UNIVERSITÉ DU QUÉBEC À RIMOUSKI

Formation de patron et modularité des canaux sensoriels de la ligne latérale chez le poisson-castor, *Amia calva* (Actinopterygii : Amiiformes)

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RÉSUMÉ

La modularité du crâne et l'intégration morphologique entre les systèmes anatomiques sont fondamentales en biologie évolutive du développement pour expliquer la diversité de plans d'organisation. Une étude récente au niveau des vaisseaux sanguins et des os du crâne a montré que la modularité est déterminée par la région anatomique et qu'elle n'est pas limitée à un système. L'interaction entre les os dermiques et les canaux de la ligne latérale est débattue depuis un siècle. Cependant, la modularité des canaux de la ligne latérale et leur intégration morphologique avec les os dermiques n'ont jamais été décrites. Une série ontogénétique de 97 spécimens de l'actinoptérygien basal *Amia calva* a permis de décrire l'ontogénie et la modularité des canaux de la ligne latérale ainsi que leur intégration avec les os crâniens. Les crânes ont été visualisés à l'aide d'un micro-CT scan. Le patterning des ramifications des groupes de pores du canal otique et de la commissure supratemporale a été décrit en codant la présence ou l'absence de chaque branche de manière à tester la présence de modules développementaux par la corrélation de Spearman dans l'ordre d'apparition des ramifications pour chaque groupe de pores. Des points de repères morphométriques ont été posés sur 25 spécimens afin de tester des modules variationnels au niveau des canaux et l'intégration entre les canaux et les os en variation individuelle et en asymétrie aléatoire. Les ramifications suivent un patterning prédéterminé, supporté par la corrélation dans l'ordre d'apparition des bifurcations entre le côté gauche et droit. Des différences dans le patterning et le test de modularité basé sur la corrélation entre les groupes de points de repères suggèrent que le canal otique et la commissure supratemporale sont deux modules séparés. L'intégration entre les os et les canaux est insuffisante pour être détectée comme un module. Cependant, les deux blocs ne sont pas indépendants ajoutant du support à l'hypothèse de l'interaction développementale entre les os dermiques et les canaux sensoriels. Les groupes de pores ont tendance à disparaître sans affecter les autres groupes de pores suggérant qu'ils sont des unités quasi-indépendantes remplissant un critère pour la modularité. La modularité des canaux sensoriels suit une organisation hiérarchique qui s'explique par les étapes et les interactions développementales de la formation du système de la ligne latérale.

Mots clés : évo-dévo, modularité développementale, modularité variationnelle, canaux sensoriels, ligne latérale, os dermiques, *Amia calva*, patterning, morphométrie géométrique

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ABSTRACT

Skull modularity and morphological integration among anatomical systems are fundamental in evolutionary developmental biology in order to explain the diversity of body plan. A recent study on cranial vasculature and the bony skull has shown that modularity is determined by the anatomic region and thus not limited to a system. The interaction between dermal bones and lateral line canals has been debated for 100 years. However, modularity of lateral line canals and their morphological integration with dermal bones have never been described. An ontogenetic series of 97 specimens of the basal actinopterygian *Amia calva* was acquired to describe the ontogeny and modularity of lateral line canals and their integration with supporting cranial bones. Skulls were visualized with a micro-CT scan. Ramification patterning of groups of pores in the otic canal and the supratemporal commissure were described by coding the presence or absence of each branch in order to test for developmental modules by calculating Spearman rank correlations in order of appearance of ramifications between groups of pores. Landmarks were digitized on 25 specimens to test for variational modules at the level of canals and integration between canals and bones in individual variation and fluctuating asymmetry. Ramifications follow a particular patterning as supported by significant positive correlation in the order of appearance of bifurcation between sides. Differences in patterning and the test of modularity based on correlation between subset of landmarks suggest that the otic canal and the supratemporal commissure are two separate modules. The integration between bones and canals was insufficient to be detected as a module. However, the two blocks were not independent adding support to the hypothesis of developmental interaction between dermal bones and sensory canals. Groups of pores tended to disappear without affecting other groups of pores suggesting that they are quasi-independent units which fill a criterion for modularity. The hierarchichal organisation of modularity in sensory canals is explained by the developmental stages and interactions of the lateral line system formation.

Keywords : evo-devo, developmental modularity, variational modularity, sensory canals, lateral line, dermal bones, *Amia calva*, patterning, geometric morphometrics

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LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

CMN   Canadian Museum of Nature, Musée canadien de la nature, Gatineau (Qc)

DFA   Discriminant function analysis, Analyse discriminante

dis2   Distance entre le groupe de pores 2 et l'intersection entre le canal otique et la commissure supratemporale

dis2.5   Distance entre le groupe de pores seul et l'intersection entre le canal otique et la commissure supratemporale

dis3   Distance entre le groupe de pores 3 et l'intersection entre le canal otique et la commissure supratemporale

Dpt   Dermoptérotique

Dsp   Dermosphénotique

ew   Extrascapular width, largeur de l'extrascapulaire

Exsc   Extrascapulaire

FA   Fluctuating asymmetry, asymétrie aléatoire

FMNH   Field Museum of Natural History, Chicago (IL, USA)

GP16   Groupe de pores 16 du canal otique

GP18   Groupe de pores 18 du canal otique

GP2   Groupe de pores 2 de la commissure supratemporale

GP3	Groupe de pores 3 de la commissure supratemporale
L	Left, côté gauche
lmk	landmarks
MDDEFP	Ministère du développement durable, de l'environnement, de la faune et des parcs, Longueuil (QC)
MOD	Analyse de modularité
otc	canal otique
p.otc	partie postérieure du canal otique
Pa	Pariétal
PLS	Partial Least Square
Po2	Postorbitaire 2
Pp	Postpariétal
Pt	Posttemporal
QTL	Quantitative trait loci, Locus de caractères quantitatifs
R	Right, côté droit
r_s	Coefficient de corrélation de Spearman
SL	Standard lenght, Longueur standard
SL_{50}	Longueur standard à laquelle 50 % des individus ont développé la structure d'intérêt
stc	commissure supratemporale

$\mathbf{V}_a$ Vertex de type a, lie deux vertex terminaux

$\mathbf{V}_b$ Vertex de type b, lie un vertex terminal

$\mathbf{V}_c$ Vertex de type c, ne lie aucun vertex terminal

INTRODUCTION GÉNÉRALE

La biologie évolutive du développement est une discipline centrale à la compréhension de l'évolution, puisque la variation disponible à la sélection naturelle est structurée par le développement en étant contrainte ou biaisée par des phénomènes tels que la modularité (Hendrikse *et al.*, 2007). La modularité est la propriété des organismes à être composés de sous-unités quasi-indépendantes : les modules (Raff, 1996). Un module se définit comme une unité quasi-indépendante ayant des composantes fortement intégrées par rapport à un processus tel que le développement ou la fonction (Wagner *et al.*, 2007). La modularité facilite l'évolution en diminuant la probabilité qu'une mutation bénéfique pour certains traits ait des effets néfastes sur les autres traits (Hendrikse *et al.*, 2007), ce qui a amené les modules à être considérés comme des unités d'évolution (Schlosser, 2002a; Schlosser, 2002b; Schlosser, 2005). De plus, les modules peuvent être dupliqués et diverger, de même que subir une cooptation, c'est-à-dire un changement de fonction (Raff, 1996). C'est ce qui explique que les modules sont souvent répétés et conservés, puisqu'il est plus probable que l'apparition d'une nouveauté découle d'une cooptation de module plutôt qu'elle apparaisse *de novo* (Winther, 2001).

Il existe plusieurs types de modularité qui sont souvent définis par le processus qui est à l'origine de l'intégration. Deux types de modularité seront abordés dans cette étude : le module développemental et le module variationnel. Le module développemental se caractérise par des traits qui sont quasi-autonomes au niveau de la formation et de la différenciation de leur patron (Raff, 1996) et il est typiquement validé par la corrélation dans l'ordre d'apparition des structures (Poe, 2004) et le patterning. Un module variationnel est quant à lui formé de traits qui covarient fortement entre eux, mais qui covarient très peu avec les éléments appartenant à d'autres modules (Wagner et Mezey, 2004). Cette définition sert aussi de critère d'identification pouvant être validé par des

méthodes basées sur la morphométrie géométrique telles que le coefficient *RV* (Klingenberg, 2009) et l'analyse des moindres carrés partiels (Rohlf et Corti, 2000). Ces méthodes font appel à l'intégration morphologique qui est fortement liée à la modularité puisqu'elle réfère à un patron de covariation (revu par Klingenberg, 2008).

La modularité a été largement étudiée relativement aux structures osseuses (ex. Hallgrímsson *et al.*, 2002; Mabee *et al.*, 2002; Ward et Brainerd, 2007; Márquez, 2008; Grünbaum *et al.*, 2012), mais très peu au niveau des tissus mous. De plus, la modularité, ou l'intégration morphologique, a été peu démontrée au niveau de l'interaction entre les tissus. Quelques auteurs (Richtsmeier *et al.*, 2006; Zelditch *et al.*, 2008; Jamniczky et Hallgrímsson, 2011) ont vérifié l'interaction entre les tissus au niveau de la tête, une structure complexe pour laquelle l'évolution ne peut être comprise que par l'intégration de toutes ses structures (Jamniczky et Hallgrímsson, 2011). À notre connaissance, aucune étude ne s'est attardée à l'intégration entre le système sensoriel et le crâne.

Le système de la ligne latérale est un système sensoriel présent chez les poissons et les tétrapodes non-amniotes (Northcutt, 1989). Il est composé d'organes, les neuromastes, pouvant être superficiels ou entourés de canaux, présents sur le crâne ou le tronc (Northcutt, 1989). Les neuromastes sont composés de cellules ciliées qui détectent les changements de pression dans l'eau associés aux mouvements variant en amplitude, de grands courants océaniques jusqu'aux mouvements du plancton (Coombs et Montgomery, 1999). Le système de la ligne latérale a une grande importance écologique. Il est associé au comportement de banc, d'évitement des prédateurs et d'alimentation (Coombs et Montgomery, 1999).

Le système de la ligne latérale n'a pas seulement une importance écologique, mais aussi une importance en phylogénie, puisque la trajectoire de la ligne latérale est utilisée pour déterminer l'homologie des os (Graham-Smith, 1978) et comme caractère phylogénétique (Cloutier et Ahlberg, 1996). Cette utilisation s'explique par le mode de croissance des os dermiques. Chez le poisson-castor *Amia calva*, l'enfoncement des

neuromastes dans le derme est suivi par l'ossification des parois formant ainsi des sections de canaux sensoriels. Il en résulte une correspondance entre la position du canal et le centre d'ossification de l'os dermique (Allis, 1889; Parrington, 1949). Les sections de canaux fusionnent et forment un pore à la surface de l'os. Chez les espèces avec des canaux ramifiés, le pore se subdivise pour former des groupes de pores ressemblant à des structures arborescentes. Très peu d'études se sont consacrées à la description des ramifications du système sensoriel, probablement à cause de son allure aléatoire. Cependant, des études dans d'autres systèmes biologiques ramifiés, tels que les cellules de Purkinje (Berry et Flinn, 1984; Berry, 1992), les végétaux (Stein, 1993; Stein et Boyer, 2006) et les bryozoaires (Kruszyński *et al.*, 2007) ont montré qu'il est possible de trouver un certain ordre dans les structures arborescentes d'apparence aléatoire. La description du développement des ramifications au niveau du système de la ligne latérale permettra de vérifier si le développement est ordonné et reproductible.

Bien que la modularité des canaux sensoriels n'ait jamais été vérifiée, plusieurs niveaux de modularité ont été reconnus à différentes échelles de la ligne latérale. Le neuromaste, la plus petite unité du système de la ligne latérale, serait un module (Franz-Odendaal et Hall, 2006). La modularité a aussi été identifiée à quelques reprises dans la partie postcrânienne de la ligne latérale basé sur les QTLs (locus de caractères quantitatifs) et les processus développementaux (Ledent, 2002; Wark *et al.*, 2012). Dans la partie crânienne, la modularité a été identifiée au niveau des placodes qui sont des épaississements de l'ectoderme responsables de la formation des neuromastes et de leurs neurones sensoriels (Schlosser, 2002c). Six paires de placodes sont présentes chez les gnathostomes (Northcutt, 1997). Puisque les placodes sont responsables de la formation des neuromastes et que ceux-ci seraient, à leur tour, responsables de la formation des canaux, nous avons posé l'hypothèse que les canaux soient aussi des modules. Dans la partie postérieure du crâne, trois placodes sont responsables de la formation des neuromastes de trois sections de canal (Northcutt, 1997). La placode otique de la ligne latérale permet le développement des neuromastes de la partie antérieure du canal otique. Les neuromastes de la partie postérieure du canal otique proviennent plutôt de la placode moyenne. Finalement,

la commissure supratemporale se forme à partir des placodes supratemporales gauche et droite. Dans la partie postérieure du crâne sous l'action de ces trois placodes, trois modules développementaux sont attendus au niveau des canaux. Ces trois modules ont été testés par des corrélations entre le patterning des ramifications appartenant aux différents canaux. Le patron de correlation entre des groupes de points de repère (*landmarks*) sera aussi utilisé pour valider cette hypothèse de modularité, mais il permettra de la tester en tant que module variationnel. Des liens développementaux se traduisent par une forte covariation à l'intérieur des éléments d'un module (Klingenberg, 2008). Les deux types de modules ne sont donc pas indépendants.

L'intégration entre le crâne et les artères a été validée chez la souris suggérant que la modularité est délimitée par la région plutôt que par les processus développementaux (Jamniczky et Hallgrímsson, 2011). L'interaction entre le crâne et les canaux sensoriels a été débattue et les liens développementaux entre les neuromastes et les os dermiques sont inconnus (Graham-Smith, 1978; Webb et Noden, 1993; Tarby et Webb, 2003). La connaissance de la nature de cette interaction est essentielle afin d'expliquer la variation dans la présence/absence des canaux et la variation dans leur association avec différents éléments dermiques. De plus, l'évaluation de cette interaction est essentielle dans un contexte phylogénétique où les éléments sont caractérisés selon leur homologie (Graham-Smith, 1978; Tarby et Webb, 2003). Elle sera vérifiée par le patron de correlation entre les groupes de points de repères évalué par le coefficient RV (Klingenberg, 2009) et l'analyse des moindres carrés partiels (Rohlf and Corti, 2000).

Cette étude est la première à quantifier le patterning des ramifications du système de la ligne latérale, à identifier la modularité au niveau des canaux sensoriels, à vérifier l'intégration entre le système sensoriel et le crâne et à utiliser deux méthodes pour vérifier la modularité. Les objectifs principaux de cette étude sont de décrire la modularité au niveau des canaux sensoriels et la modularité entre les canaux sensoriels et les os dermiques. Secondairement, la vérification du patterning au niveau des ramifications vise à favoriser leur utilisation en phylogénie. Cette étude apportera aussi de l'information quant à

la nature de l'interaction entre les différents niveaux hiérarchiques de la modularité (placodes vs. canaux) et entre la modularité développementale et variationnelle.

Le développement des ramifications a été décrit par une méthode d'identification développée dans le cadre du présent mémoire. La corrélation dans l'ordre d'apparition des ramifications a permis de vérifier que le développement des ramifications est symétrique. L'utilisation du patterning et du test de modularité à partir de points de repères soit par le coefficient RV et l'analyse de moindres carrés partiels ont permis d'identifier deux modules : le canal otique et la commissure supratemporale. L'analyse des moindres carrés partiels n'a pas validé l'hypothèse des modules basés sur l'interaction entre les os dermiques et les canaux, mais elle a permis de détecter une covariation entre les deux composantes.

CHAPITRE 1
LES BLOCS DE CONSTRUCTION DE LA TÊTE D'UN POISSON : MODULARITÉ DÉVELOPPEMENTALE ET VARIATIONNELLE DANS UN SYSTÈME COMPLEXE

Caroline Lehoux et Richard Cloutier

1.1 RESUME EN FRANÇAIS DE L'ARTICLE

La modularité du crâne et l'intégration entre les systèmes anatomiques crâniens sont fondamentales en biologie évolutive du développement afin d'expliquer la diversité de plans d'organisation. L'interaction entre les os dermiques et les canaux de la ligne latérale a été débattue durant des décennies, mais la modularité du système sensoriel n'a jamais été testée. Une série ontogénétique de 97 juvéniles et adultes du poisson-castor *Amia calva* (Actinopterygii : Amiiformes) a été utilisée afin de décrire l'ontogénie et la modularité des canaux sensoriels de la ligne latérale et leur intégration avec les os crâniens. Le patterning des ramifications des groupes de pores de deux canaux sensoriels (le canal otique et la commissure supratemporale) a été décrit de façon à tester la présence de modules développementaux par la corrélation dans l'ordre d'apparition des ramifications pour chaque groupe de pores. Des points de repères ont été posés sur 25 spécimens afin de tester la présence de modules variationnels au niveau des canaux et l'intégration entre les canaux et les os. Les ramifications suivent un patterning particulier qui est supporté par la corrélation dans l'ordre d'apparition des bifurcations entre le côté gauche et droit. Des

différences dans le patterning entre le canal otique et la commissure supratemporale et les tests de modularité par la morphométrie géométrique suggèrent que les deux canaux sont deux modules séparés. L'intégration entre les os et les canaux est insuffisante pour être détectée comme un module. Cependant, les deux blocs ne sont pas indépendants. Les groupes de pores ont tendance à disparaître sans affecter les autres groupes de pores suggérant qu'ils sont des unités quasi-indépendantes remplissant un critère pour la modularité. La modularité du système sensoriel pourrait être utilisée pour expliquer la variation dans le patron des canaux et leur association avec les os dermiques entre les espèces.

L'article sera soumis durant l'hiver 2014 au *Journal of Experimental Zoology: Part B Molecular and Developmental Evolution*. En tant que premier auteur, j'ai réalisé l'ensemble des analyses. J'ai élaboré et choisit les méthodes d'analyses en collaboration avec mon directeur. Mon directeur, le deuxième auteur, a eu l'idée originale de ce projet et a révisé cet article.

1.2 Building blocks of a fish head: Developmental and variational modularity in a complex system

1.3 Abstract

Skull modularity and integration among cranial anatomical systems is fundamental in evolutionary developmental biology in order to explain the diversity of body plan. The interaction between the dermal bones and lateral line canals has been debated for decades but modularity has never been tested in the sensory system. An ontogenetic series of 97 juvenile and adult *Amia calva* (Actinopterygii: Amiiformes) was used in order to describe the ontogeny and modularity of sensory lateral line canals and their integration with supporting cranial bones. Ramification patterning of groups of pores in two sensory canals (otic canal and supratemporal commissure) was described in order to test for developmental modules by computing correlations in appearance order between groups of pores. Landmarks were digitized on 25 specimens to test for variational modules at the level of canals and integration between canals and bones. Ramifications follow a particular patterning as supported by significant positive correlation in the order of appearance of bifurcations between sides. Differences in patterning between the otic canal and the supratemporal commissure and tests of modularity with geometric morphometrics suggest that the two canals are two separate modules. The integration between bones and canals was insufficient to be detected as a module. However, the two blocks were not independent. Groups of pores tended to disappear without affecting other groups of pores suggesting that they are quasi-independent units acting as modules. Modularity of the sensory system could be used to explain variation of pattern of canals and their association with dermal bones among species.

1.4 INTRODUCTION

Modularity is an important property of organisms responsible for a considerable amount of the diversity of body plans (Raff, '96). Modularity has been the focus of many studies taking into account primarily osteological structures such as fins (Mabee et al., 2002; Grünbaum et al., 2012), limbs (Hallgrímsson et al., 2002; Goswami et al., 2009), vertebrae (Ward and Brainerd, 2007), skull (Hallgrímsson et al., 2004; Goswami, 2006), and jaw (Márquez, 2008; Zelditch et al., 2008). However, studies regarding interactions between osteological structures and soft tissues are scarce and limited to the muscular (Zelditch et al., 2008), vascular (Jamniczky and Hallgrímsson, 2011) and nervous (Richtsmeier et al., 2006) systems. All of these integrative studies focused on the head; a very complex system whose evolution can only be understood by the integration of all its components (Jamniczky and Hallgrímsson, 2011). Indeed, modularity is not limited to tissue type but is instead defined by epigenetic interactions within a region as supported by the interaction between the skull and cranial arteries in mice (Jamniczky and Hallgrímsson, 2011). However, morphological integration of the sensory system and the skull has never been tested. The lateral line system is incorporated in the skull by sensory canals that go through dermal bones in lower vertebrates.

The lateral line system of lower vertebrates has a mechanoreceptive function performed by neuromasts whose hair cells register water movements (Webb, 2000). Neuromasts were consequently described as distant-touch receptors (Dijkgraaf, '63). Neuromasts can be either superficial or embedded to form lines, either pits or canals on the head and trunk most frequently associated to dermal elements, such as certain cranial bones and scales (Northcutt, '89). The lateral line comprises two major topological components including neuromasts and their associated sensory neurons: (1) the anterior lateral line present on the head region and (2) the posterior lateral line present on the trunk and caudal fin (Gompel et al., 2001). The anterior lateral line includes a series of six pairs of main cranial lines and two commissures in gnathostomes: supraorbital, infraorbital, otic

(including post-otic), oral, mandibular and preopercular lines, and supratemporal and ethmoid commissures (Northcutt, '89).

The sensory system including the lateral line develops from ectodermal thickenings called placodes. Six pairs of lateral line placodes are responsible for neuromasts and sensory nerves formation in gnathostomes (Northcutt, '97). The migrating primordium which originated from the placode deposits clusters of cells fated to become neuromasts at regular intervals (Ledent, 2002). A sequence of three further developmental stages is necessary to form cranial and postcranial canals: (1) embryonic patterning of neuromasts and sensory neurons, (2) differentiation and growth of neuromasts and (3) morphogenesis of lateral line canals (Webb, 2000).

Modularity has been suggested at multiple levels of the lateral line system. Firstly, lateral line placodes are recognized as developmental modules because they have autonomous patterning, morphogenesis and differentiation even at ectopic locations (Schlosser, 2002a). Lateral line placodes also behave as units of evolutionary variation because their derivatives are coherently modified (Schlosser, 2002b). Secondly, modularity has been suggested in the zebrafish, *Danio rerio,* because the postembryonic growth, during which secondary neuromasts develop, occurs with the reiteration of the same elementary process as the embryonic growth (Ledent, 2002). Thirdly, modularity has been identified in the lateral line system of sticklebacks, *Gasterosteus aculeatus*, because QTLs (quantitative trait loci) that affect lateral line morphology differ between lines of neuromasts (Wark et al., 2012). However, no study tested for modularity in cranial canals.

Canal development has long been suggested to be related to neuromasts (Pehrson, '22). While not observed in all fishes, canal position usually corresponds with the center of ossification of dermal bones which suggests a close integration between the dermal and sensory systems (Parrington, '49; Cloutier, '97). However, the relation between dermal bones and the lateral line system has been debated in some teleosts (Westoll, '41; Graham-Smith, '78) because ossification patterns of canals differ among phylogenetic groups

(Tarby and Webb, 2003). In some fishes such as the bowfin *Amia calva* and some teleosts, ossification begins around neuromasts to form a canal, the tubular lateral line component. Later in development, lateral extensions expend to form the dermal bone (Allis, 1889; Pehrson, '22; Pehrson, '40). In some teleosts such as the zebrafish, the neuromasts sit on underlying dermal bone that extend upwards and encloses neuromasts to form a canal (Webb and Shirey, 2003). Despite the ossification patterns being described, the developmental influence of neuromasts remains untested (Webb and Noden, '93). Moreover, canals form without neuromast as it is in the whitespotted greenling, *Hexagrammos stelleri*, suggesting that neuromasts are not required for ossification (Wonsettler and Webb, '97). Nevertheless, Wada et al. (2010) suggested that patterns of secondary neuromasts produced by serial budding during postembryonic growth in zebrafish are controlled by underlying dermal structures. The presence of modularity could confirm a close association in development between the lateral line component and dermal bones.

Following the ossification of canal walls, canal sections fuse to form pores that open to the bone surface (Allis, 1889). In species with branched canals, this pore subdivides and forms ramifications (Webb, 2000). The ramifications in *A. calva* are divided by groups of pores formed by the junction between two canal segments. Allis (1889) observed that ramifications have a constant relation to dermal bones as the canals. Ramifications were briefly described in *A. calva* (Allis, 1889), coelacanth *Latimeria chalumnae* (Hensel and Balon, 2001), and the Japanese Spanish mackerel *Scomberomorus niphonius* (Nakae et al., 2013) but ramification development is unknown probably because of the seemingly random appearance of branching patterns. Nevertheless, ramifications of other biological systems have been described. Berry ('84; '92) described the ramification growth in Purkinje cells based on the ratio between the terminal and link branches. In plants, algorithms have been used to reproduce branching patterns of leaf venation (Runions et al., 2005), stellar architecture (Stein, '93) and branches (Stein and Boyer, 2006). Simulation results in these studies were reminiscent of patterns observed in extinct and extant species. Moreover, the simulated leaf venation pattern was obtained by the reiteration of the same elementary

process involving auxin distribution (Runions et al., 2005) These studies conducted at the cellular to organismal levels suggest that an underlying order can be found in apparent chaotic structures. Thus, an apparent random bifurcation of sensory canals could be refuted by the description of their patterning.

The developmental origin of sensory canals and their position into different dermal bones offer an opportunity to test a combination of hypotheses concerning developmental and variational modularity in the postembryonic growth of the lateral line system. We investigated modularity in the otic canal and supratemporal commissure of *A. calva* (Fig. 1). The otic canal has two parts that are delimited by the preopercular canal. The posterior section of the otic canal has also been named the temporal (Northcutt, '97) or post-otic canal (Jarvik, '80; Northcutt, '89). The two parts of the otic canal come from two different lateral line placodes: the otic and the middle lateral line placodes (Northcutt, '97). The supratemporal commissure originates from the supratemporal lateral line placodes (Northcutt, '97). We expected those three sections of canals developing from different placodes to be three different modules since placodes were recognized as developmental modules (Schlosser, 2002a), where a developmental module is characterized by traits that are quasi-independent in terms of pattern formation and differentiation (patterning) (Raff, '96). Developmental modularity is tested by measuring correlation in ramification patterning (Poe, 2004). The hypothesis of three modules according to placodes was also tested as variational modules which include phenotypic traits that are integrated and quasi-independent of other such clusters resulting in high covariation among elements of a module (Wagner et al., 2007). Variational modularity is typically measured by geometric morphometrics (Wagner and Mezey, 2004) which was also used to test for integration between dermal bones and canals. Our study is the first, to our knowledge, to test for two different kinds of modularity by using two different methods.

1.5 MATERIAL AND METHODS

1.5.1 SPECIMENS AND IMAGING

An ontogenetic series of 97 juvenile and adult *A. calva* specimens was acquired through museum collections and commercial fisheries (Table 1). Since development of the lateral line system is correlated with size rather than age (Münz, '86; Ledent, 2002), standard length (SL) was used to reconstruct the ontogenetic series. Specimens were photographed in lateral view in order to measure the SL with CTAn (Version 1.11.4.2, SkyScan, Bruker-microCT, 2011, Belgium). SL varied between 15 mm and 545 mm ($\bar{x}$ = 194 mm; SD = 100 mm). Measurement error was less than one millimetre between two measurements for each specimen. Specimens acquired through fisheries were measured unfrozen, whereas collection specimens were already fixed in formalin and preserved in ethanol when measured. SL of one dry skull specimen was estimated using the linear regression between parietal bones (Pa) length and SL for five specimens of similar size (SL = 0.0705*Pa + 5.93; R^2 = 0.95). Specimens from fisheries were decapitated, fixed in 4 % neutralized formalin for 24 hours and preserved in 70 % ethanol.

Bones, sensory canals, and related ramifications were imaged with a micro-CT scan, SkyScan 1173 (Bruker-microCT, 2011, Belgium). Specimens were preferably scanned while fresh but 42 specimens were scanned after fixation/preservation and three were dry skulls. Fixation/preservation did not affect relative position of components inside the bones (see Appendix 1). Because of important differences in specimen size, the scanning protocol had to be adapted for each skull. For example, 200-mm SL specimens were scanned with an aluminium filter at 100 kV, 80 µA, with an exposure of 830 ms and a rotation step of 0.2°. The scanner was set to 55 kV, 100 µA, 1400 ms with no filter for smaller specimens (< 100 mm) and set to 100 kV, 98 µA, 1600 ms with a brass filter for larger specimen (> 300 mm). Resolution was set between 15 and 30 µm. Projection images were reconstructed using NRecon (Version 1.6.6.0, SkyScan, Bruker-microCT, 2011, Belgium).

1.5.2 PATTERNING OF RAMIFICATIONS

This study focuses on groups of pores (GP) 16 and 18 of the otic canal, and GP2-3 of the supratemporal commissure (Fig. 1); nomenclature of groups of pores follows Allis (1889). These groups of pores allow testing modularity hypotheses regarding their developmental identity via placodes while taking into account their proximity and their interactions with dermal bones. GP1 of the supratemporal commissure was not included in the analyses because it is not comparable to other groups of pores. It has very few ramifications until an advance stage of development, and according to Allis (1889), it develops as a single median group of pores that subdivides to form one group of pores on each extrascapular. Individuals that expressed variation in the presence/absence of certain groups of pores associated with the supratemporal commissure were pooled in order to test for the identity of the missing group of pores. When one of the GP2-3 was absent, the remaining group of pores was identified as 2.5. The distance between the group of pores and the otic canal-supratemporal commissure junction (dis3, dis2 and dis2.5), and the extrascapular width (ew) were measured for both sides of the skull. Measurements were made with CTAn on 2D images digitized with CTVox (Version 2.2.0, SkyScan, Bruker-microCT, 2011, Belgium). To remove size and magnification effect, statistical analyses were performed on the dis/ew ratio for GP2, GP3 and GP2.5. The effect of allometry was tested by a multiple regression of the dis/ew ratio on size. The null hypothesis of isometry was not rejected by the analysis of variance of the regression ($P = 0.6$). A Kruskal-Wallis analysis was used to test for differences in relative position of GP2, GP3 and GP2.5. To verify if GP2.5 occupies an intermediate position between GP2 and GP3, a Student t-test was used to compare its position with the mean position of GP2 and GP3. In order to test if groups of pores tended to be absent on both sides equally, the G-test was used, with expected frequencies of 0.5 for both sides.

In order to describe the development of ramifications, the groups of pores were schematized. Specimens > 400 mm were viewed with Seg3D (SCI Institute, 2007), a freeware that provides tools for volume segmentation. Ramifications were segmented by a

paintbrush tool and the isosurface was computed to describe their pattern. Ramifications were fewer in smaller specimens and were thus visualized in sections with CTAn and in volume rendering with CTVox. Because size classes were not equally represented, two separate analyses were conducted. Patterning was described for specimens ≤ 411 mm, whereas the spatial layout was described for specimens ≥ 411 mm. Moreover, because ramifications are visualised by X-rays, terminal branches in skin could not be visualized.

For specimens ≤ 411 mm, each bifurcation was numbered clockwise starting at the root and progressing toward terminal orders (Fig. 2). Ramifications are branching structures that can be treated as trees. An order is the number of branches that separates a given branch from the root. A ramification of a given order that bifurcated to the left was numbered $2n\text{-}1$, and $2n$ if it bifurcated to the the right, where n is the number of the ramification of the previous order. The right side of each specimen was inverted so that numbers were equivalent on both sides. Each numbered bifurcation was then coded 1 if present and coded 0 if absent. Bifurcation was taken into account from the moment that there was a constriction between two pores in formation. Fourteen specimens were described twice to compute the error. Schematization error was less than 5 % and it was lower in specimens with fewer ramifications.

Development was described with logistic regressions to estimate the SL at which 50 % of the specimens (SL_{50}) have the bifurcation of interest (Fischer-Rousseau et al., 2009; Grünbaum et al., 2012). Significance of logistic regression was tested using the G^2 statistic (Quinn and Keough, 2002). The Bonferroni correction was applied to take into account multiple comparisons of regression (significance level adjusted to 0.001). Spearman rank correlation coefficients (r_s) were computed to test for shared patterning between sides and between groups of pores. Results from the right and left sides were pooled once symmetry of patterns has been verified.

Ramification patterns for specimens ≥ 411 mm were described with a method initially developed for Purkinje cell ramifications (Berry, '92). This method quantifies vertexes and segments in large dendritic structures. Vertexes are the bifurcation points between branches

called segments. Vertexes can be of three types according to the number of terminal segments they link. Type *a* vertexes (V*a*) link two terminal segments, type *b* vertexes (V*b*) link one terminal segment and type *c* vertexes (V*c*) link none. The ratio between the different vertex types provides information about the ramification growth and their spatial layout (Berry, '92). It is expected that groups of pores with a more linear structure will have a higher number of V*b* than groups of pores with a more compact structure resulting from a completely dichotomous growth. A Kruskal-Wallis analysis was used to test for differences in types of vertexes between groups of pores. The significance level was adjusted by a Bonferroni correction to take into account comparison of the three types of vertexes (significance level adjusted to 0.02). All statistical analyses were performed with R (Version 2.15.2).

1.5.3 GEOMETRIC MORPHOMETRICS

As a result of incomplete ossification of the bones in younger specimens, absence or duplication of groups of pores, and in order to limit the analysis to one population (Lac St-Pierre), only 25 specimens out of 97 were used to verify *a priori* hypotheses of modularity. Since most specimens were immature, differences in integration between sexes was not taken into account. Raw data obtained with the micro-CT scan were resized and converted to DICOM. 3D landmarks were digitized with Morphome3cs (Version 2.0, Charles University, Prague). Skull landmarks were digitized on the surface and canal landmarks were digitized on sections of DICOM images (Fig. 1 and Table 2). Landmarks were digitized twice on different days by the same user (C.L.).

Preliminary analyses and modularity tests were conducted with MorphoJ (Klingenberg, 2011). Each landmark configuration was Procrustes transformed with a join Procrustes fit. Discriminant function analysis (DFA) was used to test for differences in configuration of landmarks between days. Directional and fluctuating asymmetry were assessed with Procrustes ANOVA (Klingenberg and McIntyre, '98). Following assessment

of measurement error, observations were averaged and covariance matrices were computed for the symmetric and asymmetric components of the complete dataset. Regression of shape on centroid size was tested for allometry (Monteiro, '99). Covariance matrices were computed from regression residuals when allometry was significant.

We tested three *a priori* hypotheses of modularity regarding the interaction between canals and bones and the interaction among canals (Fig. 3): the dermopterotic and extrascapular and their respective canals are two separate modules (hypothesis 1), the bones and canals are two separate modules (hypothesis 2), the otic canal and the supratemporal commissure are two separate modules (hypothesis 3). *A priori* hypotheses were tested using Escouffier's *RV* coefficient (Escoufier, '73) as proposed by Klingenberg (2009). Delawney triangulation was used to restrict modules to spatially contiguous landmarks (Klingenberg, 2009). Landmarks were subdivided in subsets of similar size according to hypotheses, and the *RV* coefficient was computed between subsets. The observed *RV* coefficient was then compared to a distribution of all other possible *RV* coefficients when subsets are permuted inside the configuration of landmarks. If the observed *RV* coefficient is at the lower end (5 %) of the distribution, the partition of landmarks corresponds to the true module boundaries. This method of modularity analysis (MOD) is based on the premise that covariance between modules is low and that covariance is high among elements belonging to a module (Klingenberg, 2009).

Two blocks Partial Least Square (PLS) analysis (Rohlf and Corti, 2000) was used to test hypotheses of (1) the dermopterotic and extrascapular and their respective canals as two separate modules and (2) bones and canals as two separate modules (Fig. 2). The *RV* coefficient measured the correlation between the two sets of landmarks. A permutation test (10,000 rounds) was used to test the hypothesis of complete independence between the two landmark partitions. Although the hypotheses are the same in both approaches (MOD and PLS), PLS analysis differs from MOD because it uses a separate Procrustes fit for each subset. The separate Procrustes fit method is considered more conservative than the join

Procrustes fit used in MOD because it ignores covariation related to differences in size between blocks (Klingenberg, 2009).

1.6 RESULTS

1.6.1 PATTERNING OF RAMIFICATIONS

The numbers of groups of pores vary among individuals although a generalized condition is found. Half of the specimens (48) had the arrangement of groups of pores as described by Allis (1889), including GP16, GP18, GP2 and GP3. GP16 was missing on one side in one specimen and on both sides in another specimen. Group of pores 18 was missing on both sides in one specimen. The most common type of variation was the absence of one or more groups of pores on the supratemporal commisure. A total of 31 specimens were missing either GP2 or GP3 on the left or right side (19 missing on the left and 12 on the right); the single group of pores was identified as 2.5. The frequencies of missing groups of pores on the right and left side was insufficient to investigate a lateralization process ($G = 3.25$, $P = 0.07$). One group of pores was missing for both sides of the supratemporal commissure in ten specimens. A supplementary group of pores was present in four specimens. Allis (1889) suggested that when one group of pores was missing on one extrascapular, there was an additional one on the other extrascapular; only one out of 97 specimens fitted this description.

The Kruskall-Wallis analysis on 37 specimens with at least one group of pores missing in the supratemporal commissure showed that the position of GP2, GP3 and GP2.5 relative to the intersection between the otic canal and the supratemporal commissure are significantly different ($P < 0.0001$). Post-hoc test showed differences between all three groups of pores. As groups of pores form at the convergence of sections of canals growing around neuromasts, one might expect that when either one of GP2 or GP3 is missing, the single group of pores would occupy an intermediate position. GP2.5 occupies a range of

positions that differs from the mean of the positions of GP2 and GP3 ($P = 0.04$). This shift in position is confirmed by the observation of the group of pores position relative to the suture between the dermopterotic and the postparietal. GP2.5 could not be identify as either GP2 or GP3 and is treated independently hereafter.

Groups of pores exhibited similarities in their patterning (Table 3). The patternings for the left and right sides of GP16 ($r_s = 0.89$, $P = 0.03$) and GP18 ($r_s = 1$, $P = 0.0004$) were significantly correlated. Although they did not develop at exactly the same size, there is a similarity between the curve shape for GP16 and GP18 (Fig. 4). This similarity was supported by the significant positive correlation between their patterning (L: $r_s = 0.86$, $P = 0.02$; R: $r_s = 0.94$, $P = 0.02$). Correlations were not significant for other combinations of groups of pores because of missing values related to the absence of certain groups of pores. Nevertheless, constancy in patterning was observed for GP2 and GP3. Appearance order of bifurcations for GP16 and GP18 is different from GP3 (Fig. 4). Moreover, GP2 and GP3 tend to develop bifurcations of equivalent order simultaneously (Fig. 5A). On the other hand, GP16 and GP18 tend to develop ramifications of equivalent order in an antero-posterior direction. Bifurcation 4 develops before bifurcation 3, both of third order, and bifurcation 8 develops before bifurcation 5, both of fourth order (Fig. 5B).

Comparison of the spatial layout among groups of pores shows a trend toward less Vb vertexes in the supratemporal commissure than the otic canal (Fig. 6). Due to important variation among individuals, differences among groups of pores were not significant for Va ($P = 0.1$), Vb ($P = 0.1$) and Vc ($P = 0.09$). However, ratio of Vc vertexes was different between the otic canal and the supratemporal commissure ($P = 0.01$). Composition in Va vertexes and Vb vertexes was not different between the two canals after the Bonferroni correction (significance level adjusted to 0.02; Va. $P = 0.05$; Vb. $P = 0.03$).

1.6.2 GEOMETRIC MORPHOMETRICS

Landmarks configuration did not differ significantly according to the day they were digitized ($P = 0.68$) and observations could not be assigned to either session by DFA. Procrustes ANOVA revealed significant individual effect, directional and fluctuating asymetry ($P \leq 0.0001$) for the complete (Table 4) and bone datasets. However, there was no directional asymmetry when only canal landmarks were considered ($P = 0.07$). Regression of shape on centroid size was significant for the symmetric component of variation ($P = 0.004$), but not significant for the asymmetric component ($P = 0.83$). Residuals from regression were used in the covariation matrix for the symmetric component only. The test of modularity for hypotheses 1 (the dermopterotic and extrascapular and their respective canals as two separate modules) and 2 (bones and canals as separate modules) by the MOD resulted in RV coefficients that were not at the lower end of the distribution (Table 5). Hypothesis 3 for which the otic canal and the supratemporal commissure are considered as separate modules was supported in symmetry ($RV = 0.266$, proportion lower = 0.05) and asymmetry ($RV = 0.214$, proportion lower = 0.04, Table 5). Analyses with a separate Procrustes fit (PLS) showed similar results (Table 5). Bones and canals were not independent (hypothesis 2) as were the two blocks (1) dermopterotic and (2) extrascapular, including their respective canals (hypothesis 1). These blocks do not correspond to modules.

1.7 DISCUSSION

Skull evolution can only be explained by the integration of all its components (Jamniczky and Hallgrímsson, 2011). Yet, this study is the first to test for modularity of lateral line canals and their integration with their supporting skull bones. Developmental modularity was tested at the level of groups of pores and variational modularity was tested

at the level of canals and bones. Modularity was detected at two hierarchical levels: (1) groups of pores and (2) canals. However, MOD and PLS analyses did not provide evidence for a module that integrates canals and bones.

1.7.1 GROUPS OF PORES PATTERNING, VARIATION AND MODULARITY

Sensory canal ramification was overlooked for more than a century. As Allis (1889) observed, ramifications have a constant pattern. In our study, ramification development was similar between the left and right sides for GP16 and GP18 suggesting that ramifications follow a pre-established patterning (Table 3). Although the development for the groups of pores in the supratemporal commissure was not correlated, we observed that their development is not random. The absence of a significant correlation may result from the absence of groups of pores in this region. To the best of our knowledge, the functional importance of the ramifications has never been tested. However, Janssen (2004) suggested that ramifications serve as filters for small hydrodynamic disturbances which must affect many branches in order to be recorded by the neuromasts. We suggest that the branches must have a constant configuration in order to obtain a reliable signal. However, the position of ramifications could not be investigated with geometric morphometrics due to the close proximity of putative landmarks and variation in the number of landmarks.

Some groups of pores were missing in about half the specimens, which is more abundant than what Allis (1889) had observed. The absence of a group of pores did not influence the presence or absence of other groups of pores which meet the quasi-independent criterion for modularity; elements of a module are expected to be coherently modified or lost without affecting other structures (Winther, 2001). Moreover, we demonstrated that the two canal sections join in a position (dis2.5) different from the two original positions (dis2 and dis3). Allis (1889) observed that groups of pores were absent when neuromasts were absent. We suggest that the single group of pores develops from canal sections corresponding to neuromasts different from the generalized condition. Franz-

Odendaal and Hall (2006) recognized the neuromast as a morphological module. The neuromasts could thus be the module and its modularity is detectable through the development of groups of pores.

The number of groups of pores is more variable in the supratemporal commissure than in the otic canal. Because canals and dermal bones may interact in development, it was expected that the extrascapulars, which are the bones associated with the supratemporal commissure, would also vary in number. Among basal actinopterygians [e.g., Lepisosteiformes (Grande, 2010), Acipenseriformes (Hilton and Bemis, '99)] the number of extrascapulars is frequently variable among and within species. The extrascapular series is also variable among species in different groups of sarcopterygians [e.g., actinistians (Cloutier, '91), dipnoans (Cloutier, '97)]. A great disparity in number of bones and groups of pores could be the result of developmental instability in this region. Moreover, in crown tetrapods, the extrascapulars are lost simultaneously with the supratemporal commissure (Cloutier and Ahlberg, '96). The joined phylogenetic variation of lateral line canals and dermal bones brings further evidence for their close developmental interaction.

1.7.2 MODULARITY OF SENSORY CANALS

Differences in the patterning of groups of pores and the pattern of covariation suggest that the otic canal and the supratemporal commissure are two different modules. This partition was observed (1) qualitatively with differences in the direction of ramification growth, (2) by vertex ratios in larger specimens, (3) by correlations in the order of appearance of ramifications, and (4) by the MOD with geometric morphometrics.

First, although there is variation among individuals, ramifications tended to develop simultaneously on left and right sides in the supratemporal commissure whereas they grow from an antero-posterior direction for ramifications of a given order in the otic canal (Fig. 5). In fish fins, congruence in developmental direction of skeletal elements has been

used to identify patterning modules (Mabee et al., 2002; Grünbaum et al., 2012). Thus, we suggest that similarity in the patterning of ramifications support the otic canal and the supratemporal commissure modules.

Second, vertex ratios tended to differ among groups of pores (Fig. 6). By comparing the pattern of ramification of the otic canal and the supratemporal commissure we expected groups of pores that belong to the supratemporal commisure to have a lower Vb ratio and higher Va and Vc ratios than groups of pores from the otic canal because a completely dichotomous growth does not produce any Vb vertexes (Fig. 6B). Because of the low sample size and the variation among individuals, we only detected a difference between Vc vertexes which was, as expected, higher in the supratemporal commissure. Vertex ratios were compared to a distribution (see Appendix 2) of theoretical vertex ratios for each type of growth described by Berry and Flinn ('84). Vertex ratios in the supratemporal commissure are concordant with a non-random symmetrical growth. Ramifications of the otic canal develop according to a random terminal growth, meaning that terminal vertexes of higher order have a higher probability of bifurcating (Berry and Flinn, '84). This type of growth was also observed in Purkinje cells (Berry and Flinn, '84) suggesting that developmental signals controlling ramifications could be duplicated or shared by the two different systems. On the other hand, growth differs between the otic canal and the supratemporal commissure most likely because of differences in developmental signals which would suggest the presence of two developmental modules. However, they could also differ because of bone space constraint. The otic canal is closer to the bone margin than the supratemporal commissure. Ramifications cannot be symmetrical in the otic canal because they would occupy an area larger than the bone. Vertex ratios should be interpreted carefully because expected ratios for each type of growth vary according to the number of terminal vertexes. The number of specimens is limited to large specimens who have a higher number of terminal vertexes. Because we used a limited number of individuals with varying number of vertex, our results should be used as further evidence for modularity, but not as a test for modularity itself.

Third, we tested for developmental modules by correlating the sequence of appearance of successive branching. A significant correlation between groups of pores means that ramifications develop in the same order. No correlation was detected between groups of pores of the otic canal and the supratemporal commissure and among groups of pores of the supratemporal commissure (Table 3). We could not conclude in the presence of modularity within the supratemporal commissure because half of the values were missing due to the absence of groups of pores. However, comparison of patterning and growth described previously (Fig. 5 and 6) shows that GP2 and GP3 in the supratemporal commissure are more similar with each other than groups of pores in the otic canal suggesting that GP2 and GP3 are indeed a module. Moreover, patterning of GP16 and GP18 were strongly correlated suggesting that the two sections of the otic canal are one single module. This is not what was expected according to their developmental origin because the two groups of pores develop from two different placodes (Northcutt, '97). A similar patterning could be found if a placode was initially duplicated. Modules are known to be duplicated and differentiated which facilitates evolution (Raff, '96). On the other hand, placode identity may have little influence on the lateral line system once neuromasts have been deposited. What we expected based on developmental interactions during embryogenesis (placode differentiation) may not be transferable to postembryonic development (ramification patterning). Also, the module may encompass two groups of pores if epigenetic interactions are the same in both groups of pores. Epigenetic interactions can be detected with geometric morphometrics using fluctuating asymmetry (Klingenberg, 2003).

Fourth, MOD supported the hypothesis that both part of the otic canal form a module (Table 5). However, we could not test for GP2 and GP3 as two different modules because landmark partitions have to be of similar size (Klingenberg, 2009). The modularity hypothesis was supported with individual variation and fluctuating asymmetry. Fluctuating asymmetry removes genetic and environmental influences leaving only direct developmental perturbations (Klingenberg, 2003). The two parts of the otic canal are most

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likely subjected to the same epigenetic interactions and thus form a developmental and a variational module.

Modules are quasi-independent meaning that they can be modified without affecting other structures. Since we detected two modules, the otic canal and the supratemporal commissure, we expect that these two canals vary independently throughout evolution. On the other hand, most amphibians have lost the otic lateral line placode (Schlosser, 2002b) suggesting some independence between the two parts of the otic canal. A phylogenetic investigation of the distribution of canals could validate if the two detected modules vary independently during evolution.

1.7.3 Bone and canal integration

Modularity between dermal bones and canals was not detected. PLS revealed that canals and bones are not independent. However, the covariation was not strong enough to qualify the pair as a module. The otic canal goes through two bones. The interaction between canals is most likely stronger than it is between bones and canals which obscure the bone-canal module. Although the lateral line system has a close relationship to dermal bones because of their formation around canals, other processes are predominant in determining bone shape and sutures. For exemple, feeding was associated with constraint on the skull and variation in suture pattern in the bichir, *Polypterus* (Markey et al., 2006). A different suture pattern resulting from environmental interaction would shift the position of landmarks and reduce their covariation with canal landmarks. Integration has been shown to change with age (Fischer-Rousseau et al., 2009) and our results may not hold for larval and young juveniles as they would not have gone through the same developmental and environmental interactions. Moreover, modularity is a hierarchical concept and developmental processes may act as a palimpsest which can make it difficult to detect modularity when *a priori* hypotheses are based on only one developmental process (Hallgrímsson et al., 2009).

1.7.4 A HIERARCHICAL MODEL OF MODULARITY

We propose a hierarchical model of modularity based on our results and developmental interactions described in literature. Each group of pores is a module based on their property to disappear without affecting each other. Groups of pores could be divided in modules according to their placodal origin (Module GP16, Module GP18, Module GP2 and GP3; Northcutt, '97). GP16 and GP18 are part of one developmental and variational module based on their shared patterning and covariation. Groups of pores GP18, GP2 and GP3 may be parts of a module with the extrascapular to a lesser extent. It has been suggested that placodes share a common developmental precursor: the preplacodal region (Baker and Bronner-Fraser, 2001; Schlosser, 2002a). Placode precursors have been recognized to be fated as lens in early development (Bailey et al., 2006). This common developmental origin could lead to a module that integrates all groups of pores and other sensory organs. A description of the patterning of other groups of pores is however needed to confirm the common origin.

1.7.5 COMPARISONS AND FUTURE WORK

To our knowledge, this is the first study to test for two types of modularity simultaneously with two different methods. Variational and developmental modularity are not independent since developmental pathways create a covariation structure that can be detectable as a variational module (Wagner et al., 2007). The developmental modules we detected were also variational modules adding support to the hypothesis of congruence between the two types of modularity. Moreover, Klingenberg (2003) argued that patterns of fluctuating asymmetry can be used to detect developmental modules, because it removes genetic and environmental variation leaving only perturbation in shared developmental

pathways. Our study supports but does not prove that the methods of patterning and geometric morphometrics detect the same developmental modules.

Our results support a relationship between dermal bones and the lateral line system but the nature of this interaction is still unknown. Its comprehension is essential to explain the phylogenetic association of bones with canals especially considering that lateral line canals are used as phylogenetic characters (e.g., Cloutier and Ahlberg, '96). The description of modularity in lateral line canals is thus necessary to explain the phylogenetic disparity in the presence/absence and morphology of canals. Modules have been recognized as units of evolution at the level of placodes because their derivatives tend to be coherently modified or lost (Schlosser, 2002b). In order to verify if groups of pores behave as units of evolution, ramification patterning is currently under description in fossil sarcopterygians. Considering the importance of sensory canals as phylogenetic characters, the description of ramifications in a phylogenetic context could be an underestimated tool.

1.8 ACKNOWLEDGEMENTS

1.9 TABLES

Table 1. Specimens used in the study listed by size

Locality	Collection	Specimen number	N	SL (mm)
Québec	MDDEFP	ST2-VD 20-05-2009	1	~ 15
New York	CMN	CMNFI 1993-0007	1	28
Québec	MDDEFP	SP1VP-12-06-2009	3	37-42
Québec	MDDEFP	ST1VP-16-06-2009	5	52-63
Richelieu Creek, Qc	MDDEFP	AMCA-25135	1	65
Des Prairies River, Qc	MDDEFP	AMCA-4330	4	70-85
New York	FMNH	11068	2	75, 83
St-Jean Creek, Qc	MDDEFP	AMCA-26385	1	87
Châteauguay River, Qc	MDDEFP	AMCA-13118	1	95
Châteauguay River, Qc	MDDEFP	AMCA-796	1	108
Richelieu Creek, Qc	MDDEFP	AMCA-4785	1	145
Lake St-Pierre, Qc*	UQAR	Amia008-Amia083	69	160-284
Ontario	CMN	CMNFI 1977-0309	1	~ 378 (TL = 444)
Unknown	UQAR	Amia098, -	2	408, ~ 545
Ontario	CMN	CMNFI 1977-0324	1	~ 411 (TL = 483)
Lake Champlain, Qc*	UQAR	Amia093-095	3	502, 525, 529

N. number of specimens, SL. Standard length, TL. Total length
* Specimens provided by commercial fisheries, now in collection at UQAR
MDDEFP. Ministère du Développement Durable, de l'Environnement, de la Faune et des Parcs (Section Montérégie, Longueuil, Québec), UQAR. Université du Québec à Rimouski (Rimouski, Québec), CMN. Canadian Museum of Nature (Gatineau, Québec), FMNH. Field Museum of Natural History (Chicago, Il)

Table 2. Landmarks (lmk) position for each bone or canal for the left (L) and right (R) side

Lmk		Elements	Description
L	R		
1	18	dermopterotic	Tip of the external posterior process
2	19	dermopterotic	Intersection point among the dermopterotic, postparietal and extrascapular
3	20	dermopterotic	Intersection point among the dermopterotic, postparietal and parietal
4	21	dermopterotic	Anterior tip
5	22	extrascapular	Anterior median tip
6	23	extrascapular	Posterior median tip
7	24	extrascapular	Junction between the extrascapular and the posttemporal
8	25	extrascapular	Junction between the extrascapular and the dermopterotic
9	26	otic canal	Anterior exit from the dermopterotic
10	27	otic canal	Root of GP16
11	28	otic canal	Junction between the otic and preopercular canals
12	29	posterior otic canal	Intersection point among the posterior otic canal, extrascapular and dermopterotic
13	30	posterior otic canal	Root of GP18
14	31	posterior otic canal	Posterior exit from extrascapular
15	32	supratemporal commissure	Junction between the posterior otic canal and supratemporal commissure
16	33	supratemporal commissure	Root of GP3
17	34	supratemporal commissure	Root of GP2

Table 3. Correlations in the order of appearance of bifurcation for GP16-18 (otic canal) and GP2-3 (supratemporal commissure). Bold values indicate significant correlation. L: left side patterning, R: right side patterning

Comparisons	r_s	P
16L-16R	**0.89**	**0.03**
18L-18R	**1.00**	**0.0004**
3L-3R	0.80	0.1
2L-2R	0.50	1
16L-18L	**0.86**	**0.02**
16R-18R	**0.94**	**0.02**
16L-3L	0.90	0.08
16R-3R	0.80	0.1
18L-3L	0.90	0.08
18R-3R	0.90	0.08
3L-2L	0.72	0.2
3R-2R	0.50	1

Table 4. Procrustes ANOVA for the complete dataset, including bones and canals
(FA: fluctuating asymmetry)

Effect	SS	MS	df	F	P
Individual	0.05136	0.00004554	1128	2.08	< 0.0001
Side	0.00208	0.00004323	48	1.98	0.0001
Ind * Side (FA)	0.02518	0.00002185	1152	5.14	< 0.0001
Error	0.01011	0.00000426	2375		

Table 5. Results of modularity tests for three hypotheses for both symmetric and asymmetric components of variation using join or separate Procrustes fit. Bold numbers indicate the supported hypothesis. p: proportion of configurations with a lower RV coefficient than the hypothesis, P: p-value. / separates modules

	Join Procrustes fit				Separate Procrustes fit			
	Symmetric		Asymmetric		Symmetric		Asymmetric	
	RV	p	RV	p	RV	P	RV	P
H1 dermopterotic/extrascapular and respective canals	0.399	0.2	0.377	0.5	0.274	< 0.001	0.236	0.002
H2 canal/bone	0.424	0.3	0.335	0.2	0.294	< 0.001	0.209	0.01
H3 otic/supratemporal canal	**0.266**	**0.05**	**0.214**	**0.04**	-	-	-	-

1.10 FIGURES
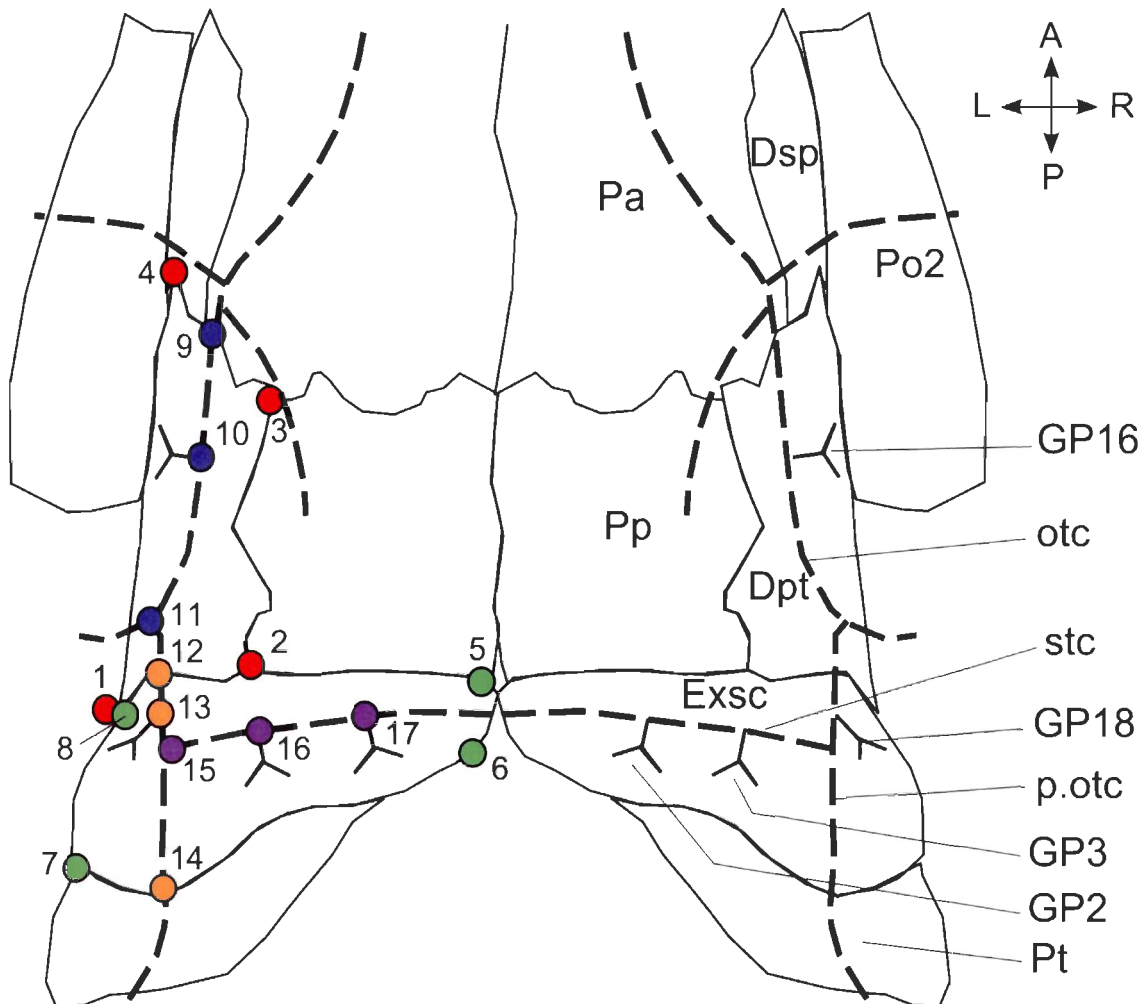
Figure 1. Dorsal view of a posterior partial skull of *Amia calva*. Position of dermal bones, canals (dotted lines) and associated groups of pores. Digitized landmarks are shown by colored circles and numbered according to Table 2. Colors correspond to subsets presented as elements in Table 2. Red: Dermopterotic; Green: Extrascapular; Blue: Otic canal; Orange: Posterior part of the otic canal; Purple: Supratemporal commissure; Exsc: extrascapular; Dpt: dermopterotic; Dsp: dermosphenotic; Pa: parietal; Po2: postorbital 2; Pp: postparietal; otc: otic canal, Pt: posttemporal; p.otc: posterior part of the otic canal; stc: supratemporal commissure; GP2, 3, 16, 18: Groups of pores 2, 3, 16 and 18.
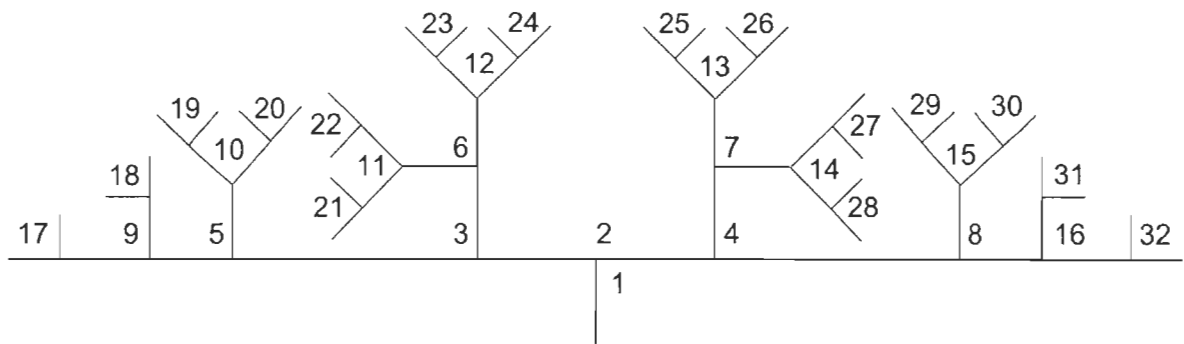
Figure 2. Schematisation of a completely symmetrical group of pores with numbered bifurcations. Bifurcations are numbered clockwise, progressing towards extremities.
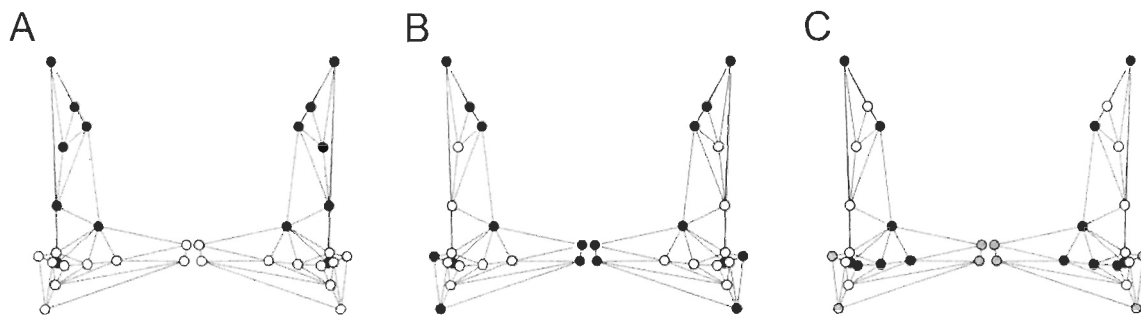
Figure 3. Modularity hypotheses tested by geometric morphometric analyses. Each shade corresponds to a module. **A.** Dermopterotic and extrascapular and respective canals as two separate modules. Black: dermopterotic and otic canal (landmarks 1-4, 9-11), White: extrascapular, posterior otic canal and supratemporal commissure (landmarks 5-8, 12-14, 15-17), **B.** Canals and bones as separate modules. Black: bones (landmarks 1-8), White: canals (landmarks 9-17), **C.** Otic canal and supratemporal commissure as separate modules. Black: dermopterotic (landmarks 1-4), White: otic canal (landmarks 8-11), Light grey: extrascapular (landmarks 5-8), Dark grey: Supratemporal commissure (landmarks 15-17)
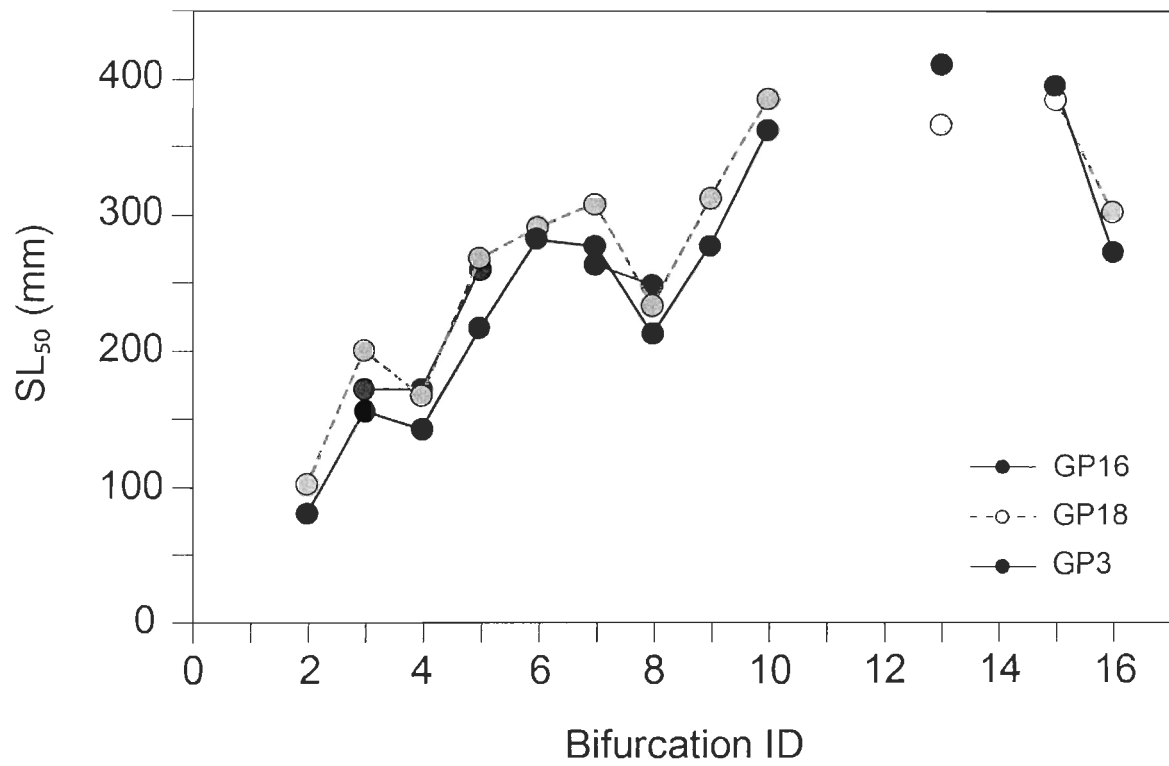
Figure 4. Patterning comparison of group of pores 16 (GP16), 18 (GP18) and 3 (GP3).

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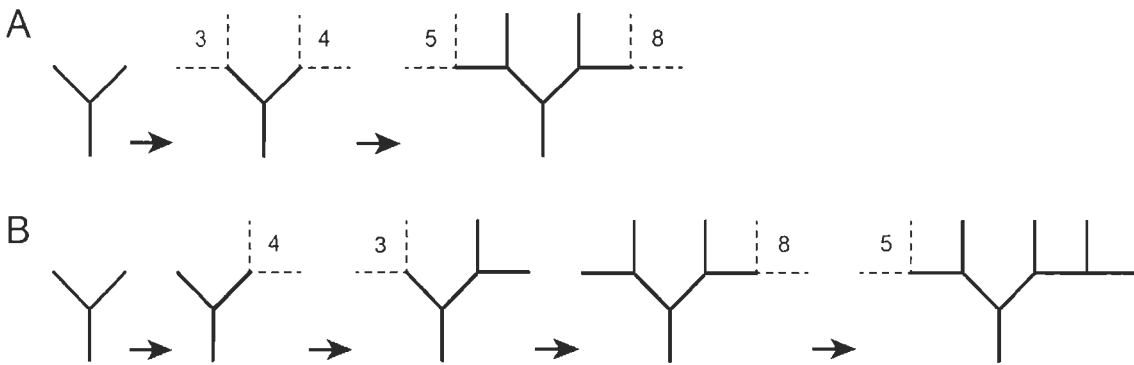
Figure 5. Comparison of ramification development for (A) the supratemporal commissure (GP2 and GP3) and (B) the otic canal (GP16 and GP18). Ramifications of equivalent order develop simultaneously in the supratemporal commissure and in an anterio-posterior direction in the otic canal. Dotted segments represent developing ramifications and numbers show bifurcation identification.
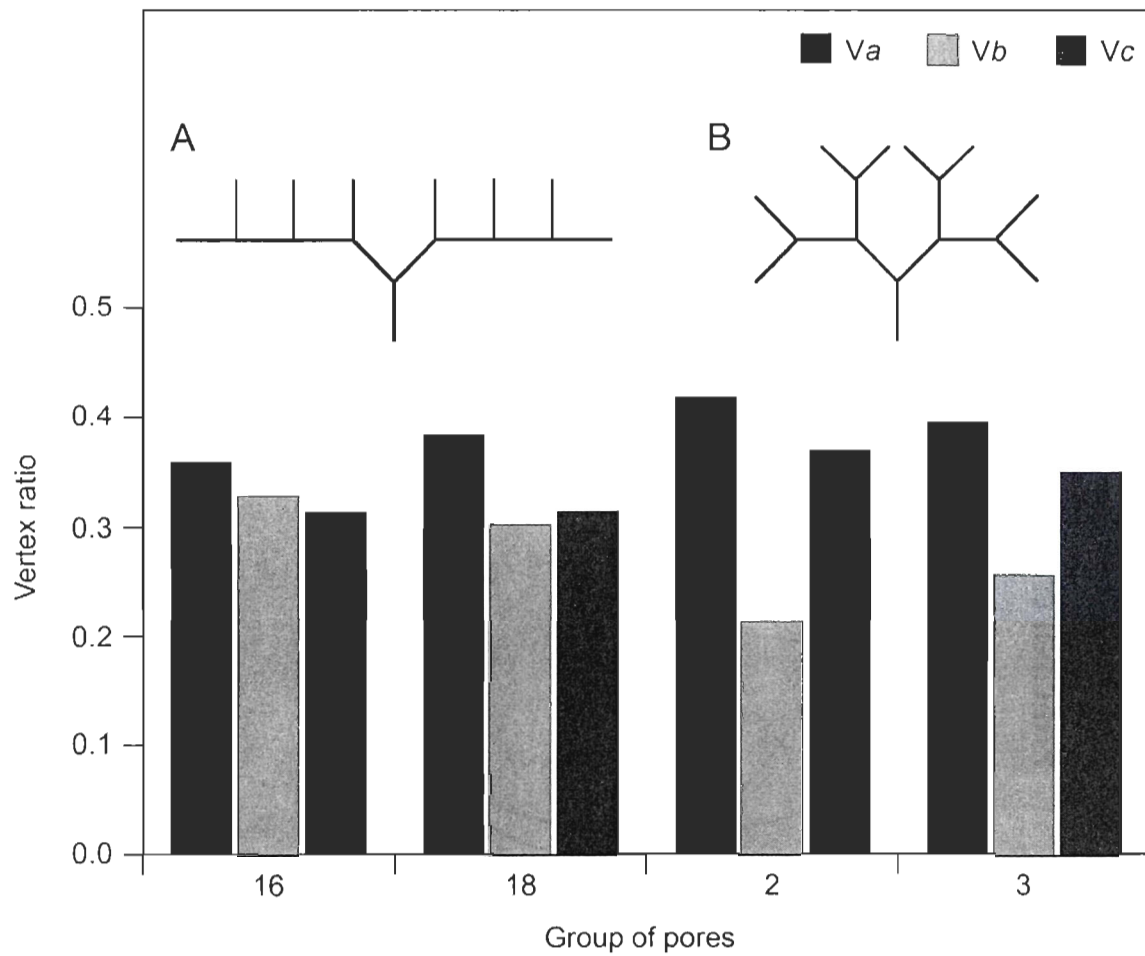
Figure 6. Comparison of spatial layout as indicated by vertex ratios for GP16, 18, 2 and 3. GP16 and GP18 exhibit a growth with a higher probability of bifurcation for ramifications of higher order (A) which is consistent with a higher Vb ratio. GP2 and GP3 grow symmetrically (B) which is consistent with a low Vb ratio.

1.11 APPENDIX 1

The analysis was conducted with geometric morphometrics (MorphoJ). Discriminant function analysis between specimens fixed before and after scanning showed no difference between their shape (P = 0.5825) as did confidence ellipses from PCA (supplementary figure 1).
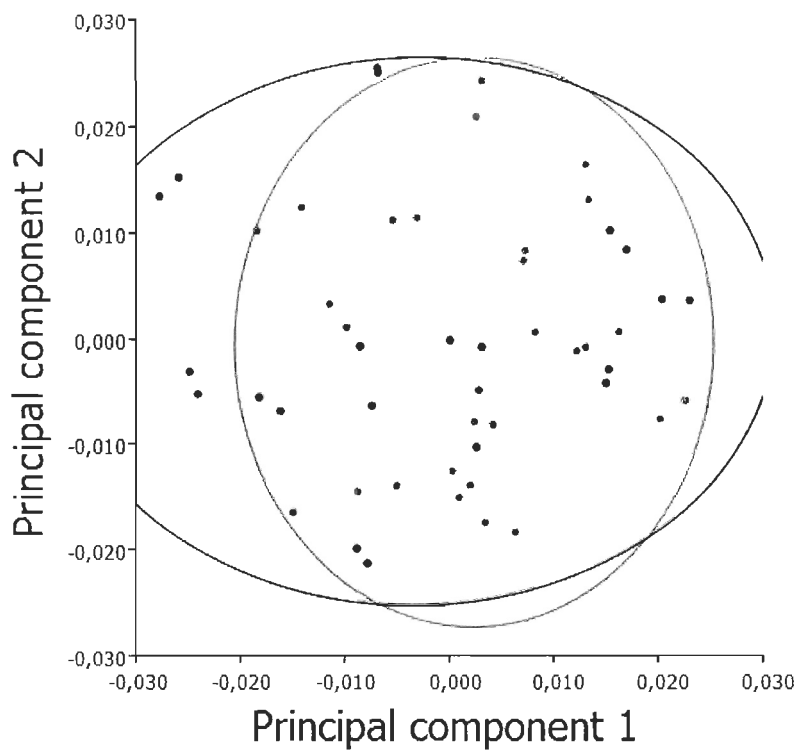
Supplementary figure 1. PCA comparing Procrustes shape of specimens scanned before fixation (grey) and specimens scanned after fixation (black). PC1 = 16 % of variance and PC2 = 14 % of variance. 90 % confidence ellipses.

1.12 Appendix 2

Vertex ratios expected according to type of growth and number of pendent vertexes (V*p*). Ratios were calculated by equations from Berry and Flinn (1984).

V*p*	random terminal growth			random segmental growth			random terminal and segmental growth		
	V*a*	V*b*	V*c*	V*a*	V*b*	V*c*	V*a*	V*b*	V*c*
10	0.37	0.37	0.26	0.67	0.44	-0.11	0.32	0.46	0.21
11	0.37	0.37	0.27	0.65	0.43	-0.08	0.32	0.46	0.22
12	0.36	0.36	0.27	0.63	0.42	-0.05	0.32	0.45	0.23
13	0.36	0.36	0.28	0.62	0.41	-0.03	0.32	0.45	0.23
14	0.36	0.36	0.28	0.61	0.41	-0.01	0.31	0.45	0.24
15	0.36	0.36	0.29	0.60	0.40	0.00	0.31	0.45	0.24
16	0.36	0.36	0.29	0.59	0.40	0.01	0.31	0.44	0.24
17	0.35	0.35	0.29	0.59	0.39	0.02	0.31	0.44	0.25
18	0.35	0.35	0.29	0.58	0.39	0.03	0.31	0.44	0.25
19	0.35	0.35	0.30	0.58	0.38	0.04	0.31	0.44	0.25
20	0.35	0.35	0.30	0.57	0.38	0.05	0.31	0.44	0.25
21	0.35	0.35	0.30	0.57	0.38	0.05	0.31	0.44	0.26
22	0.35	0.35	0.30	0.56	0.38	0.06	0.31	0.44	0.26
23	0.35	0.35	0.30	0.56	0.37	0.07	0.30	0.44	0.26
24	0.35	0.35	0.30	0.56	0.37	0.07	0.30	0.43	0.26
25	0.35	0.35	0.31	0.56	0.37	0.07	0.30	0.43	0.26
26	0.35	0.35	0.31	0.55	0.37	0.08	0.30	0.43	0.26
27	0.35	0.35	0.31	0.55	0.37	0.08	0.30	0.43	0.26
28	0.35	0.35	0.31	0.55	0.37	0.08	0.30	0.43	0.27
29	0.35	0.35	0.31	0.55	0.36	0.09	0.30	0.43	0.27
30	0.34	0.34	0.31	0.55	0.36	0.09	0.30	0.43	0.27
31	0.34	0.34	0.31	0.54	0.36	0.09	0.30	0.43	0.27
32	0.34	0.34	0.31	0.54	0.36	0.10	0.30	0.43	0.27
33	0.34	0.34	0.31	0.54	0.36	0.10	0.30	0.43	0.27
34	0.34	0.34	0.31	0.54	0.36	0.10	0.30	0.43	0.27
35	0.34	0.34	0.31	0.54	0.36	0.10	0.30	0.43	0.27
36	0.34	0.34	0.31	0.54	0.36	0.10	0.30	0.43	0.27
37	0.34	0.34	0.31	0.54	0.36	0.11	0.30	0.43	0.27
38	0.34	0.34	0.32	0.54	0.36	0.11	0.30	0.43	0.27
39	0.34	0.34	0.32	0.53	0.36	0.11	0.30	0.43	0.27
40	0.34	0.34	0.32	0.53	0.36	0.11	0.30	0.43	0.27

V_p	Non random symmetrical growth			Non random asymmetrical growth		
	V_a	V_b	V_c	V_a	V_b	V_c
10	0.45	0.00	0.55	0.11	0.89	0.00
11	0.45	0.00	0.55	0.10	0.90	0.00
12	0.44	0.00	0.56	0.09	0.91	0.00
13	0.44	0.00	0.56	0.08	0.92	0.00
14	0.44	0.00	0.56	0.08	0.92	0.00
15	0.43	0.00	0.57	0.07	0.93	0.00
16	0.43	0.00	0.57	0.07	0.93	0.00
17	0.43	0.00	0.57	0.06	0.94	0.00
18	0.43	0.00	0.57	0.06	0.94	0.00
19	0.43	0.00	0.57	0.06	0.94	0.00
20	0.43	0.00	0.57	0.05	0.95	0.00
21	0.42	0.00	0.58	0.05	0.95	0.00
22	0.42	0.00	0.58	0.05	0.95	0.00
23	0.42	0.00	0.58	0.05	0.95	0.00
24	0.42	0.00	0.58	0.04	0.96	0.00
25	0.42	0.00	0.58	0.04	0.96	0.00
26	0.42	0.00	0.58	0.04	0.96	0.00
27	0.42	0.00	0.58	0.04	0.96	0.00
28	0.42	0.00	0.58	0.04	0.96	0.00
29	0.42	0.00	0.58	0.04	0.96	0.00
30	0.42	0.00	0.58	0.03	0.97	0.00
31	0.42	0.00	0.58	0.03	0.97	0.00
32	0.42	0.00	0.58	0.03	0.97	0.00
33	0.42	0.00	0.58	0.03	0.97	0.00
34	0.41	0.00	0.59	0.03	0.97	0.00
35	0.41	0.00	0.59	0.03	0.97	0.00
36	0.41	0.00	0.59	0.03	0.97	0.00
37	0.41	0.00	0.59	0.03	0.97	0.00
38	0.41	0.00	0.59	0.03	0.97	0.00
39	0.41	0.00	0.59	0.03	0.97	0.00
40	0.41	0.00	0.59	0.03	0.97	0.00

1.13 REFERENCES

Allis EP. 1889. The anatomy and development of the lateral line system in *Amia calva*. *J Morphol* 2:463-566.

Bailey AP, Bhattacharyya S, Bronner-Fraser M, Streit A. 2006. Lens specification is the ground state of all sensory placodes, from which FGF promotes olfactory identity. *Dev Cell* 11:505-517.

Baker CVH, Bronner-Fraser M. 2001. Vertebrate cranial placodes I. Embryonic induction. *Dev Biol* 232:1-61.

Berry M. 1992. Vertex and segment analysis of dendritic fields. In: Stewart MG, editor. Quatitative Methods in Neuroanatomy. Chichester New York: John Wiley & Sons Inc. p. 325-339.

Berry M, Flinn R. 1984. Vertex analysis of Purkinje cell dendritic trees in the cerebellum of the rat. *Philos Trans R Soc Lond B Biol Sci* 221:321-348.

Cloutier R. 1991. Patterns, trends, and rates of evolution within the Actinistia. *Environ Biol Fish* 32:23-58.

Cloutier R. 1997. Morphologie et variations du toit crânien du dipneuste *Scaumenacia curta* (Whiteaves) (Sarcopterygii), du Devonien supérieur du Québec. *Geodiversitas* 19:61-105.

Cloutier R, Ahlberg PE. 1996. Morphology, characters, and the interrelationships of basal sarcopterygians. In: Stiassny ML, Parenti LR, Johnson GD, editors. Interrelationships of Fishes. San Diego: Academic Press. p. 445-479.

Dijkgraaf S. 1963. The functioning and significance of the lateral-line organs. *Biol Rev* 38:51-105.

Escoufier Y. 1973. Le traitement des variables vectorielles. *Biometrics*:751-760.

Fischer-Rousseau L, Cloutier R, Zelditch ML. 2009. Morphological integration and developmental progress during fish ontogeny in two contrasting habitats. *Evol Dev* 11:740-753.

Franz-Odendaal TA, Hall BK. 2006. Modularity and sense organs in the blind cavefish, *Astyanax mexicanus*. *Evol Dev* 8:94-100.

Gompel N, Cubedo N, Thisse C, Thisse B, Dambly-Chaudière C, Ghysen A. 2001. Pattern formation in the lateral line of zebrafish. *Mech Dev* 105:69-77.

Goswami A. 2006. Cranial modularity shifts during mammalian evolution. *Am Nat* 168:270-280.

Goswami A, Weisbecker V, Sánchez-Villagra MR. 2009. Developmental modularity and the marsupial–placental dichotomy. *J Exp Zool* 312B:186-195.

Graham-Smith W. 1978. On the lateral lines and dermal bones in the parietal region of some crossopterygian and dipnoan fishes. *Philos Trans R Soc Lond B Biol Sci* 282:41-105.

Grande L. 2010. An empirical synthetic pattern study of gars (Lepisosteiformes) and closely related species, based mostly on skeletal anatomy. The resurrection of Holostei. *Copeia* 2A:1-863.

Grünbaum T, Cloutier R, Vincent B. 2012. Dynamic skeletogenesis in fishes: Insight of exercise training on developmental plasticity. *Dev Dyn* 241:1507-1524.

Hallgrímsson B, Jamniczky H, Young NM, Rolian C, Parsons TE, Boughner JC, Marcucio RS. 2009. Deciphering the palimpsest: studying the relationship between morphological integration and phenotypic covariation. *Evol Biol* 36:355-376.

Hallgrímsson B, Willmore K, Dorval C, Cooper DML. 2004. Craniofacial variability and modularity in macaques and mice. *J Exp Zool* 302B:207-225.

Hallgrímsson B, Willmore K, Hall BK. 2002. Canalization, developmental stability, and morphological integration in primate limbs. *Yearb Phys Anthropol* 119:131-158.

Hensel K, Balon EK. 2001. The sensory canal systems of the living coelacanth, *Latimeria chalumnae*: A new instalment. *Environ Biol Fish* 61:117-124.

Hilton EJ, Bemis WE. 1999. Skeletal variation in shortnose sturgeon (*Acipenser brevirostrum*) from the Connecticut River: implications for comparative osteological studies of fossil and living fishes. In: Arratia G, Schultze H-P, editors. Mesozoic Fishes 2 - Systematics and Fossil. München: Verlag. p. 69-94.

Jamniczky HA, Hallgrímsson B. 2011. Modularity in the skull and cranial vasculature of laboratory mice: Implications for the evolution of complex phenotypes. *Evol Dev* 13:28-37.

Janssen J. 2004. Lateral line sensory ecology. In: von der Emde G, Mogdans J, Kapoor BG, editors. The Senses of Fish. Netherlands: Springer. p. 231-264.

Jarvik E. 1980. Basic Structure and Evolution of Vertebrates. London, New York: Academic Press p. 575.

Klingenberg CP. 2003. Developmental instability as a research tool: using patterns of fluctuating asymmetry to infer the developmental origins of morphological integration. In: Polak M, editor. Developmental instability: causes and consequences. New York: Oxford University Press. p. 427-442.

Klingenberg CP. 2009. Morphometric integration and modularity in configurations of landmarks: tools for evaluating a priori hypotheses. *Evol Dev* 11:405-421.

Klingenberg CP. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Mol Ecol Resour* 11:353-357.

Klingenberg CP, McIntyre GS. 1998. Geometric morphometrics of developmental instability: Analyzing patterns of fluctuating asymmetry with Procrustes methods. *Evolution* 52:1363-1375.

Ledent V. 2002. Postembryonic development of the posterior lateral line in zebrafish. *Development* 129:597-604.

Mabee PM, Crotwell PL, Bird NC, Burke AC. 2002. Evolution of median fin modules in the axial skeleton of fishes. *J Exp Zool* 294:77-90.

Markey MJ, Main RP, Marshall CR. 2006. *In vivo* cranial suture function and suture morphology in the extant fish *Polypterus*: implications for inferring skull function in living and fossil fish. *J Exp Biol* 209:2085-2102.

Márquez EJ. 2008. A statistical framework for testing modularity in multidimensional data. *Evolution* 62:2688-2708.

Monteiro LR. 1999. Multivariate regression models and geometric morphometrics: the search for causal factors in the analysis of shape. *Syst Biol* 48:192-199.

Münz H. 1986. What influences the development of canal and superficial neuromasts. *Ann Kon Mus Mid Afr Zool Wetensch* 251:85-89.

Nakae M, Shinohara G, Miki K, Abe M, Sasaki K. 2013. Lateral line system in *Scomberomorus niphonius* (Teleostei, Perciformes, Scombridae): Recognition of 12 groups of superficial neuromasts in a rapidly-swimming species and a comment on function of highly branched lateral line canals. *Bull Natl Mus Nat Sci, Ser A* 39:39-49.

Northcutt RG. 1989. The phylogenetic distribution and innervation of craniate mechanoreceptive lateral lines. In: Coombs S, Görner P, Münz H, editors. The Mechanosensory Lateral Line. New York: Springer. p. 17-78.

Northcutt RG. 1997. Evolution of gnathostome lateral line ontogenies. *Brain Behav Evol* 50:25-37.

Parrington FR. 1949. A theory of the relations of lateral lines to dermal bones. *Proc R Soc Lond B Biol Sci* 119:65-78.

Pehrson T. 1922. Some points in the cranial development of teleostomian fishes. *Acta Zool* 3:1-63.

Pehrson T. 1940. The development of dermal bones in the skull of *Amia calva*. *Acta Zool* 21:1-50.

Poe S. 2004. A test for patterns of modularity in sequences of developmental events. *Evolution* 58:1852-1855.

Quinn GP, Keough MJ. 2002. Experimental design and data analysis for biologists. Cambridge: Cambridge University Press. 537 p.

Raff RA. 1996. The shape of life: genes, development, and the evolution of animal form. Chicago: The University of Chicago Press. 544 p.

Richtsmeier JT, Aldridge K, DeLeon VB, Panchal J, Kane AA, Marsh JL, Yan P, Cole TM. 2006. Phenotypic integration of neurocranium and brain. *J Exp Zool* 306B:360-378.

Rohlf FJ, Corti M. 2000. Use of two-block partial least-squares to study covariation in shape. *Syst Biol* 49:740-753.

Runions A, Fuhrer M, Lane B, Federl P, Rolland-Lagan A-G, Prusinkiewicz P. 2005. Modeling and visualization of leaf venation patterns. *ACM T Graphic (TOG)* 24:702-711.

Schlosser G. 2002a. Development and evolution of lateral line placodes in amphibians I. Development. *Zoology* 105:119-146.

Schlosser G. 2002b. Development and evolution of lateral line placodes in amphibians. II. Evolutionary diversification. *Zoology* 105:177-193.

Scientific Computing and Imaging Institute. "Seg3D" Volumetric Image Segmentation and Visualization, http://www.seg3d.org.

Stein WE. 1993. Modeling the evolution of stelar architecture in vascular plants. *Int J Plant Sci* 154:229-263.

Stein WE, Boyer JS. 2006. Evolution of land plant architecture: beyond the telome theory. *Paleobiology* 32:450-482.

Tarby ML, Webb JF. 2003. Development of the supraorbital and mandibular lateral line canals in the cichlid, *Archocentrus nigrofasciatus*. *J Morphol* 255:44-57.

Wada H, Ghysen A, Satou C, Higashijima S, Kawakami K, Hamaguchi S, Sakaizumi M. 2010. Dermal morphogenesis controls lateral line patterning during postembryonic development of teleost fish. *Dev Biol* 340:583-594.

Wagner GP, Mezey JG. 2004. The role of genetic architecture constraints in the origin of variational modularity. In: Schlosser G, Wagner GP, editors. Modularity in Development and Evolution. Chicago: The University of Chicago Press. p. 338-358.

Wagner GP, Pavlicev M, Cheverud JM. 2007. The road to modularity. *Nat Rev Genet* 8:921-931.

Ward AB, Brainerd EL. 2007. Evolution of axial patterning in elongate fishes. *Biol J Linn Soc* 90:97-116.

Wark AR, Mills MG, Dang L-H, Chan YF, Jones FC, Brady SD, Absher DM, Grimwood J, Schmutz J, Myers RM, Kingsley DM, Peichel CL. 2012. Genetic architecture of variation in the lateral line sensory system of threespine sticklebacks. *G3: Genes|Genomes|Genetics* 2:1047-1056.

Webb JF. 2000. Mechanosensory lateral line: Microscopic anatomy and development. In: Ostrander GK, editor. The Laboratory Fish. Washington: Academic Press. p. 463-470.

Webb JF, Noden DM. 1993. Ectodermal placodes contributions to the development of the vertebrate head. *Am Zool* 33:434-447.

Webb JF, Shirey JE. 2003. Postembryonic development of the cranial lateral line canals and neuromasts in zebrafish. *Dev Dyn* 228:370-385.

Westoll TS. 1941. Latero-sensory canals and dermal bones. *Nature* 148:168-168.

Winther RG. 2001. Varieties of modules: Kinds, levels, origins, and behaviors. *J Exp Zool* 291:116-129.

Wonsettler AL, Webb JF. 1997. Morphology and development of the multiple lateral line canals on the trunk in two species of *Hexagrammos* (Scorpaeniformes, Hexagrammidae). *J Morphol* 233:195-214.

Zelditch ML, Wood AR, Bonett RM, Swiderski DL. 2008. Modularity of the rodent mandible: Integrating bones, muscles, and teeth. *Evol Dev* 10:756-768.

CHAPITRE 2
CONCLUSION

Cette étude est la première depuis plus d'un siècle à se pencher sur le développement des ramifications du système de la ligne latérale. Les corrélations du patterning des groupes de pores entre les côtés gauche et droit ont montré que le développement des ramifications ne procède pas de façon aléatoire. Les ramifications des groupes de pores ont été utilisées dans cette étude afin d'identifier des modules au niveau des canaux sensoriels. La modularité a été identifiée au niveau des groupes de pores et des canaux. Contrairement aux trois modules attendus au niveau des canaux, seulement deux modules ont été détectés : le canal otique et la commissure supratemporale. La modularité n'a cependant pas été détectée entre les os et les canaux.

La disparition de groupes de pores, principalement dans la commissure supratemporale, n'affecte pas la présence des autres groupes de pores. Ce phénomène répond au critère de quasi-indépendance des modules ; les modules sont modifiés ou disparaissent sans affecter les autres modules (Winther, 2001; Schlosser, 2002a). Chaque groupe de pores se comporte donc comme un module. Dans la commissure supratemporale, si un groupe de pores disparaît d'un côté, celui restant occupe une position qui lui est propre. Le changement dans la position pourrait provenir de la disparition d'un neuromaste (Allis, 1889). Puisque les sections de canaux se forment autour des neuromastes (Allis, 1889), elles se rencontrent à une position différente lorsqu'un groupe de pores est manquant.

Les patternings des ramifications du canal otique et de la commissure supratemporale diffèrent et supportent la modularité de ces deux canaux. Les premières ramifications au

niveau du canal otique se développent dans une direction antéro-postérieure, alors qu'elles bifurquent simultanément du côté gauche et droit au niveau de la commissure supratemporale. Une similitude dans la direction de développement a été interprétée comme un module de patterning au niveau des nageoires de poissons (Mabee *et al.*, 2002). La différence dans le mode de croissance de ces deux canaux a aussi été démontrée par la méthode des vertex traditionnellement appliquée aux cellules de Purkinje (Berry et Flinn, 1984; Berry, 1992). Le canal otique se développe selon une croissance aléatoire terminale, c'est-à-dire avec une plus grande probabilité de bifurquer par les extrémités, tout comme les cellules de Purkinje, alors que la commissure supratemporale se rapproche plutôt d'une croissance symétrique. La similarité entre le développement des canaux sensoriels et les cellules de Purkinje suggère que les signaux développementaux qui causent la bifurcation sont partagés par les systèmes nerveux et sensoriel. Comme les placodes sont à la fois impliquées dans la formation du neuromaste et des nerfs sensoriels, les éléments sensoriels et nerveux du système du la ligne latérale partagent les mêmes signaux développementaux (Northcutt, 1997). Il est donc tout aussi probable que les ramifications des canaux sensoriels suivent un patron de bifurcation semblable aux nerfs sensoriels des neuromastes voisins.

La corrélation dans l'ordre d'apparition des bifurcations supporte aussi les modules développementaux du canal otique et de la commissure supratemporale. Les patternings des deux sections du canal otique sont corrélés positivement. Le canal otique qui est séparé en deux sections se développant à partir de deux placodes différentes forme un seul module développemental. La présence d'un seul module développemental associé à deux placodes suggère qu'une des deux placodes provient de la duplication de l'autre. Ainsi, bien qu'il y ait deux précurseurs développementaux, ils fourniraient tous les deux le même signal développemental au canal. La duplication de module a été observée au niveau des nageoires de poissons (Mabee *et al.*, 2002) et les éléments en série comme les dents, les vertèbres et les côtes (Raff, 1996). Il est aussi possible que les processus durant la phase embryonnaire ne soient pas transposables à la phase post-embryonnaire pour identifier les modules, c'est-

à-dire que les placodes n'influencent pas les processus de ramification. La commissure supratemporale serait, quant à elle, un seul module développemental et provient d'une seule placode. La disparition de groupes de pores au niveau de la commissure supratemporale chez la moitié des individus a cependant limité l'analyse statistique. Il a été impossible de détecter des corrélations significatives qui impliquent des groupes de pores de la commissure supratemporale. Cependant, les données obtenues à partir de la description du développement et des vertex suggèrent que cette commissure est quasi-indépendante du canal otique.

Les modules du canal otique et de la commissure supratemporale ont aussi été détectés à l'aide du coefficient RV utilisée en morphométrie géométrique. L'hypothèse est supportée à la fois en variation individuelle et en asymétrie aléatoire. L'asymétrie aléatoire a la fonction de soustraire toute information de la variation qui est liée à l'environnement ou à la génétique (Klingenberg, 2008). L'information restante n'a trait qu'aux perturbations à l'intérieur d'une voie développementale. Si une covariation est détectée entre l'asymétrie aléatoire de certains traits, c'est qu'ils partagent une voie développementale (Klingenberg, 2008). La méthode de géométrie morphométrique basée sur l'asymétrie aléatoire permet donc, comme la méthode du patterning, de détecter des modules développementaux. Cependant, le qualitatif développemental est utilisé par très peu d'auteurs. Un consensus sur les types de modularité est nécessaire. Une congruence entre la modularité variationnelle et développementale dans cette étude suggère que le développement est à la base de l'intégration dans le système de la ligne latérale. Le développement crée des corrélations entre les structures (Klingenberg, 2008). De plus, la congruence entre les modules détectés par le patterning et le coefficient RV en asymétrie aléatoire suggère que les deux méthodes sont complémentaires pour détecter les modules développementaux.

La morphométrie géométrique utilisée avec le coefficient RV et l'analyse des moindres carrés partiels n'a toutefois pas permis de supporter l'hypothèse de modularité entre les os dermiques et les canaux. Cependant, une covariation entre les deux éléments a

été détectée suggérant qu'ils ne sont pas strictement indépendants. Cela supporte l'hypothèse selon laquelle le système de la ligne latérale et les os dermiques interagissent durant le développement. La modularité au niveau du canal otique qui traverse deux os est probablement plus forte que la modularité entre les canaux et les os et elle vient obscurcir le signal. Une méthode développée par Márquez (2008) permettrait de résoudre ce problème de détection, puisqu'elle permet de combiner plusieurs processus développementaux dans les hypothèses de modularité. Cette méthode ne permet cependant pas d'utiliser des données 3D à l'heure actuelle. De plus, même si le neuromaste est impliqué dans la formation d'os dermiques, d'autres processus sont responsables de la délimitation des os, ce qui explique que l'intégration entre les canaux et les os peut être faible à l'âge adulte. Par exemple, le patron de suture des os du crâne chez *Polypterus* dépend des contraintes biomécaniques associées à l'alimentation (Markey *et al.,* 2006).

Les modules sont considérés par certains auteurs comme les unités de l'évolution (Schlosser, 2002b) ou de la sélection (Brandon, 1999). La compréhension des unités qui constituent le système de la ligne latérale pourrait permettre d'expliquer la variation entre les groupes phylogénétiques. Les composantes d'un module tendent à être perdues ou modifiées de façon cohérente comme il a été observé au niveau des dérivés des placodes chez les amphibiens (Schlosser, 2002a). De plus, le système de la ligne latérale a une grande importance en phylogénie, puisqu'il est utilisé pour déterminer l'homologie des os et comme caractère phylogénétique (Graham-Smith, 1978; Cloutier et Ahlberg, 1996). Mieux comprendre l'interaction entre les os, les canaux sensoriels et les neuromastes est essentiel afin d'expliquer l'évolution. Cette relation entre les os et les neuromastes a été au centre de quelques études descriptives dans les dernières années (Tarby et Webb, 2003; Webb et Shirey, 2003). Cependant, la nature et le mécanisme développemental de cette interaction restent inconnus.

Cette étude est la première à montrer une organisation modulaire des canaux sensoriels. Elle est aussi la première à utiliser deux méthodes différentes afin de tester la

modularité. Elle valide la concordance entre la modularité développementale et variationnelle ainsi qu'entre les méthodes de détection des modules développementaux (patterning et morphométrie géométrique en asymétrie aléatoire). La modularité des canaux sensoriels permet d'expliquer les interactions entre différents processus développementaux : développement des placodes, déposition de neuromastes et formation des canaux. Cependant, la modularité au niveau d'autres canaux qui proviennent d'autres placodes devrait être décrite. De plus, la comparaison entre les ramifications chez *A. calva* et celles d'autres groupes phylogénétiques permettrait de vérifier si le patron de ramification est conservé dans l'évolution et s'il est modifié en cohérence avec l'appartenance des groupes de pores à un module.

LISTE DE RÉFÉRENCES BIBLIOGRAPHIQUES

Allis EP. 1889. The anatomy and development of the lateral line system in *Amia calva*. Journal of Morphology 2:463-566.

Berry M. 1992. Vertex and segment analysis of dendritic fields. In: Stewart MG, editor. Quatitative Methods in Neuroanatomy. Chichester, New York: John Wiley & Sons Inc. p. 325-339.

Berry M, Flinn R. 1984. Vertex analysis of Purkinje cell dendritic trees in the cerebellum of the rat. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences 221:321-348.

Brandon RN. 1999. The units of selection revisited: the modules of selection. Biology and Philosophy 14:167-180.

Cloutier R, Ahlberg PE. 1996. Morphology, characters, and the interrelationships of basal sarcopterygians. In: Stiassny ML, Parenti LR, Johnson GD, editors. Interrelationships of Fishes. San Diego: Academic Press. p. 445-479.

Coombs S, Montgomery JC. 1999. The enigmatic lateral line system. In: Fay RR, Popper AN, editors. Comparative hearing: fish and amphibians. New York: Springer. p. 319-362.

Franz-Odendaal TA, Hall BK. 2006. Modularity and sense organs in the blind cavefish, *Astyanax mexicanus*. Evolution & Development 8:94-100.

Graham-Smith W. 1978. On the lateral lines and dermal bones in the parietal region of some crossopterygian and dipnoan fishes. Philosophical Transactions of the Royal Society of London Series B, Biological Sciences 282:41-105.

Grünbaum T, Cloutier R, Vincent B. 2012. Dynamic skeletogenesis in fishes: Insight of exercise training on developmental plasticity. Developmental Dynamics 241:1507-1524.

Hallgrímsson B, Willmore K, Hall BK. 2002. Canalization, developmental stability, and morphological integration in primate limbs. American Journal of Physical Anthropology 119:131-158.

Hendrikse JL, Parsons TE, Hallgrímsson B. 2007. Evolvability as the proper focus of evolutionary developmental biology. Evolution & Development 9:393-401.

Jamniczky HA, Hallgrímsson B. 2011. Modularity in the skull and cranial vasculature of laboratory mice: Implications for the evolution of complex phenotypes. Evolution & Development 13:28-37.

Klingenberg CP. 2008. Morphological integration and developmental modularity. Annual Review of Ecology, Evolution, and Systematics 39:115-132.

Klingenberg CP. 2009. Morphometric integration and modularity in configurations of landmarks: tools for evaluating a priori hypotheses. Evolution & Development 11:405-421

Kruszyński KJ, Kaandorp JA, van Liere R. 2007. A computational method for quantifying morphological variation in scleractinian corals. Coral reefs 26:831-840.

Ledent V. 2002. Postembryonic development of the posterior lateral line in zebrafish. Development 129:597-604.

Mabee PM, Crotwell PL, Bird NC, Burke AC. 2002. Evolution of median fin modules in the axial skeleton of fishes. Journal of Experimental Zoology 294:77-90.

Markey MJ, Main RP, Marshall CR. 2006. In vivo cranial suture function and suture morphology in the extant fish *Polypterus*: implications for inferring skull function in living and fossil fish. Journal of Experimental Biology 209:2085-2102.

Márquez EJ. 2008. A statistical framework for testing modularity in multidimensional data. Evolution 62:2688-2708.

Northcutt RG. 1989. The phylogenetic distribution and innervation of craniate mechanoreceptive lateral lines. In: Coombs S, Görner P, Münz H, editors. The Mechanosensory Lateral Line. New York: Springer. p. 17-78.

Northcutt RG. 1997. Evolution of gnathostome lateral line ontogenies. Brain, Behavior and Evolution 50:25-37.

Parrington FR. 1949. A theory of the relations of lateral lines to dermal bones. Proceedings of the Zoological Society of London 119:65-78.

Poe S. 2004. A test for patterns of modularity in sequences of developmental events. Evolution 58:1852-1855.

Raff RA. 1996. The shape of life: genes, development, and the evolution of animal form. Chicago: The University of Chicago Press. 544 p.

Richtsmeier JT, Aldridge K, DeLeon VB, Panchal J, Kane AA, Marsh JL, Yan P, Cole TM. 2006. Phenotypic integration of neurocranium and brain. Journal of Experimental Zoology 306B:360-378.

Rohlf FJ, Corti M. 2000. Use of two-block partial least-squares to study covariation in shape. Systematic Biology 49:740-753.

Schlosser G. 2002a. Development and evolution of lateral line placodes in amphibians. II. Evolutionary diversification. Zoology 105:177-193.

Schlosser G. 2002b. Modularity and the units of evolution. Theory in Biosciences 121:1-80.

Schlosser G. 2002c. Development and evolution of lateral line placodes in amphibians I. Development. Zoology 105:119-146.

Schlosser G. 2005. Amphibian variations–the role of modules in mosaic evolution. In: Callebaut W, Rasskin-Gutman D, editors. Modularity: Understanding the development and evolution of natural complex systems. Cambridge: The MIT Press. p. 143-179.

Stein WE. 1993. Modeling the evolution of stelar architecture in vascular plants. International Journal of Plant Sciences 154:229-263.

Stein WE, Boyer JS. 2006. Evolution of land plant architecture: beyond the telome theory. Paleobiology 32:450-482.

Tarby ML, Webb JF. 2003. Development of the supraorbital and mandibular lateral line canals in the cichlid, *Archocentrus nigrofasciatus*. Journal of Morphology 255:44-57.

Wagner GP, Mezey JG. 2004. The role of genetic architecture constraints in the origin of variational modularity. In: Schlosser G, Wagner GP, editors. Modularity in Development and Evolution. Chicago: The University of Chicago Press. p. 338-358.

Wagner GP, Pavlicev M, Cheverud JM. 2007. The road to modularity. Nature Reviews Genetics 8:921-931.

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Ward AB, Brainerd EL. 2007. Evolution of axial patterning in elongate fishes. Biological Journal of the Linnean Society 90:97-116.

Wark AR, Mills MG, Dang L-H, Chan YF, Jones FC, Brady SD, Absher DM, Grimwood J, Schmutz J, Myers RM, Kingsley DM, Peichel CL. 2012. Genetic architecture of variation in the lateral line sensory system of threespine sticklebacks. G3: Genes| Genomes| Genetics 2:1047-1056.

Webb JF, Noden DM. 1993. Ectodermal placodes contributions to the development of the vertebrate head. American Zoologist 33:434-447.

Webb JF, Shirey JE. 2003. Postembryonic development of the cranial lateral line canals and neuromasts in zebrafish. Developmental Dynamics 228:370-385.

Winther RG. 2001. Varieties of modules: Kinds, levels, origins, and behaviors. Journal of Experimental Zoology 291:116-129.

Zelditch ML, Wood AR, Bonett RM, Swiderski DL. 2008. Modularity of the rodent mandible: Integrating bones, muscles, and teeth. Evolution & Development 10:756-768.