1   **Evolution of median fin patterning and modularity in living and fossil osteichthyans**

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3   **Short title:** Median fin patterning in osteichthyans

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Abstract

Morphological and developmental similarities, and interactions among developing structures are interpreted as evidences of modularity. Such similarities exist between the dorsal and anal fins of living actinopterygians: (1) both fins differentiate in the same direction [dorsal and anal fin patterning module (DAFPM)], and (2) radials and lepidotrichia differentiate in the same direction [endoskeleton and exoskeleton module (EEM)]. To infer the evolution of these common developmental patternings among osteichthyans, we address (1) the complete description and quantification of the DAFPM and EEM in a living actinopterygian (the rainbow trout *Oncorhynchus mykiss*) and (2) the presence of these modules in fossil osteichthyans (coelacanths, lungfishes, porolepiforms and 'osteolepiforms'). In *Oncorhynchus*, sequences of skeletal elements are determined based on (1) apparition (radials and lepidotrichia), (2) chondrification (radials), (3) ossification (radials and lepidotrichia), and (4) segmentation plus bifurcation (lepidotrichia). Correlations are then explored between sequences. In fossil osteichthyans, sequences are determined based on (1) ossification (radials and lepidotrichia), (2) segmentation, and (3) bifurcation of lepidotrichia. Segmentation and bifurcation patterns were found crucial for comparisons between living and extinct taxa. Our data suggest that the EEM is plesiomorphic at least for actinopterygians, and the DAFPM is plesiomorphic for osteichthyans, with homoplastic dissociation. Finally, recurrent patterns suggest the presence of a Lepidotrichia Patterning Module (LPM).

Introduction

42

43   In the past two decades, the median fins [i.e., dorsal, anal, and caudal fins] of fishes

44   have been the focus of an overwhelming body of research in evolutionary developmental

45   biology. Primary interest for these so-called unpaired fins lies in their locomotor functions

46   [1–3], ecological implications [4,5], comparative morpho-anatomy [6], molecular [7] and

47   developmental [8] patterning, as well as morphological disparity [9]. Indeed, among

48   median fins, the dorsal and anal fins of piscine osteichthyans show a great morphological

49   disparity, reflecting the evolvability of this system [9–11].

50   Osteichthyans primitively display two dorsal fins and a single anal fin [12].

51   Independently and repeatedly in actinopterygians and sarcopterygians, the number of dorsal

52   fins is reduced by loss or fusion with the caudal fin [9,11,13]. The loss of the anal fin is less

53   frequent in piscine osteichthyans, although loss or fusion with the caudal fin occurs in some

54   teleosts and dipnoans [11,14]. Among 'elpistostegalians', the extinct transitional taxa

55   between fishes and tetrapods, the condition is poorly documented (e.g., *Panderichthys,*

56   *Tiktaalik*). However, *Elpistostege*, considered a basal tetrapod, has lost the dorsal fin while

57   an anal fin is present [15]. And finally, the absence of both the dorsal and anal fins is

58   considered a synapomorphy shared by aquatic and terrestrial tetrapods [14] with the

59   exception of *Elpistostege* [15]. Even with such morphological disparity, the structure and

60   development of the median fins is expected to be broadly similar among osteichthyans

61   because these fins have similar constituents.

62   In a forerunner comparative study, Mabee et al. [16] revealed the recurrence of

63   similar developmental patterning (i.e., sequences and direction of development among

3

64   endoskeletal and exoskeletal elements) in the dorsal and anal fins among living

65   actinopterygians, which they interpreted as evidence of modularity. They found out that the

66   similar patterning of these fins might be indicative of two modules: (1) the Dorsal and Anal

67   Fin Patterning Module (DAFPM), where the skeletal elements (radial bones and

68   lepidotrichia) of both fins differentiate in the same direction and (2) the Endoskeleton and

69   Exoskeleton Module (EEM), where the directions of development of the endoskeleton

70   (radials) and exoskeleton (lepidotrichia) are similar. The DAFPM and EEM are considered

71   to be maintained during actinopterygian phylogeny [16] but a phylogenetic inference across

72   osteichthyans was not possible owing to the absence of comparative data on early

73   actinopterygians and sarcopterygians. A broad phylogenetic sampling of actinopterygians

74   and sarcopterygians is thus necessary to document the patterns of developmental similarity

75   between dorsal and anal fins throughout osteichthyan evolution. However, in order to

76   validate the prospective existence and distribution of median fin modules among

77   osteichthyans (i.e., specifically the DAFPM and EEM *sensu* [16]), it is mandatory to

78   include extant as well as extinct taxa within a comparative framework.

79   The rarity of fossilized ontogenies [17] and a bias toward the preservation of hard

80   tissues (bones *versus* cartilages) limit our assessment of early developmental patterns in

81   extinct taxa. However, the presence of fossilized individual ontogenies (i.e., anatomical

82   structures found in adults that have recorded individual developmental patterns), can

83   potentially broaden the phylogenetic sampling. The complex structure of osteichthyan fin

84   rays (e.g., [18,19]) provides such developmental data because fin rays are accretional

85   structures for which structural elements are added without substantial remodelling, thus

86   allowing the preservation of early developmental patterning.

87   The main objectives of this study are (1) to provide a complete description of the

88   patterns of developmental similarity of the dorsal and anal fins in a living actinopterygian,

89   the rainbow trout (*Oncorhynchus mykiss*), (2) to describe the dorsal and anal fins patterning

90   in fossil osteichthyans, and (3) to compare the similarity of the developmental patterns in

91   order to discuss the prospective existence of the DAFPM and EEM modules within

92   osteichthyans. Patterns of developmental similarity were investigated by using relative

93   developmental sequences and direction of development among endoskeletal and

94   exoskeletal elements of the dorsal and anal fins. We expect developmental sequences to be

95   (1) significantly congruent between dorsal and anal fins (i.e., indicative of DAFPM), and

96   (2) significantly congruent between the endoskeleton and exoskeleton within each fin (i.e.,

97   indicative of EEM).

98

99   Nomenclature

100   In order to facilitate comparisons during the description of fin morphologies and its

101   constituents, a brief review of the main elements composing the median fins of

102   osteichthyans is presented here. We will detail the different types of fins rays encountered

103   in osteichthyans (i.e., lepidotrichia and actinotrichia) and the events used to define the

104   median fin developmental patterning.

105   Lepidotrichia

106   Lepidotrichia are osseous fin rays of dermal origin. Each lepidotrichium is

107   composed of two parallel and symmetrical elements called hemirays. Lepidotrichia are

108   usually segmented (i.e., "jointed" lepidotrichia). The "joints" correspond to very narrow,

109   non-mineralized spaces between adjacent segments connected by collagenous ligaments

110   called Sharpey's fibres. Adjoining segments are sequentially added distally during growth

111   [20] before the ossification of the ray, which begins proximally. The most proximal

112   segment is always longer than the others from the very first stages of development. This

113   pattern has been observed in many living and fossil species (e.g., the actinopterygians

114   *Gobius*, *Pygosleus*, *Cottus*, and *Blennius*, among others, and the sarcopterygian *Miguashaia*

115   and *Eusthenopteron* [21–23]. The lepidotrichia articulate with the most distal endoskeletal

116   elements (e.g., radials or pterygiophores; or the phalanges as in *Elpistostege* [15]) in the

117   paired and median fins. The hemirays are contralaterally arranged on both sides of the

118   endoskeleton. Usually, each radial bone carries more than one lepidotrichium, however

119   certain derived taxa (e.g., teleosts and coelacanths) show a 1:1 ratio between the radials and

120   the fin rays in the median fins. In their most distal portion, the lepidotrichia usually

121   bifurcate (i.e., branched lepidotrichia) as if the ray was split in two. Numerous episodes (or

122   orders) of bifurcation can occur in a single ray. Lepidotrichia represent a synapomorphy of

123   crown osteichthyans [24]. Extant dipnoans (i.e., *Neoceratodus*, *Lepidosiren* and

124   *Protopterus*) display a unique kind of partially ossified lepidotrichia termed camptotrichia

125   [25].

126   **Actinotrichia**

6

127   Actinotrichia are flexible fin rays formed by long fibres of collagen known as

128 elastoidine [19]. The actinotrichia form the main support of the osteichthyan fins in larval

129 and juvenile stages of the ontogeny and are found in the most distal part of the adult fins

130 arranged in contralateral palisades. During the formation of the lepidotrichia, actinotrichia

131 are progressively resorbed, both within hemirays and between lepidotrichia, leaving only a

132 narrow distal fringe. The formation of actinotrichia is followed by the apparition and

133 development of the endoskeletal elements (e.g., radials), and the formation of the

134 lepidotrichia [16,26–28]. Mesenchymal cells (osteoblasts) may then use the actinotrichia as

135 a scaffold during the initial stages of formation of the lepidotrichia [19,26,29].

136 **Median fin patterning**

137   Median fin patterning can be defined in terms of a series of events rather than solely

138 on formation (or differentiation, *sensu* [16]). An event defines a unit of transformation with

139 concomitant phenotypic changes (e.g., lepidotrichia ossification) and an event may have

140 different properties (e.g., onset, offset, duration) [30]. The chronological order of events

141 corresponds to a sequence. Fins being composed of different elements, we referred to a

142 developmental sequence when comparing the same developmental state (e.g., apparition,

143 chondrification, ossification) among elements. We referred to an ontogenetic sequence

144 when comparing different developmental states for a single element [31].

145   Of the numerous developmental events associated with fin formation in living

146 actinopterygians [8,28,32–38], our study focused on 11 skeletogenic events: (1) apparition

147 (i.e., collagenous matrix precursor) of actinotrichia, (2) apparition (i.e., mesenchymal

148 condensation) of proximal radials, (3) chondrification of proximal radials, (4) apparition of

149 lepidotrichia, (5) apparition (i.e., mesenchymal condensation) of distal radials, (6)

150 chondrification of distal radials, (7) segmentation of lepidotrichia, (8) ossification of

151 lepidotrichia, (9) bifurcation of lepidotrichia, (10) ossification of proximal radials, and (11)

152 ossification of distal radials. The establishment of these developmental events is a powerful

153 tool that could be used to uncover patterns of developmental similarity and shed light on

154 patterns of modularity across osteichthyan evolution.

155

Material and methods

Living material

158   Developmental sequences of the dorsal and anal fins were obtained from embryo-

159 juvenile specimens of the rainbow trout (*Oncorhynchus mykiss*) ranging from 5 days pre-

160 hatching to 100 days post-hatching (dph). Alevins-juveniles were reared in swimming

161 channels under constant water velocity (0.4 cm/s) in 2005 [see [39] for rearing conditions].

162 Specimens were sampled every day up to 34 dph, every other day from 34 to 80 dph, and

163 every four days up to 100 dph. Samples were fixed in neutral buffered formalin for 48h,

164 and then preserved in 70% ethanol. One specimen for each sampling day, plus one or two

165 replicates for specimens between 0 to 24 dph, were cleared-and-double stained with

166 Alizarin red S for bones and Alcian blue for cartilages [40]. Replicates were used to palliate

167 with staining problems [41]. Pre-hatching specimens were removed from their egg capsule

168 prior to clearing and Alcian blue staining. Digital pictures were taken before staining and 5-

169 10 days after staining to avoid interpretive errors owing to destaining. In total, eighty

170   specimens were used to reconstruct developmental sequences of serial skeletal elements

171   from the dorsal and anal fins (Figs 1 and 2) for all the events associated with fin

172   development. All specimens were reared and used for a previous experiment [39], for

173   which protocols were approved by the Université du Québec à Rimouski's animal care and

174   use committee.

175

176   **Fig 1. Details of the dorsal and anal fins of the rainbow trout (*Oncorhynchus mykiss*)**

177   **based on a 31.33 mm long juvenile specimen.** The main morphological features of these

178   fins are identified. The serial elements, the proximal radials (PR), the distal radials (DR)

179   and the lepidotrichia (L), are numbered from the anterior to the posterior of the fins. Act,

180   actinotrichia; an.f, anal fin; bif, bifurcation; caud.f, caudal fin; DR, distal radial; dors.f,

181   dorsal fin; L, lepidotrichia; PR, proximal radial; pect.f, pectoral fin; pelv.f, pelvic fin; seg,

182   segmentation.

183

184   **Fig 2. Cleared and stained specimens of the rainbow trout (*Oncorhynchus mykiss*).** The

185   cartilage is stained in red and the cartilages in blue. A: Dorsal fin showing the beginning of

186   lepidotrichia ossification (specimen SL = 28.36 mm); B: Anal fin showing the beginning of

187   lepidotrichia ossification (specimen SL = 19.80 mm); C: Dorsal fin showing the beginning

188   of radial ossification (specimen SL = 24.90 mm); D: Anal fin showing the beginning of radial

189   ossification (specimen SL = 24.90 mm). Arrows point anteriorly. L, lepidotrichia; PR,

190   proximal radial; seg, segmentation.

191

192 Observations were made under a Leica MZ16A binocular mounted with a digital

193 camera. Standard length (SL) was measured prior to staining with Northern Eclipse

194 Software (Version 6.0). Since SL and dph are highly correlated ($r^2 = 0.952$; $P < 0.001$) and

195 SL is recognized as a better proxy for morphological development in fishes [42], SL was

196 used for all statistical analyses.

197 Event coding was based on colour uptake by skeletal elements. In this study, three

198 states are recognized for radials: (1) present (mesenchymal cell condensations without stain

199 uptake), (2) cartilaginous (blue), and (3) ossified (red). Four developmental states are

200 recognized for lepidotrichia: (1) present (collagenous matrix), (2) ossified (red), (3)

201 segmented (number of segments per lepidotrichium), and (4) bifurcated (position of the

202 bifurcation along the lepidotrichia). Surveyed specimens with their size (SL) and event

203 coding of the skeletal elements are listed in S1 File.

204 To manage with the inter-individual variation in the number of radials and

205 lepidotrichia, positional homologies and numbering of elements were inferred *a posteriori*

206 by lining up all specimens with the third radial (variation being more important in

207 peripheral areas) and by comparing similarities among sequences of similar-sized

208 specimens. Myomere counts (from cranial to caudal) were used in the earliest stages as a

209 topographical criterion to identify the first proximal radials to differentiate. The dorsal and

210 anal fins are positioned at the level of myomeres 21-32 and 40-50, respectively.

211 Logistic regressions were used to estimate the SL at which 50% (SL_{50}) of the

212 specimens have reached a given developmental state (i.e., present, cartilaginous, ossified,

213 segmented and bifurcated) for each skeletal element (see [30] for further details).

214   Significance of the logistic regressions were tested using the Likelihood ratio statistic [43].

215   To interpret a regression for a given element, the significance level was calculated using the

216   Bonferroni correction; the collective significance level of 0.05 was divided by the number

217   of elements to get the nominal significance level for each regression. Statistical analyses

218   were performed with R Studio for Windows v. 1.3.1093 (library: MASS R; [44]).

219   In order to investigate the median fins patterning and developmental similarity, the

220   SL_{50} values of each skeletal element (i.e., derived from the logistic regressions) were used

221   to order the serial elements in relative developmental sequences within a fin. The relative

222   order of a skeletal element within a developmental sequence was then converted by

223   attributing a rank value. Spearman rank correlation coefficients were then used to describe

224   the relations between the developmental sequences in the dorsal and anal fins, and in the

225   endoskeleton and exoskeleton (see [30,45] for the detailed procedure). Only the elements

226   for which the logistic model was significant (under the nominal significance level) were

227   considered for Spearman correlations. Logistic regressions do not produce SL_{50} when the

228   elements are present in all specimens. The actual sizes of the smallest specimens were

229   included in the Spearman correlations involving the apparition of the proximal radials in

230   the dorsal and anal fins, since few skeletal elements were already present in these

231   specimens. These already present proximal radials are ranked as the first appeared in the

232   developmental sequence for the Spearman correlations.

233   **Fossil material**

234   The phylogenetic sampling includes six Palaeozoic (Devonian-Carboniferous)

235   species of osteichthyans comprising a 'palaeonisciform' actinopterygian (*Elonichthys*

11

236 *peltigerus*) and five sarcopterygians including coelacanths (*Miguashaia bureaui* and

237 *Rhabdoderma exiguum*), lungfishes (*Dipterus valenciennesi*), porolepiforms (*Quebecius*

238 *quebecensis*) and 'osteolepiforms' (*Eusthenopteron foordi*). Specimens were chosen

239 according to their exceptional state of preservation (articulated postcranial material and

240 undistorted fins) and, whenever possible, availability of ontogenetic series. Specimens of *E.*

241 *peltigerus* and *R. exiguum* come from the Upper Carboniferous (middle Pennsylvanian)

242 Francis Creek Shale (Mazon Creek area, Illinois) and studied specimens are housed in the

243 Field Museum of Natural History (FMNH; Chicago, IL, USA). Specimens of *M. bureaui*,

244 *Q. quebecensis* and *E. foordi* come from the Upper Devonian (middle Frasnian) Escuminac

245 Formation (Miguasha, Quebec, Canada); studied specimens are housed in the Musée

246 d'Histoire Naturelle de Miguasha (MHNM, parc national de Miguasha, Quebec, Canada),

247 the American Museum of Natural History (AMNH, New York, NY, USA), the Musée de

248 géologie René-Bureau from Université Laval (ULQ, Quebec, Canada), and The University

249 of Kansas Biodiversity Institute and Museum of Natural History, Division of Vertebrate

250 Paleontology (KUVP, Lawrence, KS, USA). Finally, specimens of *D. valenciennesi* come

251 from the Middle Devonian (Givetian) Achanarras beds (Scotland, UK); studied specimens

252 are housed in the Natural History Museum (BMNH, London, UK). A complete list of

253 surveyed specimens with their size [SL or total length (TL)] are listed in S1 Table.

254 Fossil specimens were examined under a Leica MZ9.5 binocular equipped with a

255 drawing tube and were photographed with an Olympus Camedia C5060. Developmental

256 states include (1) ossification (presence; radials and lepidotrichia), (2) segmentation

257 (lepidotrichia), and (3) bifurcation (lepidotrichia).

258

259

Results

Developmental patterning of the dorsal and anal fins of

Oncorhynchus

263   The dorsal and anal fins of juvenile *Oncorhynchus* are similar in their anatomy and

264   shape but differ slightly in meristic counts of skeletal elements and fin size (Fig 1; Table 1).

265   Generally, the dorsal and anal fins are composed of 29 (i.e., 15 proximal and 14 distal

266   radials) and 25 (i.e., 13 proximal and 12 distal radials) endoskeletal elements, and 18 and

267   15 exoskeletal elements (i.e., lepidotrichia), respectively. In both fins, proximal radials

268   (PR), distal radials (DR), and lepidotrichia (L) are organized in a one-to-one relationship,

269   with the exception of the first proximal and distal radials that support four and three

270   lepidotrichia in the dorsal and anal fins, respectively, while the last proximal and distal

271   radials support two lepidotrichia in both fins. Nine of the eleven developmental events

272   previously described (see Nomenclature) were analysed in *Oncorhynchus*; the relative

273   developmental sequence in each fin is ordered based on SL_{50} (Table 2). The presence of

274   actinotrichia (event 1) was not analysed except for its initial position in the sequence of

275   events. The ossification of distal radials (event 11) occurs after 100 dph, beyond the

276   timeframe of our study thus no data were available.

277

278 **Table 1. Meristic counts for the skeletal elements, proximal radials (PR), distal radials**

279 **(DR) and lepidotrichia (L) and proportions of the dorsal (D) and anal (A) fins in living**

280 **and extinct osteichthyans.**

281

Taxa	Dorsal fin			Anal fin			Proportion
	PR	DR	L	PR	DR	L	
Oncorhynchus	12-15	12-14	15-18	11-13	11-13	12-16	D > A
Elonichthys	5-12	1-17	34-40	3-9	1-17	40-47	D < A
Miguashaia	1	NA	21-28	1	NA	19-25	D = A
Rhabdoderma	?	?	12-21	?	?	9-21	D = A
Quebecius	?	?	30-35	?	?	31-36	D = A
Dipterus	1[1]	5[1]	42-48	1[1]	4[1]	22-28	D > A
Eusthenopteron	1	3	19-26	1	3	20-25	D = A

282 NA, non applicable

283 ?, endoskeletal elements are unknown

284 [1] Following Ahlberg and Trewin [46]; there is more than one row of distal radials; data are

285 for one row.

Table 2. Values of the SL_{50} for the serial skeletal elements, i.e. the proximal radials (PR), the distal radials (DR) and the lepidotrichia (L) during nine out of eleven developmental events of the developmental sequence of the dorsal and anal fins of *Oncorhynchus*. See Nomenclature for the complete list of events. The serial skeletal elements are ordered from the anterior to the posterior of the fins and their column is representative of their position in the fin (see Fig 1). A PR or DR is found in more than one column when more than one L is articulated with it. The sites of initiation of the different events are identified with a grey shading.

Events	Serial elements (SL_{50} mm)																	
Dorsal fin																		
2. Apparition of PR[1]	PR1	PR1	PR1 (15.249)	PR2 (12.692)	PR3 (10.478)	PR4 (8.608[ns])	PR5 (NA)	PR6 (NA)	PR7 (NA)	PR8 (NA)	PR9 (NA)	PR10 (9.362)	PR11 (9.362)	PR12 (9.362)	PR13 (11.553)	PR14 (12.573)	PR15 (12.080[ns])	PR15
3. Chondrification of PR	PR1	PR1	PR1 (19.422)	PR2 (15.613)	PR3 (13.490)	PR4 (12.608)	PR5 (12.608)	PR6 (12.608)	PR7 (12.608)	PR8 (12.608)	PR9 (12.608)	PR10 (12.890)	PR11 (13.390)	PR12 (14.580)	PR13 (15.128)	PR14 (17.288)	PR15 (16.993[ns])	PR15

	L01	L02	L03	L04	L05	L06	L07	L08	L09	L10	L11	L12	L13	L14	L15	L16	L17	L18
4. Apparition of L	L01 (18.315)	L02 (16.908)	L03 (15.488)	L04 (14.579)	L05 (13.949)	L06 (13.722)	L07 (13.285)	L08 (13.285)	L09 (13.285)	L10 (13.285)	L11 (13.285)	L12 (13.722)	L13 (14.561)	L14 (14.932)	L15 (15.191)	L16 (16.030)	L17 (18.085)	L18 (16.962^{ns})
5. Apparition of DR				DR1 (23.756)	DR2 (16.045)	DR3 (15.535)	DR4 (15.059)	DR5 (15.059)	DR6 (14.815)	DR7 (14.815)	DR8 (14.815)	DR9 (14.815)	DR10 (15.308)	DR11 (16.058)	DR12 (16.564)	DR13 (16.901)	DR14	DR14 (17.332)
6. Chondrification of DR[2]				DR1 (34.430^{ns})	DR2 (18.541)	DR3 (17.142)	DR4 (17.002)	DR5 (16.744)	DR6 (16.947)	DR7 (17.489)	DR8 (17.489)	DR9 (17.489)	DR10 (17.786)	DR11 (18.459)	DR12 (18.459)	DR13 (18.601)	DR14	DR14 (18.848)
7. Segmentation of L[3]	L01 (NA)	L02 (42.618)	L03 (39.910)	L04 (25.307)	L05 (19.511)	L06 (18.160)	L07 (17.782)	L08 (17.555)	L09 (17.671)	L10 (17.671)	L11 (17.671)	L12 (17.173)	L13 (17.896)	L14 (17.947)	L15 (17.947)	L16 (17.927)	L17 (18.792)	L18 (16.962^{ns})
8. Ossification of L	L01 (26.217)	L02 (22.830)	L03 (21.657)	L04 (19.913)	L05 (19.509)	L06 (19.374)	L07 (19.374)	L08 (19.374)	L09 (19.777)	L10 (19.913)	L11 (19.913)	L12 (20.696)	L13 (21.856)	L14 (22.920)	L15 (24.634)	L16 (27.151)	L17 (26.971^{ns})	L18 (NA)
9.	L01	L02	L03	L04	L05	L06	L07	L08	L09	L10	L11	L12	L13	L14	L15	L16	L17	L18

Bifurcation of L								(31.085)	(28.338)	(28.119)	(24.798)	(27.177)	(24.067)	(23.598)	(27.883)	(30.672)	(32.704[ns])	(NA)
10. Ossification of PR	PR1	PR1	PR1 (30.096)	PR2 (27.485)	PR3 (25.880)	PR4 (25.520)	PR5 (25.894)	PR6 (26.254)	PR7 (29.754)	PR8 (30.937)	PR9 (31.206)	PR10 (31.206)	PR11 (32.016)	PR12 (34.553[ns])	PR13 (34.607[ns])	PR15 (34.945[ns])	PR15	PR15 (NA)

Anal fin

2. Apparition of PR[1]	PR1	PR1	PR1 (15.066)	PR2 (13.290)	PR3 (12.309)	PR4 (10.507[ns])	PR5 (10.507[ns])	PR6 (NA)	PR7 (NA)	PR8 (NA)	PR9 (7.465[ns])	PR10 (7.462[ns])	PR11 (12.484)	PR12 (13.997)	PR13 (15.353[ns])	PR14 (NA)	PR14	
3. Chondrification of PR	PR1	PR1	PR1 (19.674)	PR2 (16.550)	PR3 (14.463)	PR4 (13.569)	PR5 (13.569)	PR6 (12.852)	PR7 (12.852)	PR8 (12.846)	PR9 (14.159)	PR10 (15.016)	PR11 (16.395)	PR12 (17.488)	PR13 (18.591)	PR14 (NA)	PR14	
4. Apparition of L	L01 (19.135)	L02 (17.783)	L03 (15.523)	L04 (14.405)	L05 (13.138)	L06 (12.884)	L07 (12.884)	L08 (12.884)	L09 (12.884)	L10 (13.451)	L11 (14.059)	L12 (15.084)	L13 (16.331)	L14 (16.870)	L15 (17.184)	L16 (17.257[ns])	L17 (NA)	
5.				DR1	DR2	DR3	DR4	DR5	DR	DR7	DR8	DR9	DR1	DR1	DR1	DR1	DR1	

Apparition of DR				(40.547[ns])	(16.698)	(15.485)	(15.762)	(15.762)	6 (15.762)	(15.762)	(16.057)	(16.057)	0 (16.057)	1 (16.325)	2 (17.347)	3 (18.404)	3
6. Chondrification of DR[2]				DR1 (40.917[ns])	DR2 (18.705)	DR3 (18.195)	DR4 (18.195)	DR5 (18.195	DR 6 (18.440)	DR7 (18.440)	DR8 (18.597)	DR9 (18.597)	DR1 0 (18.751)	DR1 1 (18.865)	DR1 2 (19.209)	DR1 3 (19.830)	DR1 3
7. Segmentation of L[3]	L01 (NA)	L02 (NA	L03 (35.789[ns])	L04 (20.827)	L05 (17.996)	L06 (17.996)	L07 (17.671	L08 (17.671	L09 (17.671)	L10 (17.671)	L11 (17.844)	L12 (17.996)	L13 (18.143)	L14 (18.235)	L15 (18.645)	L16 (19.170[ns])	L17 (NA)
8. Ossification of L	L01 (28.357)	L02 (24.287)	L03 (20.417)	L04 (19.938	L05 (19.515)	L06 (19.515)	L07 (19.516)	L08 (19.646	L09 (19.646)	L10 (20.168)	L11 (20.168)	L12 (20.988)	L13 (21.697)	L14 (22.650)	L15 (23.927)	L16 (25.310[ns])	L17 (30.071[ns])
9. Bifurcation of L	L01	L02	L03	L04	L05	L06 (35.399[ns])	L07 (29.525)	L08 (26.506)	L09 (25.230)	L10 (25.174)	L11 (26.800)	L12 (26.147)	L13 (26.286)	L14 (27.036)	L15 (29.923)	L16 (43.001[ns])	L17 (30.071[ns])
10. Ossificat	PR1	PR1	PR1	PR2 (30.483	PR3 (29.349)	PR4 (28.255)	PR5 (28.635	PR6 (29.680)	PR7 (29.680)	PR8 (32.897)	PR9 (33.131	PR1 0	PR1 1	PR1 2	PR1 3	PR1 4	PR1 4

ion of PR			(37.937[ns])									(32.834	33.117)	(32.640)	(46.057[ns]	(NA)		

NA, non applicable

[ns], these SL_{50} results are not significant

[1] Few proximal radials of the dorsal and anal fins were present in the smallest specimens (8.650 mm and 10.894 mm in SL, respectively).

[2] Events 6 and 7 are inverted in the anal fin, i.e. lepidotrichia segmentation (event 7) occurs prior to distal radial chondrification (event 6).

[3] Event 7 corresponds to the overall initiation of segmentation; the following events of segmentation (up to 6 events of segmentation; data not shown) are not included in the series of 11 events.

301   The apparition of proximal radials (event 2) begins before hatching. Few proximal

302   radials, located centrally, are present in the dorsal and anal fins of all the specimens

303   examined, even in the smallest pre-hatching specimens (8-10-mm SL), thus, no SL_{50} were

304   obtained for these elements (Table 2; Fig 3A). These proximal radials are interpreted as the

305   initiation sites of the development of the dorsal (PR 5-9) and anal (PR 6-8) fin

306   endoskeletons. These sites are congruent in the dorsal and anal fins (Table 2; Fig 3A). In

307   both fins, proximal radials appear by proceeding bilaterally from the initiation site (Table

308   3). This indicates a bidirectional pattern for the development of the proximal radials; the

309   most peripherally located proximal radials are the last to appear. The relative

310   developmental sequences are not simultaneous but are significantly correlated between fins

311   (Table 4). All following events occur after hatching.

312

313   **Fig 3. Comparisons between nine out of eleven developmental sequences for the dorsal**

314   **(black) and anal (gray) fins of the rainbow trout (*Oncorhynchus mykiss*).** See

315   Nomenclature for the complete list of events. Skeletal elements are ordered from anterior to

316   posterior. Filled and empty symbols represent significant and non-significant results for

317   SL50, respectively.

318

319   **Table 3. Directions of developmental sequences of ten out of eleven events for the**

320   **dorsal (D) and anal (A) fins in living and extinct osteichthyans.** See Nomenclature for

321   the complete list of events.

322

Events	Oncorhynchus		Elonichthys		Miguashaia		Rhabdoderma		Quebecius		Dipterus		Eusthenopteron	
	D	A	D	A	D	A	D	A	D	A	D	A	D	A
2. Apparition of proximal radials	B	B	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]
3. Chondrification of proximal radials	B	B	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]
4. Apparition of lepidotrichia	B	B	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]
5. Apparition of distal radials	B	B	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]
6. Chondrification of distal radials	B	B	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]	NA[1]
7. Segmentation of lepidotrichia	B	B	B	B	B	B	B	B	?	?	B	B	B	B
8. Ossification of lepidotrichia	B	B	?	?	?	?	B	B	?	?	?	?	?	?
9. Bifurcation of lepidotrichia	B	B	NA[2]	NA[2]	B	B	NA[2]	NA[2]	B	B	B	B	B	B

10. Ossification of proximal radials	B	B	?	?	NA^3	NA^3	?	?	?	?	?	?	NA^3	NA^3
11. Ossification of distal radials	NA^4	NA^4	$B\text{-}AP^5$	$B\text{-}AP^5$	NA^3	NA^3	?	?	?	?	?	?	PA	PA

323   B, Bidirectional; AP, Antero-posterior; PA, Postero-anterior

324   ?, the skeletal elements and/or sequence are unknown

325   NA: non-applicable

326   [1] This event is not observable in fossil taxa

327   [2] Bifurcation is unknown

328   [3] Only one endoskeletal element (for *Miguashaia*, the condition is unknown for the anal

329   fin, however, the dorsal fin has at least three distal radial and one large basal plate)

330   [4] The ossification occurs after 100 dph.

331   [5] Data suggest an antero-posterior direction but a bidirectional direction is possible (see the

332   text)

333

334

335   **Table 4. Spearman correlations between developmental sequences of (a) the dorsal**

336   **and anal fins and (b) fin exoskeleton and endoskeleton of the dorsal (D) and anal (A)**

337   **fins of *O. mykiss* to validate the presence of the DAFPM and the EEM respectively.**

338   See Nomenclature for the complete list of events.

Events	n	r_s	P
Dorsal and anal fin patterning module			
2. Proximal radial differentiation	8	0.8553628	<0.01
3. Proximal radial chondrification	13	0.9185589	<0.001
4. Lepidotrichia differentiation	15	0.8604663	<0.001
5. Distal radial differentiation	12	0.6288943	<0.05
6. Distal radial chondrification	12	0.9019622	<0.001
7. Lepidotrichia segmentation	12	0.6691664	<0.05
8. Lepidotrichia ossification	15	0.9576292	<0.001
9. Lepidotrichia bifurcation	8	-0.40476	ns
10. Proximal radial ossification	10	0.9268293	<0.001
11. Distal radial ossification	NA	NA	NA
Endoskeleton and exoskeleton module			
Events 2 and 4: Proximal radial and lepidotrichia differentiation (D)	14	0.903	< 0.001
Events 2 and 4: Proximal radial and lepidotrichia differentiation (A)	12	0.730	< 0.01
Events 4 and 5 Lepidotrichia and distal radial differentiation (D)	14	0.972	< 0.001
Events 4 and 5 Lepidotrichia and distal radial differentiation (A)	12	0.891	< 0.001

Events 8 and 10 Lepidotrichia and proximal radial ossification (D)	11	0.854	< 0.001
Events 8 and 10 Lepidotrichia and proximal radial ossification (A)	11	0.839	< 0.01

339   NA, non applicable: The ossification of distal radials occurs after 100 dph

340   ns, non significant

341

342   The chondrification of the proximal radials (event 3) quickly follows their

343   appearance. In both fins, the chondrification sequences show similar patterns as to their

344   sequences of apparition (S1 Table); chondrification starts from the same initiation site (i.e.,

345   the most centrally located proximal radials) and further proceeds bidirectionally (Table 3).

346   The bidirectional sequences of chondrification are significantly correlated and are almost

347   simultaneous between dorsal (PR 4-9) and anal (PR 8) fins (Fig 3B, Table 4).

348   Lepidotrichia appear (event 4) slightly after the differentiation of the first proximal

349   radial and subsequently articulate with the distal radials. The initiation site of the

350   lepidotrichia is similar in both fins (L7-11 in the dorsal fin and L6-9 in the anal fin) and

351   corresponds in position to the early apparition of the proximal radials (S1 Table). Similar to

352   proximal radials, new lepidotrichia appear by proceeding bidirectionally from the initiation

353   site (Table 2). The sequences of apparition of lepidotrichia are highly correlated between

354   fins and almost simultaneous (Fig 3C, Table 4).

355   The first distal radials (event 5) to appear differ in position between the dorsal

356   (DR6-9) and anal (DR3) fins (Table 2). In both fins, the sequences of apparition proceed

24

357   bidirectionally from the initiation site. The sequences are significantly correlated (Fig 3D,

358   Table 4).

359   The chondrification of distal radials (event 6) follows rapidly, starting from similar

360   initiation sites, DR5 in the dorsal fin and DR3-5 in the anal fin (Table 2). Starting from the

361   initiation site, the chondrification sequences proceed bidirectionally and are almost

362   simultaneous as well as highly correlated between fins (Fig 3E, Table 4).

363   Lepidotrichia grow by distal addition of new segments, a process called

364   segmentation (event 7). Through growth, five and six segmentation events occur in the anal

365   and dorsal fins, respectively. The lepidotrichia with the higher numbers of segments in

366   most specimens are the L10-14 in the dorsal fin and L8-10 in the anal fin and the number of

367   segments on the adjacent lepidotrichia decreases in a bidirectional pattern. The first

368   segmentation event occurs slightly posteriorly in the dorsal fin (L12) comparatively to the

369   anal fin (L7-10) (Fig 3F). The site for the initiation of segmentation corresponds to the

370   lepidotrichia with the highest number of segments, suggesting that the longest lepidotrichia,

371   in terms of number of segments, are the first lepidotrichia to segment in the developmental

372   sequence (Fig 1). All sequences of segmentation are bidirectional (Table 3). The sequences

373   of the first segmentation are almost simultaneous and significantly correlated between fins

374   (Fig 3F, Table 4).

375   The initiation site for the ossification of lepidotrichia (event 8) is similar in both fins

376   (L6-8 in the dorsal fin and L5-6 in the anal fin) (Fig 3G). Sequences of ossification are

377   bidirectional, simultaneous, and highly correlated (Tables 3 and 4). There are small,

378   positional differences for the initiation site of apparition, segmentation and ossification of

379   the lepidotrichia (Fig 3G). Nevertheless, sequences of apparition are significantly correlated

380   with sequences of segmentation of the lepidotrichia in the dorsal (0.670; p<0.01) and anal

381   (0.768; p<0.01) fins, while the results differ between sequences of segmentation and

382   ossification of the lepidotrichia (dorsal, 0.098; p>0.05; anal, 0.485; p>0.05) (Table 4).

383   The bifurcation of the lepidotrichia (event 9) is initiated at different positions in the

384   dorsal (L14) and anal (L10) fins (Fig 3H). The bidirectional sequences of bifurcation

385   between fins are not significantly correlated and not simultaneous (Fig 3H, Table 4).

386   Generally, in the dorsal fin, the lepidotrichia located at the initiation site show more

387   proximal bifurcations comparatively to the lepidotrichia located bilaterally of the initiation

388   site; this pattern is not as clear in the anal fin. A single order of bifurcation is present.

389   The ossification of proximal radials (event 10) is initiated from PR4, located in the

390   same anterior portion of both fins (Fig 3I). In both fins, the sequences of ossification

391   proceed in a bidirectional direction, and are highly correlated, but not simultaneous (Fig 3I,

392   Table 4).

393   All nine developmental events analysed show a certain degree of congruence

394   between the dorsal and anal fins (e.g., similar initiation site and/or similar direction) and

395   eight events are significantly correlated between fins (events 2-8 and 10; Table 4).

396   Moreover, in congruence with the one-to-one relationship observed between radials and

397   lepidotrichia in both fins, the first lepidotrichia to appear are related to the first apparition

398   of proximal and distal radials (Fig 4); this is corroborated by the strong correlations

399   between sequences of apparition of the endoskeletal and exoskeletal elements within each

26

400   fin (Table 4). Highly significant correlations are also found for sequences of ossification of

401   the endoskeleton and exoskeleton within both fins (Fig 4, Table 4).

402

403   **Fig 4. Comparisons between developmental sequences (apparition and ossification) of**

404   **the endoskeleton (radials; black) and exoskeleton (lepidotrichia; grey) of the dorsal**

405   **(A, C, E) and anal fins (B, D, F) of the rainbow trout (*Oncorhynchus mykiss*).** Skeletal

406   elements are ordered from anterior to posterior. Filled and empty symbols represent

407   significant and non-significant results for SL50 respectively.

408

409   # Developmental patterning in the dorsal and anal fins of fossil

410   # osteichthyans

411   For the six fossil osteichthyans surveyed (Table 1, S1 Table), only the events

412   dealing with the lepidotrichia [i.e., segmentation (event 7), ossification (event 8), and

413   bifurcation (event 9)] and the ossification of endochondral elements [i.e., proximal radials

414   (event 10) and distal radials (event 11)] are available to study due to the nature of fossils

415   and the rarity of fossilized ontogenies. Meristic counts (Table 1) are based on mean values

416   from all specimens examined for each taxon, including both juveniles and adults whenever

417   possible. Congruence of events between the dorsal and anal fins, and between the

418   endoskeleton and exoskeleton are inferred based on initiation sites, and when available, the

419   direction of sequences.

420

421 ## Actinopterygians

422 ### *Elonichthys peltigerus*

423 The dorsal and anal fins of *Elonichthys* are of similar shape but differ slightly in size (Table

424 1; Fig 5) [47]. The dorsal fin is composed of 16-19 distal radials whereas the anal fin has

425 19-23 proximal radials. The general relationship between radials and lepidotrichia is 1:2,

426 with some supernumerary lepidotrichia occurring in the anterior and posterior margins.

427

428 **Fig 5. The Carboniferous actinopterygian *Elonichthys peltigerus*.** A, Fossil specimen

429 (FMNH PF 7502) and drawings of its dorsal (B) and anal (C) fins. Arrows point anteriorly.

430 An. f, anal fin; caud. f, caudal fin; Dors. f, dorsal fin; DR, distal radial; L, lepidotrichia; Pect.

431 f, pectoral fin; Pelv. f, pelvic fin; PR, proximal radial; seg, segmentation.

432

433 Lepidotrichia are the first structures to ossify in both fins. Segmented lepidotrichia

434 (event 7) are present in all specimens. The number of segments varies little with respect to

435 SL (from 4-5 to 6-7 segments per lepidotrichium). In both fins, lepidotrichia from the

436 anterior part of the fin are usually the longest (L7-15 in the dorsal and L10-13 in the anal

437 fin) and articulate with distal radials 5-6, which seem among the first ones to ossify (see

438 below). The number of segments decreases bilaterally from these lepidotrichia.

439 Lepidotrichia are already numerous in the smallest specimen (38 dorsal and 46 anal

440 lepidotrichia); therefore, it is not possible to infer a sequence of ossification (event 8). None

441 of the specimens show bifurcated lepidotrichia (event 9).

442   Ossification sequences are difficult to reconstruct for the proximal (event 10) and

443   distal radials (event 11). Proximal radials are visible at the anterior portion of the dorsal and

444   anal fins; remaining radials are probably hidden under the scale cover. Distal radials have

445   only been clearly identified in the dorsal fin (FMNH PF 7502) (Fig 5). When present, both

446   ossified proximal and distal radials do not reach the posterior margin of both fins.

447

Actinistians

449   *Miguashaia bureaui*

450   The second dorsal and anal fins of *Miguashaia* are overly similar in size and shape

451   (Table 1; Figs 6 and 7) with narrow, rectangular bases and pointed anterior corners ([22],

452   fig. 1B). Only the lepidotrichia can be described (27-28 in the dorsal fin and ca. 25 in the

453   anal fin), a single specimen (MHNM 06-1809) partially shows the radials articulating with

454   the second dorsal basal plate.

455

456   **Fig 6. The Devonian coelacanth *Miguashaia bureaui*.** A, Juvenile specimen (ULQ 120)

457   and drawings of its second dorsal (B) and anal (C) fins. Arrows point anteriorly. An. f, anal

458   fin; bif, bifurcation; Caud. f, caudal fin; Dors. f, dorsal fin; L, lepidotrichia; seg,

459   segmentation.

460

461   **Fig 7. The Devonian coelacanth *Miguashaia bureaui*.** A, Adult specimen (MHNM 06-41)

462   and drawings of its second dorsal (B) and anal (C) fins. Note the occurrence of merging

463   segments (m.seg) at the base of the fins. Arrows point anteriorly. An. f, anal fin; bif,

464   bifurcation; Caud. f, caudal fin; Dors. f, dorsal fin; L, lepidotrichia; Pect. f, pectoral fin; Pelv.

465   f, pelvic fin; PR, proximal radial; seg, segmentation.

466

467   Numerous segmentations (up to five) are visible in the lepidotrichia (event 7) of the

468   dorsal and anal fins of the smallest specimen (MHNM 06-1633, 64.5 mm TL). The longest

469   lepidotrichia are L4-7 in the dorsal fin and L5-8 in the anal fin in specimen MHNM 06-41

470   (Fig 7) and the number of segments decreases gradually bilaterally from these sites. The

471   basal proximal segment is always longer than the others in all specimens examined. In

472   specimen MHNM 06-41, the segment immediately distal to the first proximal segment

473   appears to be half-fused with this basal segment (Fig 7; L11, m.seg), thus evidencing that

474   the increase in length of the basal segment during growth is the result of its merging with

475   other proximal segments.

476   Ossified lepidotrichia are already numerous (28 dorsal and ca. 22 anal lepidotrichia)

477   in the smallest specimen (MHNM 06-1633); it is thus not possible to infer an ossification

478   sequence (event 8).

479   Few bifurcations (event 9) are seen on L15 and L14-17, respectively in the dorsal

480   and anal fins of the small specimen ULQ 120 (85 mm TL; Fig 6). The most proximal

481   bifurcations are positioned approximately between L12-15 in all specimens (Figs 6 and 7).

482   Bifurcations are present anteriorly and posteriorly to L12-15 in large specimens.

483   Bifurcations are gradually displaced distally anterior to the most proximally bifurcated

484   lepidotrichium (L15) resulting in a higher proportion of proximally bifurcated lepidotrichia

485   in the posterior portion of the fins. Up to three orders of bifurcation have been observed in

486   specimen MHNM 06-494 (400 mm TL).

487   Ossified endoskeletal supports (events 10 and 11) are poorly documented. A single

488   basal plate in the first dorsal fin is present in one partial specimen (MHNM 06-1232), while

489   the basal plate of the second dorsal fin shows articulating surfaces for three radials, the

490   posterior one being well-preserved (MHNM 06-1809).

491

492   **_Rhabdoderma exiguum_**

493   The second dorsal and anal fins of _Rhabdoderma_ are almost identical in size and

494   shape (Table 1, Fig 8). They display a narrow-based fan-like outline and, as for

495   _Miguashaia_, only the lepidotrichia are preserved.

496

497   **Fig 8. The Carboniferous coelacanth _Rhabdoderma exiguum_.** A, Small specimen (FMNH

498   PF 9954) and drawings of its second dorsal (B) and anal (C) fins. Arrows point anteriorly.

499   An. f, anal fin; Caud. f, caudal fin; Dors. f, dorsal fin; L, lepidotrichia; Pelv. f, pelvic fin; seg,

500   segmentation.

501

502   All specimens show segmented lepidotrichia (event 7). The number of segments

503   increases with SL (from 5-6 to 13-14 segments). In both fins, the longest lepidotrichia

504   occur between L9-12 and the number of segments gradually decreases bilaterally from this

505   site.

506   The number of lepidotrichia increases up to 21 in both fins during growth (event 8).

507   Lepidotrichia at the anterior and posterior extremities are absent in the dorsal and anal fins

508   of small specimens, suggesting that central lepidotrichia might have ossified before the

509   anterior and posterior ones (Fig 8). This fin comparison between small (less than 21

510   lepidotrichia; e.g., FMNH PF7528; 33 mm SL) and larger specimens (21 lepidotrichia; e.g.,

511   FMNH PF7338; 48 mm SL) was done by lining up their respective longest lepidotrichia.

512   None of the specimens show bifurcation of the lepidotrichia (event 9), and no

513   ossified endoskeletal supports are preserved in embryos and larvae examined (events 10

514   and 11).

515

516   **Porolepiforms**

517   *Quebecius quebecensis*

518   The second dorsal and anal fins of *Quebecius* have a similar size and shape (Table

519   1) [48 (figs 1, 7), 49 (fig. 2)]. Endoskeletal supports remain unknown in this taxon and only

520   the lepidotrichia can be described (between 30 and 40 lepidotrichia in both fins).

521   Segmented lepidotrichia (event 7) are already visible in the smallest specimen

522   (MHNM 06-1474a); however, the number of segments is unclear in all specimens owing to

523   preservation issues. Lepidotrichia are well developed, and their number is similar in the

524   small as well as in the larger specimens (35 dorsal lepidotrichia and 34 anal lepidotrichia),

525   it is thus not possible to infer an ossification sequence (event 8).

526   The first bifurcation (event 9) is seen in L22 of the anal fin (MHNM 06-1474a). The

527   most proximal bifurcations occur between L18-21 in longer specimens, with other

528 branched lepidotrichia located anteriorly and posteriorly to this area. In both fins, the

529 posterior lepidotrichia display more proximal bifurcations than the anterior lepidotrichia.

530

531 **Dipnoans**

532 ***Dipterus valenciennesi***

533 The second dorsal and anal fins of *Dipterus* differ in size and shape (Table 1; Fig 9), with

534 the second dorsal fin being longer and higher than the somewhat pointed, leaf-shaped anal

535 fin. Lepidotrichia are more numerous than their supporting radials, which are known to be

536 grossly similar between fins with minor differences in terms of the number and shape [46].

537

538 **Fig 9. The Devonian dipnoan *Dipterus valenciennesi.*** A, Fossil specimen of (BMNH

539 P.22187) and drawings of its second dorsal (B) and anal (C) fins. Note the development of

540 scales (Sc) covering the proximal portion of the lepidotrichia. Arrows point anteriorly. An.

541 f, anal fin; bif, bifurcation; Caud. f, caudal fin; Dors. f, dorsal fin; L, lepidotrichia; Pect. f,

542 pectoral fin; seg, segmentation.

543

544

545   Lepidotrichia show multiple segmentations (event 7). The longest lepidotrichia are

546 not found at the same position in the dorsal (L16-26) and anal (L8-12) fins; these areas are

547 interpreted as different initiation sites of segmentation. In both fins, the number of

548 segments gradually decreases bilaterally from these sites. The basal segment is

549 considerably long, comprising one third of each lepidotrichium total length.

550   Even the smallest specimens show numerous well ossified lepidotrichia (44 dorsal

551   and 25 anal), it is thus not possible to infer a sequence of ossification (event 8).

552   Up to three orders of bifurcation (event 9) are present. Bifurcations are present

553   between L14-18 and L39-42 in the dorsal fin and between L11-12 and L17-22 in the anal

554   fin. Specimen BMNH P22187 (Fig 9A) is informative concerning the sequence of

555   bifurcation: (1) two orders of bifurcation are present in the dorsal fin: 1st order: L15-42 (bif

556   1), 2nd order: L28-39 (bif 2) (Fig 9B), and three orders in the anal fin: 1st order: L10-20 (bif

557   1); 2nd order: L11-18 (bif 2); 3rd order: L13-16 (bif 3) (Fig 9C); (2) the most proximal

558   bifurcations are in L36-37 in the dorsal fin and in L13-16 in the anal fin (Figs 9B and 9C),

559   and (3) bifurcated lepidotrichia are found anteriorly and posteriorly to these sites. Thus, in

560   both fins, the posterior-most lepidotrichia show more proximal bifurcations whereas the

561   anterior-most lepidotrichia are more distally branched.

562   Ossified endoskeletal supports (events 10 and 11) were not observed due to the

563   scale cover (Sc, Figs 9B and 9C). Their sequence of ossification is unknown.

564

565 'Osteolepiforms'

566 *Eusthenopteron foordi*

567   The second dorsal and anal fins of *Eusthenopteron* are similar in size and shape

568   (Table 1; Figs 10 and 11), displaying a narrow-based and posteriorly pointed profile [50].

569   The median fins display a broad basal plate on which generally three distal radials

570   articulate, carrying numerous lepidotrichia (up to 25 in each fin).

571

572 **Fig 10. The Devonian 'osteolepiform'** *Eusthenopteron foordi.* A, Very small specimen

573 (MHNM 06-1754) and a drawing of its anal fin (B). Arrows point anteriorly. An. f, anal fin;

574 bif, bifurcation; Caud. f, caudal fin; Dors. f, dorsal fin; DR, distal radial; L, lepidotrichia;

575 Pect. f, pectoral fin; Pelv. f, pelvic fin; seg, segmentation.

576

577

578 **Fig 11. The Devonian 'osteolepiform'** *Eusthenopteron foordi.* A, Small specimen (MHNM

579 06-1769) and drawings of its second dorsal (B) and anal (C) fins. Arrows point anteriorly.

580 Note the preservation of a basal plate (BPL) and a reduced scale covering (Sc) at the base of

581 the fins. An. f, anal fin; bif, bifurcation; Caud. f, caudal fin; Dors. f, dorsal fin; DR, distal

582 radial; L, lepidotrichia; Pect. f, pectoral fin; Pelv. f, pelvic fin; PR, proximal radial; seg,

583 segmentation.

584

585   The first segmentations (event 7) are seen in L11-14 in the dorsal fin and in L8-13

586 in the anal fin (MHNM 04-1293p10-Ef1; 40.8 mm SL). The position of these first

587 segmented lepidotrichia is congruent with the location of the longest lepidotrichia in larger

588 specimens. In these specimens, most lepidotrichia are segmented and the number of

589 segments gradually decreases bilaterally from L8-14.

590   Lepidotrichia are the first structures to ossify in both fins (event 8); they are

591 numerous in the smallest specimen (MHNM 04-1293p9-Ef1, 27.4 mm SL; 18 and 21

592 lepidotrichia in dorsal and anal fins, respectively) and the highest number of lepidotrichia is

593   reached early (MHNM 06-1754; 49.3 mm SL; Fig 10), thus it is not possible to infer an

594   ossification sequence.

595   Bifurcations (event 9) are restricted between L6-7 to L25 in large specimens and up

596   to five orders of bifurcation are present in both fins. First bifurcations are present in L13

597   and 17 in the dorsal fin and in L18-19 in the anal fin (MHNM 06-1754; Fig 10B) Most

598   proximal bifurcations and subsequent orders of bifurcation are initiated in this area (e.g.,

599   MHNM 06-1769: 2nd order: L9-17 in the dorsal fin and L13-18 in the anal fin; 3rd order:

600   L15-17 in the anal fin; Figs 11B and 11C).

601   Radial ossification (DR; event 11) occurs prior to the ossification of the basal plate

602   (event 10) (BPL, Figs 11B and 11C). A postero-anterior pattern of ossification for dorsal

603   and anal distal radials is suggested because few small specimens (43-56-mm SL) show only

604   the posterior, or the posterior and central radials in the dorsal or anal fins. The basal plate is

605   first seen at 88.9-mm SL and 69.6-mm SL in the dorsal and anal fins, respectively.

606

Discussion

608   Our study described a series of 11 skeletogenic events in the formation of the endo-

609   and exoskeletal components of the dorsal and anal fins of the living actinopterygian

610   *Oncorhynchus mykiss* between 5 days pre-hatching up to 100 days post-hatching. We also

611   documented some of these events in one Carboniferous actinopterygian species, and five

612   Devonian-Carboniferous sarcopterygian species. For the first time, we quantified the

613   segmentation and bifurcation of lepidotrichia supporting a lepidotrichial patterning module.

614   The similarity and synchronicity of these developmental events between the dorsal and anal

615   fins support the hypothesis that these two fins form a developmental module.

616

Median fins in vertebrates

618   The fins of vertebrates can be described as membranous lateral outgrowths of the

619   body walls reinforced internally by elongated elements, which can be of endoskeletal (e.g.,

620   radial bones) or dermal (e.g., fin rays) origin.

621   Median fins are present in stem vertebrates, such as myllokunmingiids, in the form

622   of dorsal and ventral skin folds reminiscent of the median larval fin fold observed during

623   the early ontogeny of more advanced fishes (e.g., [6,9,11,51]). The earliest 'agnathans'

624   display well-developed median fins, which include, in most cases, a caudal fin and elongate

625   dorsal and ventral fins. A separate anal fin has been confidently identified in

626   petromyzontiforms, anaspids [52,53], the anaspid-like *Euphanerops,* and in certain

627   thelodonts (e.g., *Loganellia*) [54]. In *Euphanerops*, the anal fins are paired fins [55,56].

628   The presence of an anal fin in hagfishes has been suggested in *Myxinikela* [57], but

629   it is currently difficult to confirm given the poor fossil record of Myxiniformes. An anal fin

630   could be a plesiomorphic characteristic of vertebrates if the ventral fin of *Myxinikela* is

631   resolved as homologous to the anal fin of anaspids and gnathostomes. However, the

632   distribution of the anal fin is variable among vertebrates [11]. The differentiation of an anal

633   fin from a ventral fin fold might have occurred early in vertebrate history since anal fins

634   supported by endoskeletal elements have been identified in fossil lampreys (e.g.,

635   *Hardistiella* and possibly *Mayomyzon*), despite its absence in extant forms (e.g,

636   *Petromyzon, Lampreta*). In these, an anal 'crest' made of a skin fold devoid of fin rays

637   occurs, but an anal fin may develop in certain atavistic specimens [54,58]. There is no

638   evidence of an anal fin in heterostracans [12] or arandaspids [59] so its absence can be

639   considered a derived loss from the primitive condition of fossil lampreys. Janvier (2007;

640   [54]) suggested that the 'horizontal caudal lobe' of osteostracans might be a modified anal

641   fin. Galeaspids and pituriaspids appear to lack an anal fin [60,61] but uncertainty is due to

642   the poor preservation of their postcranial skeleton. In gnathostomes, the anal fin is not

643   preserved usually in 'placoderms', probably due to its reduced size and the cartilaginous

644   nature of the radials, but has been illustrated in some cases (e.g., arthrodires like

645   *Africanaspis* and *Dunkleosteus*) [62,63]). The anal fin is primitively present in all crown

646   gnathostomes but may be absent in certain chondrichthyans [9,11]. Living and fossil

647   actinopterygians (with only a few exceptions in Osteoglossiformes, Anguilliformes,

648   Lampridiformes, Siluriformes, and Syngnathiformes) and most piscine sarcopterygians

649   (with exception in derived dipnoans) possess an anal fin. Among 'elpistostegalians',

650   *Elpistostege* is known to retain an anal fin [15], which is definitely lost in tetrapods, while

651   *Tiktaalik* might have lost the anal fin.

652   In the case of dorsal fins, paleontological and developmental evidence reveal that it

653   is not constrained in its anterior extent and position, as opposed to the anal fin (which

654   cannot extend anteriorly to the position of the anus), resulting in a variable occurrence of

655   long-based and short-based dorsal fins in the earliest vertebrates [9]. *Pikaia* and

656   Myllokunmingiida are the first and oldest early vertebrates in which a separate dorsal fin

657   occurs. Lampreys display elongate dorsal fins that are not supported by radials. Extant

658   lampreys display two dorsal fins, separated by a gap but the 'posterior dorsal fin' is now

659   assumed to corresponds to the anterior extension of the caudal epichordal lobe seen in

660   fossil lampreys (e.g., *Mesomyzon*; [64]). Fossil lampreys (e.g., *Hardistiella* and

661   *Mayomyzon*) show no gap separating the anterior and posterior dorsal fins as in extant

662   lampreys, suggesting that the double dorsal fins of lampreys and gnathosthomes is a

663   convergent feature [54]. Duplication of short-based dorsal fins is also a common case in the

664   evolution of vertebrates [9]. Several osteostracans have two dorsal fins (e.g., *Ateleaspis*,

665   *Aceraspis*, and *Hirella*), but the anterior one lacks a fin web and resembles more a scale-

666   covered hump than a proper fin [54]. On the other hand, the second dorsal fin of

667   osteostracans clearly displays a fin web made of small scales arranged in a lepidotrichial

668   pattern overlying numerous delicate radials. However, it is still debated whether this fin

669   should be considered homologous to the posterior dorsal fin of gnathostomes or to the

670   anterior part of the epichordal lobe of the caudal fin of lampreys, anaspids, and thelodonts.

671   Among gnathostomes, 'placoderms' can either display single (e.g., antiarchs, stensionellids,

672   rhenanids, and arthrodires) or double (e.g., ptyctodontids) dorsal fins [9]. Nevertheless, the

673   plesiomorphic condition for crown gnathostomes, including chondrichthyans,

674   'acanthodians', and osteichthyans, is the occurrence of two dorsal fins supported by radials,

675   fin rays, and sometimes associated spines [65–67]. Actinopterygians loss the anterior dorsal

676   fin (from *Cheirolepis* onwards) but may regain a second dorsal fin either spinous (e.g.,

677   acanthomorphs) or adipous (e.g., euteleosts) [9]. Many sarcopterygians primitively retain

678   two dorsal fins, with the exception of the derived loss of the dorsal fins in post-Devonian

679   dipnoans and 'elpistostegalians' + tetrapods.

680

Comparison of the median fin developmental patterning in osteichthyans

683   Fin and fin ray development have been well studied by developmental biologists

684 since the middle of the 19th century (e.g., [68–70]) and numerous studies have dealt with

685 the morphological and molecular features of fin ray development and regeneration (see a

686 review in [7] and references therein). Indeed, fin rays are a good tool to better understand

687 vertebrate ontogenetic development [17] and the connections between gene expression

688 (during normal development, regeneration, and mutagenesis) and morphological and

689 structural variation of anatomical traits.

690   Fin and fin ray development has been thoroughly surveyed in osteichthyans through

691 the zebrafish *Danio rerio* [7]. Other studies on fin anatomy and development have been

692 performed mostly in extant actinopterygians such as *Salmo* [26], *Medaka* [71], *Tilapia* [72],

693 *Amia* [73], *Polyodon* [6,74], and *Acipenser* [74], but also in sarcopterygians such as the

694 dipnoans *Neoceratodus* [75,76], *Protopterus* [25], and *Lepidosiren* [77], and the coelacanth

695 *Latimeria* [78,79]. Our new data on extant (*Oncorhynchus*) and extinct (*Elonichthys,*

696 *Miguashaia, Rhabdoderma, Dipterus, Quebecius,* and *Eusthenopteron*) osteichthyans allow

697 us to accurately depict similarities in the developmental patterning of the median fins of

698 bony fishes dealing with the morphological and temporary characteristics of appearance,

699 chondrification, and ossification of endoskeletal elements (proximal and distal radials) and

700 dermal fin rays (actinotrichia and lepidotrichia).

Apparition, chondrification, and ossification of radials and lepidotrichia

701

702   In extant actinopterygians (e.g., *Oncorhynchus*, *Salvelinus*, *Danio*) ([8,37,80]; this

703   study) all developmental sequences (apparition, chondrification, and ossification) of the

704   endoskeleton and exoskeleton for the dorsal and anal fins are bidirectional. This

705   bidirectional pattern is corroborated by (1) common initiation sites for most corresponding

706   events, (2) significant correlations between developmental sequences in both fins (dorsal

707   and anal), and between the endoskeleton and exoskeleton (proximal/distal radials and

708   lepidotrichia), and (3) a certain degree of simultaneity between sequences of

709   apparition/chondrification (radials and lepidotrichia) and for the ossification of

710   lepidotrichia. Despite little discrepancies among the initiation sites for

711   apparition/chondrification/ossification of radials seen in *Oncorhynchus*, and between

712   apparition/ossification of lepidotrichia, all these events can be confidently considered to be

713   initiated from a unique initiation site. Unfortunately, the ossification patterns of many fossil

714   osteichthyans are difficult to infer due to the preservation biases associated with the nature

715   of fossilisation. However, in certain exceptional cases, ossification patterns can be

716   tentatively reconstructed, such as in the coelacanth *Rhabdoderma* in which a bidirectional

717   sequence occurred for the lepidotrichia Table 3, Fig. 8), whereas in the 'osteolepiform'

718   *Eusthenopteron* ossification of the distal radials proceeded postero-anteriorly [17,81]

719   (Table 3); however, the narrowness of the fin and the reduced number of radials in

720   *Eusthenopteron* might explain why the pattern is unidirectional.

721   In the case of lepidotrichia, fossil evidence confirms that dermal fin rays always

722   ossify relatively early during ontogeny and before the endoskeletal radials ([82]; this

723   study), a condition identical to that of *Oncorhynchus* and other extant osteichthyans.

724   However, as for the radials, it is not easy to infer an ossification sequence for the

725   lepidotrichia in immature fossil specimens.

Segmentation of lepidotrichia

727   Lepidotrichial growth is achieved by successive addition of distal segments at the

728   extremity of the forming lepidotrichia [28]. The process of segmentation is congruent

729   between the dorsal and anal fins in *Oncorhynchus* in terms of: (1) similar initiation sites,

730   (2) common bidirectional sequences of segmentation, (3) simultaneity, and (4) significantly

731   correlated sequences. The correlations among the lepidotrichia apparition, segmentation,

732   and ossification sequences within both fins suggest that all three sequences are initiated

733   from the same site and proceed in a bidirectional sequence. This congruence also matches

734   the apparition/chondrification/ossification patterns described in radials.

735   In fossil taxa, an important aspect concerns the identification of the initiation site of

736   segmentation. Considering the observation made in *Oncorhynchus* (i.e. longer lepidotrichia

737   are the ones for which segmentation started earlier), the location of the longest lepidotrichia

738   in the dorsal and anal fins can be confidently identified as the initiation site of segmentation

739   in fossil specimens. This scenario has been proposed in all extinct osteichthyans surveyed

740   (i.e., *Elonichthys*, *Miguashaia*, *Rhabdoderma*, *Quebecius*, *Dipterus,* and *Eusthenopteron*)

741   (Table 3).

742   These similarities in patterning imply that sequences of segmentation may as well

743   be generally used as proxies for sequences of ossification in osteichthyans. Considering the

744   impracticality to observe an ossification sequence for lepidotrichia in fossil specimens,

745   segmentation patterns are essential for comparisons between living and extinct taxa.

Bifurcation of lepidotrichia

747   Bifurcations are the results of the distal branching of an individual lepidotrichial

748   segment after the intersegmental joint [38]. As for segmentation, bifurcation patterns can be

749   compared in extant and extinct taxa. However, the bifurcation pattern is not clear in

750   *Oncorhynchus*: (1) the sequences of bifurcation of both fins are bidirectional but not

751   correlated, (2) the initiation site for bifurcation is posterior to the other initiation sites

752   (apparition, ossification, segmentation) in the dorsal fin but similar in the anal fin, and (3)

753   the initiation site corresponds to the lepidotrichia with the most proximal bifurcations in the

754   dorsal fin but not in the anal fin. These results might be artefactual owing to small sample

755   size and inter-individual variation. However, despite these potential biases, bifurcation

756   sequences are likely similar between fins.

757   The bifurcation patterns found in both fins of extinct sarcopterygians (e.g.,

758   *Miguashaia*, *Quebecius*, *Dipterus*, *Eusthenopteron*) corroborate the observations made on

759   the dorsal fin of *Oncorhynchus*: (1) an initiation site for bifurcation located posteriorly to

760   the initiation site for segmentation, (2) concordance between the initiation site and the

761   lepidotrichia with the most proximal bifurcations, and (3) a bidirectional sequence of

762   bifurcation. These similarities between extant and extinct osteichthyans suggest a shared

763   pattern of bifurcation in which bifurcation is initiated in a different position than the other

764   events for the lepidotrichia.

765

Median fin modularity and evolution in osteichthyans

766

767   Modularity is a fundamental property of organisms playing an important role in

768 their evolution [83–85]. Anatomical modules refer to an internal organization of anatomical

769 structures into distinct units, or modules, which develop and vary in quasi-autonomy, but

770 within which the constituents interact and vary together [83,85,86]. This quasi-autonomy

771 among modules allows three main evolutionary processes: dissociation,

772 duplication/divergence, and co-option [83]. Different categories of modularity, and

773 modules, have been defined over the past few decades. Among these categories, Zelditch &

774 Goswami [85] emphasized the intricate relationship between developmental and functional

775 modularity. Developmental modules are often represented as networks depicting their

776 physical location, spatial extent and genetic specification, while functional modules are

777 represented by anatomical elements integrated as structural components of a functional (or

778 physiological) system [85]. However, developmental and functional modules have not been

779 investigated methodologically as thoroughly as variational and evolutionary modules (see

780 [85] for an exhaustive critical review of the methods). Thus, the identification of

781 developmental and functional modules is frequently only suggested without being tested.

782 Herein, we have proposed a method to compare similarities among sequences of

783 developmental events helping to assess developmental modules.

784   Building on the original idea of Mabee et al. [16] of comparing sequence of

785 formation (e.g., chondrification and ossification; e.g., in [87]), few studies have proposed to

786 quantify the phenotypic patterning using correlation. Previous examples include correlation

787 of relative sequence of events [88] and correlation of neural branching patterns in the skull

788   [89]. Patterning is closely associated to the concept of developmental modularity [83,89].

789   Thus, methodologically one has to compare similar patterning to infer developmental

790   modules.

791   In our study, we are comparing our results that are methodologically constrained

792   with previous developmental modules proposed by Mabee et al. [16] for actinopterygians:

793   (1) the Endoskeleton and Exoskeleton Module (EEM) and (2) the Dorsal and Anal Fin

794   Patterning Module (DAFPM). We will discuss the evidences supporting the occurrence of

795   these modules in the median fins of the surveyed taxa, in osteichthyans, and in vertebrates

796   as a whole.

797

798   Endoskeleton and Exoskeleton Module

799   The median and paired fins of osteichthyans are constituted of two skeletons, or

800   modules, formed by distinct developmental processes: (1) the endoskeleton and (2) the

801   exoskeleton [90–92]. In extant vertebrates, and more particularly in gnathostomes, the

802   Endoskeleton and Exoskeleton Module (EEM) explains the similarities in the direction of

803   development of the endoskeleton from the paired and median fins (fin radials and girdles)

804   and the exoskeleton (fin rays). The EEM is thus composed of two interacting submodules

805   (SM): (1) the endoskeleton submodule (EnSM) and (2) the exoskeleton submodule

806   (ExSM). The EnSM as a whole probably originated at the base of the Gnathostomata with

807   the evolution of pelvic fins in 'placoderms', homologous to those of osteichthyans [54,93],

808   while the ExSM is related to the origin of lepidotrichia in osteichthyans [24,94] (Fig 12).

809

810 **Fig 12. Vertebrate phylogeny illustrating the evolution of fin related characters and**

811 **median fin modules.** A, Interrelationships of the main groups of vertebrates and

812 distribution of fin characters. Taxa in bold have living representatives. Fins have been

813 plotted in the tree according to their definition as membranous outgrowths of the body

814 walls internally supported by endoskeletal (e.g., radial bones) or exoskeletal (e.g., fin rays)

815 elements and based on consensual hypotheses of homology (see main text for a discussion

816 on the distribution of fins in the selected taxa). Preferred tree topology combined from [95–

817 97] for non-gnathostomes, [98,99] for actinopterygians, and [15,100] for sarcopterygians;

818 B, Distribution of median fin modules (DAFPM and EEM) in the studied species. DAFPM,

819 dorsal and anal fin patterning module; EEM, endoskeleton exoskeleton module; LPM,

820 lepidotrichia patterning module.

821

822   The endoskeletal components of a vertebrate fin includes series of radial bones,

823 which support both paired (pectoral and pelvic) and median (dorsal, anal and caudal) fins.

824 Endoskeletal radials have been proposed to be present in the median fins of the earliest

825 vertebrates (e.g., myllokunmingiids like *Haikouichthys*) [16,101]. However, these

826 supposedly cartilaginous structures have been reinterpreted as either epidermal folds or

827 collagenous structures [11,54,102] and the presence of true radials is thus now restricted to

828 crown vertebrates as present in the caudal fin of hagfishes, lampreys, and even conodonts

829 [11,54,103]. Radial elements (either osseous or cartilaginous) have been identified in the

830 caudal fin of many other subsequent groups of vertebrates (e.g., heterostracans, anaspids,

831 osteostracans and gnathostomes) [52], but it is not always clear whether the other median

46

832   fins (i.e., dorsal and anal) were also supported by radials. The ribbon-shaped anteroventral

833   paired fins of certain 'agnathans' (e.g., *Euphanerops*, anaspids) present numerous thin,

834   parallel radials, lacking any fin support or girdle [55], whereas in osteostracans, possibly

835   pituriaspids, and gnathostomes a few large radials articulate with a massive girdle forming

836   stout, paddle-shaped paired fins [11,54].

837   Dermal fin rays are absent in cyclostomes (hagfishes and lampreys), but they are

838   known in non-osteichthyans (e.g., in 'agnathans' like *Euphanerops* and in gnathostomes

839   like 'acanthodians' and chondrichthyans) [55,63,104,105]. However, the structure and

840   histological nature of the fin rays in early vertebrates is difficult to decipher. In

841   *Euphanerops*, the paired anteroventral fins are composed of 'fin rays' made of stacked

842   chondrocytes, which articulate with the cartilaginous radials [55]. In anaspids (e.g.,

843   *Birkenia*), the anal and caudal fin are covered with small scales and in the epichordal lobe

844   of the caudal fin the scales are arranged in rows, recalling the jointed structure of the

845   lepidotrichia in osteichthyans [52]. The small median fins of thelodonts (e.g, *Phlebolepis*)

846   are also covered by minute scales, closely stacked together and sometimes forming a fin

847   web [106], similar to the configuration of the caudal fin of the arandaspid *Sacabambaspis*

848   [58]. In osteostracans (e.g., *Escuminaspis*), the second dorsal fin is supported by numerous

849   narrow radials and covered by small scales arranged in rows [52,107]. Among

850   gnathostomes, chondrichthyans possess ceratotrichia [104,108–111], large fibrous fin rays

851   but homologous in all senses to the actinotrichia of osteichthyans [19,78,112,113].

852   Ceratotrichia have also been identified in 'placoderms' [e.g., *Bothriolepis* [114–117],

853   dunkleosteids [118], stensionellids [115]. 'Acanthodians' possess dermal fin rays with an

47

854   ossified proximal portion and a distal, non-ossified portion, which, according to Géraudie

855   and Meunier [78] correspond to large fibrous rays, similar to ceratotrichia. Wide

856   ceratotrichia may thus represent the primitive condition from which the slender

857   actinotrichia evolved in osteichthyans. Completely ossified fin rays (lepidotrichia) are

858   solely present in osteichthyans.

859   The exoskeleton submodule (ExSM) might thus have been present in the last

860   common ancestor of chondrichthyans and osteichthyans, since partially ossified fin rays

861   (i.e., potential lepidotrichia) may be present in the caudal fin of certain 'acanthodians'

862   [119,120], currently considered as stem chondrichthyans [65, 121,122]. On the other hand,

863   this module is lost in the limbs of tetrapods [91,123], in which dermal fin rays are absent

864   from the paired fins but still retained in the caudal fin of Devonian forms (e.g.,

865   *Acanthostega, Ichthyostega*) [124,125]. This pattern of lepidotrichial reduction at the

866   transition between fishes and tetrapods has been documented in *Tiktaalik* by looking at the

867   dorso-ventral asymmetry of hemirays [126]. The origin of the EEM most likely coincides

868   with the common presence of both submodules (EnSM and ExSM) in crown gnathostomes

869   (Fig. 12). Interactions between these two modules resulted in the morphological disparity in

870   terms of relative size, shape, and position of the paired and median fins.

871   Based on the sequence of nine events surveyed during *Oncorhynchus* fin

872   development, the patterning of the EEM in living actinopterygians is composed of five

873   events, all starting from the same initiation site and following the same direction: (1)

874   apparition/chondrification of radials, (2) apparition of lepidotrichia, (3) segmentation of

875   lepidotrichia, (4) ossification of lepidotrichia, and (5) ossification of proximal radials. The

48

876   ossification of distal radials is probably the sixth step, but it was not observed in our

877   juvenile specimens. Based on our observations, the EEM can thus be confidently

878   considered plesiomorphic at least in actinopterygians, since it was not possible to confirm

879   its presence in fossil sarcopterygians.

880   **Dorsal and Anal Fin Patterning Module**

881   The anatomical composition, disparity, phylogenetic distribution, and modularity of

882   fins in all orders of extinct and living fishes have been surveyed by Larouche et al. [9,11].

883   Larouche et al. [9] recognized that the dorsal and anal fins form an evolutionary module

884   nested within a median fin module. A Dorsal and Anal Fin Module (DAFM) most likely

885   evolved early in stem-gnathostomes (Fig. 12). Larouche et al. [11] suggested that fin

886   modules, including DAFM, re-expressed within the topographic boundaries of fin-forming

887   morphogenetic fields.

888   Mabee et al. [16] described the DAFPM based on a similar direction of

889   differentiation (i.e., apparition of the skeletal elements) for the dorsal and anal fins of

890   actinopterygians. However, our data allow to expand Mabee et al.'s original description of

891   the DAFPM to also include the patternings of chondrification, ossification, segmentation,

892   and bifurcation (Tables 2 and 3; Fig 3). Further evidences of the DAFPM are given on

893   extant actinopterygians by similar coordination of phenotypic plastic response between

894   both fins [37] and similar pattern of correlated shape between both fins [127]. Mabee et al.

895   [16] considered the bidirectional direction of development as plesiomorphic at least for

896   teleosts. Our data show that the sequences of all events are effectively bidirectional in

897   *Oncorhynchus* and most fossil osteichthyans (Table 3) thus suggesting that developmental

49

898   bidirectionality is most likely plesiomorphic for osteichthyans. This general bidirectional

899   development may differ in some sarcopterygians (e.g., radials in *Eusthenopteron*), most

900   likely owing to a reduction of size in both fins, but does not necessarily compromise the

901   presence of the primitively shared DAFPM among osteichthyans.

902   The Dorsal and Anal Fin Patterning Module (DAFPM) is the developmental module

903   corresponding to the anatomical DAFM of Larouche et al. [9]. In addition, Mabee et al.

904   [16] suggested that the DAFPM originated from a Dorsal and Anal Fin Positioning Module

905   (DAFPoM), one in which the antero-posterior position of the dorsal and anal fins are

906   correlated. Most likely the basal condition of the DAFPoM in actinopterygians is a

907   condition in which these median fins occupy a symmetrical position [16]; this condition is

908   observable in Devonian actinopterygians (e.g., *Dialipina, Pickeringius, Howqualepis,*

909   *Mimipiscis* [128–130]), while the anal fin is located slightly anterior to the dorsal fin in

910   *Cheirolepis* [131] or the opposite (e.g., *Gogosardina, Moythomasia, Limnomis* [132–134]).

911   Mabee et al. [16] mentioned that there was a high level of dissociability of the positioning

912   module from the patterning module among actinopterygians. Outside actinopterygians, the

913   generalized condition in actinistians (*Miguashaia*; [22]), dipnomorphs (e.g., *Holoptychius,*

914   *Quebecius, Glyptolepis, Uranolophus*; [48,49]) and tetrapodomorphs (e.g., *Cabonnichthys,*

915   *Eusthenopteron, Gyroptychius, Heddleichthys*; [50,135,136]) also corresponds to the

916   symmetrical positioning. Not only the position of the dorsal and anal fins is symmetrical,

917   but the shape, size and number of endoskeletal elements correspond to a mirror image. The

918   DAFPM of osteichthyans implies that the skeletal elements (either of endochondral origin

919   such as the radials or dermal origin such as the lepidotrichia) of both median fins (anal and

50

920   dorsal) differentiate in the same direction and thus share common developmental properties

921   [16].

922   The DAFPM is confirmed by our data in actinopterygians (*Oncorhynchus*,

923   *Elonichthys*), as well as in sarcopterygians like coelacanths (*Miguashaia*, *Rhabdoderma*),

924   porolepiforms (*Quebecius*), and 'osteolepiforms' (*Eusthenopteron*) (Tables 2 and 3). The

925   DAFPM can also be recognised in early lungfishes (e.g., *Dipterus, Barwickia*) based on

926   morphological similarities between these fins, particularly with respect to the oar-shaped

927   fin support supporting the distal radials [137]. However, due to the heterogeneity of fin

928   morphologies in dipnoans it is difficult to confidently reconstruct the evolution of the

929   DAFPM across lungfishes [17]. Consequently, a dissociation of the DAFPM is inferred

930   during dipnoan evolution. Multiple dissociations have also occurred in actinopterygians

931   every time either one of the dorsal or anal fin is absent or fused to the caudal fin (e.g.,

932   Osteoglossiformes, Anguiliiformes, Siluriformes, Lampridiformes, Sygnathiformes) [9].

933   Among other sarcopterygians non-surveyed in our study, the DAFPM can be certainly

934   inferred in onychodonts (e.g., [138]) and rhizodonts (e.g., [139]) based on the size and

935   shape similarities between both median fins. The DAFPM is lost definitely at the basis of

936   the clade including the 'elpistostegalians' and tetrapods with the loss of the dorsal fins (Fig

937   12).

Lepidotrichial Patterning Module

939   Molecular mechanisms and grafting experiments in zebrafish suggest that a

940   lepidotrichium can be grafted to a new location and grows quite normally by the addition of

941   new segments [140,141]. However, interactions among lepidotrichia are necessary to

51

942   achieve the original morphology because the position of segmentation and bifurcation are

943   dependant of the position of the lepidotrichia within fins [140,141]. This suggests a smaller

944   modular unit within the exoskeleton module (ExM), the lepidotrichium itself. The hemi-

945   lepidotrichium (or hemiray) could be the smallest unit of regeneration (and development) in

946   the hierarchical modular organization of fins [142], because developmental interactions are

947   recognized to control coordination of segmentation and bifurcation between hemirays

948   [141]. Segmentation and bifurcation patterning similarities found in this study may be

949   indicative of molecular mechanism conservatism in osteichthyans.

950   Lepidotrichia patterning in *Oncorhynchus* includes: (1) apparition, (2)

951   segmentation, (3) ossification, and (4) bifurcation. Data from fossil specimens agree with

952   this sequence. Therefore, the patterning of the lepidotrichia appears conserved in

953   osteichthyans and a "Lepidotrichia Patterning Module" (LPM) may be generalized in all

954   the fins of all osteichthyans.

955   The molecular basis behind the establishment of segmentation has been explored by

956   using regenerative experiments in zebrafish. Part of the molecular machinery involved in

957   segmentation is recruited for lepidotrichia bifurcation [18,143]. Molecular transcripts

958   involved in segmentation are: (1) *evx1* acts as an on/off switch defining the putative

959   boundaries between two successive segments [28], mutant zebrafish for this gene grow

960   normal lepidotrichia, but joint formation between successive segments is impaired [38]; (2)

961   three genes of the Sonic Hedge Hog signalling pathway (i.e., *shh, bmp2, ptc1*) are thought

962   to be involved in the patterning of the lateral limits of segments [18]; (3) *hoxa13b* may

963   participate in the elaboration of the next segment [144]; and (4) *cx43*, a particular gene

964   allowing intercellular communication involved in joint position [145].

965   Segmentation and bifurcation processes might be independently regulated. In *evx1*

966   zebrafish mutants, bifurcation occurs normally while joint formation is down-regulated

967   [38]. At least two genes are expressed during the bifurcation process: (1) prior to

968   bifurcation, *shh* is expressed centrally where a new segment is forming, then laterally in the

969   presumptive twin-segments [18,143], and (2) *bmp2* (required for bone synthesis in the

970   central region of the segment) is restricted in the two lateral domains copying *shh* prior to

971   branching [18]. Fossil evidence shows that bifurcation likely originated at the base of

972   osteichthyans. One notable exception is the puzzling *Dialipina salgueiroensis,* which lacks

973   bifurcation (RC, pers. obs.); *Dialipina* is either considered a stem actinopterygian [146] or

974   a stem osteichthyan [24]. The absence of bifurcation is highly homoplastic in living

975   [32,147] and fossil actinopterygians as well as in some sarcopterygians (e.g., it constitutes a

976   common derived feature of post-Devonian coelacanths) [148]. Because bifurcation is the

977   last event of the lepidotrichia developmental sequence, it might be more susceptible to be

978   affected by epigenetic phenomena [37].

979

Conclusions

981   Our analysis of median fin development in *Oncorhynchus* has allowed the

982   quantification and validation of two median fin modules in a living actinopterygian: the

983   Dorsal and Anal Fin Patterning Module (DAFPM) and the Endoskeleton and Exoskeleton

984   Module (EEM). Comparison with other extinct osteichthyans, comprising both

985   actinopterygians and sarcopterygians (coelacanths, dipnoans, porolepiforms and

986   'osteolepiforms') has corroborated the data on extant taxa, but highlighted the difficulties

987   of confidently identifying developmental sequences based on fossil specimens. The

988   DAFPM and EEM modules incorporate all the events associated with fin patterning

989   including the sequences of segmentation and bifurcation of the lepidotrichia that are crucial

990   for comparisons and inferences of developmental sequences in fossil osteichthyans.

991   We suggest that: (1) the EEM includes the apparition, segmentation, and

992   ossification sequences, and, based on our results, is plesiomorphic at least for

993   actinopterygians; (2) the DAFPM includes the apparition, chondrification, and ossification

994   sequences plus the segmentation and bifurcation sequences, and is plesiomorphic for

995   osteichthyans with multiple dissociations along osteichthyan phylogeny. Additionally, the

996   recurrence of the developmental pattern of the lepidotrichia in living and fossil

997   osteichthyans suggests an additional developmental module within fins, the Lepidotrichia

998   Patterning Module (LPM), where the constitutive units, the hemirays, have a synchronous

999   and similar development. The median fins of osteichthyans have thus been shown to be

1000   important representatives for the study of modularity across the evolution of vertebrates.

1001

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54

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1012

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Supporting information

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1403

1404 **S1 Table. Fossil specimens examined given in size order.** For each species, the size

1405 series is given with SL or TL (mm) and specimen number. Only *D. valenciennesi* is not

1406 represented by immature specimens.

1407

1408 **S1 File. Coding of the serial skeletal elements, i.e. the proximal radials (PR), the distal**

1409 **radials (DR) and the lepidotrichia (L), during nine out of eleven developmental events**

1410 **of the developmental sequence of the dorsal and anal fins of *Oncorhynchus mykiss*.** For

1411 each specimen, the size is given in SL (mm) and the skeletal elements are coded with 0, 1 or

1412 NA. The definitions of these codes are different for each developmental event, they are

1413 provided.

1414

A

L07
L06
L05
PR01
PR14
10 mm

B

PR01
L04
L05
L06
PR12
5 mm

C

seg
PR3
PR4
5 mm

D

PR3
PR4
5 mm

A

Caud. f

Dors. f (b)

An. f (c)

Pelv. f   Pect. f

5 mm

B

seg

DR

PR

L

1 mm

C

PR

L

seg

1 mm

A

Dors. f 1

Dors. f 2 (b)

Caud. f

An. f (c)

5 mm

B

L15 (bif)

seg

1 mm

C

L17 (bif)

L15 (bif)

seg

L14 (bif)

1 mm

A

Dors. f 1

Dors. f 2 (b)

Caud. f

Pect. f.

Pelv. f.

An. f (c)

10 mm

B

L4 (bif)

seg

L12 (bif)

L14(bif)

5 mm

C

L10 (bif)

L11 (m.seg)

seg

L6 (bif)

5 mm

A

Dors. f (b)

Caud. f

Pelv. f   An. f (c)

5 mm

B

seg

1 mm

C

seg

L

1 mm

A

Dors. f 1 Dors. f 2 (b) Caud. f

An. f (c)

Pect. f

10 mm

B

seg L19 (bif 1)

Sc

L34 (bif 2)

L34 (bif 1)

L37 (bif)

C

Sc

5 mm

L16 (bif)

L13 (bif 1)

L13 (bif 2)

L13 (bif 3)

seg L11

5 mm

A
Caud. f
Dors. f 2 (b)
Dors. f 1
An. f (c)
Pelv. f
Pect. f
10 mm

B

seg
L1
DR
Sc
BPL
L17(bif 2)
L17 (bif 1)
5 mm

C
DR
L17 (bif 3)
L17 (bif 2)
L17 (bif 1)
BPL
Sc
L1
seg
5 mm

A

dors. f (b)

caud. f

pect. f

pelv. f

an. f (c)

B

seg

Act

L01

DR1

PR1

PR2

bif

DR13

L17

PR14

C

PR14

DR12

L16

PR2

PR1

DR1

L01

Act

seg

bif