another level of complexity to
31 discussions of modularity and integration in the skull of bony fishes.

32

33 **KEY WORDS:** Cichlidae; neuromast; lateral line; heterochrony; modularity, dermatocranium,
34 hair cell

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38 **INTRODUCTION**

39 The mechanosensory lateral line system of fishes detects unidirectional and low frequency
40 oscillatory water flows and plays critical roles in prey detection and other behaviors (reviewed in
41 Webb, et al., 2008). Directionally sensitive neuromast receptor organs are distributed on the skin
42 (superficial neuromasts) as well as in canals (canal neuromasts) on the head, trunk and tail. The
43 cranial lateral line canals, which are integrated into a conserved subset of the dermatocranial
44 elements of bony fishes, demonstrate well-defined morphological variation among bony fishes
45 and among teleosts in particular (narrow, widened, reduced and branched canals; Webb, 1989b).
46 Narrow canals, the most common of the four canal morphologies, are well-ossified with small
47 pores that connect the fluid within the canal with the outside environment. In contrast, widened
48 canals have evolved convergently in only about a dozen families of typically benthic or deep-
49 water marine and freshwater teleosts. Widened canals are larger in diameter than narrow canals,
50 may cover much of the head, and typically contain large neuromasts. The canal roof is weakly
51 ossified and dominated by large bony canal pores, which are covered by an epithelium that is
52 pierced by very small “epithelial pores” that provide the connection between the fluid within the
53 canal and the external environment. Narrow and widened cranial lateral line canals have been
54 shown to be functionally distinct (Webb, et al., 2008; Denton and Gray, 1988, 1989) and it has
55 been suggested that the evolution of canal morphology among teleosts is the result of
56 heterochrony, or evolutionary changes in developmental timing (Webb 1989a). The study of
57 closely related species with narrow and widened canals provide an interesting context for the
58 integrative study of the adaptive evolution of the lateral line system, but it requires detailed
59 analyses of lateral line development.

60 The development of the lateral line system has been studied in detail in only a small number
61 of species, all of which have narrow canals. It has been described as occurring in three phases
62 [5]. Migration of neuromast primordia from the cranial lateral line placodes establishes spatial
63 patterning of neuromasts in embryos and early larvae (as elegantly detailed in the posterior
64 lateral line system of zebrafish, *Danio rerio*; reviewed in Nunez et al., 2009; Aman and
65 Piotrowski, 2011; Chitnis et al., 2011). Then development continues with neuromast growth
66 (increase in size, change in shape) revealing distinctions between presumptive canal neuromasts
67 (those that will eventually become enclosed in canals) from other superficial neuromasts (that
68 will remain on the skin; e.g., Webb and Shirey, 2003). Finally, in late stage larvae,
69 morphogenesis of the lateral line canals is initiated around individual canal neuromasts to
70 initially form tubular canal segments, a process that occurs in four stages (Webb and Shirey,
71 2003; Tarby and Webb, 2003): Stage I - neuromast differentiates in the epithelium, Stage II -
72 neuromast sinks into an epithelial depression and then canal walls emerge from the dermal bone
73 below the neuromast and ossify, Stage III - epithelium encloses the neuromast forming a canal
74 segment, and Stage IV - ossified canal walls meet over the neuromast forming the ossified canal
75 roof. As they are forming, canal segments are increasing in diameter (Tarby and Webb, 2003;
76 Moore and Webb, 2008). Adjacent segments are also growing towards one another and fuse
77 leaving a common pore between them (e.g., Allis, 1889), thus accounting for the alternating
78 positions of neuromasts and pores along the length of the cranial canals in most bony fishes
79 (Webb and Northcutt, 1997).

80 The hypothesis that heterochrony can explain phenotypic evolution in the lateral line system
81 of bony fishes has been posed (Webb, 1989b; Webb, 1990), but not explicitly tested. The
82 evolution of reduced and branched cranial lateral line canals from narrow canals has been

83 hypothesized to be the result of the simple truncation/deceleration (paedomorphic trend) or
84 extension/acceleration (peramorphic trend) of canal morphogenesis, respectively (Webb, 1989b).
85 In contrast, the evolution of widened canals appears to be the result of “dissociated
86 heterochrony”, defined as a mixture of the evolution of peramorphic and paedomorphic features
87 (McNamara, 1997). For instance, the larger neuromasts and larger diameters that characterize
88 widened canals (reviewed in Webb, 2013) are hypothesized to be peramorphic features, while
89 the reduction in canal ossification that results in the large canal pores bounded by bony bridges
90 (as opposed to a solid canal roof pierced by small pores) of widened canals are hypothesized to
91 be a paedomorphic feature. The mechanisms underlying observed heterochronic change likely
92 include changes in osteoblast and osteoclast activity that alter the timing and/or pattern of
93 ossification of the canals, and/or changes in rates of hair cell differentiation from support cells
94 that result in differences in the size and shape of hair cell populations, and thus neuromast
95 morphology. Changes in gene expression and/or the action of gene products involved in these
96 processes could explain differences in adult canal and neuromast morphology. Such differences
97 are hypothesized to occur via heterochrony, but alternatively, changes in gene expression (or
98 action of gene products) could cause dramatic morphological differences in early larvae followed
99 by isometric increases in canal diameter and neuromast size relative to fish size.

100 Any study of the developmental basis for evolutionary change in phenotype requires the
101 availability of complete ontogenetic series from closely related species that have the phenotypes
102 of interest. The study of the development of widened lateral line canals, in particular, has been
103 hampered by the fact that the small number of taxa with widened canals (Webb, 2014) are
104 largely inaccessible for study and/or are particularly difficult to rear in the laboratory. The
105 speciose and diverse cichlid fishes provide an important opportunity to test a hypothesis of

106 heterochrony in the evolution of the cranial lateral line system. They typically have narrow
107 cranial lateral line canals (Tarby and Webb, 2003; Branson, 1961; Peters, 1973; Webb, 1989c),
108 but among the endemic Lake Malawi cichlids, *Aulonocara*, *Alticorpus* and *Trematocranus* have
109 widened lateral line canals (Konings, 1990, 2007). Like other Lake Malawi cichlids that have
110 proven to be excellent subjects for comparative analyses of functional morphology and
111 development (Albertson and Kocher, 2001, 2006; Albertson, et al., 2001, Streebman, et al.,
112 2003; Hulsey et al., 2005; Sylvester et al., 2010), *Aulonocara* spp. (peacock cichlids; Meyer et
113 al., 1987) are particularly easy to maintain and rear under laboratory conditions. Furthermore, in
114 contrast to other cichlids, which are generally considered to be visual predators, *Aulonocara* uses
115 its lateral line system to detect water flows generated by benthic invertebrate prey living in sandy
116 substrates (Konings, 1990; Schwalbe, et al., 2012). In this study, a comparison of the pattern and
117 timing of development of cranial lateral line canals and canal neuromasts in *Labeotropheus*
118 *fuelleborni* and *Metriaclima zebra* (narrow canals) with *Aulonocara baenschi* (widened canals)
119 were used to test the hypotheses that the evolution of widened canals is the result of
120 heterochrony.

121

122 **MATERIALS AND METHODS**

123

124 The three study species, *Labeotropheus fuelleborni*, *Metriaclima zebra*, and *Aulonocara*
125 *baenschi* (referred to by genus throughout), were reared at 27.8°C with a 12:12 light cycle in a
126 multi-tank re-circulating system. For each species, one male was placed in a tank with 4-5
127 females to facilitate breeding and fish were fed 2x/day with commercial flake food. Fry were
128 extracted from the mouths of brooding females a few days after hatch and reared in small

129 containers supplied with a constant flow of tank water and after yolk absorption were fed high-
130 quality Spirulina flake food (as per Albertson and Kocher, 2001). Fish were sampled periodically
131 over two months yielding developmental series that were prepared for histological analysis,
132 SEM, and clearing and staining (Fig. 1). All fish were anaesthetized in MS222 and fixed in 10%
133 formalin in phosphate-buffered saline (PBS). All procedures followed an approved IACUC
134 protocol.

135

136 **Histological Analysis**

137 Histological material was prepared from one brood each of *Labeotropheus* (n=9, 11-70 dpf,
138 7.5-21.5 mm SL), *Metriaclima* (n=9, 11-70 dpf, 8-23 mm SL), and *Aulonocara* (n=18, 5-53 dpf,
139 <5.0-23 mm SL; Fig. 1). *Labeotropheus* and *Metriaclima* >11 mm SL were decalcified in Cal-Ex
140 (Fisher) for 4 hours (11-12 mm SL), or overnight (\geq 16 mm SL), then rinsed in phosphate buffer,
141 and placed for one hour each, in cold 5%, 10% and 20% sucrose solutions in PBS. *Aulonocara*
142 was decalcified for 2 hours (6.0-7.5 mm SL), 3.5 hours (8.0-8.5 mm SL) or 8 hours (>8.5 mm
143 SL). All fish were dehydrated in an ascending ethanol and t-butyl alcohol series and embedded in
144 Paraplast Plus (Fisher). Serial transverse sections were cut at 8 μ m, mounted on slides subbed
145 with 10% albumen in 0.9% NaCl, and stained with a modification of the HBQ stain (Hall, 1986)
146 to accomplish differential staining of cell nuclei, cartilage and bone. The supraorbital (SO),
147 mandibular (MD), preopercular (PO) and infraorbital (IO) canals were easily observed in
148 histological sections.

149 The supraorbital (SO) and mandibular (MD) canals run rostro-caudally in the nasal and
150 frontal bones (dorsal surface of skull) and in the dentary and anguloarticular bones (lower jaw)
151 were particularly conducive to quantitative analysis histological material (also see Tarby and

152 Webb, 2003; Webb and Shirey, 2003). First, a complete inventory of serial transverse sections of
153 the head in each specimen revealed canal neuromast location, and names (SO1-5, MD1-5) were
154 assigned based on their location. Then, the pattern and timing of the development of the canal
155 segments forming around each canal neuromast were assessed using the developmental stages
156 defined for *Amatitlania nigrofasciata* (= *Archocentrus nigrofasciatus*, Stages I-IV; Tarby and
157 Webb, 2003).

158 A quantitative analysis of the rate of neuromast growth (length and width of SO1-5, MD1-5)
159 and increase in canal diameter (at the level of each canal neuromast) was carried out for the SO
160 and MD canals in larvae and juveniles (5-25 mm) of all three species. This allowed a test of the
161 hypothesis that heterochronic changes in the rates of increase in neuromast size (length, width)
162 and canal diameter among species can explain the evolution of a widened lateral line canal
163 system from a narrow lateral line canal system. In addition, a comparison of canal and
164 neuromast development in the SO and MD canals in the three species was used to determine if
165 there is evidence for regional (or local) heterochrony between the MD and SO canals. It was
166 predicted that the MD canal would be wider, which would be consistent with the notion that the
167 MD is an adaptation, in particular, for detection of benthic prey in *Aulonocara* (Schwalbe, et al.,
168 2012).

169 Neuromast length was determined by counting the number of sections in which neuromast
170 tissue (hair cells surrounded by thickened epithelium composed of mantle cells) was present and
171 multiplying by section thickness ($8\mu\text{m}$; measurement error $\pm 16\mu\text{m}$). Neuromast width was
172 measured (to nearest $0.1\mu\text{m}$) at the rostro-caudal midpoint of each canal neuromast by digitally
173 tracing the curve defined by the apical surface of the cells composing the neuromast around the
174 inner circumference of the canal. Internal canal diameter (defined by internal surface of ossified

175 canal bone) was measured (to nearest $0.1\mu\text{m}$) at the level of each neuromast in the same section
176 as neuromast width, across the canal, at its widest point above the neuromast. Canal diameter
177 could not be measured until canal morphogenesis had commenced, so canal diameter was
178 determined only in those canal segments that were already at Stage II-IV. Canal diameter is
179 known to fluctuate such that canal diameter tends to be larger between neuromast positions than
180 at neuromast positions along the canal, especially in some (but not all) species with widened
181 canals (Webb, 2014). Thus, by measuring diameter at the level of each neuromast, comparisons
182 among species with narrow and widened canals are more consistent and provide a conservative
183 measure of interspecific differences in canal diameter.

184 All measurements were obtained digitally using Spot software (v. 5.0, Diagnostic
185 Instruments, Sterling Heights, MI USA) on an Olympus BH-2 or Zeiss AxioVision software (v
186 4.6.3, Carl Zeiss MicroImaging GmbH, Gottingen, Germany) on a Zeiss AxioImager1 compound
187 microscope. Left-right means of values for each parameter (canal diameter, neuromast length,
188 neuromast width) were calculated to reduce the effects of asymmetry arising with variation in
189 plane of section among individuals. Analysis of Covariance (ANCOVA; JMP, v.10.0.2, SAS
190 Institute, Inc.) was used to detect differences in slopes for each parameter (canal diameter,
191 neuromast length, neuromast width) among the three species after data were tested for normality,
192 and log transformed if needed. If slopes for a given parameter were determined to be
193 heterogeneous (statistically different), then the Johnson-Neyman technique (Johnson and
194 Neyman, 1936) was performed to determine the range of X values (in this case, fish size) in
195 which there is no significant difference in the parameter of interest (“region of non-significance”;
196 White, 2003) between two species and by extension, the range of fish sizes in which a significant
197 difference is present. A similar analysis was then performed to detect differences ontogenetic

198 trends in neuromast size (length, width) and canal diameter in the SO versus MD canals in each
199 of the three study species using the same approach. Significance was defined a priori as $P < 0.05$
200 in all analyses. The graphic representation of data is derived from raw (not log transformed data)
201 to illustrate biologically (as opposed to statistically) relevant measurements.

202

203 **Scanning Electron Microscopy**

204 Specimens of *Labeotropheus* (from two broods including that used for histological analysis,
205 7-70 dpf, 7.5-26 mm SL, n=17; Fig. 1) were dehydrated in an ascending series of ethanol, critical
206 point dried in liquid CO₂, coated with Au-Pd alloy, and mounted on carbon-coated stubs in order
207 to visualize as many of the lateral line canals as possible. Specimens were imaged with a Hitachi
208 S5-7 SEM and acquired using 4x5 Polaroid film. Photos were scanned at high resolution and
209 minimally post-processed using Adobe Photoshop 4.0 (Adobe Systems, Inc., San Jose, CA,
210 USA).

211

212 **Clearing and Staining and μ CT Imaging**

213 *Aulonocara baenschi* (12-39 dpf, 7-19 mm SL, n=12, from one brood; Fig. 1) were
214 enzymatically cleared and stained for both bone (Alizarin Red) and cartilage (Alcian Blue;
215 Potthoff, 1984) to visualize the lateral line canals. In addition, micro-computed tomographic
216 (μ CT) imaging was carried out on a formalin-fixed specimen of *Aulonocara baenschi* (87 mm
217 SL). The fish was imaged in air using tube settings of 45 kVp and 177 μ A, integration time of
218 300 ms, and scan resolution (voxel size) of 6 μ m (smaller fish) or 16 μ m (larger fish) using a
219 μ CT 40 (Scanco Medical AG, Brütisellen, CH). Once reconstructed, 3-D image volumes were

220 exported as DICOM image stacks and reconstructed using volume and surface rendering
221 protocols in OsiriX (Pixmeo, Geneva Switzerland; <http://www.osirix-viewer.com/>).

222

223 **RESULTS**

224 The timing of major developmental events was similar in all three species. Hatching occurred
225 at <7 days post-fertilization (dpf, at <5 mm TL) and newly hatched fry had a large ovoid yolk sac
226 (Fig. 2). Caudal fin flexion started quickly, just a few days post-hatch, and was complete at 7-8
227 dpf (<7.5 mm SL) in *Labeotropheus* and *Metriaclima* and a few days later in *Aulonocara* (11
228 dpf, ~7 mm SL). In all three species, the yolk sac was not absorbed until well after flexion was
229 complete, at 18-21 dpf (11-12 mm SL; Balon, 1977; C. Albertson, pers. comm.), just prior to the
230 normal time of release from the mother's mouth.

231

232 **Distribution of Canals and Canal Neuromasts**

233 *Labeotropheus*, *Metriaclima* and *Aulonocara* have the same number of canal neuromasts and
234 the same complement of cranial lateral line canals. Five canal neuromasts are present in the
235 supraorbital canal (SO1-5). SO1 is located in the portion of the canal in the tubular nasal bone
236 just medial to the naris (Fig. 4C, 5A, C) and neuromasts SO2-5 are located in the portion of the
237 canal embedded in the frontal bone. Five canal neuromasts (MD1-5) are present in the
238 mandibular canal (Fig. 3A-C). Neuromasts MD1-4 are in the portion of the MD canal in the
239 dentary bone, and neuromast MD5 is located in the short canal segment in the angulo-articular
240 bone (Fig. 3A-C, and in illustrations of *Labeotropheus* and *Metriaclima* in Albertson and Kocher
241 (2001). In addition, the preopercular (PO) canal, which is contiguous with the MD canal, is
242 contained in the L-shaped preopercular bone (Fig. 3A-C; Fig. 4A, B). The infraorbital (IO) canal

243 is contained within the lacrimal bone just under the rostro-ventral border of the orbit (Fig. 4C)
 244 and continues caudally in the series of infraorbital ossicles that follow the circumference of the
 245 orbit (Fig. 3A-C). The difference in the diameter of the IO canal in *Labeotropheus* and
 246 *Metriaclima* (Fig. 3A, B) versus *Aulonocara* (Fig. 3C). is particularly noticeable. On the dorsal
 247 surface of the head, the pore between neuromasts SO3 and SO4 in each of the SO canals extend
 248 medially to form a pore in the dorsal midline, joining the right and left SO canals (Fig. 5C, D).
 249 Finally, caudal to the orbit the post-otic canal continues through the pterotic, extrascapular, post-
 250 temporal, and supracleithral bones and is contiguous with the trunk canal contained in the lateral
 251 line scales.

252

253 **Pattern and Timing of Canal Morphogenesis**

254 In all three species, the pattern of development of individual canal segments was the same
 255 (e.g., Stages I-IV; Tarby and Webb, 2003) despite differences in canal morphology (narrow vs.
 256 widened). Initiation of canal morphogenesis, marked by the formation of longitudinal
 257 depressions or grooves in the vicinity of individual canal neuromasts (Stage II; Fig. 6B, C, G, H),
 258 started within two weeks of fertilization (at 7-8 mm SL). The processes of canal segment
 259 enclosure (Stage III; Fig. 6D, I) and ossification (Stage IV; Fig. 6E, J) then continued for many
 260 more weeks through metamorphosis (larval-to-juvenile transformation; at 10-12 mm SL), and a
 261 concurrent three-fold increase in fish size.

262 A comparison of the timing of canal morphogenesis showed considerable asynchrony among
 263 canals. In *Labeotropheus*, SO, MD, and PO canal grooves (Stage II) were visible as early as ~10-
 264 11 dpf (7-8 mm SL; Fig. 4A, C). Within a day (at 12 dpf), the SO and MD canals started to
 265 enclose (Stage III). Several days later (17-18 dpf, 11-12 mm SL), the PO and MD canals were

266 partially or completely enclosed, and the infraorbital (IO) canal (the portion in the lacrimal bone,
267 but not the remainder of the IO canal contained within in the infraorbital ossicles) was enclosed
268 (Fig. 4C, D). Within two days (19-20 dpf, 12 mm SL, when fish are normally ready to be
269 released from the mother's mouth), some or all of the segments that compose each of the canals,
270 with the exception of the portion of the IO canal in the infraorbital ossicles caudal to the lacrimal
271 bone, had enclosed and ossification (Stage IV) had started (Fig. 5A, B). After several weeks (by
272 42 dpf, 16 mm SL), the SO, MD and PO canals had all ossified (Stage IV), and the IO canal
273 segments in the infraorbital ossicles were finally enclosed (Stage III). SEM illustrated the pores
274 in one juvenile at 56 dpf (~19 mm SL) in which the pores of adjacent canal segments that
275 compose the IO canal had still not fused, leaving double pores (Fig. 5E) and at 70 dpf (~23 mm
276 SL) the double pores had fused to form the single pores characteristic of adult fishes (Fig. 5F).

277 The timing of canal morphogenesis in *Metriaclima* appears to be similar to that in
278 *Labeotropheus*. The SO, MD and PO grooves (Stage II) were apparent in young larvae just after
279 flexion was complete (~11 dpf, 7-8 mm SL), and most or all of the SO and MD canal segments
280 were enclosed (Stage III) about a week later (17-20 dpf; 11 mm SL). By 20-22 dpf (11-12 mm
281 SL), the portion of the IO canal in the lacrimal (containing three canal neuromasts) was enclosed,
282 but the enclosure of the remainder of the IO canal (in the infraorbital ossicles) was delayed for
283 many weeks (Fig. 4C, 6E, F). The SO and MD canal segments were all ossified (Stage IV) in
284 juveniles between 42 and 56 dpf (~19-20 mm SL).

285 In *Aulonocara*, SO grooves (Stage II) were present a bit earlier (at 8 dpf, ~5 mm SL) and
286 some MD grooves were already present at 11 dpf (~7 mm SL) as in *Labeotropheus* and
287 *Metriaclima*. Enclosure of the SO and MD canals (Stage III) started in slightly older individuals
288 (15-17 dpf, 9-11 mm SL) than in *Labeotropheus* and *Metriaclima*, as yolk absorption had begun.

289 At 20-26 dpf (11-12 mm SL), the SO and MD canals, and the portion of the IO canal in the
290 lacrimal bone were enclosed and had begun to ossify (Stage IV); the SO and MD canals were
291 ossified several weeks later (47 dpf; 21-22 mm SL).

292 The onset of canal enclosure (Stage III) and canal ossification (Stage IV) in the SO and MD
293 canals showed some interesting contrasts between the species with narrow canals
294 (*Labeotropheus* and *Metriaclima*) and widened canals (*Aulonocara*). First enclosure in the SO
295 canal occurred by 11-12 dpf in *Labeotropheus* and *Metriaclima*, but occurred over a longer
296 interval (11 to 15 dpf) in *Aulonocara*. First ossification in the SO canal occurred between 12 and
297 17 dpf in *Labeotropheus* and *Metriaclima*, and a few days later (17 to 20 dpf) in *Aulonocara*.
298 Similarly, first enclosure in the MD canal occurred by ~11-12 dpf in *Labeotropheus* and
299 *Metriaclima*, and a few days later (15 to 17 dpf) in *Aulonocara*. First ossification in the MD
300 canal occurred at 12 to 17 dpf in *Labeotropheus* and *Metriaclima*, but several days later (23 to 26
301 dpf) in *Aulonocara*.

302

303 **Order and Timing of Development of Segments within Canals**

304 Asynchrony in development was obvious among canal segments within a canal, but a
305 particular canal segment was not observed at all four of the developmental stages (I-IV) in
306 different individuals due to the rapid progression of canal development and the size and age of
307 individuals available for analysis. Thus, mean fish size at first canal enclosure (Stage III) and
308 canal ossification (Stage IV) for each canal segment was used to approximate the relative order
309 and timing of the development of canal segments within the SO and MD canals (Table 1).

310 The development of the segments of the SO and MD canals did not occur in a simple rostro-
311 caudal (or caudo-rostral) direction within a canal. Nevertheless, a consideration of the mean fish

312 size at which a particular canal segment enclosed and ossified among the individuals analyzed
 313 revealed trends that allowed some generalizations to be made (see Table 1). In the SO canal, the
 314 SO4 canal segment appeared to be the first to enclose (at ~8-9 mm SL) and the first to ossify in
 315 all three species. The other segments then enclosed in a roughly caudal to rostral direction, with
 316 the more caudal segments (SO3-5) tending to enclose before the more rostral segments (SO1-3).
 317 Subsequent ossification occurred in roughly the same order among segments. The order and
 318 timing of the enclosure and ossification of individual canal segments appears to be a bit different
 319 in the MD canal. The MD2 segment tended to enclose first in *Labeotropheus*, but the MD3
 320 segment tended to enclose first in *Metriaclima* and *Aulonocara*. The order of ossification did not
 321 reveal any particular pattern in *Labeotropheus*, but in *Metriaclima* and *Aulonocara*, the more
 322 caudal segments (MD3-5) enclosed before the more rostral segments (MD1-2).

323

324 **Canal Diameter at Enclosure and Ossification**

325 Canal diameter could be measured as soon as a neuromast had sunk into a depression or
 326 groove (Stage II, Fig. 6B, G). Canal diameter continued to increase as bone ossified to form the
 327 canal walls (Fig. 6C, H), as the canal enclosed (Stage III, Fig. 6D, I), and as the canal roof
 328 ossified (Stage IV; Fig. 6E, J). The minimum canal diameters for each SO and MD canal
 329 segment at first enclosure (Stage III) and ossification (Stage IV) provided insights into the
 330 functional implications of canal growth during larval and juvenile development (Table 1). In
 331 *Labeotropheus* and *Metriaclima* (narrow canals) the SO and MD canal segments were first
 332 enclosed at diameters of at least ~65 and ~95 μm , respectively, but in *Aulonocara* (widened
 333 canals) they enclosed at diameters of at least ~70 and ~115 μm , respectively. Thus, the MD
 334 canal segments tended to enclose at larger diameters than those of the SO canal in all three

335 species and the MD canal in *Aulonocara* tended to enclose at larger diameters than in either
336 *Labeotropheus* or *Metriaclima*. Ossification occurred at diameters of at least 20 μm , but in some
337 cases, 60 μm greater than the diameters at which enclosure was observed in a particular MD
338 canal segment. The minimum diameter at which the five MD segments ossified was 83-109 μm
339 in *Labeotropheus* (versus 78-120 μm for its five SO segments), 108-170 μm in *Metriaclima*
340 (versus 81-119 μm for its SO segments) and 136-194 μm in *Aulonocara* (versus 93-183 μm for
341 its SO segments).

342

343 **Quantitative Analysis of Neuromast and Canal Development**

344 An ANCOVA (SO and MD canal data combined) revealed that rates of increase in canal
345 diameter and neuromast size (length, width) varied significantly among species (Fig. 7, Table 2).
346 However, significant interactions (species x fish size) were found for neuromast size (length and
347 width) and canal diameter, so the Johnson-Neyman technique (White, 2003) was used to
348 determine the range of fish sizes in which each parameter was not statistically different (between
349 species pairs), and thus by extension when it was statistically different ($P < 0.05$).

350 The ontogenetic rate of increase in canal diameter was not statistically different (equal
351 slopes) in *Labeotropheus* and *Metriaclima*, but canal diameters were consistently larger in
352 *Metriaclima* (Table 2). The rate of increase in canal diameter in *Aulonocara* was 1.5 and 1.9
353 times that in *Labeotropheus* or *Metriaclima*, respectively (Fig. 7A; Table 2, 3). As a result, canal
354 diameter was already significantly larger in *Aulonocara* than in either *Labeotropheus* or
355 *Metriaclima* larvae at lengths >4.5 and >7.8 mm SL, respectively.

356 Similarly, the ontogenetic rate of increase in neuromast length was not statistically different
357 in *Labeotropheus* and *Metriaclima*, but neuromasts were consistently longer in *Labeotropheus*

358 than in *Metriaclima* (Fig. 7B; Table 2, 3). In *Aulonocara*, neuromast length increased at a rate
359 that was 2.7 or 2.8 times that in *Labeotropheus* or *Metriaclima*, respectively (Table 3) and
360 neuromast length was significantly greater in *Aulonocara* than in either *Labeotropheus* or
361 *Metriaclima* larvae at lengths >12.3 and >9.0 mm SL, respectively. The ontogenetic rate of
362 increase in neuromast width was not statistically different in *Labeotropheus* and *Metriaclima*,
363 but neuromast width was consistently greater in *Metriaclima* (Fig. 7C). Neuromast width in
364 *Aulonocara* increased at a rate 2.2 or 1.6 times that in *Labeotropheus* or *Metriaclima*,
365 respectively (Table 2) and was significantly greater in *Aulonocara* larvae than in either
366 *Labeotropheus* or *Metriaclima* larvae at lengths >8.3 and >7.6 mm SL, respectively.

367 Another ANCOVA revealed differences in rates of increase in canal diameter and neuromast
368 size (length, width) in the supraorbital (SO) versus the mandibular (MD) canal in the three study
369 species (Table 4, 5). In *Labeotropheus*, canal diameter and neuromast length increased at rates
370 that were not statistically different in the SO and MD canals, but SO canals were wider and SO
371 neuromasts were longer than the MD canal and MD canal neuromasts (Table 4). The rate of
372 increase in neuromast width was 1.8 times greater in the SO canal than in the MD canal (Table
373 5), such that neuromasts were significantly wider in the SO canal than in the MD canal in larvae
374 >9.7 mm SL. In *Metriaclima*, neuromast length in the two canals increased at rates that were not
375 statistically different (Table 4), but neuromasts were consistently longer in the SO canal. The
376 rates of increase of neuromast width and canal diameter were greater in the SO canal in
377 *Metriaclima* by a factor of 2.1 and 1.6, respectively (Table 5), such that SO neuromast width was
378 significantly greater in larvae >16.0 mm SL, and SO canal diameter was significantly greater in
379 larvae >14.1 mm SL. In *Aulonocara*, canal diameter and both neuromast length and width all
380 increase faster in the SO canal than in the MD canal (Table 4), which was unexpected. Canal

381 diameter increased 1.3 times faster in the SO canal than in the MD canal (Table 5), such that the
382 SO canal was significantly wider than the MD canal in larvae >15.2 mm SL. Neuromast length
383 and width both increased 1.2 times faster in the SO canal (Table 5) such that SO neuromasts
384 were significantly longer and wider than MD neuromasts in larvae at lengths >12.3 mm SL and
385 >9.6 mm SL, respectively. The differences in developmental rate between the SO and MD canal
386 can be attributed to regional (local) heterochrony.

387

388 **DISCUSSION**

389 This study has provided the first detailed description of the development of widened lateral
390 line canals in a teleost, and the first detailed comparison of the development of narrow and
391 widened canals. It has shown that: 1) canal neuromast number and the pattern of canal
392 morphogenesis are conserved, regardless of adult canal morphology, 2) the evolution of widened
393 canals from narrow canals can occur via dissociated heterochrony (a combination of peramorphic
394 and paedomorphic trends) and regional (local) heterochrony in canal diameter and neuromast
395 size between canals accounts for variation among canals within a species, and 3) the morphology
396 of the lateral line canals and the dermal bones in which they are found (e.g., the mandibular canal
397 contained within the dentary and anguloarticular bones of the mandible) can evolve
398 independently of each other.

399

400 **Pattern and timing of development in narrow versus widened canals**

401 The three study species (*Labeotropheus fuelleborni*, *Metriaclima zebra*, *Aulonocara*
402 *baenschi*) had the same complement of canal neuromasts in the SO and MD canals. This is
403 evidence of a conserved process of neuromast patterning that is independent of the subsequent

404 development of the lateral line canals. In addition, the same pattern of development was
405 observed in the three study species and occurs in four stages as described in a South American
406 cichlid with narrow canals (*Amatitlania nigrofasciata* = *Archocentrus nigrofasciatus*; Tarby and
407 Webb, 2003), and an unrelated teleost, the zebrafish, *Danio rerio* (Webb and Shirey, 2003).
408 Thus, the pattern of groove formation (Stage II), enclosure (Stage III) and canal roof ossification
409 (Stage IV) that results in the formation of lateral line canal segments appears to be conserved
410 among teleosts with both narrow and widened canals.

411 The timing of the different stages of canal development varies among species (Tarby and
412 Webb, 2003; Webb and Shirey, 2003) and may be related to functional demands in developing
413 fishes (Webb, 2013). For instance, the morphogenesis of the cranial lateral line canals progresses
414 quickly to Stage IV (ossification of enclosed canal segments) in both the African cichlids
415 examined in this study and the South American convict cichlid examined in a prior study
416 (*Amatitlania nigrofasciata*; Tarby and Webb, 2003). The result is that in *Labeotropheus*,
417 *Metriaclima* and *Aulonocara*, canal enclosure (Stage III) and ossification (Stage IV) is well-
418 underway after a prolonged process of yolk absorption when transforming juveniles are normally
419 released from the mother's mouth, and must start to feed (at ~ 21 days post-fertilization). In
420 contrast, the cranial lateral line canals in larval and juvenile zebrafish demonstrates a prolonged
421 stage II, in which presumptive canal neuromasts sit in open grooves (Webb and Shirey, 2003).
422 This morphology is predicted to facilitate detection of prey at the water's surface in their native
423 habitat, as has been demonstrated in killifish (Schwarz, et al., 2011).

424 The quantitative ontogenetic analysis presented here has demonstrated that canal diameter
425 and neuromast size are initially similar among the three study species, but that significantly
426 different rates of increase in these parameters result in the development of narrow canals

427 (*Labeotropheus* and *Metriaclima*) versus widened canals (*Aulonocara*). In addition, the process
428 of canal enclosure in the SO and MD canals commences a bit later and the process is a bit more
429 prolonged (over a longer growth interval) and occurs at larger canal diameters in *Aulonocara* in
430 contrast to the narrow canals of *Labeotropheus* and *Metriaclima*. Nevertheless, by the time
431 larvae are normally released from the mother's mouth (~ 21 dpf; 11-12 mm SL), canal diameter
432 (Fig. 4e, j) and neuromast length and width already distinguish *Aulonocara* (widened canals)
433 from *Labeotropheus* and *Metriaclima* (narrow canals).

434 Interspecific differences in lateral line morphology are correlated with differences in feeding
435 habit. *Labeotropheus* feeds on filamentous algae from rocks and *Metriaclima* brushes loose
436 plant matter from algae beds, but also plucks plankton from the water column (Albertson and
437 Kocher, 2006), and it is likely that the lateral line system is not critical for feeding in these taxa.
438 However, *Aulonocara stuartgranti* uses its widened lateral line canal system to detect benthic
439 invertebrate prey as it swims and glides over sandy substrates (Schwalbe et al., 2012). Its
440 feeding behavior suggests that the MD canal, lower arm of the PO canal, and perhaps the portion
441 of the IO canal ventral to the orbit (whose pores are obvious in ventral view, Fig. 3) are critical
442 for prey detection. Surprisingly, the SO canal increases in diameter faster than the MD canal
443 such that the SO canal is wider in diameter and its canal neuromasts are longer and wider than
444 those in the MD canal in all three study species, regardless of canal morphology. Thus, the
445 prediction that canal diameter and neuromast size in the MD canal would be greater than in the
446 SO canal as the result of adaptive function for benthic prey detection was not borne out.
447 Nevertheless, in *Aulonocara*, the MD canal enclosed and was ossified at diameters $>100 \mu\text{m}$ and
448 canal diameter and was already larger than in *Labeotropheus* or *Metriaclima* in early larvae
449 (lengths >7.8 mm SL), such that the widened MD canal becomes morphologically

450 distinguishable from the narrow MD canal well before feeding commences. The timing of the
451 onset of lateral line-mediated feeding behavior (as described in adults, Schwalbe et al., 2012) is
452 not yet known, but it is predicted that it will be dependent on the development of their typical
453 prey search strategy (three phase cycle: swim, glide, pause) as well as favorable hydrodynamic
454 properties of the lateral line canals that will allow stimulation of canal neuromasts by the water
455 flows generated by prey.

456 It is concluded that the evolution of widened canals and their larger canal neuromasts are the
457 result of dissociated heterochrony. High rates of increase in canal diameter and neuromast size
458 are interpreted as peramorphic trends and the delay of SO and MD canal enclosure and the
459 prolonged duration of this process in *Aulonocara* in contrast to *Labeotropheus* and *Metriaclima*
460 are interpreted to be paedomorphic trends. In addition, the initial process of canal roof
461 ossification and fusion of adjacent canal segments to form a common pore is followed by a
462 decrease in pore size in *Labeotropheus* (Fig. 5C, D and Fig. 5E, F), a process noted by Allis
463 (1889) in *Amia calva*. Thus, the evolution of large canal pores characteristic of widened canals
464 (Webb, 1989b) appears to be the result of a paedomorphic trend - either a slower rate in, or
465 truncation of the process of ossification of the canal roof in comparison to that in species with
466 well-ossified narrow canals that have smaller canal pores.

467

468 **The Mandibular Lateral Line Canal as a Component of the Mandible**

469 *Labeotropheus* and *Metriaclima* are distinguished by the morphology and genetics of the oral
470 jaw apparatus, including differences in the length and width of the lower jaw (Albertson and
471 Kocher, 2006), which are attributed to directional selection (Albertson, et al., 2003). A QTL
472 analysis had indicated that several aspects of mandibular morphology critical for feeding are

473 inherited as modules, thus supporting the notion of a degree of morphological integration in the
474 cichlid mandible (Albertson and Kocher, 2006). However, the function of the mandible is not
475 limited to feeding and it is thus subjected to other selective pressures, such as those associated
476 with lateral line function. This study has shown that the diameter of the MD canal and size of the
477 canal neuromasts contained within it are not significantly different in *Labeotropheus* and
478 *Metriaclima* in mid-stage larvae and older or larger individuals, despite significant differences in
479 overall mandibular morphology (Konings, 1990). The mandibular canal is narrow and well-
480 ossified in both species, with the same number of canal neuromasts and small canal pores.
481 Interestingly, the canal pores in the shortened mandible of *Labeotropheus* appear to be more
482 closely positioned to each other than those in *Metriaclima* (as in Fig. 3 and illustrations in
483 Albertson and Kocher, 2001). This difference in inter-pore distance, presumably related to
484 differences in mandibular length, may have unappreciated consequences for the lateral line
485 function (discussed in Coombs and van Netten, 2006).

486 If the mandible can respond to directional selection with respect to feeding while the MD
487 canal does not change in diameter, then it follows that the morphology of the MD canal could
488 evolve independently of lower jaw morphology in response to selection for modified water flow
489 detection. For example, the morphology of the lower jaw of *Aulonocara* spp. (Fig. 3a, b) appears
490 to be similar to that in *Metriaclima*, but this study has shown that while neuromast patterning
491 (number of canal neuromasts) does not differ between these two taxa, canal width and neuromast
492 size diverge in larval *Aulonocara* and *Metriaclima*, to become the widened and narrow lateral
493 line canals characteristic of juveniles and adults (Fig. 6E, J). The evolution of widened canals
494 from narrow canals for the enhancement of prey detection capabilities may also have impacts on
495 lower jaw function. Examination of dried skeletons revealed that the mandible of *Aulonocara*

496 appears particularly “delicate” (Webb and Kocher, unpubl. observ.) due to reduced ossification
497 of the canal roof resulting in their characteristically large pores, but the consequences of these
498 features for feeding mechanics have not been considered. A consideration of the lateral line
499 canals as components of the dermatocranial bones, with functional roles (e.g., ventrally directed
500 canals in benthic feeders) or more subtle architectural or constructional roles (e.g., the dorsal SO
501 canal in the frontal bone), is an aspect of the analysis of the integration and modularity in the
502 skull of fishes that deserves more attention.

503

504 **Summary**

505 This study has demonstrated that simple, correlated changes in developmental rates
506 (heterochrony) in the lateral line canals contained within dermal bones (a component of the
507 dermatocranium) and in canal neuromasts (a component of the peripheral nervous system) can
508 explain the evolution of an adaptive phenotype, widened lateral line canals. In particular, it
509 revealed “dissociated heterochrony” among species with narrow vs. widened canals (a
510 combination of peramorphic and paedomorphic shifts), as well as regional (local) heterochrony,
511 differences in rates between canals (and between their respective neuromasts) within individuals.
512 The genetic basis of these changes deserve further study and will need to consider the processes
513 of intramembranous bone ossification and dynamics of hair cell populations in neuromasts. This
514 study also demonstrated that heterochronic change in canal diameter and neuromast morphology
515 can occur without a change in other aspects of lateral line development (e.g., neuromast
516 patterning [canal neuromast number] or the pattern/process of neuromast-centered canal
517 morphogenesis). With reference to the life history of the mouth brooding cichlid fishes used in
518 this study, the divergence in canal phenotype (narrow vs. widened) has already occurred in

519 young larvae, so that by the time they are released from the mother's mouth and exogenous
520 feeding commences, canal diameter and neuromast size already distinguish *Aulonocara*
521 (widened canals) from *Labeotropheus* and *Metriaclima* (narrow canals), which is likely to have
522 interesting implication for the ontogeny of prey detection capabilities. Finally, the ability of the
523 lower jaw to evolve independently of lateral line canal morphology (*Labeotropheus* vs.
524 *Metriaclima*), and the ability of the lateral line canals (and neuromasts) to evolve independently
525 of the lower jaw (*Aulonocara* vs. *Metriaclima*), demand that the canals of the mechanosensory
526 lateral line system become a part of the conversation concerning integration and modularity in
527 the skull of fishes.

528

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539

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- 627

628 **Figure Captions**

629 Fig. 1. Fish age and size for specimens of *Labeotropheus fuelleborni* (n=25), *Metriaclima*
630 *zebra* (n= 8), and *Aulonocara baenschi* (n= 57) used for histological analysis, and for SEM and
631 clearing and staining, as noted.

632

633 Fig. 2. Larval and juvenile *Aulonocara baenschi*. **A:** Yolk sac larvae (pre-flexion) on day of
634 hatch (5.5 mm SL, 5 dpf). **B:** Yolk sac larvae (post-flexion) several days after hatch (5.5 mm
635 SL, 8 dpf). **C:** Older yolk sac larva - cranial lateral line canals have started to develop (7 mm SL,
636 12 dpf). **D:** Juvenile after release from mother's mouth (14 mm SL, 29 dpf).

637

638 Fig. 3. MicroCT images of adults of the three study species. **A:** *Labeotropheus fuelleborni*
639 (~80 mm SL, narrow canals; 1 μm resolution). **B:** *Metriaclima zebra* (~92 mm SL, narrow
640 canals; 18 μm resolution). **C, D:** *Aulonocara baenschi* (86 mm SL, widened canals; 16 μm
641 resolution). **A-C:** 3-D volume rendering in ventral view showing the MD, PO and IO canals.
642 Asterisks (*) indicate the location of the canal neuromasts in the MD canal, which is contained in
643 the dentary and angulo-articular bones (in A and B) and the canal neuromasts in the MD canal as
644 well as in the lower arm of the L-shaped PO canal (in C). **D:** Transverse slice (16 μm thickness)
645 of *Aulonocara baenschi* at the level of the lens of the eye (e), indicating the lumen of the SO
646 canal and the PO, IO and SO canals.

647

648 Fig. 4. Scanning electron microscopic (SEM) images illustrating canals and neuromasts of
649 larval and juvenile *Labeotropheus fuelleborni*. **A:** Yolk sac larva (10 dpf) with very small
650 presumptive canal neuromasts (arrows), and grooves (Stage II) of developing mandibular (MD)

651 and preopercular (PO) canals, n = naris. **B:** Enlargement of PO groove in A indicating oval canal
 652 neuromasts (white arrows) and two neuromasts that will remain superficial (black arrows). **C:**
 653 Early juvenile (17 dpf) with rostral portion of IO canal (in lacrimal bone), SO and PO canals that
 654 are enclosed. The other neuromasts of the IO canal series along the ventral and caudal
 655 boundaries of the orbit are still superficial (arrows). The supraorbital (SO) and preopercular (PO)
 656 canals are already enclosed and have pores. Lower jaw had been removed. **D:** Rostral-most IO
 657 neuromast (at asterisk in C) demonstrating oval shape and narrow sensory strip containing
 658 sensory hair cells each with a long kinocilium and shorter stereocilia (white strands). Double
 659 arrow indicates hair cell orientation. Neuromast is surrounded by squamous epithelial cells with
 660 prominent microvillar ridges. Scale bars in A, C = 500 μm ; B = 100 μm ; D = 10 μm .

661
 662 Fig. 5. Scanning electron microscopic (SEM) images illustrating enclosure of canals and
 663 pores in *Labeotropheus fuelleborni*. **A:** Dorsal view of the head in a late stage larva (12 dpf),
 664 showing grooves of partially enclosed bilateral supraorbital (SO) canals. n=naris. **B:** Ventral
 665 view of mandible in a young juvenile (17 dpf) showing neuromast (left most arrow) and
 666 developing canal. **C:** Dorsal view of head of young juvenile (17 dpf) showing naris (n) and SO
 667 canal pores medial to naris and orbit (arrows). Superficial neuromasts (sn) are visible between
 668 the left and right SO canals. Note double pore at dorsal midline (mp). **D:** Dorsal view of head in
 669 larger juvenile (20 dpf), with labeling as in C. The fusion of the double pore (in C) forms a single
 670 median pore (mp). **E:** Lateral view of enclosed SO PO, and IO canals with single and double
 671 pores (arrows) in a 56 dpf juvenile. **F:** Lateral view of a 70 dpf juvenile in which the double
 672 pores in E (arrows) have fused to form smaller, single pores. Scale bars in A = 300 μm ; B = 150
 673 μm ; C = 250 μm ; D, E = 500 μm ; F = 600 μm .

674

675 Fig. 6. Development of individual canal segments at the level of canal neuromasts in the MD
676 canal in two species with narrow and widened lateral line canals. See text for more explanation.
677 **A-E:** *Labeotropheus fuelleborni* (narrow canals, 17-70 dpf), **F-J:** *Aulonocara baenschi* (widened
678 canals, 17-47 dpf). **A, F:** Stage I - neuromasts sits flush with epithelium, b, g) Stage IIa -
679 neuromast sits in epithelial depression. **C, H:** - Stage IIb - neuromast sits in epithelial groove
680 between ossified canal walls (pink = ossified bone). **D, I:** - Stage III - neuromast enclosed by soft
681 tissue, E, J) - Stage IV - neuromast enclosed in ossified canal segment. m -Meckel's cartilage
682 (turquoise) in A and other images. Arrows point to center of hair cell population in each
683 neuromast. Note the much larger canal diameter in J (*Aulonocara*, 47 dpf) when compared to E
684 (*Labeotropheus*, 70 dpf). Scale for all images (as in J) = 100 μm .

685

686 Fig. 7. Rates of increase in **A:** canal diameter, **B:** neuromast length, **C:** neuromast width
687 relative to fish size (SL) in *Labeotropheus fuelleborni* and *Metriaclima zebra* (both with narrow
688 canals) and *Aulonocara baenschi* (widened canals) derived from histological material (raw data,
689 not log transformed is illustrated). Each data point is the mean of left and right for each
690 neuromast or canal diameter, and data for SO and MD canals are combined. See Tables 2 and 3
691 for statistical analyses of log transformed data (where warranted).

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TABLE 1. Mean fish size and minimum canal diameter at which individual canal segments in the supraorbital (SO) and mandibular (MD) canals are enclosed (Stage III) and ossified (Stage IV) derived from histological material. Ascending values of mean fish size at enclosure and ossification among segments within a canal series are used to infer the order of canal enclosure and ossification within that canal (see text for additional details).

Species	NM	Enclosure (Stage III)		Ossification (Stage IV)	
		Mean Fish Size (mm SL)	Min. Canal Diameter (μm)	Mean Fish Size (mm SL)	Min. Canal Diameter (μm)
<i>Labeotropheus fuelleborni</i>	SO1	11.8	78.4	19.0	99.1
	SO2	10.9	79.9	14.8	119.4
	SO3	10.3	106.8	14.2	77.5
	SO4	8.5	103.3	13.9	81.1
	SO5	12.0	66.8	14.2	86.5
	MD1	11.6	98.6	16.3	82.8
	MD2	10.9	36.7*	15.5	102.9
	MD3	12.0	93.9	16.0	109.4
	MD4	11.5	109.7	16.7	94.3
	MD5	11.8	93.0	19.0	97.2
<i>Metriaclima zebra</i>	SO1	13.2	70.4	19.2	83.9
	SO2	12.3	102.0	21.0	176.1
	SO3	11.8	69.1	21.1	182.7
	SO4	9.3	70.1	16.9	89.7
	SO5	11.5	113.1	16.9	86.7
	MD1	13.7	89.1	17.0	108.4
	MD2	12.0	85.9	21.0	169.9
	MD3	10.8	97.3	20.7	113.6
	MD4	11.3	124.8	20.7	122.7
	MD5	11.4	121.3	20.7	161.3
<i>Aulonocara baenschi</i>	SO1	16.1	125.9	19.0	98.2
	SO2	14.8	130.3	19.9	249.4
	SO3	13.7	111.5	18.5	183.2
	SO4	9.0	67.1	16.8	93.7
	SO5	9.3	93.0	17.5	154.8
	MD1	16.2	134.7	21.7	194.0
	MD2	16.9	115.5	15.7	166.0
	MD3	10.5	118.8	18.2	136.3
	MD4	13.7	131.1	19.9	157.4
	MD5	13.5	128.8	20.2	160.5

* obvious outlier

TABLE 2. Results of ANCOVA's for mean (left/right) canal diameter, neuromast length and neuromast width (μm) for both SO and MD canals (combined) in the three study species. All data was log transformed to achieve normality. SL = Standard length (fish size) in mm. Significance = $P < 0.05$. See Table 3 for ANOVA results. If the interaction term for the ANCOVA was significant (indicating heterogeneity of slopes), the Johnson-Neyman technique was used to determine the region of non-significance for fish size (SL). See text for additional details.

	<i>N</i>	<i>R</i> ²	<i>F</i>	<i>d.f.</i>	<i>P</i> -value
Canal Diameter	280	0.77			
Species			104.0364	2, 274	<0.0001
SL			360.3079	1, 274	<0.0001
Species x SL			17.9530	2, 274	<0.0001
Neuromast Length	312	0.70			
Species			59.6912	2, 306	<0.0001
SL			221.3197	1, 306	<0.0001
Species x SL			46.1167	2, 306	<0.0001
Neuromast Width	312	0.75			
Species			81.7744	2, 306	<0.0001
SL			356.7013	1, 306	<0.0001
Species x SL			24.2879	2, 306	<0.0001

TABLE 3. Results of ANOVA showing ontogenetic trends for mean (left/right) canal diameter, neuromast length and neuromast width (μm) of SO and MD canals combined in the three study species. All data was log transformed to achieve normality. SL = standard length (fish size) in mm. See Table 2 for results of ANCOVA for these data. Significance level = $P < 0.05$.

	<i>N</i>	Regression	R^2	<i>P</i>
Canal Diameter				
<i>Labeotropheus</i>	77	$\log Y = 1.759 + 0.022 * SL$	0.36	<0.0001
<i>Metriaclima</i>	80	$\log Y = 1.858 + 0.018 * SL$	0.53	<0.0001
<i>Aulonocara</i>	123	$\log Y = 1.781 + 0.033 * SL$	0.80	<0.0001
Neuromast Length				
<i>Labeotropheus</i>	77	$\log Y = 1.718 + 0.016 * SL$	0.19	<0.0001
<i>Metriaclima</i>	85	$\log Y = 1.623 + 0.015 * SL$	0.27	<0.0001
<i>Aulonocara</i>	150	$\log Y = 1.425 + 0.043 * SL$	0.80	<0.0001
Neuromast Width				
<i>Labeotropheus</i>	77	$\log Y = 1.574 + 0.021 * SL$	0.34	<0.0001
<i>Metriaclima</i>	85	$\log Y = 1.497 + 0.029 * SL$	0.59	<0.0001
<i>Aulonocara</i>	150	$\log Y = 1.427 + 0.045 * SL$	0.78	<0.0001

TABLE 4. Results of the ANCOVA for comparison of canal diameter, neuromast length and neuromast width (μm) in the supraorbital (SO) and mandibular (MD) canals in each of the three study species. Data was log transformed where appropriate to achieve normality. SL (Standard Length) = fish size in mm. Significance level = $P < 0.05$. See Table 5 for ANOVA results. If the interaction term was significant (indicating heterogeneity of slopes), the Johnson-Neyman technique was used to determine the region of non-significance for fish size (SL). See text for additional details.

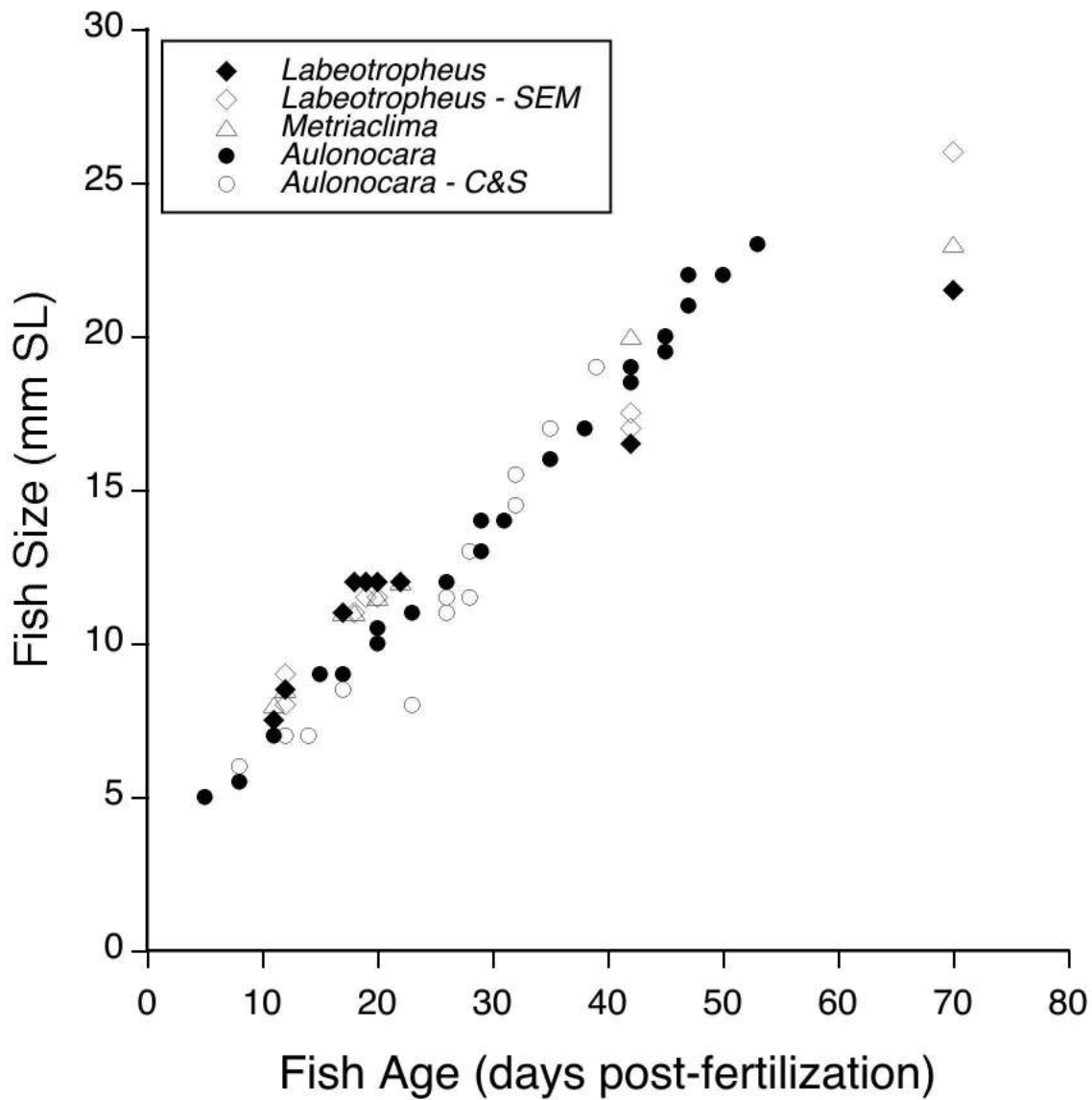
	<i>N</i>	<i>R</i> ²	<i>F</i>	<i>d.f.</i>	<i>P</i> -value
<i>Labeotropheus</i>					
Canal Diameter	77	0.403			
SL			45.3922	1,73	<0.0001
Canal			5.2392	1,73	0.0250
SL x Canal			0.0406	1,73	0.8408
Neuromast Length	77	0.407			
SL			25.8146	1,73	<0.0001
Canal			24.2579	1,73	<0.0001
SL x Canal			1.4813	1,73	0.2275
Neuromast Width	77	0.558			
SL			61.6011	1,73	<0.0001
Canal			26.4280	1,73	<0.0001
SL x Canal			5.3971	1,73	0.0230
<i>Metriaclima</i>					
Canal Diameter	80	0.575			
SL			94.7149	1,76	<0.0001

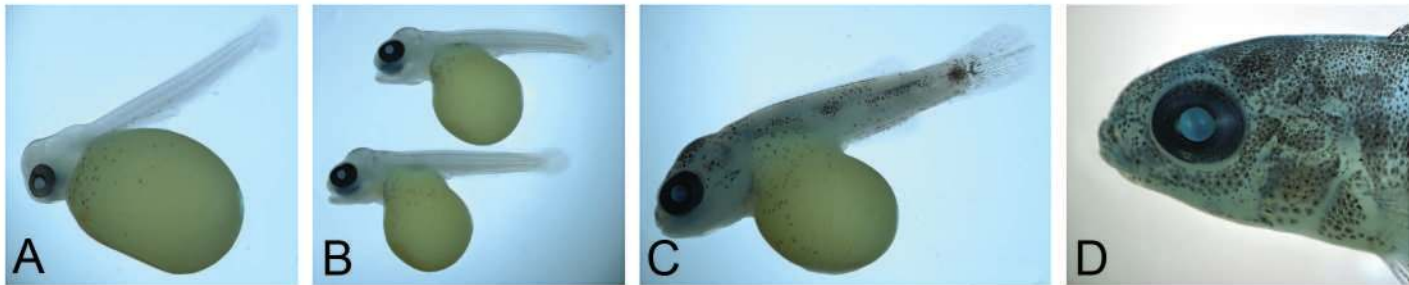
Canal			3.6308	1,76	0.0605
SL x Canal			4.6918	1,76	0.0334
Neuromast Length	85	0.302			
SL			32.039	1,81	<0.0001
Canal			2.8567	1,81	0.0948
SL x Canal			0.3114	1,81	0.5784
Neuromast Width	85	0.639			
SL			129.9772	1,81	<0.0001
Canal			0.7738	1,81	0.3816
SL x Canal			10.7873	1,81	0.0015
<i>Aulonocara</i>					
Canal Diameter	123	0.851			
SL			643.0620	1,119	<0.0001
Canal			5.8674	1,119	0.0169
SL x Canal			8.4776	1,119	0.0043
Neuromast Length	150	0.868			
SL			946.9223	1,146	<0.0001
Canal			9.2902	1,146	0.0027
SL x Canal			6.3914	1,146	0.0125
Neuromast Width	150	0.826			
SL			668.7916	1,146	<0.0001
Canal			19.1464	1,146	<0.0001
SL x Canal			4.4107	1,146	0.0374

TABLE 5. ANOVAs showing ontogenetic trends for mean (left/right) measurements of canal diameter, neuromast length and neuromast width in the supraorbital (SO) versus the mandibular (MD) canals of each of the three study species. Data was log transformed where necessary to achieve normality. See Table 4 for results of ANCOVA for these data. SL = standard length (fish size) in mm. Significance level = $P < 0.05$.

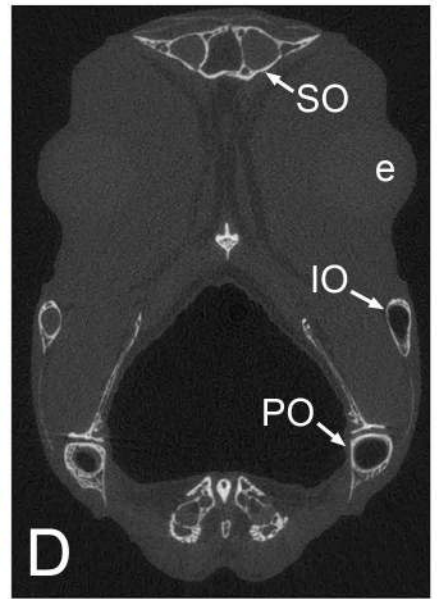
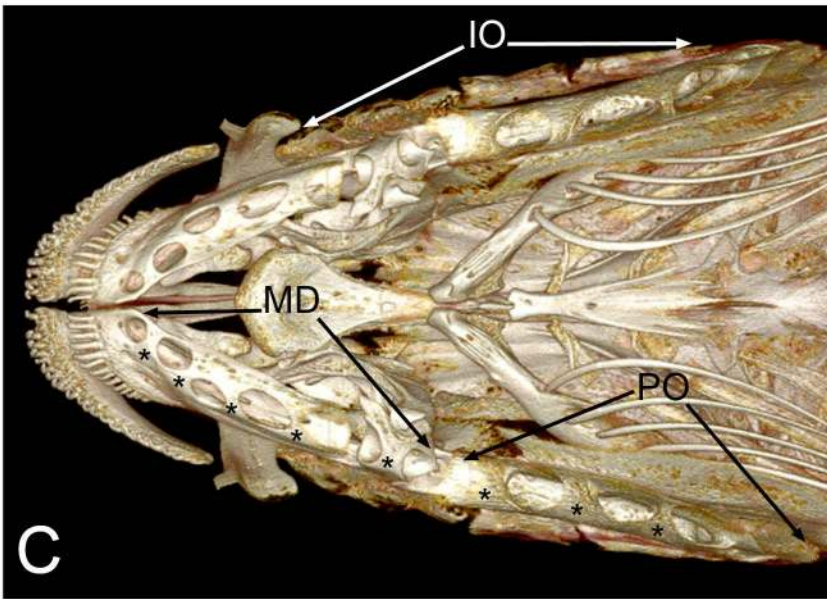
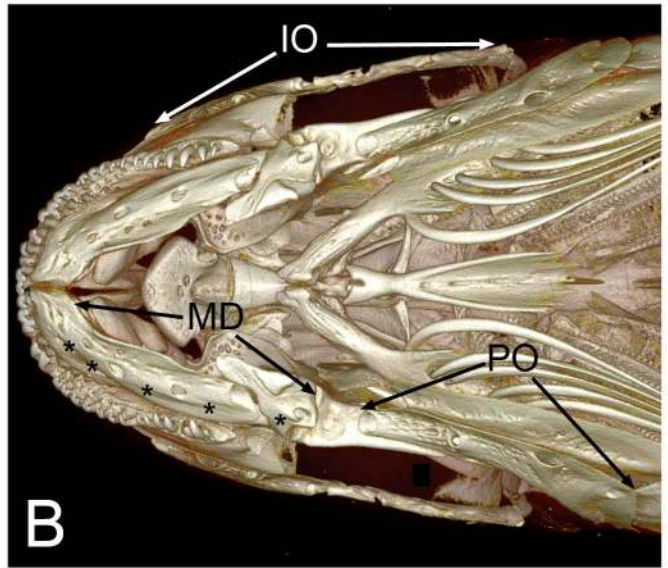
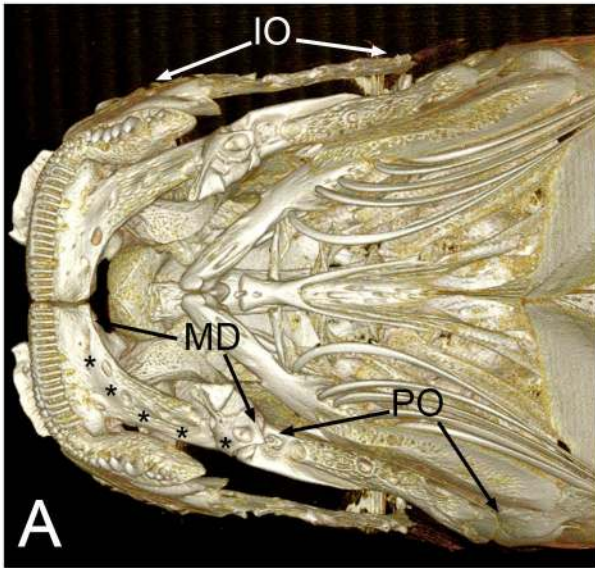
		<i>N</i>	Regression	R²	P
<i>Labeotropheus</i>					
Canal Diameter	SO	40	$\log Y = 1.790 + 0.022 * SL$	0.537	<0.0001
	MD	37	$\log Y = 1.715 + 0.023 * SL$	0.297	<0.0005
Neuromast Length	SO	40	$Y = 43.766 + 4.294 * SL$	0.281	<0.0004
	MD	37	$Y = 41.119 + 2.635 * SL$	0.274	<0.0009
Neuromast Width	SO	40	$Y = 19.519 + 4.754 * SL$	0.524	<0.0001
	MD	37	$Y = 30.783 + 2.582 * SL$	0.387	<0.0001
<i>Metriaclima</i>					
Canal Diameter	SO	39	$\log Y = 1.78 + 0.021 * SL$	0.583	<0.0001
	MD	41	$\log Y = 1.93 + 0.014 * SL$	0.530	<0.0001
Neuromast Length	SO	43	$\log Y = 1.624 + 0.017 * SL$	0.297	<0.0002
	MD	42	$\log Y = 1.620 + 0.014 * SL$	0.272	<0.0004
Neuromast Width	SO	43	$\log Y = 1.396 + 0.037 * SL$	0.776	<0.0001
	MD	42	$\log Y = 1.605 + 0.020 * SL$	0.395	<0.0001
<i>Aulonocara</i>					

Canal Diameter	SO	65	$Y = -29.894 + 15.846 * SL$	0.841	<0.0001
	MD	58	$Y = 8.986 + 12.582 * SL$	0.875	<0.0001
Neuromast Length	SO	75	$Y = -26.736 + 11.026 * SL$	0.922	<0.0001
	MD	75	$Y = -13.939 + 9.352 * SL$	0.801	<0.0001
Neuromast Width	SO	75	$Y = -30.647 + 12.927 * SL$	0.833	<0.0001
	MD	75	$Y = -15.264 + 10.984 * SL$	0.807	<0.0001

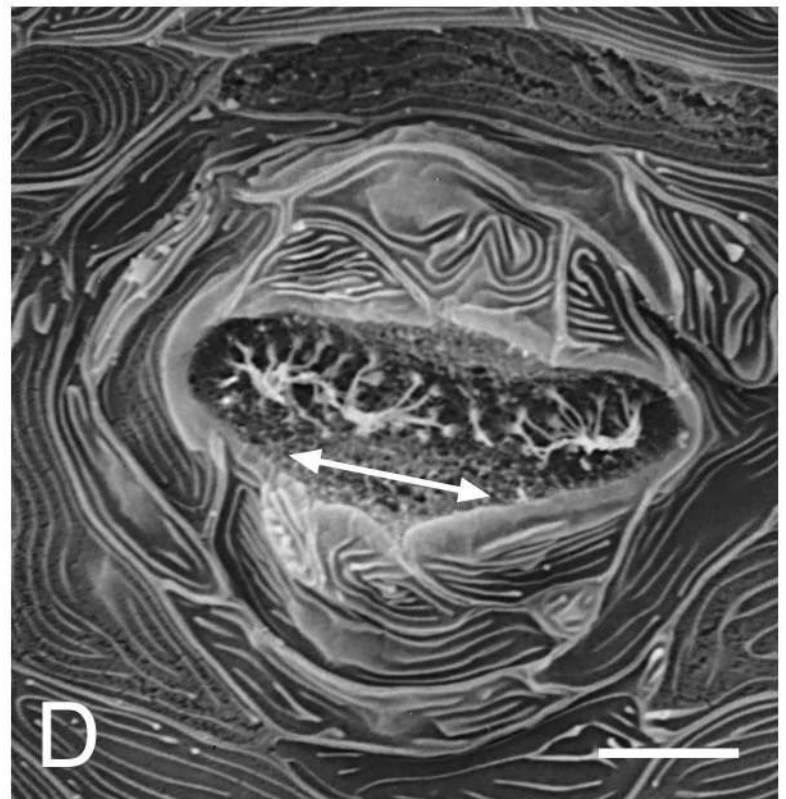
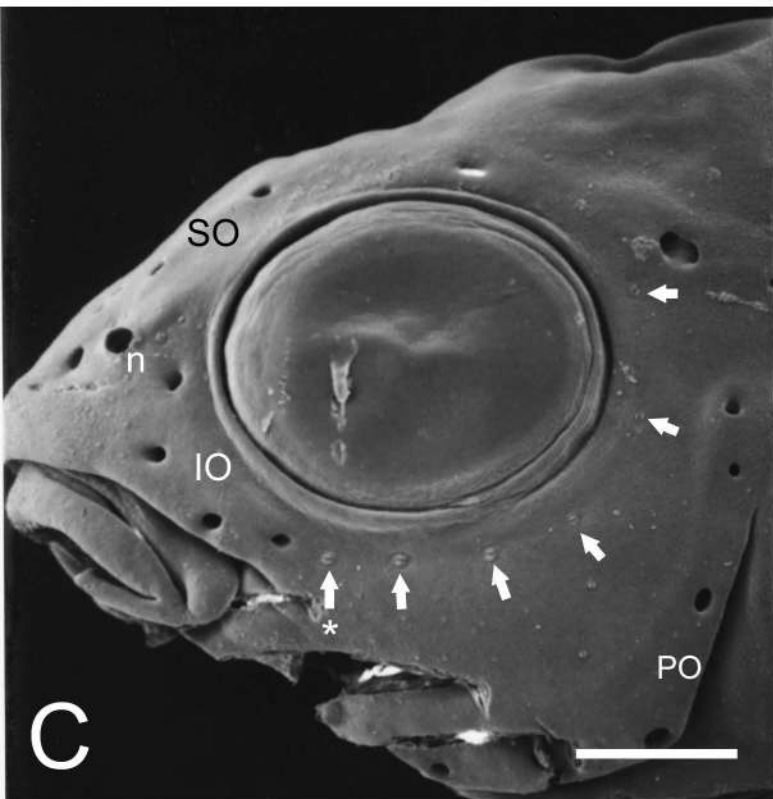
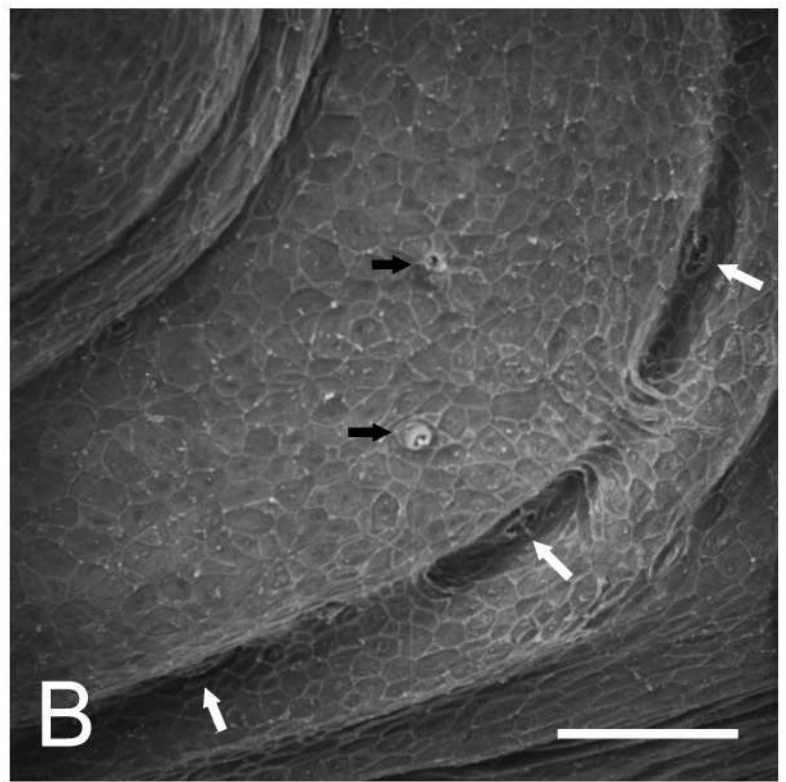
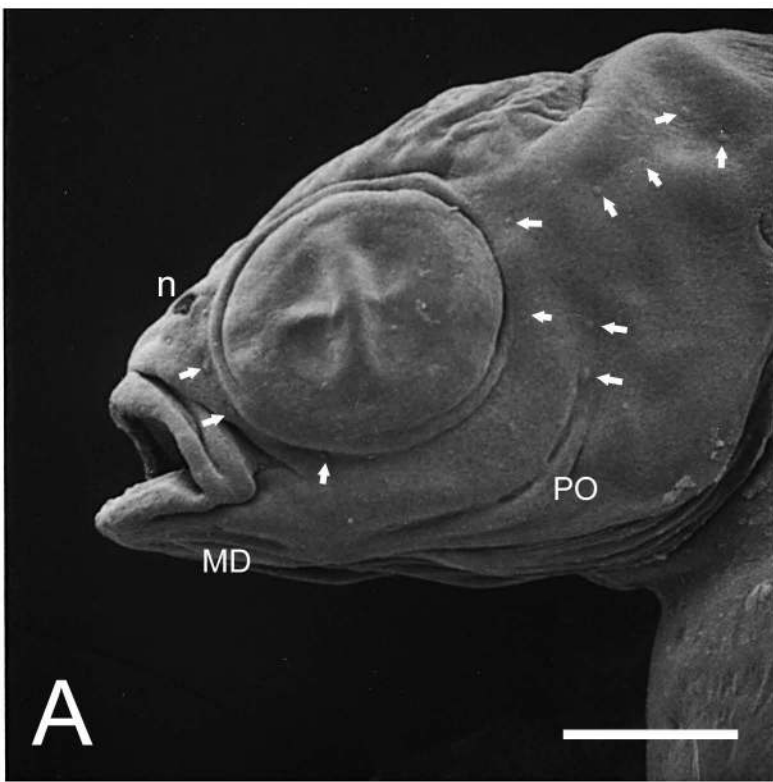




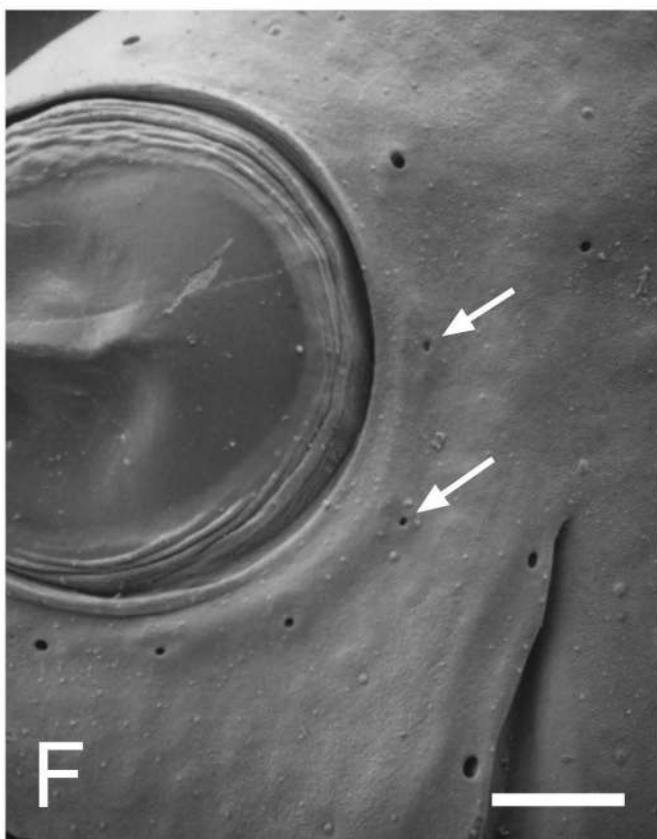
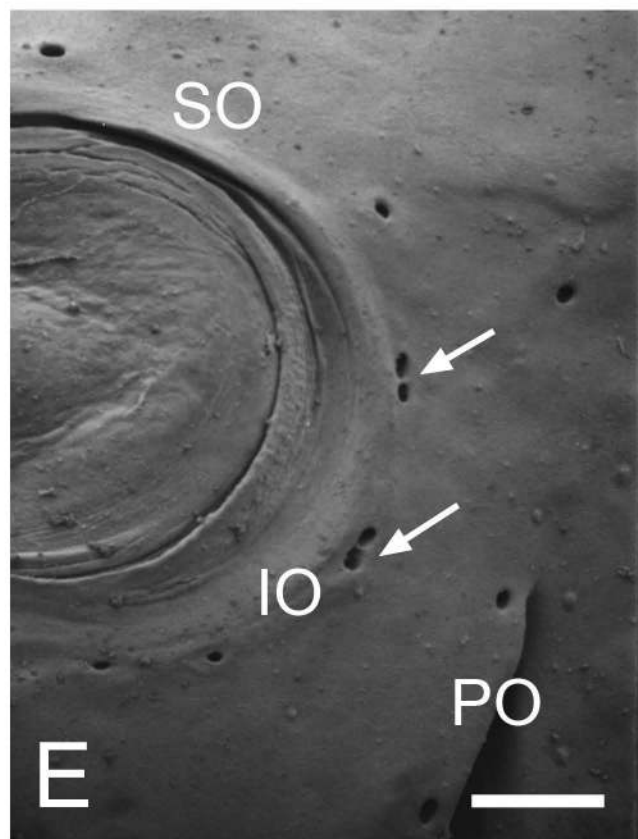
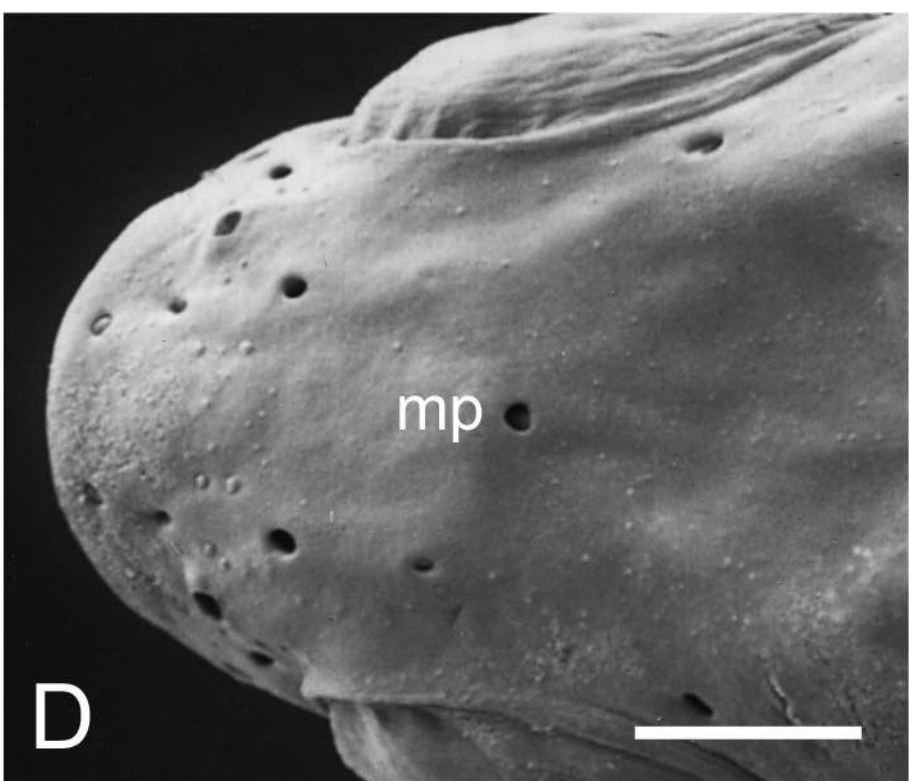
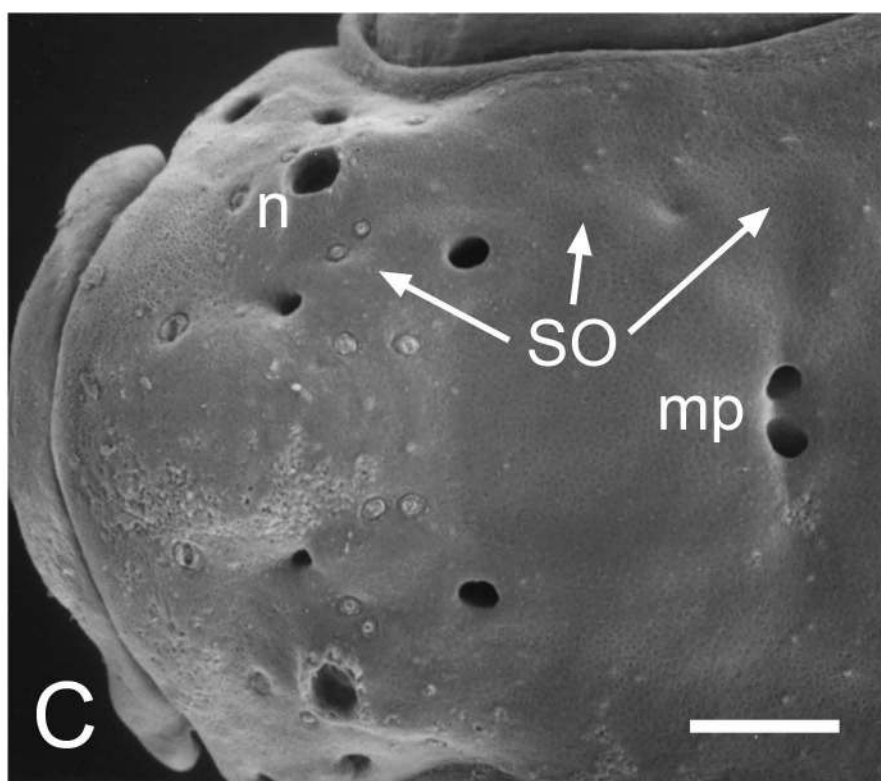
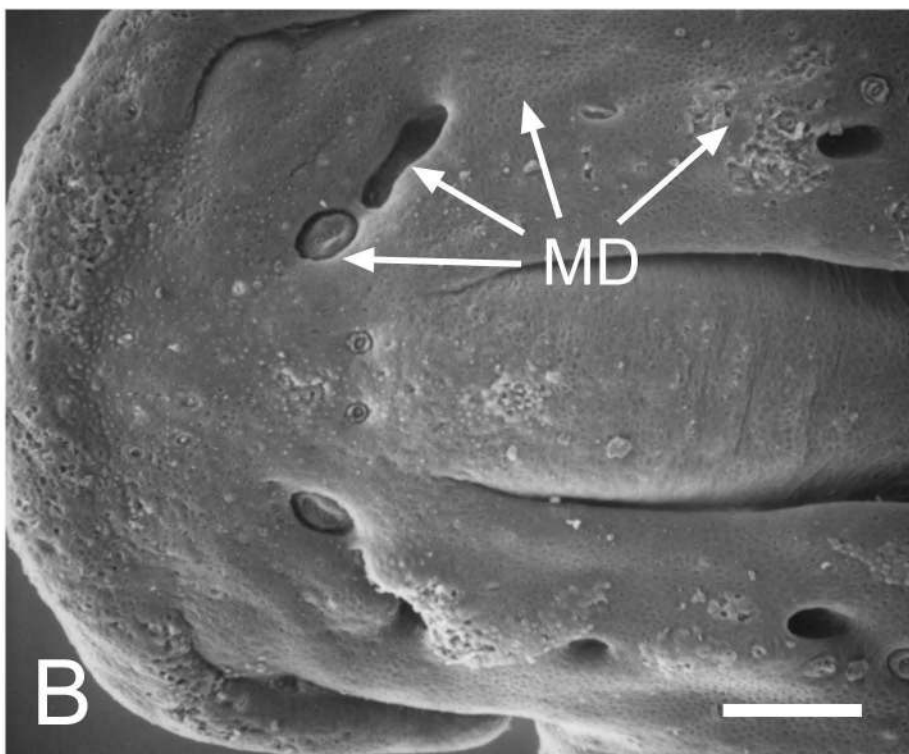
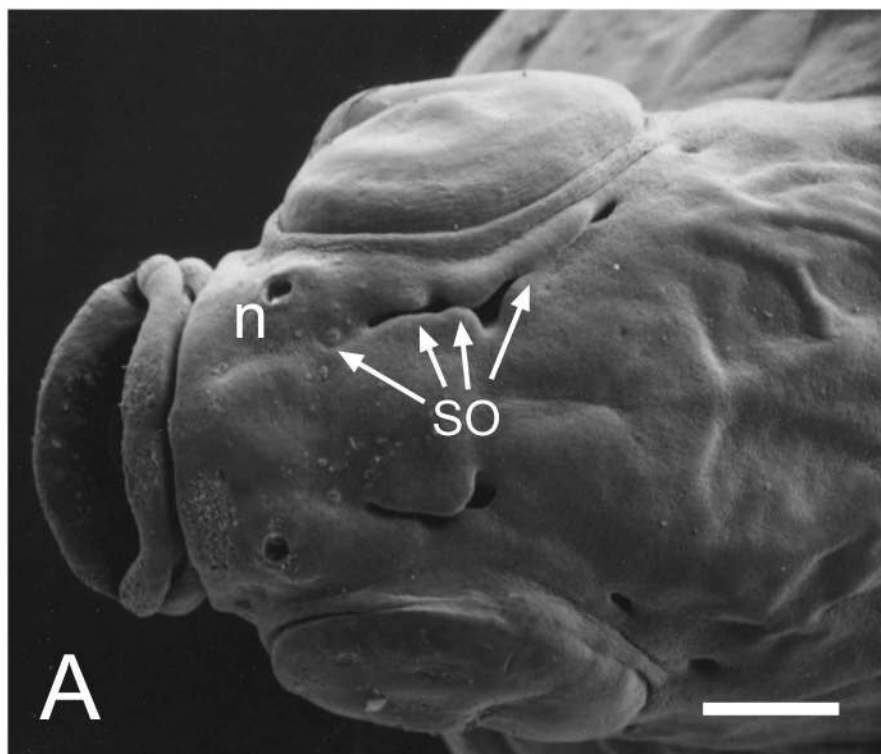
Webb et al., Figure 2.

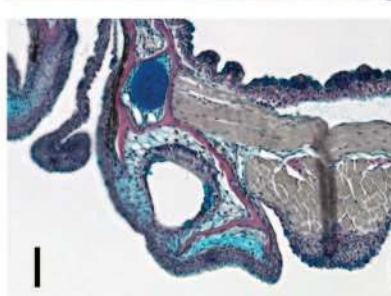
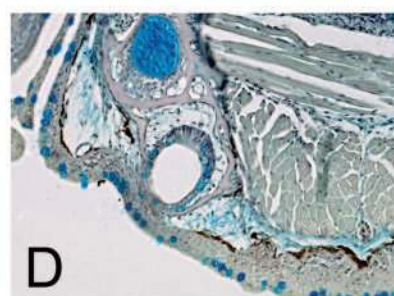
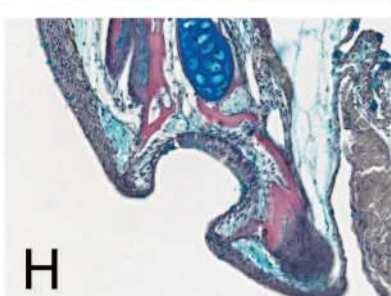
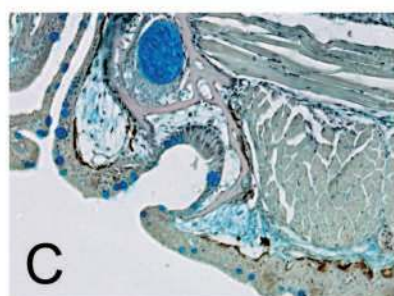
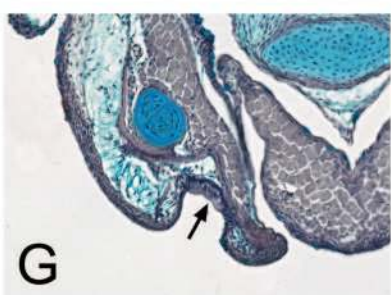
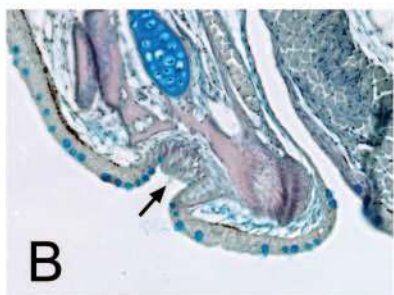
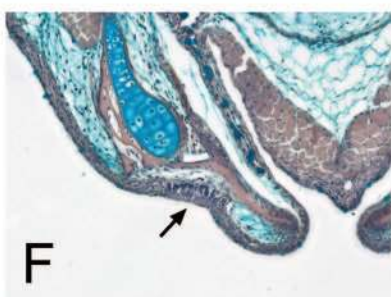
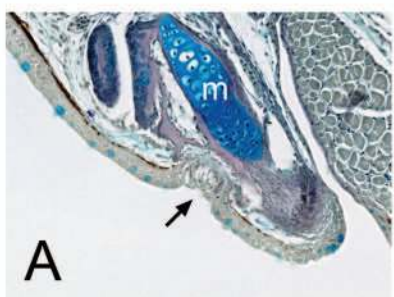


Webb et al., Figure 3



Webb et al., Figure 4





Webb et al., Figure 6

