

ERC Consolidator Grant 2020 Part B2

A. STATE-OF-THE-ART AND OBJECTIVES

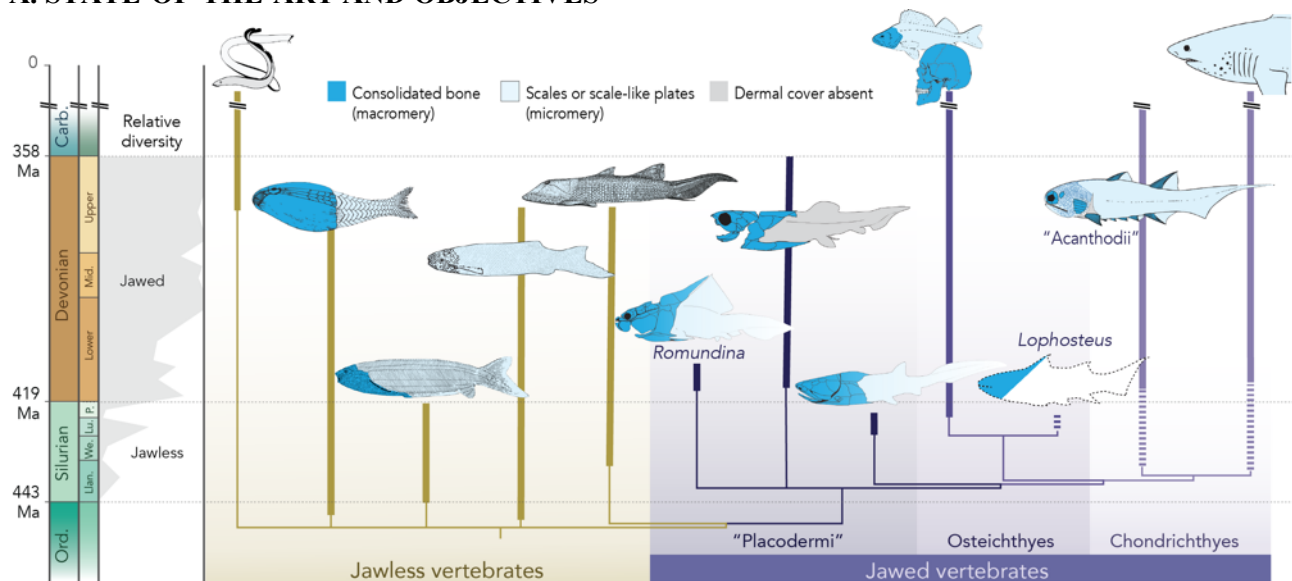


Figure 1. Phylogenetic relationships of early gnathostomes and the evolution of exocranial armour (shown in shades of blue and grey).

Jawed vertebrates (gnathostomes) represent over 60,000 species comprising more than 99% of living vertebrate diversity. The group originated from a common ancestor that lived more than 430 million years ago (Brazeau and Friedman, 2015) during an early phase in the evolutionary establishment of modern animal biotas (Klug et al., 2010). The gnathostome body plan is characterized by a skull with jaws and teeth and paired sensory organs, as well as by paired appendages, and specialised skeletal hard tissues. These traits formed the functional anatomical basis of the great morphological disparity of gnathostome form, permitting the evolution of diverse feeding, respiratory, and locomotory mechanisms. Consequently, the origins of these structures have been the targets of nearly 200 years of research in comparative biology, from palaeontology to developmental genetics. In spite of such a long history of intensive investigation, key questions in the origin of gnathostomes—especially the origins of jaws themselves—remain enduring mysteries and targets of intensive investigation in the study of developmental evolution (Kuratani et al., 2001; e.g. Depew et al., 2002; Kuratani et al., 2012; Miyashita, 2015).

The lack of clear living or fossil intermediates between jawless and jawed fishes has left room for abundant speculation and theorizing in attempts to understand the comparative basis for the origins of jaws and the patterning of the gnathostome head. The antiquity of the events means inferences based on extant taxa alone are poorly constrained—nearly a billion cumulative lineage years separate any living jawed vertebrate from its nearest living relative (Brazeau and Friedman, 2015). The fossil record of Palaeozoic (approx. 500 to 370 million year ago) gnathostomes provides an essential source of data for reconstructing the foundations of modern vertebrate diversity. However, this presents its own problems not only in its incompleteness but in the necessity of using morphological data to reconstruct phylogeny. Consequently, **both developmental and palaeontological research programmes remain tied to hypothetical model ancestors that are difficult to test without either new data or fundamentally new theoretical and methodological insights**. Furthermore, it remains unclear what such hypothetical model ancestors can deliver in terms of evolutionary insights about novelty and the origin of functional diversity. A more complete synthesis of macroevolutionary and microevolutionary theory will need to focus on the origins and maintenance of *variation* (Shubin and Marshall, 2000) and what can be learned about it from palaeontological and ‘palaeo-developmental’ records.

The goals of this project are to: 1) reconstruct the evolutionary foundations of gnathostome craniofacial diversity through new, expeditionary fieldwork in the Canadian Arctic and studies of exceptionally preserved fossils transitional between jawless and modern jawed vertebrate cranial constructions; 2) propose and test **a new model of the origin of cranial modularity in gnathostomes and a functional-developmental basis of cranial variability**; 3) devise new phylogenetic methods better adapted for the analysis of complex morphological data; 4) test new hypotheses about the links between skeletogenetic modes and craniofacial and jaw disparity in establishment of early jawed vertebrates.

A1. Phylogenetic and palaeontological background

While modern taxa leave the origins of jaws and skull poorly constrained on their own, the fossil record of the earliest gnathostomes in the Silurian and Devonian can populate the naked stem branches separating modern lineages (Donoghue, 2005; Brazeau and Friedman, 2014; 2015). The palaeontological, zoological, and molecular phylogenetic records of gnathostomes agree broadly on the deep phylogenetic relationships of extant jawless and major jawed vertebrate clades (Meyer and Zardoya, 2003; Chen et al., 2012; Brazeau and Friedman, 2014). There is widespread agreement that modern jawless fishes are outgroups of modern jawed vertebrates. The living jawed vertebrates comprise two major clades—the Osteichthyes (bony fishes and tetrapods) and the Chondrichthyes (sharks, skates and rays, and chimaeras). The subjects of more considerable phylogenetic debate are the earliest fossil representatives which lived during the Early to Middle Palaeozoic. In addition to early representatives of the Osteichthyes and Chondrichthyes, two extinct assemblages—the **Acanthodii** and the **Placodermi** have been the subjects of continuing debate concerning their respective monophyly and relationships to extant lineages (Maisey, 1986; Brazeau, 2009; Young, 2010; Davis et al., 2012; King et al., 2016). Alongside these taxa are a host of diverse jawless ‘ostracoderms’—armoured jawless fishes with an array of skeletonization patterns (Fig. 1). A major advance in our understanding of early gnathostomes was achieved in the latter part of the last century with the numerical phylogenetic analysis of these groups showing that they form a jawed vertebrate stem-group (Forey and Janvier, 1993; Donoghue et al., 2000). Together, this assemblage of Palaeozoic gnathostomes and their relatives can be interrogated for deeper understanding of the evolution of the modern vertebrate body plan (Brazeau and Friedman, 2015; Janvier, 2015).

Evolution of the gnathostome skull and skeleton

The origins of skeletonization was a major innovation in animal evolution (Vermeij, 1989). Of all the skeletonising hard tissues that have been evolved by animals, none quite match the complexity and versatility of vertebrate hard tissues, especially bones and teeth. Despite its static, rigid appearance, bone is a complex tissue with a highly dynamic life history (Hall, 2005). It is unlike other animal hard tissues in its capacity for both mechanical strength and ability to undergo ‘pre-programmed’ remodelling throughout the life. Furthermore, bone growth can be continuous while avoiding risky behaviours like moulting (e.g. arthropods). Bone may thus form the substrate for unique functional and life history innovations. All vertebrates possess an endoskeleton formed (or preformed) of cartilage. However, jawed vertebrates and many extinct jawless vertebrates possess an endoskeleton and an exoskeleton, the latter always made of mineralised tissue. Only osteichthyans (the so-called ‘bony vertebrates’) possess an ossified endoskeleton, but jawed vertebrates of all major lineages may possess a rind of superficial mineralisation (perichondral bone) around their endoskeletal cartilage. Finally, **dermal bone** is bone that forms in the skin from ectodermal mesenchyme. The early fossil record of vertebrates—jawed and jawless—is characterised by an extensive diversity of dermal bone tissues.

Evolution of the dermal skeleton (or exoskeleton) of vertebrates has been a long-standing research programme. The structure of dermal bone is primitively composed of repeating units called **odontodes**—tooth-like structures that arise from a dermal papilla (Ørvig, 1980; Reif, 1982). Early vertebrate dermal bone can be generalised into three or four layers (Ørvig, 1980; Giles et al., 2013; Keating et al., 2015; Keating and Donoghue, 2016): a dentinous outer layer, which may possess tubercular structures that result from odontogenic papillae, a middle cancellous layer that contains spaces for blood vessels that supplied nutrients and **odontocytes** (bone-depositing cells), and a basal lamellar layer on the internal surface {see, Sire:2009wc}. The microstructure of bone, when studied either in thin-sections or computed microtomography (CT) scanning, can reveal details of the processes that grow and shape bone. We can detect how a bone grows through the accretion of new layers, as evidenced by the ‘burial’ or previous bone generations or the superposition of some layers over others. Evidence of resorption and remodelling can be observed in the ‘cross-cutting’ relationships between different bone layers from different generations, along with the presence of tissue-level pre-requisites such as vascularisation canals (which provided a blood supply that could supply new bone resorption and development cells). Because of these signatures, much has been learned about the developmental processes that shaped early bone (Donoghue, 2002). Phylogenetic work has similarly permitted reconstruction of the stepwise evolutionary history of these same processes (Donoghue et al., 2006; Sire et al., 2009).

The armoured face: the primitive gnathostome condition

The fossil record of early gnathostomes and their jawless relatives indicates that the dermal skull likely coalesced from a tessellated, or micromeric condition (Figs. 1, 2). This latter condition—perhaps owing to presumptions of the primordial nature of sharks—was long considered the likely primitive condition of early

gnathostomes, characterizing the last common ancestor of living (Miles, 1973). Within this framework, the transition from jawless to jawed vertebrates would have taken place in an ancestor covered in a tessellate exoskeleton (Fig. 2, ‘micromery’)—consistent with a ‘shark-like’ model of ancestral gnathostomes. This was furthermore consistent with outgroup comparisons using living taxa alone. However, the introduction of numerical phylogenetic techniques to this question coupled with some highly unexpected discoveries of Silurian and very early Devonian taxa have overturned this view by establishing homology between the armoured skeletons of the placoderms and osteichthyans (Zhu et al., 2013; Dupret et al., 2014; Brazeau and de Winter, 2015; Giles et al., 2015; Zhu et al., 2016). The shark-like model of the ancestral gnathostome has now been deposed.

A possible intermediate stage in the transition from tessellated to macromeric armour in placoderms is exhibited by a dubiously monophyletic assemblage called ‘acanthothoracids’. These taxa are of central significance to this work for several reasons. 1) ‘Acanthothoracids’ demonstrate a mode of bone growth comprising expansive dermal plates covered in a superficial coating of polygonal tesserae (Westoll, 1967; Goujet, 1984; Vařkaninová and Ahlberg, 2017); 2) Members of the group are generally

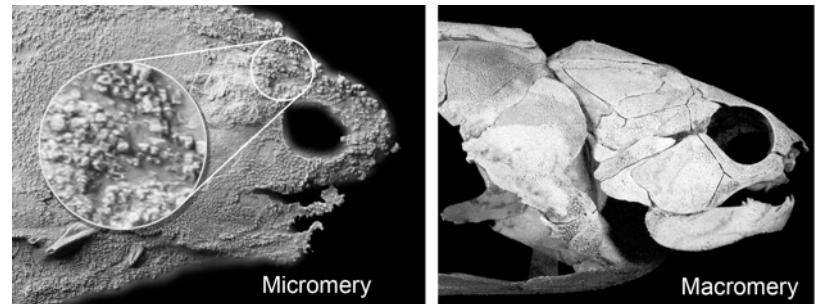


Figure 2. Two modes of skull construction in early gnathostomes: Left: micromeric condition formed of numerous, small undifferentiated plates. Right: macromeric condition formed of differentiated plate. In gnathostomes, the plates are interlocking at overlapping joints.

considered to be ‘primitive’ placoderms or even intermediate between jawless fishes and other placoderms (Dupret et al., 2014); Acanthothoracids very often lack evidence of open, growing sutures (and therefore very likely had to orchestrate their bone growth with complex programs of remodeling). Acanthothoracids may reveal an intermediate condition in the origin of differentiated armour, but there is some notable conflict in how they are interpreted and potential links to crown-group gnathostomes. This includes the fact that their exoskeletons strongly resemble exoskeletons of putative stem-group osteichthyans (Friedman and Brazeau, 2010; Cunningham et al., 2012). Furthermore, unpublished ERC-funded work by myself and collaborators has uncovered a highly unexpected acanthothoracid in Mongolia that has an endoskeleton formed of endochondral bone—the major osteichthyan synapomorphy (Giles et al., n.d.). Thus, there remains deep phylogenetic uncertainty about the ‘acanthothoracids’, but they are major determinants of prevailing phylogenetic theory. There is no easy way to reconcile all of this information in light of current gnathostome phylogenies **and a wholesale reinvestigation of deep gnathostome relationships will be required. However, new methods and new, high-quality data will be necessary to resolve this background.**

A2. Evolution of the vertebrate craniofacial diversity: a new model and synthesis

What remains missing from the study of gnathostome origins is a synthesis that underscores its evolutionary importance beyond the construction of hypothetical model ancestors. Although some significant strides have been made in the phylogenetic understanding of fossil gnathostomes, it would appear that the problem of the origins of jaws themselves still remains out of reach. Here I present a novel synthetic model of the evolutionary assembly of the gnathostome skull. While individual components of this theory have been well recognized, they have so far not been subject to a holistic synthesis. I propose a shift in perspective, focusing on the sub-functionalisation of the gnathostome skull that promoted both the origins and diversification of jaws.

A2.1. Jaws and cranial ‘evolvability’: the missing piece of the ‘key innovation’ hypothesis

Jaws present a classic ‘key innovation’ hypothesis—an evolutionary novelty thought to underwrite the adaptive radiation of gnathostomes and potentially the competitive displacement of Palaeozoic jawless fishes (Blieck, 2011; 2016). To explore this hypothesis, recent works have used quantitative analysis of jaw shape and character variables to construct morphospaces with superimposed taxonomy and geologic time (Anderson et al., 2011; Hill et al., 2018). Support for the ‘key innovation’ hypothesis has been somewhat mixed (Anderson et al., 2011; Blieck, 2011). However, another underlying pattern has been overlooked: **The first major radiations and excursions into extreme shifts in jaw morphology are achieved independently in gnathostome lineages capable of expansive exocranial ossifications: placoderms and osteichthyans** (Fig. 1; Anderson et al. 2011). It is only after the end-Devonian extinction—where the placoderms and a large proportion of the lobe-finned osteichthyans go extinct—that chondrichthyans achieve greater morphological

disparity in jaw shape (Hill et al., 2018) and invade ecological space previously filled by placoderms and some osteichthyans (Sallan and Coates, 2010).

What underlies the diversification potential of gnathostome exocranial? The sutures of the gnathostomes skull have long attracted the attention of comparative morphologists seeking to decode the ‘common plan’ of the gnathostome skull and seek out characters for phylogenetic and taxonomic study. **I propose a different perspective on sutures that considers their jointly developmental and functional significance and I propose that their origin was an evolutionary innovation.** The functional importance of sutures has become increasingly recognised by vertebrate morphologists and their diversity of form is now the subject of investigations in both fishes and tetrapods (Markey and Marshall, 2006; Markey et al., 2006; Moazen et al., 2008; Curtis et al., 2013). Here I propose that one of the major innovations in gnathostomes is the evolution of integral cranial sutures—boundaries between bones that allowed uninterrupted growth whilst maintaining the functional integrity of the skull. I am proposing this as an innovation because I contend here that it was integral to the evolution of cranial disparity from the earliest gnathostomes to the present day.

A macromeric skull evolved multiple times in the early history of vertebrates. Among these examples, even the ‘jawless’ heterostracans are known to have evolved jaw-like oral openings (Purnell, 2002; Elliott et al., 2004). **What evolved uniquely in the gnathostome exoskeleton is the generation of mechanically static or integral interfaces between large neighbouring plates that could remain unfused and yet capable of continued growth.** In this way, the gnathostome skull (and shoulder girdle) became composed of multiple, quasi-independent units that nevertheless maintained a high degree of integration. Broad overlapping or interdigitating skull bone margins are currently unknown in any jawless fishes. The earliest integral sutures were simple ‘scarf joints’ seen in placoderm (Downs and Donoghue, 2009). These become considerably more complex in ‘primitive’ osteichthyans (Porro et al., 2015) and complex patterns of interdigitation of bones are seen in the earliest tetrapods (Clack, 2007). Knowledge of the biomechanical function and diversity of suture morphologies (Markey and Marshall, 2006; Markey et al., 2006; Moazen et al., 2008; Curtis et al., 2013) underwrites the potential that they provide information on key aspects of function and cranial differentiation. Importantly, they are directly observable in fossil material and their developmental context can be obtained from high-resolution histological study (Downs and Donoghue, 2009). **What remains unknown is their diversity across the transition from jawless fishes to the earliest representatives of living gnathostome lineages.** However a study of this diversity would open up new possibilities for understanding a *simultaneously functional and developmental* diversification in gnathostomes.

However, previous works have not considered sutures as a functional and developmental innovation in their own right. This establishes a new perspective on the origins of jaws as a ‘key innovation’—hypotheses that are notoriously difficult to test rigorously but require phylogenetic replication (Rabosky, 2014). The origin of gnathostome jaws was, unfortunately, a singular event. However, it remains unknown how often complex interlocking sutures evolved. They are not ubiquitous among placoderm-grade early gnathostomes. Furthermore, placoderms appear to show only simple overlapping scarf-joints, while more complex interdigitating morphologies evolved in early osteichthyans and tetrapods. In this proposal I argue that we should test whether they were of fundamental importance in the generation of vertebrate craniofacial disparity and that their origins and early diversification can be reconstructed in the fossil record. This is important because it links the generation of functional diversity to developmental novelties that can be recorded in fossils. Because early bone development in vertebrates laid down a record of its developmental history, the developmental processes such as accretion and superposition of tissue, remodeling and resorption can be studied directly in fossils without the need for ontogenetic series. **By linking these novelties to functional diversification and craniofacial disparity, we can isolate novel developmental mechanisms that genuinely led to variation and diversity, thus establishing a more complete evolutionary synthesis.**

A2.2. A new cranial growth model for the evolution of the gnathostome skull

The origin of integral sutures occurred during the transition from a tessellated (‘micromeric’) skull roof to a macromeric (Fig. 2) skull seen in placoderms and osteichthyans. Understanding this as a potential innovation requires dissecting its evolutionary history. There lacks a clear empirical documentation of the transition from a tessellated, largely undifferentiated morphology in jawless fishes to the macromeric condition. The spectrum of Palaeozoic gnathostomes and their jawless relatives provide scope for testing theories on the origin of the gnathostome exoskeleton. I propose the following provisional theory on the assembly of the gnathostome skull and integral sutures and how it can be reconstructed in fossil groups. This is a principally three-stage process (Fig. 3):

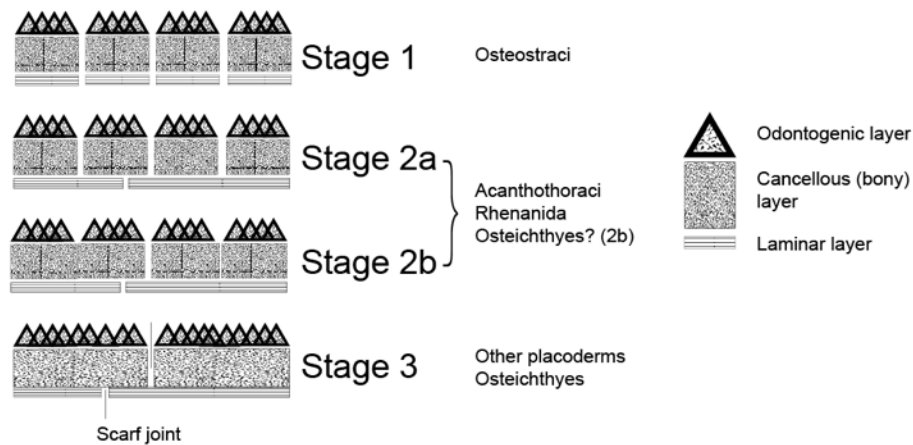


Figure 3. A model of the evolution of a suture-linked gnathostome skull.

- **Stage 1:** Exoskeleton composed of multiple, mostly undifferentiated platelets. Condensations are linked and each tessera possesses a single condensation point for each layer.
- **Stage 2a:** Superficial bony and tubercular layers retain developmental independence (growing as tessellated modules). Multiple superficial tesserae share basal layer originating from a
- **Stage 2b:** Superficial layers grow centripetally from a condensation point near centre of laminated layer. Superficial layers may overlap neighbouring basal layers. In Stage 2 bone growth, skull roof patterns are unstable and may show *strong taxic and individual variation*.
- **Stage 3:** Superficial layers are fixed to basal laminar layer condensation point. Scarf joints are formed where the accretionary margins of bone occur. These provide strong mechanical links between neighbouring bones. However, they occur at the accretionary zones of plates, so they free individual bones to grow semi-independently and differentiate structurally.

This model therefore proposes that Stage 2 laid the foundation for the development of modern-type cranial sutures. Notably, taxa with Stage 3 growth (and modifications thereof) are the most functionally and morphologically diverse Palaeozoic gnathostome taxa (Anderson et al., 2011). Therefore, I propose the following **hypothesis: the origin of sutures was a key innovation that led to the diversification** of several early gnathostome groups. **The key to testing this hypothesis** is the documentation and analysis of cranial variation in key early gnathostome taxa and the accurate resolution of their phylogenetic relationships. Stages 1 and 2 are not theoretical: they are observable in many early gnathostome taxa, such as jawless osteostracans and the enigmatic ‘acanthothoracid’ and rhenanid placoderms. Stage 2b and 3 will require fresh observational tests in the analysis of well-preserved placoderm and early osteichthyan fossils. This project will generate this data through the collection of new material and the analysis of fossil data in existing collections.

A2.3. Morphological phylogenetics in the molecular age

This project will rely on the use of morphological data to reconstruct phylogenetic relationships as it is impossible to recover molecular sequence data from Palaeozoic fossils. Morphology presents challenges from the level of character analysis to the problem of abundant missing data. **Unfortunately, computational phylogenetic tools for morphological data have not kept up with advances in molecular sequence analysis.** While there is evidence that model-based approaches may improve phylogenetic estimates from morphology (Wright and Hillis, 2014; O'Reilly et al., 2016), ‘good’ models of morphological evolution remain elusive and what would make such a model is perhaps what remains most unclear. Most advances in model-based approaches to morphology have focused on the model itself (Wright et al., 2015). However, the application of these models still fails to capture the elaborately hierarchical information contained in morphological data because all characters are treated independently. This failure to capture hierarchical information in a two-dimensional matrix representation manifests itself in the ‘inapplicable data problem’, known to have an analytical cost when the number of characters in a hierarchy are sufficient (Maddison, 1993; Brazeau et al., 2018). However, the inapplicable data problem belies a wider problem of failing to recover hierarchically structured information in morphology. The morphological systems and extensive amount of morphological novelty to be confronted in this project severely break the ‘2D’ matrix assumptions of phylogenetic analyses. The challenge for phylogeneticists is to find a method that minimises (or eliminates) redundant information and avoids logically inconsistent calculations (such as the reconstruction of tail colour on nodes where tails are absent).

Until recently, phylogenetic software had never been adapted to handle morphological character hierarchies. Investigators have had to rely on crude approximations using coding ‘tricks’ that attempt to minimise distortions resulting from hierarchical characters (i.e. autocorrelated transformations that can over-estimate tree scores). Recent work in my research group has developed a reasonably fast approximation for ancestral states reconstructions under hierarchical dependency using a parsimony approach (Brazeau et al., 2018). Alongside this, more exact (but slower) parsimony algorithms have been developed and made available by De Laet (De Laet, 2018). However, both of these approaches are restricted to the use of parsimony and morphologists are now beginning to more routinely adopt probabilistic methods that employ likelihood calculations. Tarasov (2019) recently proposed an approach using hidden Markov models for use in a probabilistic framework. However, the complexity of this grows exponentially with the number of sub-dependent characters. **There is thus a need for a more generalised, computationally efficient approach to studying discrete morphological traits phylogenetically.** Such methods would have the potential to capture the more elaborately hierarchical information in morphological data. Given the problems of incomplete data sampling, investigator bias, and the lack of clear homology priors across many gnathostome groups, there is also a need for methods that could allow objective tests of competing hypotheses—either prior to or informed by the process of phylogenetic analysis itself.

A3. Objectives

The objectives of this work are to generate a refreshed understanding of the evolution of modern gnathostomes through new phylogenetic insights, new theories of the origin of cranial disparity, a new reconstruction of the assembly of the gnathostome skull, and the development of new numerical tools for studying morphology comparatively and phylogenetically. The project will deliver the following concrete outcomes:

- New comparative data of exceptionally preserved early gnathostome fossils from the Canadian Arctic.
- New data on the development and evolution of cranial integration and modularisation mechanisms in the earliest vertebrates.
- New methodological tools for exploring comparative relationships between discrete body parts.
- Phylogenetic methods for analysing contingent and hierarchical characters in likelihood framework.
- New perspectives on the role of developmental novelties recordable in the fossil record and how they link to skeletal innovation.

B. METHODOLOGY

I am proposing a three-part research programme that combines classical palaeontological techniques and modern digital imaging technologies with new developments in the application of numerical phylogenetics to morphological data. Fieldwork will be used to attack gaps and biases in the record of early gnathostomes and expose the early diversity of cranial exoskeletons. Specimen-based work (derived from both fieldwork and existing collections) will be used to generate a detailed reconstruction of the evolutionary assembly of the earliest gnathostome exoskeletons and how they transformed from tessellate (‘micromeric’) conditions to macromeric armour, how and where sutures evolved, and how these transformations map to the stabilisation of skull architecture. At the centre of this study is a new phylogenetic framework for understanding the origin of the modern gnathostome skull and exoskeleton. Because fossils are the targets of the research, morphological data will be our only source of data for phylogenetic reconstruction. However, these data are considered problematic for their incompleteness, subjectivity of analysis, and lack of satisfactory models for their analysis. In this work, we will help overcome these issues with the development of a new numerical toolkit for character analysis and capturing morphology’s intricate hierarchical structure.

B1. Exposing the diversity of early gnathostome head morphology—new insights from the Canadian Arctic

A key objective is to resolve the problematic relationships of the placoderm fishes and their links to early osteichthyan skull construction. **The greatest insights into the evolution of the gnathostome head from fossils have been driven by exceptionally preserved fossils of armoured gnathostomes from the late Silurian and Early Devonian periods** (Zhu et al., 2013; Dupret et al., 2014; Giles et al., 2015; Zhu et al., 2016). However, fossils from the Silurian are especially rare. The Canadian Arctic hosts one of the oldest fossil faunas of armoured gnathostomes known outside of China. Fossils from this region have been pivotal in our understanding of deep gnathostome relationships from some of the earliest placoderms (Ørvig, 1975; Dupret et al., 2014) and bony fishes (Jessen, 1980). Furthermore, there is yet evidence of an even older gnathostome fauna in the Canadian Arctic. This includes fragmentary remains of the enigmatic, and presumably transitional

form between placoderm-grade fishes and osteichthyans known as *Lophosteus* (Märss et al., 1998; Schultze and Märss, 2004). There is also a report of placoderms from the Silurian on Ellesmere Island (Poey, 1988).

Not only does the Canadian Arctic host one of the oldest and most important early gnathostome faunas, but it presents the ideal factors for palaeontological fieldwork. The region hosts over 700,000 square kilometres of exposed marine Silurian bedrock (de Freitas et al., 1999). The vast quantities of exposed bedrock provide nearly limitless opportunity for prospecting. Based on my own preliminary work, reports from previous works, and past geological investigations, fieldwork in this area has the potential to deliver transformative insights into the evolution of the earliest gnathostomes, the radiation of the two major living lineages, and the transformations leading to the modern gnathostome skull. However, the remoteness of these localities and the logistical difficulties of accessing them prevented any sustained field campaigns in recent years. Most recent work has either focused on microfossil biostratigraphy (Märss et al., 1998) or some of the very well-preserved jawless fishes (Elliott and Loeffler, 1989; Elliott and Dineley, 1991; Märss et al., 2002; Elliott et al., 2015).

Field season 1. Eastern Cornwallis Island and Devon Island.

In 2018, I led a preliminary field expedition to eastern Cornwallis Island to reinvestigate the source of fragmentary fossils of *Lophosteus* (Märss et al., 1998; Schultze and Märss, 2004). This work uncovered a new set of beds in the Barlow Inlet Formation that yield extremely well preserved—even articulated—jawless fish heads (Fig. 4). Upon processing our samples from this locality, we discovered previously unrecognized macrofossil material of armoured gnathostome (Fig. 4C-F). This represents one of the oldest records of armoured gnathostomes outside of China and significant potential for well-preserved skeletal material. While there is considerable disagreement regarding the identity and even taxonomic coherence of *Lophosteus* (Botella et al., 2007; Friedman and Brazeau, 2010; Cunningham et al., 2012; Chen et al., 2017), it is nevertheless agreed to represent at least one or more late Silurian gnathostomes. Because the late Silurian represents such a sizeable gap in the record of early gnathostomes, new morphological data have the potential to deliver profound changes in understanding of deep gnathostome phylogeny. Most significantly is the fact that, although it is attributed to the gnathostome crown-group, its most strongly resembles ‘acanthothoracid’ placoderms—that group most widely considered to be the most ‘primitive’ placoderms. This highlights profound character conflict that would be resolvable with more complete knowledge of the anatomy of *Lophosteus*. Not only is it of considerable phylogenetic significance, but if the earliest osteichthyans possessed an acanthothoracid-like mode of skeletogenesis, then this signals the possibility that the stabilized skull roof patterns of placoderms and osteichthyans may be at least partially independently derived. Therefore, new data from *Lophosteus* will be central to a detailed reconstruction of the assembly of modern gnathostome skull architecture and how it links to placoderm morphologies.

We will return to the Barlow Inlet Formation on eastern Cornwallis Island to excavate our newly discovered locality and recover new, well-preserved fossils of the exoskeleton of *Lophosteus*. We will extend this work with exploratory prospecting of lateral equivalent beds on eastern Cornwallis and the extraordinary exposures of the Barlow Inlet Formation on western Devon Island (Thorsteinsson and Uyeno, 1980).

Field season 2. Drake Bay, Prince of Wales Island. Acanthothoracids are pivotal in prevailing hypotheses of placoderm relationships and I propose that they may show an intermediate stage in the assembly of a macromeric skull. A deeper appreciation of their diversity and variability is essential. It is vital that

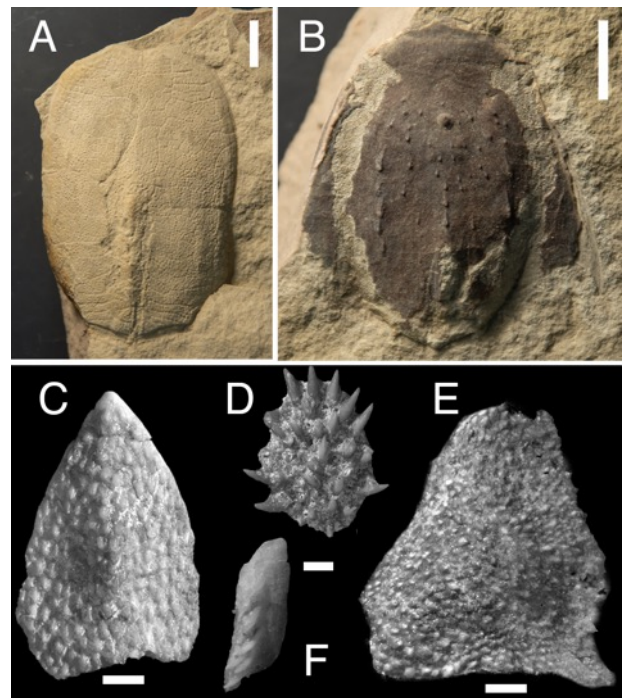


Figure 4. Preliminary results: vertebrate fossils from the late Silurian of eastern Cornwallis Island collected by the PI and colleagues at a newly discovered locality in 2018. A and B: Well-preserved jawless head shields showing high-quality preservation of fish material in newly discovered beds. C-F: *Lophosteus* macro- and microfossils from these beds C: Mid-dorsal plate; D: tooth plate; E: cleithrum (shoulder plate); F: body scale. Scale bars: A, B: 1 cm; C, E: 2mm; D, F: 1 mm.

phylogenetic interpretations of ‘acanthothoracids’ are not restricted to the small sample sizes currently available. The Drake Bay locality on western Prince of Wales Island boasts a rich fauna of three-dimensionally preserved ‘acanthothoracid’ and arthrodire placoderms, along with abundant heterostracan jawless fishes. The fossils are extremely well preserved and three-dimensional, they respond very well to chemical preparation techniques that extract the fossils from their host rock with minimal damage to the material. This locality presents an ideal, low-risk target for palaeontological investigation in the Canadian Arctic. The ‘acanthothoracid’ *Romundina* is well known from this locality on the basis of isolated braincases, skull roofs, and shoulder elements. However, the locality demonstrates clear potential for articulated material (Fig. 5). Such material would provide a major step-change in early gnathostome phylogenetics because articulated body fossils remain virtually unknown.

By revisiting the Drake Bay locality, we will create an expanded sample of phylogenetically key early gnathostome taxa. The exceptional, three-dimensionally preserved material responds extremely well to acid preparation and will therefore deliver valuable insights into the structure of ‘primitive’ gnathostome head morphology and cranial bone construction. The opportunity to collect articulated or semi-articulated material will provide fundamentally deeper phylogenetic insights by filling in missing data and exposing character (in)congruencies that can be the targets of rigorous character analysis. We will prospect the Drake Bay locality over the period of three weeks to collect both ‘high-grade’ material for detailed macro-anatomical investigation and fragmentary material for destructive sampling analysis.

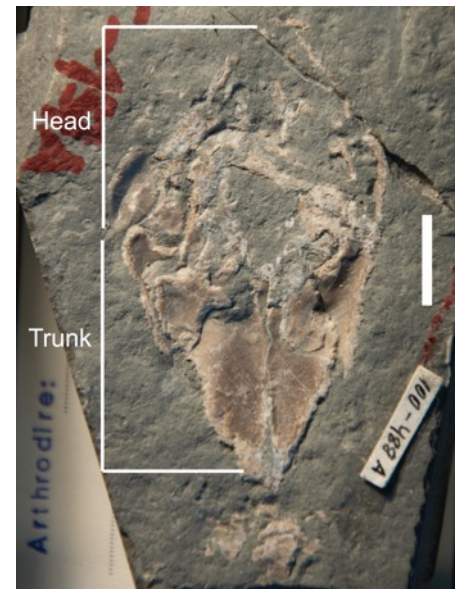


Figure 5. Articulated head and trunk of a placoderm fish from the Drake Bay Locality, Prince of Wales Island (Museum of Geology, University of Göttingen). Scale: 1 cm.

Field seasons 2 & 3. Southern and central Ellesmere Island. This represents the highest risk component of the fieldwork and seeks an entirely exploratory investigation based on geological priors and stratigraphical report data. We will target the late Silurian Cape Phillips and Devon Island Formations exposed on southern and central Ellesmere Island. Silurian localities in the Baumann Fiord area have been reported as yielding placoderm material (Poey, 1988). The Devon Island Formation is known to preserve vertebrate fossils (Mayr and Packard, 1994) but is largely unexplored by vertebrate palaeontologists. What is most enticing about this formation is that the rock types and depositional environment show all of the hallmark features of exceptional fossil preservation (Seilacher et al., 1985; McCoy et al., 2015a; 2015b): the rocks are organic-rich shales with authigenic pyrite deposition suggesting anoxic bottom conditions; they lower units are finely laminated and host syngedimentary concretions (Mayr and Packard, 1994). As with the Late Devonian Gogo Formation—famed for its exceptional vertebrate fossils (Long and Trinajstić, 2010)—the formation is deposited in an inter-reef basin. Articulated fish material are already known from the lower part of the Cape Phillips Formation (Poey, 1988; Märss et al., 2002). However, definitive gnathostomes are not yet known. There is risk here in failing to recover significant material of gnathostomes, but the risks are well worth it in light of the potential for outstanding levels of fossil preservation. Articulated gnathostomes from this interval would be among the oldest and most complete in the world and would add vital new data in illuminating phylogenetic relationships.

B2. Morphological and palaeo-developmental analysis of gnathostome cranial diversity

In this project I will derive a rich set of comparative data from both the field and collections-based work. Collections-based work will offset some of the risks associated with fieldwork. The objective of this component of the project will be to create a comparative anatomical and palaeo-developmental record of the earliest skull assemblies across the spectrum from jawless fishes to early chondrichthyans and osteichthyans. The data generated here will supply both new phylogenetic analyses and test of the new model of cranial evolution and diversification presented here.

B2.1. Macrofossil documentation: We will use X-ray computed tomography (CT) and standard laboratory techniques to document the exoskeletal diversity of early gnathostomes and jawless relatives. We will sample a host of exceptionally preserved, acid-prepared early gnathostome fossils from the collections of the Natural History Museum, UK where I am a Scientific Associate. These fossils will include primarily acid-prepared fossils from Gogo (i.e. pachyosteomorph arthrodires, antiarchs, ptyctodonts, the osteichthyans *Mimipiscis*,

Moythomasia, and *Onychodus*), Taemas-Wee Jasper (*Shearsbyaspis*, *Parabuchanosteus*, *Goodradigbeeon*). Material of *Lophosteus* and acanthothoracid placoderms derived from fieldwork and on loan to me from the Geological Museum in Göttingen will be acid prepared and subject to computed tomography analyses. Using microtomography, we will explore the cross-sectional ‘mesostructure’ of sutures in these groups, documenting which bone layers cooperate in the formation of scarf joint and interdigitating joint margins, as well as whether or not particular bone layers are present or absent. These data will provide tests of Stages 2 and 3.

For functional analysis, we will document the three-dimensional structure of sutures and express their morphological structure and complexity in the form of discrete characters. We will score variables such as structure of simple lap joints, levels and depths of interdigitation, and distribution across different parts of the skull in order to create a dataset of sutural diversity for comparative analysis. This will be co-tabulated with morphometric and discrete functional character data on jaw structure (Anderson et al. 2011).

We previously collected and will collect additional heterostracan fossils from the Canadian Arctic and, given their extreme abundance in the Canadian Arctic, we anticipate collecting additional examples. Heterostracans appear to represent an independent origin for a macromeric exoskeleton. They similarly show a cline between ‘micro-plated’ forms and macromeric forms. We will acid-prepare several specimens and CT scan them so that we can use these for a comparative contrast with jawed vertebrate examples.

B2.2. Micro-histology: Our new field collected material will provide relative freedom to undertake destructive sampling. We will not strictly ‘high-grade’ our collecting in the Arctic, as may have been done in the past, but will focus as well on ‘low value’ fossils that can be acid-etched and thin-sectioned for high-resolution histological analysis. Complementing this, we will use phase-contrast tomography on the Zeiss Xradia Versa CT scanner at the Natural History Museum to create 3D high-resolution histology of the growth interfaces of sutural and non-sutural bone margins. We will document, for instance, patterns of open and closed sutures, the presence or absence of scarf and interdigitating joints, or closed sutures, correlate with processes such as remodeling and resorption. For instance, how did the closed sutures of acanthothoracids and some other placoderms sustain growth? Was there active resorption at the margins of cranial plates?

B3. New approaches to the phylogenetic study of morphology

B3.1. Anatomical networks as a computable method of character analysis

One of the theoretical novelties in this work is the deeper appreciation of the possibility of ancestral polymorphism in the evolution of discrete characters such as disjoint skeletal units or body segments. Character analysis of morphological data requires investigators to propose prior homology statements about their characters in order to undertake phylogenetic analysis. However, homology is a fundamentally phylogenetic question (Patterson, 1982), and character analysis may need updating in light of new phylogenetic theories. Topology and topographic relationships alongside ‘special similarity’ are the main logical methods in this analysis (Rieppel and Kearney, 2002). There is, as yet, no computational method for doing this.

Fundamentally, problems of topological homology are network problems. Networks have been pioneered in the analysis of anatomical data (Diogo et al., 2009), but they have yet to be leveraged for phylogenetic analysis. However, comparative approaches for studying gene regulatory networks is an emerging field (Thompson et al., 2015). This project asks to what extent these advances can be harnessed in character analysis to study particularly intractable problems as the homologies of the gnathostome skull (or related problems in other groups, such as the arthropod head problem; Fig. 6). Jardine (1967) demonstrated that a computable approach to character analysis and deployed this on the problem of the vertebrate skull. Such a method has not seen wider adoption among comparative biologists and has never been implemented in a modern computer program. However, Jardine’s method demonstrated that it was possible to make homology proposals computationally. However, it opens up new possibilities for character analysis of problematic topological homologies.

I propose to implement a modified version of Jardine’s method to generate a computationally objective approach to character analysis. the use of a stochastic approach to Jardine’s algorithm, injecting an iterative random component with subsampling, akin to Monte Carlo estimation as a means of comparing skull bone

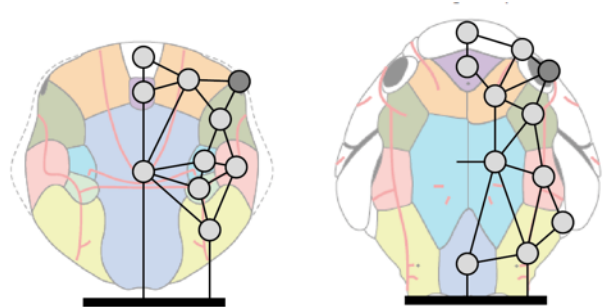


Figure 6. Two placoderm skulls showing disparate patterns and represented by a planar network. Fixed anatomical landmarks and polarities such as the eyes (dark grey discs) and posterior margin (black bar) establish computationally useful priors. Drawings from Zhu et al. (2013).

patterns. This will be used to quantify the degree of similarity between two skulls (or any set of connected, discrete anatomical parts) in terms of the number of possible equivocal interpretations as calculated by Jardine’s method. This method will identify cases where *a priori* skull bone homologies cannot be determined, adding an element of measurement of uncertainty to Jardine’s method. This will set the stage for the next set of analyses in which such character contingencies are handled in a likelihood framework.

B3.2. A maximum likelihood approach to character hierarchies

I propose will develop algorithms for hierarchical character dependencies very recently devised for parsimony analysis (De Laet, 2005; 2015; Brazeau et al., 2018), but with more powerful features and using a likelihood framework. As parsimony is a ‘special case’ of maximum likelihood (Tuffley and Steel, 1997), it should be possible to derive a maximum likelihood representation of these new methods. To accomplish this would be to develop a more generalised approach to the application of these methods that would allow the calculation of likelihoods hierarchically related characters. The newly devised methods will allow the encoding of contingent networks of characters (in the form of separate character hierarchies) that can be co-optimised with phylogenetic analysis.

Parsimony methods for character hierarchies compute the cost of a character on a tree under a constraint imposed by a parent character. As in normal parsimony methods, the total number of transformations are totalled up to form the score of the tree. However, an additional quantity—the number of ‘subcharacters’ or ‘region splits’ on the tree—needs to be calculated as well. Because maximum parsimony methods are, in fact, minimising homoplasy, this quantity ensures that the total score of the tree reflects some minimum value *plus* the number of changes that imply homoplasies. Thus these methods give scores that penalise the tree for homoplasy. De Laet’s method goes a step further than Brazeau et al. in keeping track of exact relationships between characters. Brazeau et al. use the distribution of gaps scored in the matrix to infer the hierarchy, however this is inexact. De Laet’s method requires pre-supplying these relationships. However, obtaining the score of the tree involves a complex series of calculations: the root (i.e. presence/absence character) in the hierarchy is calculated using full ancestral states. The sub-dependent characters are then mapped onto the tree under the constraint imposed by that character. This initiates a search, where nodes in the ‘absent’ state for the parent character are then ‘switched’ and the length of the tree re-tested under this new constraint. This process is continued until an optimal score is found.

De Laet’s parsimony method can be adapted for easier and more generalised use, and can be extended to a likelihood framework. Character hierarchies can be defined by specifying a (triangular) matrix of character and character-state dependencies (i.e. character *j* depends on state *x* in character *i*). For example:

$$\begin{array}{cccc} - & - & x & - \\ & - & x & x \\ & & \ddots & \vdots \\ & & & - \end{array}$$

Where the – indicates there is no sub-dependency. Given such a matrix, the data could be partitioned into a series of hierarchies. The structure of such a hierarchy is composed of a parent (or root) character, which is typically a presence vs. absence character. There can be any number of subdependent characters who can only have a logical value at a node in a tree if the parent character is in the ‘presence’ state. The likelihood of a tree given a set of *h* character hierarchies can be given as:

$$L = L_{(1)} \cdot L_{(2)} \cdot \dots \cdot L_{(h)} = \prod_{j=1}^h L_{(j)}$$

Which is simply the formula for the likelihood of a tree based on the product of individual character likelihoods—where *h* would instead enumerate the individual characters. In this case, *h* enumerates the hierarchies which may each contain one or more characters (the parent character is always in its own hierarchy).

Computing the likelihood of each $L_{(h)}$ will be more complicated than for a single character. At the base of a hierarchy will be the parent or root character, typically a simple binary presence/absence character. Following De Laet’s method, the likelihood of the parent character can be reconstructed in an ‘unconstrained’ manner over the whole tree. However, the relative likelihoods for given nodes will need to be updated in light of the conditional likelihoods for all sub-dependent characters in the hierarchy. During the calculation of likelihoods at each node, the method will need to consider the different implications of presence/absence of the parent character on the likelihoods of the sub-dependent characters over the whole tree. That is, the

sub-dependent characters will have to ‘evolve’ over the tree in some manner that respects the constraint imposed by the parent character.

A further complication is the calculation of the likelihood of a character when the parent character is assumed absent. I propose that it should be proportional to the product of the probabilities of the character appearing independently in the descendant subtrees. This reflects, in effect, the probability of drawing the states in two sub-clades completely at random and getting the same values. This will give a more natural interpretation to the ‘subcharacters’ parameter of De Laet (2015) expressed in terms of probabilities.

The process itself will be more complicated than what is presented here. The probability of a parent character given a tree and model is related to the E term in Lewis’s (2001) correction for absence of constant characters. The probability of a character in a hierarchy is conditional on the distribution of the parent character, but the converse is also true. Some modification of Tuffley & Steel’s (1998) covarion model may be adaptable for these purposes. The probabilities will need to be co-optimised between parent and dependent characters. This will require successive iteration of the presence/absence probabilities and recalculation of the likelihoods for the entire hierarchy, equivalent to the search for optimal state combinations in De Laet’s {**Anagallisaprogram:2018di*} *anagallis* program for parsimony. It will be one of the project’s objectives to determine how best to accomplish this, however I preliminarily suggest that this operation occurs during the iteration of branch lengths during a Markov chain Monte Carlo estimation as is now common practice in computational phylogenetics in a Bayesian framework.

To support this component of the work, I am collaborating Dr Peter Foster (Natural History Museum, UK) who will provide local advice on computation methods and with Dr April Wright (Southeastern Louisiana University) who is a collaborator in RevBayes project (Höhna et al., 2016) contributing developments for model-based analysis of morphological data. Our goal will be to implement these methods in RevBayes.

B4. Component 4: Phylogenetic reconstruction and analysis of diversity

B4.1. Phylogenetic analysis: fresh data and cutting-edge methods

The data gathered in sections B2 and B3 will be encoded into a phylogenetic data matrix. This matrix will be complemented with a hierarchical dependency matrix developed on the basis of prior character analyses and the new methods of character analysis outlined above. The provisional estimate for the size of the phylogenetic dataset will be between 100 and 150 terminal taxa. The estimated number of characters is between 300 and 400. However, many of these characters will be ‘bundled’ into hierarchies. We will apply both parsimony and Bayesian posterior probability analyses to infer the phylogenetic relationships of Palaeozoic gnathostome taxa. The results will deliver a new phylogenetic hypothesis (or set of hypotheses) for the interrelationships of the earliest jawed vertebrates and supply a further hypothesis-testing framework for understanding trait-dependent diversification. Our ancestral states reconstructions derived from these results and using the same methods as those used analyse the tree will be used to extract a detailed history of the evolution of the gnathostome head and other key gnathostome traits (such as paired appendages), where our new data might supply novel insights. We will initially use these results to make several key, qualitative assessments: 1) whether acanthothoracids exhibit a phylogenetically intermediate stage in the evolution of the gnathostome skull from jawless precursors (Stage 2 of my model); 2) Whether placoderms represent a clade or grade of early gnathostomes and, consequently, how many times a stabilised pattern of skull roof bones evolved (and whether the common patterns seen in arthrodires and osteichthyans are actually homologous or represent convergence based on overall skull geometry). The latter insight will be especially important as it would demonstrate the possibility that deep conservation across many groups of living gnathostomes may as well be underlain by functional constraints (something further assessed in the next section).

B4.2. Reconstructing skull construction and testing innovation hypotheses

Using the newly devised character reconstruction algorithms and our new data, we will undertake ancestral state reconstruction of gnathostome dermal evolution characters and map these alongside the morphological structure of the skull, documenting the presence or absence of open sutures, stable vs. polymorphic skull roof patterns and assessing the competing hypotheses of single or multiple origins of stable skull roof comparisons. The data generated in B1 and B2 will not simply be used to serve phylogenetic ends. We will create a discrete character database for skull construction mechanisms documenting the nature of suture structure, from variables of complexity (i.e. simple scarf joints, interdigitating joints etc.), the presence or absence of evidence of resorption, and whether or not sutures are open or closed.

To test the innovation hypothesis of gnathostome cranial growth mechanisms I propose a series of comparative analyses derived from the above results. In collaboration with Dr Lauren Sallan (U Pennsylvania) we are assembling a ‘supertree’ and trait database for total-group gnathostomes (i.e. spanning Palaeozoic jawed and jawless vertebrates). Phylogenetic trees will be time-calibrated using records of stratigraphic data for

sampled tips. We estimate between 300 and 400 terminal taxa. Combining our new data with the wealth of information available from palaeohistological studies of jawless fishes, we will use modern quantitative comparative methods to infer trait-dependent diversity patterns across various extinct and extant early vertebrate clades. Databases exist for trait data on early gnathostome jaw disparity (Anderson et al., 2011; Hill et al., 2018). We can leverage these datasets to analyse phylogenetic covariance of jaw disparity metrics with indicators of modularity in the form of the disparity of suture morphology. Secondly, we will explore how stable patterns (quantified with our new network-based approach) evolved in concert with changes to skull function. We will test covariation in stable and ‘homologizeable’ skull roofs in relation to factors such as cranial kinesis, integration between the mandibular arch and facial skeleton, the overall level of cranial ossification, and a numerical index of suture complexity. We will then apply comparative trait-based analysis of diversity methods (Rabosky 2014) to explore the evolutionary relationship between diversification and suture complexity across the early gnathostome tree.

Risks and Feasibility

I have two previous field experience in the arctic, as well as several years field experience in remote areas, including eight field seasons in western Mongolia, and an additional field season in northern Canada, for a total of 11 seasons of remote wilderness fieldwork experience. The Canadian Arctic is an unforgiving and often unyielding climate, but I have taken measures to ensure the overall feasibility and success of fieldwork. Arctic fieldwork is seasonal: possible only in the short-duration summers from about mid-June through August. All logistics in this project were designed in consultation with the Canadian Polar Continental Shelf Program (PCSP), a Government of Canada agency that coordinates and supports logistics for field operations in the Canadian Arctic. They review and approve all project designs for feasibility (in both logistics and costs) and coordinate aircraft logistics. PCSP supplies equipment free of charge as well as consumables with costs recuperated from clients (such as this project).

The project is high-risk, including a significant dependence on fieldwork. However, the modular design of the project is designed to offset any risk. Each component of the project stands to make its own impactful contributions. The new data from fieldwork will be primarily of interest to palaeontologists, but the phylogenetic outcomes will be of wider significance for comparative biologists. While there is risk that the fieldwork will not deliver any useful new data, I have minimised this in several ways. Firstly, in the first two seasons I have targeted known, productive localities. Both of these localities are known to yield fossils of taxa that are of pivotal value in the reconstruction of gnathostome phylogenies. The highest risk fieldwork is saved for a later season, by which time we should have generated a body of substantial data from two previous seasons and collections-based work. Finally, it is very easy to dismiss palaeontological fieldwork if it is perceived as merely a ‘trophy hunting’ expedition. However, a key part of our project involves destructive sampling to generate high-resolution histological data of bone interfaces. Such material—especially for acanthothoracid placoderms and stem-group osteichthyans—may be difficult to derive from existing collections and would be subject to curatorial restrictions. Fieldwork will allow us to collect ‘low-grade’ material from important taxa that are underrepresented in existing collections. Thus, we can assure results by not depending solely on the discovery of well-preserved examples of rare taxa.

There are risks that newly proposed phylogenetic advances will be too difficult, computationally intractable, or be inapplicable to realistic data. By applying approximate techniques, such as MCMC estimation we will ensure that meaningful results can be computed in reasonable times, even while inexact. If we are unsuccessful in delivering new methodologies, other parts of the project can still move forward, but will simply have to rely on older techniques.

Dividends and legacy

When this project is completed it will drive new perspectives and approaches to the study of vertebrate (and indeed animal) diversity in the fossil record. To date, palaeoecological approaches to studying diversification and function in the fossil record have focused on large-scale morphometrics as key indicators (i.e. jaw mechanics, body size, body and appendage shape). There is no question that such variables are important. However, this work will call attention to the key data in skeletogenic processes as a locus for the generation of diversity. By mapping histological patterns of bone development to large-scale patterns of skeletal construction, we will show how microscopic details of bone formation can make important predictions about diversification potential and possibly even key aspects of life history (i.e. bone resorption, growth, and replacement). Thus macroevolutionary studies will be able to leverage this data for large-scale analytical studies.

One of the major strengths of this proposal is that, while it is high-risk, it is virtually guaranteed to generate a body of valuable comparative data. The data derived from new fossil collections and new, freely

available tomography datasets will serve as a long-lasting resource for generations of comparative vertebrate palaeontologists. This will ensure that the results will have a long-lasting impact.

The new phylogenetic tools will be designed with the problems of this project in mind. However, these problems are not unique to early gnathostomes. They are part of a general class of problems in comparative biology that relate to the hierarchical nature of biological data and the problems of homology assessment. The new tools will be generally transferrable to other areas of comparative morphology and might be used (perhaps with relatively minor adaptation) in the analysis of comparative molecular development.

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