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The Origin of Tetrapod Limbs and Girdles: Fossil and Developmental Evidence

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ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2009

ISSN 1651-6214
ISBN 978-91-554-7448-5
urn:nbn:se:uu:diva-98911

Dissertation presented at Uppsala University to be publicly examined in Lindhalsalen, EBC, Norbyvägen 14, Uppsala, Thursday, April 9, 2009 at 13:00 for the degree of Doctor of Philosophy. The examination will be conducted in English.

Abstract

Boisvert, C A. 2009. The Origin of Tetrapod Limbs and Girdles: Fossil and Developmental Evidence. Acta Universitatis Upsaliensis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology* 613. 53 pp. Uppsala. 978-91-554-7448-5.

Around 375 million years ago, the first tetrapods appeared, marking one of the most important events in vertebrate evolutionary history. The fin to limb transition saw the appearance of fingers and a weight bearing pelvic girdle. While very little research has been done on the evolution of the tetrapod pelvic girdle, a fair amount has been done on the origins of fingers but some aspects remained controversial. A combination of palaeontology, developmental biology and comparative morphology was therefore used in this thesis to better understand the fin to limb transition. The pectoral fin of *Panderichthys*, a sarcopterygian fish closely related to tetrapods was CT-scanned and modeled in three dimensions and its pelvic girdle and fin were examined with traditional techniques. This information from the fossil record was integrated with comparisons of the development of the Australian lungfish, *Neoceratodus forsteri*, our closest living fish relative and the axolotl (*Ambystoma mexicanum*), a salamander representing well the condition of early tetrapods. Development of bone and cartilage was studied through clearing and staining and development of skeletal muscles through immunostaining. In situ hybridizations were performed on the lungfish to study the expression of *Hoxd13*, associated with the formation of digits in tetrapods.

This work shows that the late expression phase of *Hoxd13* is present in *Neoceratodus* and is associated with the formation of radials. Redescription of the pectoral fin of *Panderichthys* reveals that distal radials are present, which, in addition to other information, lead us to conclude that digits are not novelties in tetrapods but rather have evolved from the distal radials present in the fins of all sarcopterygian fish. The earliest tetrapods lack a full set of wrist + carpals/ankle + tarsal bones. Here, we propose that this region of the limbs evolved after fingers and toes through an expansion of the region between the proximal limb bones and the digits. As for the pelvic girdle, it is very primitive in *Panderichthys* but comparison of its development in *Neoceratodus* and *Ambystoma* suggest that the ischium evolved through the posterior expansion of the pubis and the ilium, through an elongation of the iliac process already present in sarcopterygian fishes.

The results of this thesis help to better understand the fin to limb transition and show that it is more gradual than previously believed.

Keywords: palaeontology, developmental biology, morphology, evo-devo, fish-tetrapod transition, fin to limbs, paléontologie, biologie du développement, morphologie, évo-dévo, sortie des eaux, évolution des pattes, paleontologi, utvecklingsbiologi, morfologi, evo-devo, fisk-fyrfotingsövergång, fena till ben övergången

Catherine Anne Boisvert, Evolutionary Organism Biology,

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ISSN 1651-6214

ISBN 978-91-554-7448-5

urn:nbn:se:uu:diva-98911 (<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-98911>)

À ma famille
Vous êtes toujours là pour moi

To David
You bring the best out of me

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I Johanson, Z., Joss, J. M. P., **Boisvert, C. A.**, Ericsson, R., Sutiya, M. and Ahlberg, P. E. (2007) Fish fingers: Digit homologues in Sarcopterygian fish fins. *Journal of Experimental Zoology:Part B Molecular and Developmental evolution*, 308B(2007):757-768
- II **Boisvert, C. A.**, Mark-Kurik, E. and Ahlberg, P. E. (2008) The pectoral fin of *Panderichthys* and the origin of digits. *Nature*, 456(7222): 636-638
- III **Boisvert, C. A.** (2009) The humerus of *Panderichthys* in three dimensions and its significance in the context of the fish-tetrapod transition. *Acta Zoologica* 90(Suppl. 1): 297-305
- IV **Boisvert, C. A.** (2005) The pelvic fin and girdle of *Panderichthys* and the origin of tetrapod locomotion. *Nature* 438(7071)
- V **Boisvert, C. A.**, Ahlberg, P. E. and Joss, J. M. P. Comparative pelvic development of the axolotl (*Ambystoma mexicanum*) and the Australian lungfish (*Neoceratodus forsteri*) Manuscript

The following papers were written during the course of my PhD studies but are not included in this thesis.

- VI Clément, G. and **Boisvert, C. A.** (2006) Lohest's true and false "Devonian amphibians": Evidence for the rhynchodipterid lungfish *Soederberghia* in the Famennian of Belgium. *Journal of Vertebrate Paleontology* 26(2): 276-283
- VII **Boisvert, C. A.** (2009). Vertebral development of modern salamanders provides insights into a unique event of their evolutionary history. *Journal of Experimental Zoology:Part B Molecular and Developmental evolution*, 312B(2009):1-29

In **Paper I**, CAB helped reproduce the in situ hybridization experiments and contributed to the discussion. In **Paper II**, CAB did the initial 3D modeling of the pectoral fin, produced the descriptions and most of the interpretations, wrote the article and produced the illustrations from animations produced by PEA. In **Paper V**, CAB obtained salamander material, performed all experiments, imaging, descriptions, interpretations and wrote the manuscript.

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Life in the Devonian

Biogeography and climate

Imagine a warm summer day without the smell of flowers, the buzz of a bee or the song of a cricket. Imagine a landscape without grass but with plants that seem to belong to the undergrowth. If you were to visit earth towards the end of the Devonian period, about 375 million years ago, it is this strange, silent world that you would experience. During that period, the earth looked very different. Although parts of the continents we know today were already present at that time, they were arranged very differently. The super continent of Gondwana, containing what would become South America, Africa, India, Antarctica and Australia was slowly drifting towards Laurussia (what would become North America, the Canadian arctic, the Baltic, part of Western Europe, and part of Russia) (Li et al., 1993; Dineley and Loeffler, 1993) narrowing the oceans between them and creating many shallow water, semi-marine environments on their coasts. It is only during the Permian, roughly 80 million years later, that most of the continents became united as Pangea, which split through the Mesozoic 250-65 million years ago into the continents as we know them today (Press and Siever, 1998).

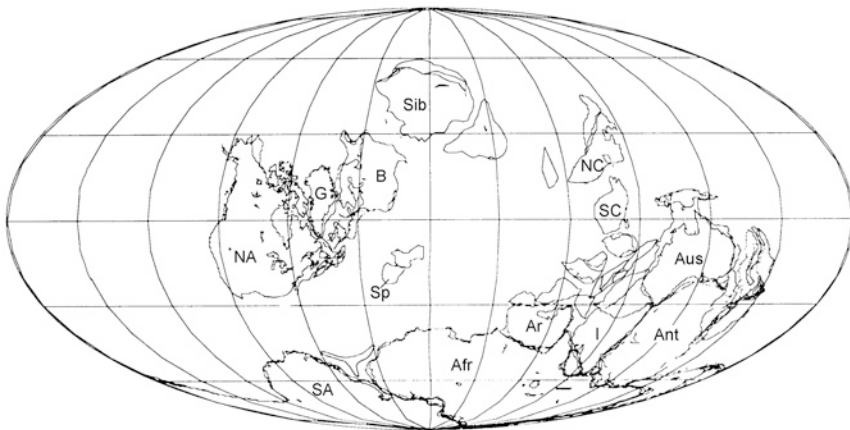


Figure 1: The earth at the end of the Devonian period, around 370 million years ago. Laurussia is on the top left and Gondwana, on the bottom right. Abbreviations: Afr: Africa, Ant: Antarctica, Ar: Arabian peninsula, Aus: Australia, B: Baltic, G: Greenland, I: India, NA: North America, NC: North China, SA: South America, Sib: Siberia, SC: South China, Sp: Spain. From Li et al (1993).

As a result of the continental arrangement in the Late Devonian, Eastern North America, Greenland, the Baltic, Scotland, Russia, China and Australia were at the equator or close to it and benefited from a tropical climate (Streel et al., 2000). It is in these localities that all the earliest tetrapods (four-legged vertebrates) have been discovered. They are found in a variety of environments, ranging from streams and rivers, alluvial plains to near shore marine environments. It is in those warm, shallow water environments, most likely on the edges of Laurussia that tetrapods evolved about 380 million years ago (Blicek et al., 2007).

Life and ecosystems

The very first multicellular organisms to conquer land were plants. During the Silurian period (445-415 million years ago), liverworts, fungi and moss-like plants started inhabiting the nearshore environments. By the beginning of the Devonian period (around 415 million years ago), terrestrial plants were becoming more complex and diverse. It is at that time that club mosses (lycopsids) appear and by the late Devonian (around 380 million years ago), coastal environments were covered with a diversified and rich flora (Clack, 2002). Relatively large lycopods, pre-ferns, horsetails and the ancestors of conifers (progymnosperms such as *Archaeopteris*) composed the forests adjacent to water bodies. In those forests thrived a plethora of arthropods, having followed the plants onto land in the middle of the Silurian. Flying insects had yet to evolve but springtails were making their first attempts at getting off ground. By the late Devonian, arthropods had diversified and scorpions, mites, centipedes, millipede-like animals and the very first silk-producing spiders were thriving in marginal forests (Clack, 2002). Despite this diversity, no herbivorous arthropods were present and vertebrates had yet to conquer land.

At this time, all of the world's vertebrate diversity could be found in the water. Jawless fishes covered with bony plates, such as osteostracans and heterostracans, were being outcompeted by a growing diversity of jawed fishes (or gnathostomes) (Janvier, 1996). Amongst those jawed fishes were the placoderms, a very diverse group of heavily armored fish who were very abundant during this period (Carroll, 1988). These fish, some of which were top predators, looked superficially like space-ships, with a head covered by bony plates and a long, flexible tail. Sharks were present in the late Devonian but were not very diverse. However, the Acanthodians (or spiny sharks) were relatively abundant at the time (Schultze, 1996). Despite their common name, these were not sharks per se but are likely to be at the very base of all jawed vertebrates (Brazeau, 2009).

One of the most important groups of fishes at that time was the osteichthyans, or bony fishes. In addition to bearing bony scales on their bodies,

they also possess a skeleton made of endochondral bone. This group is separated into two: Actinopterygii, or ray-finned fishes and Sarcopterygii, or lobe-finned fishes. Actinopterygians have long dermal rays in their fins and they represent the majority of vertebrate diversity found today (Cloutier and Arratia, 2004). However, during the late Devonian, they were relatively rare and most of them were very small (Carroll, 1988). It is the sarcopterygians, possessing fins with a long internal skeleton, who were the most abundant and diverse group of osteichthyans at the time (Trewin, 1986). This group included lungfishes and coelacanths, which are still alive today, as well as large predatory fishes such as rhizodonts and the ancestors of tetrapods (Ahlberg and Johanson, 1998). These appeared towards the end of the Frasnian period (around 375 million years ago) and later gave rise to all vertebrates who conquered land, air and even returned to water (Fig 2) (Clack, 2000).

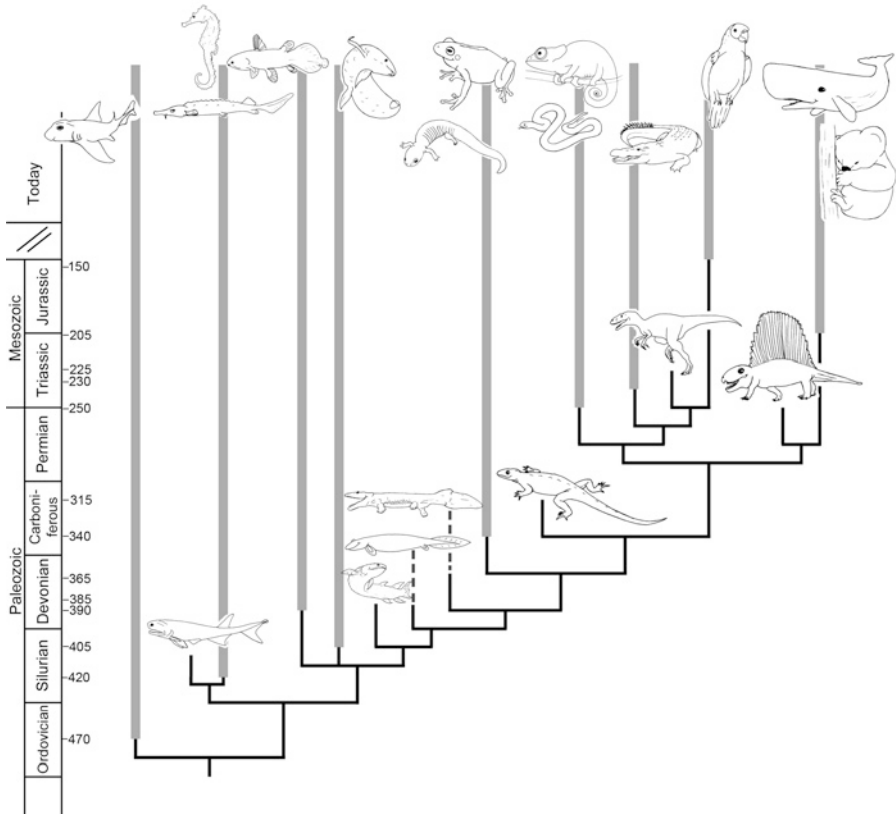


Figure 2: Phylogenetic tree of jawed vertebrates (gnathostomes). On the left, a partial time scale showing dates of the first appearance of the different taxa or groups. The base of the pale grey lines show the time of appearance of modern groups and the grey line, their continuation until today. The approximate date of appearance of extinct taxa is represented by the upper tip of the black line. The length of the line is not meant to represent an estimate of their divergence date. Taxa from left to right: **Chondrichthyans** (sharks, skates and rays) represented by *Heterodontus*; **Actinopterygians**: *Cheirolepis* and modern ray-finned fishes represented by a seahorse and a sturgeon; **Sarcopterygian** fishes: coelacanths represented by *Latimeria*, lungfishes represented by *Neoceratodus*, *Eusthenopteron*, *Panderichthys*; **Tetrapods**: *Acanthostega*, **Amphibians**: Lissamphibia represented by a frog and a salamander, **Amniotes**: *Hylonomus*, Squamata (lizards and snakes) represented by a snake and a chameleon, crocodylians, dinosaurs represented by *Megalosaurus*, birds, *Dimetrodon* (a pelycosaur), mammals represented by a koala and a whale.

We will never fully know why this fish-tetrapod transition occurred; the ancestors of tetrapods were all relatively large predators, bound to water for food and reproduction. However, it may have been competition for food and breeding as well as escape from predators that drove some of those fish towards increasingly shallow water environments. Given the fact that these environments were getting increasingly clogged by decaying vegetation and therefore were low in oxygen, fish would have needed to rely increasingly

on air breathing and perhaps seeking an escape from those hypoxic waters onto land (Clack, 2002). It has also been suggested that the ancestors of tetrapods crawled onto land to bask in the sun, thereby increasing their metabolism and making them more competitive when returning into the water (Carroll et al., 2005). The best crawlers, those with the best adaptation to support their weight and survive out of the water, would therefore be selected. Notwithstanding why tetrapods emerged, their evolution from sarcopterygian fish remains one of the biggest transitions in vertebrate history.

Fish-tetrapod transition

One of the best known sarcopterygians fish closely related to tetrapods is the tristichopterid *Eusthenopteron*. Numerous well preserved, three-dimensional specimens have been found in Miguasha, Québec, allowing in-depth analyses of its anatomy (Andrews and Westoll, 1970; Cote et al., 2002; Jarvik, 1980). *Eusthenopteron*, just like other sarcopterygians fishes, possessed lungs as well as gills and the internal skeleton of their fins had equivalents of the humerus, radius and ulna of tetrapod arms and femur, tibia and fibula of their legs (Andrews and Westoll, 1970). However, the distal ends of those fins were covered with dermal fin rays, or lepidotrichia. These, in addition to the dorsal and anal fins of fish like *Eusthenopteron* are lost in tetrapods, the only fin rays remaining being those of the tail (Clack, 2000). (Fig 3 in red). In fully terrestrial tetrapods, the fin rays of the tail are completely lost.

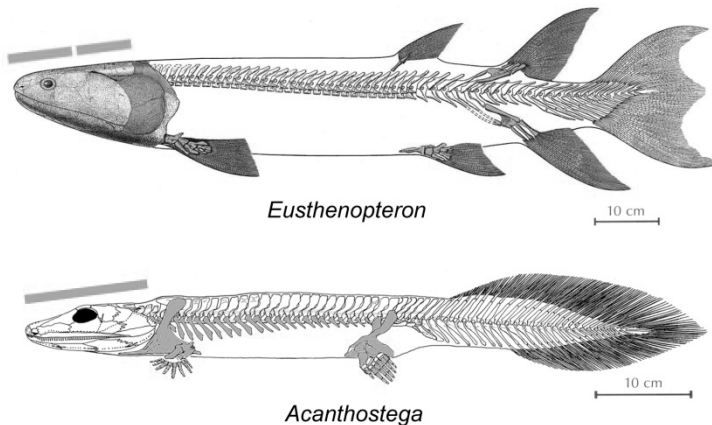


Fig 3. Morphological changes during the fish-tetrapod transition. *Eusthenopteron* exemplifies the condition of sarcopterygian fish, and the early tetrapod *Acanthostega* that of tetrapods. In red are elements that are lost during the transition, in green are changes and in yellow, new structures for tetrapods. The line above the head represents the intracranial joint in *Eusthenopteron* and its absence in tetrapods.

The skull of early tetrapods such as *Acanthostega* is also a little different from that of *Eusthenopteron*. For example, the hinge connecting the front and rear halves of the skull roof in *Eusthenopteron* is absent in *Acanthostega* which has a more consolidated skull roof (Fig 3 green line). Early tetrapods lost a series of bones on the side of the skull (the operculo-gular and supracleithrals), freeing the head from the body and thereby creating a neck. This transformation, as opposed to those mentioned above, is more gradual, the bones being present but reduced in *Panderichthys*, a fish closely related to tetrapods (Clack, 2000).

In addition to the loss of these bones, proportions of the skull change during the transition. The snout elongates, the orbits increase in size and become more dorsal. These changes are likely associated with a change in habitat from open water to the water-air interface in more shallow environments. The transformation of the hyomandibula (a bone involved in jaw and gill support in fishes) into the stapes of the middle ear (Brazeau and Ahlberg, 2006) might also have evolved hand in hand with adaptations to a new environment. Enabling or being driven by this gradual shift towards more coastal and terrestrial environments are changes in the appendages. The internal skeleton of the fins is remodeled slowly (**Papers II and III**) (Shubin et al., 2006) to become limbs with fingers and toes and there is a shift in size and locomotory reliance from the pectoral to the pelvic appendage. Fish rely primarily on the body musculature and pectoral fins for locomotion but in tetrapods, it is the hindlegs that assume most of this function (Coates et al., 2002). In connection to this, the hind-legs are larger and more powerful than the front-limbs and marked changes are observed in the pelvic girdle. In fish such as *Eusthenopteron*, the pelvic girdle is a small, crescentric structure with no connection to the vertebral column, but in tetrapods, it is a much larger, weight-bearing structure attaching itself and the hindlimbs to the vertebral column. Other changes of the appendicular skeleton include increase in rib size and the connection of vertebrae through zeugapophyses of the neural arches in tetrapods (Fig 3 in yellow). While, some fifty years ago, these changes in anatomy appeared to be very large evolutionary leaps, it is becoming increasingly clear that most of them are gradual changes. New information from fossils phylogenetically intermediate between *Eusthenopteron* and *Acanthostega* such as *Panderichthys* (**Papers II, III and IV**) as well as descriptions of new material such as *Tiktaalik* (Daeschler et al., 2006) and *Ventastega* (Ahlberg et al., 2008) are providing a much clearer picture of how the fish-tetrapod transition occurred.

Studying the fish-tetrapod transition

Palaeontology

When studying fossils, we build phylogenetic analyses based on as many characters of as many taxa as possible. This establishes a framework to understand evolution and the direction of character change. The traditional way to study those characters is through preparation techniques which aim to free or uncover bone from the matrix with the use of needles, pneumatic tools or in some cases, with acid (fossils from the Gogo locality in Western Australia can be prepared this way). This is still the most commonly used way to study fossils and a prepared specimen was examined for **Paper IV**.

In the late 1920s, the method of serial grinding was first extensively used by staff and students at the Swedish Museum of Natural History in Stockholm in order to study anatomy not accessible by traditional preparation methods (Jarvik, 1980; Chang, 1982). To do so, a fossil preserved in three dimensions was gradually ground down at 20 μ m intervals. The ground surface was photographed and drawn before the next section was to be removed. This long and arduous process, also involving the production of three dimensional wax models, produced extremely detailed data on the internal anatomy of the specimen in question but led to its complete destruction.

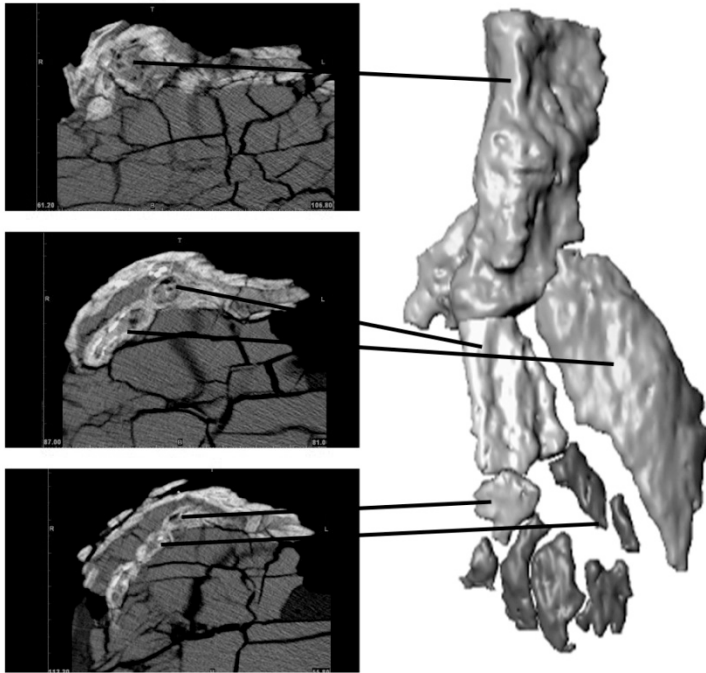


Fig 4. Images obtained from CT-scanning of the fossil *Panderichthys* (left) with their corresponding place on the three-dimensional model of the pectoral fin. The scan images show the fossil in transversal section.

Thanks to modern computer tomography methods (CT) initially developed for medical use, it is now possible to gain the same type of information without destroying the specimen. A medical scanner or, for smaller specimens, a custom-built high resolution scanner is used to image the specimen. This results in a series of black and white images where bone can be distinguished from the matrix through differences in density (Fig 4). Using these differences in density, the bone or space between bones (the cranial cavity for example) can be modeled in three dimensions with imaging software. This CT-scanning and modeling technique was used in **Papers II and III**.

Evo-Devo

Fossils provide representations of what ancient life, extinct organisms or even species still alive today looked like in the past. However, the fossil record is not perfect and the earlier the time period one wants to study, the worse the fossil record becomes. In addition to this, while series of fossil species arranged in a phylogeny describe the evolutionary pattern (ie the direction and nature of change) of a group, they do not explain the process of evolutionary change (ie the mechanisms driving changes in morphology). It

was Jean-Baptiste de Lamarck who first proposed an evolutionary mechanism explaining patterns found in the fossil record (Lamarck, 1809). However, it was Darwin who proposed the theory of natural selection as the process driving evolution (Darwin, 1859), laying the foundations for modern evolutionary biology. Darwin explained differences in animal forms as being the result of natural selection in different environments, but he explained their similarities as a result of their shared ancestry. Darwin considered comparative embryology to be the best source of information for homologies (similarities) which led many morphologists of the latter part of the 19th century to describe and compare development in different species (summarized in Hall (2003) and Gilbert (2003)). One of these morphologists was Haeckel, who suggested that “ontogeny recapitulates phylogeny”; that new species emerge from the addition of a developmental step and that studying embryology is equivalent to observing the evolution of increasingly complex species (summarized in Gilbert (2003)).

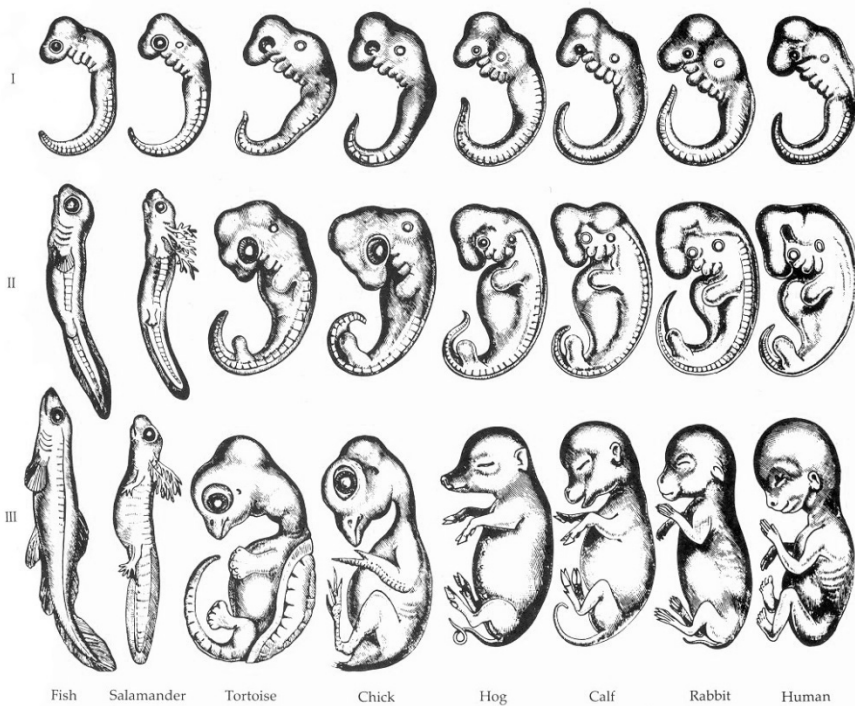


Fig 5. “Ontogeny recapitulates phylogeny” Hypothesis of Haeckel (1866) who viewed evolution as a ladder of increasingly complex developmental processes and organisms rather than as a branching event.

Haeckel's ideas gained a lot of support, partially because it could explain evolutionary processes when other explanations couldn't. However, Haeckel believed that phylogeny caused ontogeny and that old species would be removed by natural selection at the emergence of new species. The use of this

particular “law” in racist ideology (by Haeckel himself) no doubt contributed to the decline in popularity of embryology. With the advent of experimental embryology towards the end of the 19th century, comparative embryology began to be regarded as obsolete and most efforts were now focused on the experimental manipulation of certain organisms (Love and Raff, 2003). By the 1930s, the fields of genetics and embryology were already separate, with their own techniques, schools of thought and, most importantly, specialized vocabulary and study organisms. In the decade between 1936 and 1947, biologists specializing in different aspects of genetics or evolution created the modern evolutionary synthesis (Huxley, 1943). It integrated population genetics, which developed quickly with the rediscovery of Mendel’s work in 1900, to evolutionary biology, developmental and molecular genetics but not embryology (Gilbert, 2003).

Despite criticism voiced by Goldschmidt, Waddington, Schmalhausen and Leibowitz amongst others (see Gilbert (2003) for a review), embryology remained excluded from the modern synthesis until 1977. During that year, Stephen J. Gould (1977) emphasized the importance of heterochrony (change in the timing of developmental events) and comparative ontogeny in understanding evolutionary processes. Far from reviving Haeckel’s biogenetic laws, he points out that other models of evolution and development have to be proposed. One such model was elaborated by Francois Jacob (1977) who suggested that new functions occurred as the result of the use of preexisting components to make workable structures (tinkering). In this context, tinkering does not have a definite direction on its own but, directed by evolution, it can create new structures; for example, create lungs from esophagus tissue. Similarly, new gene functions can be caused by small changes in DNA in the copy of a gene that has been duplicated. The same year, publication of a new method to sequence DNA (Maxam and Gilbert, 1977) made it possible to test Jacob’s hypothesis. In the early 80’s, homeobox genes were discovered, proving Jacob’s hypothesis to be correct. The advancement of techniques and knowledge in molecular and developmental biology, genetics and phylogeny as well as an increased interest to integrate ideas from all fields associated with evolution saw the creation, in 2000, of new journals and a society dedicated to this integration. This established EvoDevo firmly as a respected field of research and encouraged specialists of different fields to collaborate more widely.

Evo Devo research is very new and is therefore being performed principally by scientists trained in one particular field. Given the historical separation of embryology and developmental genetics, the fields’ inherent vocabularies, philosophies and study organisms are so far apart that attempts at integrating all disciplines to explain a particular evolutionary event can be very difficult. This is why we are witnessing an increasing number of successful collaborations between researchers from fields traditionally very distinct. In addition to this, students are now being trained in the discipline

of Evo Devo, which will produce a new generation of researchers trained to understand and link different fields rather than specializing in a particular one.

One of the obvious advantages of this Evo Devo synthesis is the potential to gain a better understanding of the course of evolution and the mechanisms underlying it but, given that study organisms often differ widely, the data might not be directly equivalent. In the case of research presented in this thesis (**Papers I and IV**), developmental genetics and comparative ontogeny were used to complement the understanding of morphological transformations not entirely explained by transformations in the fossil record as well as to provide hypotheses about the mechanisms underlying them. For the question of the origin and evolution of fingers in tetrapods (**Paper I**), this had been attempted before (Sordino et al., 1995) but comparison of developmental mechanisms were made between two model organisms (the zebrafish and the mouse) that are so far from each other phylogenetically that a misinterpretation was made. Unfortunately, no developmental genetic data from organisms more closely related to the first tetrapods was present at the time and it is still the case today that in-depth analyses of gene interaction and expression can be well characterized, but only in model organisms (such as the mouse, zebrafish or fruitfly).

I believe that the comparison of genetic machinery between these model organisms is useful in hypothesizing ways in which evolution may have taken place, but I suggest that the comparisons should be only made for homologous structures. In order to establish these homologies, the adult morphology of extinct and extant forms filling the phylogenetic gap between model species should be well known and I believe that extra insight can be gained from comparative development. In **Paper IV**, I hypothesize mechanisms for the evolution of the pelvic girdle in tetrapods by comparing its development in the Australian lungfish and a salamander. These data points, on either side of the fish-tetrapod transition, combined with knowledge of the morphology of fossil forms and their evolution leading to the study organisms ensures that the structures compared are most likely to be homologous. Evo Devo is an exciting field which has evolved very quickly in the past decade and will continue to do so with the advent of new techniques in fields as widely separated as palaeontology and molecular biology.

Developmental biology

Depending on the type of information desired, developing organisms can be observed as a whole (prepared with whole-mount techniques) or by thin sections to study specific, often smaller areas of the animal (sectioning techniques such as histology). Because the aim of **Papers I and V** was to study changes in morphology over time, whole-mount techniques have been used.

To study the development of the skeleton, a method called clearing and staining was used. With this technique, the larvae are bleached to remove dark pigments on their skin and are then put in a solution of acetic acid and Alcian blue. This dye binds to mucopolysaccharides, proteins which are important components of connective tissues such as cartilage (Klymkowsky and Hanken, 1991). Muscles are then removed with the use of digestive enzymes which break down the proteins making up muscle fibers. This allows the skeleton to be observed more clearly. In order to visualize bone, larvae are immersed in an alkaline solution of Alizarin red. This is a natural dye extracted from roots of the Madder plant and it binds to calcium of the extracellular matrix in bone (Klymkowsky and Hanken, 1991). The blue and red larvae are then preserved in glycerol (a component of soap) and can be observed with or without a light microscope.

Immunohistochemistry is another technique that allows visualization of tissues during development but has the advantage of being a little more specific than clearing and staining. This technique takes advantage of the ability of vertebrates to produce antibodies to protect them from foreign objects (antigens) such as bacteria, viruses or foreign proteins. Antibodies are proteins which are highly specific to the antigen they were developed against and form a strong bond with them when encountered. The immune system of mice, rabbits or goats is then used to produce antibodies specific to the protein under study; in my case, skeletal muscle and acetylated tubulin present in nerves (**Paper V**). When larvae are incubated in this primary antibody, it binds to the tissue of interest (for example muscles). However, primary antibodies used in **Paper V** are not coupled with a dye and are therefore invisible. In order to detect bound antibodies, larvae are incubated in a secondary antibody coupled with a fluorescent dye. Figure 6 shows schematically how this type of immunostaining works at the molecular level.

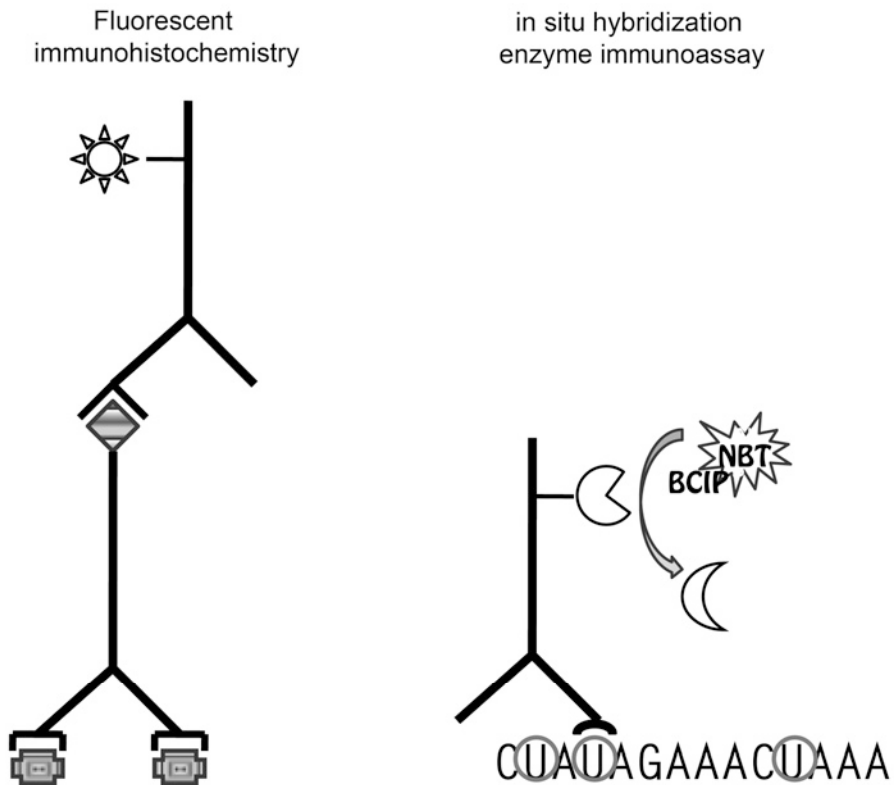


Fig 6. Representation of immunohistochemistry and in situ hybridization at the molecular level. The black sticks with a forked base are antibody molecules and the grey shapes represent antigens. On the left, the antigen is skeletal muscle. The primary antibody binds specifically to it (square “feet” of the sticks) and a second antibody, linked with a fluorophore (the sun) binds specifically to the primary antibody. On the right, the antigen is the base uracil compounded with DIG. The primary antibody is coupled with a protein (pie shape) which catalyzes the oxidation reaction of BCIP to produce a blue precipitate (moon).

The technique used to visualize gene expression (in **Paper I**) uses immunohistochemistry to visualize messenger RNA (mRNA) transcripts. When genes are expressed, the DNA encoding them (which never leaves the cell nucleus) gets transcribed into mRNA, which leaves the nucleus to be translated into a protein or to interact with other mRNAs. The principle of in situ hybridization is to produce a complementary sequence to the mRNA of the gene of interest (*Hoxd13* in the case of **Paper I**) where it is present in the cell and to visualize this compound. To do this, the gene of interest or a large part of it is sequenced and a complementary* copy is produced. When synthesizing this copy, uracil conjugated with digoxigenin (DIG) is used. Because this small molecule (a steroid) has a strong potential to produce an immune response, it can be detected with the use of an antibody. This anti-

body can in turn be visualized through a secondary antibody labeled with a fluorescent protein or with enzyme immunoassays (used in **Paper I**). In an enzyme immunoassay, the primary antibody is coupled with an enzyme which accelerates the reaction between two molecules, producing a visible precipitate. In **Paper I**, we used 5-bromo-4-chloro-3-indolyl phosphate (BCIP) as a substrate and Nitro blue tetrazolium chloride (NBT) as the oxidizer. When BCIP is oxidized by NBT through the help of the alkaline phosphatase bound to the primary antibody, it produces a blue precipitate at the antibody binding site. This allows for visualization of the mRNA sequence in the cells where it was secreted (in situ). This is a powerful tool for understanding the timing and position of gene expression during development. This technique, used preferably with fluorescent compounds, can be used to visualize several genes at the same time (Denkers et al., 2004). This provides the opportunity to understand the temporal and physical relation of gene expression during development.

* An identical copy would not bind to the mRNA sequence of interest. Because of the chemical arrangement of each nucleotide composing RNA (AUCG) and DNA (ATCG) they only anneal (form hydrogen bonds) with their complementary base. Like magnets, identical copies of the mRNA sequence of interest would repel each other while complementary copies would attract each other and bind together.

Fins to limbs

Origin of fingers

One of the readily recognizable morphological transformations during the fish-tetrapod transition is that of the origin of fingers. A defining feature of tetrapods, they were hypothesized to originate from the radials of sarcopterygian fish fins as early as 1874 (Gegenbaur). However, this was mainly based on the similarities between rhizodont fins and tetrapod limbs and it did not gain much popularity at the time given the scarcity of fossil evidence. In 1992, the pectoral fin of *Panderichthys*, a sarcopterygian fish closely related to tetrapods, was described as lacking distal radials, possessing instead two large bony plates at the extremities of its fins (Vorobyeva, 1992). In 1995, comparison of genes involved in appendage development in the zebrafish (a derived actinopterygian) and mice showed that a second, late phase of expression of the gene *Hoxd13* was associated with the appearance of digits and that it was necessary for their formation (Sordino et al., 1995). However, this late phase of expression was absent in zebrafish and it was thought to be completely absent in all fish (actinopterygian and sarcopterygian). The co-option of *Hoxd13* and the elaboration of a late phase of expression in tetrapods was hypothesized to be the mechanism behind the origin of digits as a novel structure in tetrapods (Sordino et al., 1995). This idea was widely accepted throughout the 90's and was included in the larger scheme of the mechanisms leading to the evolution of tetrapods (Shubin et al., 1997; Coates and Cohn, 1998). This hypothesis for the evolution of tetrapod fingers and toes raised a lot of interest and, in 2007, three major papers demonstrating the expression of *Hoxd13* in non-model organisms were published (Davis et al., 2007; Freitas et al., 2007; Johanson et al., 2007). The first to be published (Davis et al., 2007), demonstrated the presence of a late phase of expression of *Hoxd13* associated with the appearance of radials in the fins of the paddlefish, a basal actinopterygian. This suggested that a late phase of *Hoxd13* expression was primitive for actinopterygians and that it may have been lost in zebrafish. This can be explained by the fact that the zebrafish fin is very reduced compared to that of the basal members of the Actinopterygii (Fig 7). In sharks and primitive actinopterygians, the fin endoskeleton is composed of three parts. In teleosts, the metapterygium is lost but in sarcopterygians it is the only part retained (Mabee, 2000; Raff, 2007). It is therefore not surprising that a loss of gene function is correlated with the absence

of the radials pertaining to the metapterygium. This emphasizes the need to compare gene expression pattern of distantly related species against a strong morphological background to ensure that gene function associated with homologous structures is being compared.

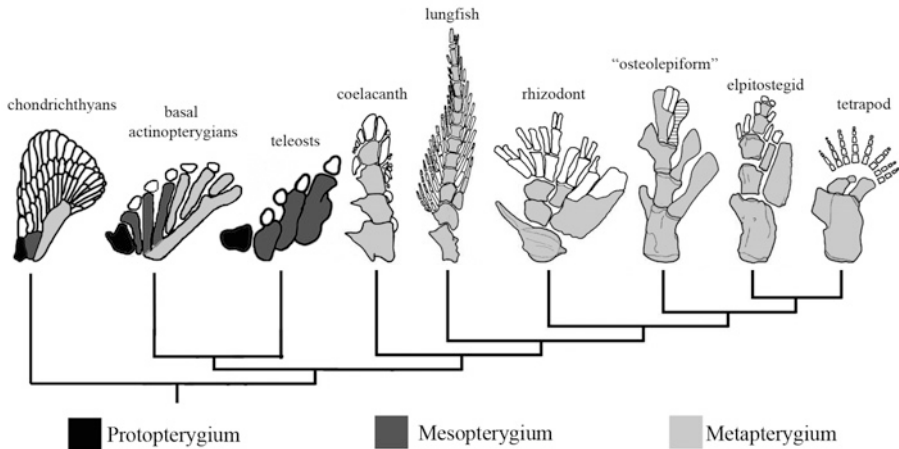


Fig 7. Gnathostome phylogeny representing fin/limb endoskeleton. In chondrichthyans and basal actinopterygians (represented by *Polyodon* here), the fin is composed of a propterygium (black), a mesopterygium (dark grey) and a metapterygium (pale grey). In the zebrafish (teleosts on the phylogeny), this region is lost whereas in sarcopterygian fish and tetrapods, it forms the entity of the fin. In the latter, it is the propterygium and mesopterygium which are lost. Given the loss of the region in which radials would develop, it is not surprising that zebrafish do not display the late phase of *Hoxd13* expression.

As in *Polyodon*, sharks have been found to have a biphasic *Hoxd13* expression associated with the presence of radials, demonstrating the primitive character of this expression pattern in gnathostomes (Freitas et al., 2007). In **Paper I**, we demonstrated the presence of a late phase of expression of *Hoxd13* also associated with the appearance of radials, showing that this pattern is also primitive for sarcopterygian fish.

In addition to this gene expression evidence, the elpistostegid fish *Tiktaalik* (a member of the group most closely related to tetrapods (Daeschler et al., 2006)) was shown to have radials at the ends of its pectoral fins (Shubin et al., 2006) (Fig 7). Being very closely related to *Panderichthys*, this called into question the previous interpretation of *Panderichthys* as lacking radials (Vorobyeva, 1992). In **Paper II**, we CT-scanned a specimen of *Panderichthys rhombolepis* preserved in three dimensions and modeled one of its pectoral fins. Comparison of the model with the material originally described showed that radials are present at the end of the fin and that the plates originally described were an artifact of preparation.

When studying the hands and feet of *Acanthostega* and *Ichthyostega*, the two earliest tetrapods with well preserved limbs, it can be noted that they have very few central bones of the hands (metacarpals) and feet (metatarsals) (referred to as mesopodium from now on). This means that digits abut almost directly onto the more proximal elements of the arm and leg (see Fig 1 in **Paper I**). In *Ichthyostega*, a few more central elements are present than in *Acanthostega* and in a more derived tetrapod, even more elements are present, both distal to the arm/leg bones and proximal to the digits. **Paper I** therefore suggests that digits evolved first from the distal radials of sarcopterygian fishes and that the mesopodium evolved later as an expansion of the zone between the proximal limb bones and the digits. This is supported by the mode of development of the fins of *Neoceratodus*. The proximal elements of the fin (equivalents of the tetrapod humerus, radius, ulna and ulnare) develop as subdivisions of a continuous field of pre-chondrogenic cells. The radials on the outside of the fin (pre-axial radials) and on the inside of the fin (post-axial radials) on the other hand, develop as separate condensations from the main axis and only form a connection to it later in development. This is very similar to the mode of development of salamander limbs, where fingers and toes develop after the proximal limb bones but before the palms (mesopodium). The same mode of development has been observed in fossil growth series of temnospondyls (group giving rise to modern amphibians) (Anderson et al., 2008) and in some seymouriamorphs (part of the group giving rise to amniotes) (Ruta et al., 2003). This suggests that a pattern of development involving the independent development of digits would be primitive for all tetrapods. Frogs as well as modern amniotes such as birds and mammals develop their limbs in a proximo-distal fashion, the digits being subdivision of a limb bud laid down early in the development of the limb. However, it has been shown that digits can develop even if they are separated from the rest of the limb by a physical barrier. This means that they retain a degree of independence from the proximal limb bones, suggesting that the primitive pattern of development is that of *Neoceratodus* and salamanders. The proximo-distal development of the limb in frogs and amniotes is therefore independently derived.

Evolution of the humerus

One of the important features associated with the origin of limbs in the first tetrapods is their orientation relative to the body, which is more lateral than in sarcopterygians. In fish such as *Eusthenopteron*, the fins are pointing towards the tail and dipping forward relative to the horizontal plane but in tetrapods such as *Acanthostega*, the limbs are parallel to the horizontal plane and pointing posterolaterally. These changes, along with the elaboration of new muscles as well as changes in the range of movement can be studied

through the morphology of the humerus. The humeri of the sarcopterygian fishes *Gogonasmus* and *Eusthenopteron* are cylindrical in shape and the angle between the entepicondyle (flange post-axial to the humerus) and the edge of the humerus is very large whereas tetrapods have flat, L-shaped humeri (Fig 8). Other remarkable changes are the reorientation of ridges for muscle attachment. On the dorsal surface, the ectepicondyle is diagonal to the preaxial margin in *Eusthenopteron* but parallel to it in tetrapods. On the ventral face, the humeral ridge is diagonal in *Eusthenopteron* but almost perpendicular to the preaxial margin in *Acanthostega*.

Panderichthys is a sarcopterygian fish from the Middle Devonian of Latvia and was, until recently, the closest tetrapod relative represented by complete fossils. Its humerus has been described in detail (Vorobyeva, 2000) and was characterized as being intermediate in morphology between that of *Eusthenopteron* and *Acanthostega*. Since then, *Gogonasmus*, a tetrapodomorph fish (Long et al., 2006), *Tiktaalik*, a fish more closely related to tetrapods than *Panderichthys* (Daeschler et al., 2006; Shubin et al., 2006) as well as an isolated humerus from the Catskill formation likely to belong to a very primitive tetrapod (Shubin et al., 2004) have been described (Fig 8). Given the breadth of new data available, I redescribed the humerus of *Panderichthys* from a three-dimensional model, compared it to the different specimens originally described (Vorobyeva, 2000) and analyzed it within this new framework (**Paper III**).

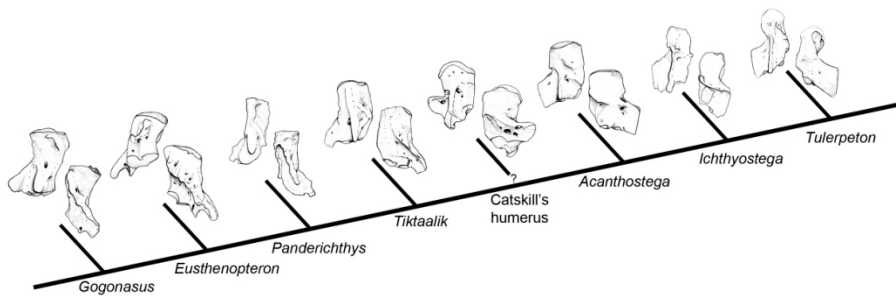


Fig 8. Humeri of sarcopterygian fish (*Gogonasmus*, *Eusthenopteron*, *Panderichthys* and *Tiktaalik*) and early tetrapods (Catskill's animal, *Acanthostega*, *Ichthyostega* and *Tulerpeton*) mapped on a phylogeny (from Clack (2004), *Gogonasmus* added according to Friedman et al. (2007)). This shows the progressive transformation of sarcopterygian cylindrical humeri into the flat and L-shaped ones typical for early tetrapods. The top drawing for each taxa is the dorsal view, and bottom, the ventral view.

In general, the morphology of the three-dimensional model agrees well with the previous description and discrepancies can be attributed to flattening of the originally described specimen. What emerges from this study is that the humerus of *Panderichthys* displays a combination of primitive (fish-like), derived (tetrapod-like or towards the tetrapod condition) and unique features. The fins of *Panderichthys* would have dipped forward, just like those of less

crownward tetrapodomorph fishes but their orientation relative to the body is intermediate between that of fish and tetrapods. Similarly, the humerus of *Panderichthys* is intermediate in shape between that of fish and tetrapods: it is flatter than in other fish and displays a reduced angle between the entepicondyle and the post-axial margin of the humerus, tending towards the L-shape characteristic of tetrapods. What is a little surprising with this analysis is that, although the morphology in *Panderichthys* is similar to that of *Tiktaalik*, when it differs from it, it is often more derived, despite the more basal phylogenetic position of *Panderichthys*. Several traits described for the humerus of *Panderichthys* in this analysis seem to be unique to this taxon. These include a short ectepicondyle (dorsal view) and a short humeral ridge (ventral view), both likely to be linked to peculiarities of the fin musculature. This detailed comparison of humeral morphologies across the fin to limb transition provides a more gradual view of the changes than previously described. However, the idiosyncrasies observed in the different taxa studied point to a wide range of morphological specializations most likely related to the breadth of ecological adaptations already present at the time.

Transformation of the pelvic girdle

Tetrapods are distinct from sarcopterygian fishes in that they rely more heavily on their hindlimbs than on their forelimbs for locomotion. This is the opposite from sarcopterygian fishes and this shift in locomotory dominance was made possible by the evolution of a weight-bearing pelvic girdle in tetrapods. In sarcopterygian fishes such as *Eusthenopteron*, the pelvic girdle is crescent shaped, the two halves joined at the middle by a weak cartilaginous bridge. In tetrapods, the surface area for the connection of the left and right sides of the girdle is much larger, thanks to a more rectangular pubis and the evolution of an ischium posterior to it. In fish, the pelvic girdle is attached to the body wall musculature but is free from the vertebral column (Fig 9, left panel). Because tetrapod pelvic girdles are connected to the vertebral column through the ilium and sacral rib, the tetrapod morphology allows for a weight-bearing function (Fig 9, right panel). In addition to this, the acetabulum of sarcopterygian fishes is posteriorly positioned on the pubis, meaning that the fin points backwards whereas in tetrapods, the acetabulum is laterally positioned on the girdle, the limbs therefore projecting to the sides of the animal.

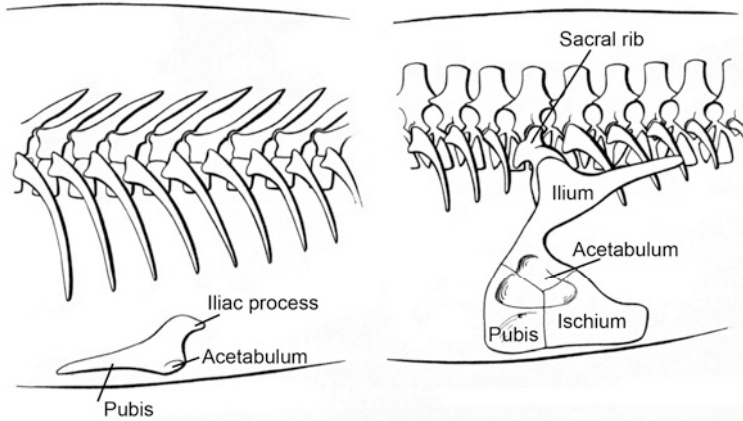


Fig 9. Transformations of the pelvic girdle through the fish-tetrapod transition. The pelvic girdle of *Eusthenopteron* (left panel) exemplifies the condition in sarcopterygian fishes. The girdle is crescent shaped in ventral view (not shown), the acetabulum is at the back of the girdle and an iliac process extends towards the vertebral column but does not connect with it. In tetrapods (right panel), a new structure, the ischium, is present posteriorly and is fused to the pubis. The left and right sides of the pubis + ischium fuse together in ventral view (not shown) and the pelvic girdle is connected to the vertebral column through contact between the ilium and the sacral rib, allowing for a weight-bearing function. In addition, the acetabulum is oriented lateral to the body in tetrapods. Anterior to the left, from Romer (1986).

Despite the large changes in size and morphology of the pelvic girdle, it is one of the least studied aspects of the fin to limb transition. The morphology of the girdle, as described above, is well defined for *Eusthenopteron* and for early tetrapods such as *Acanthostega* and *Ichthyostega* but nothing had been described for fossils phylogenetically intermediate between them. In **Paper IV**, I studied and described the only known pelvis and pelvic fin endoskeleton of *Panderichthys*. Unfortunately, the primitive, fish-like morphology of the pelvic girdle is not very informative in understanding how the transition occurred. In order to do this, I compared the development of the pelvic girdle and associated musculature in modern representatives of sarcopterygians (the Australian lungfish) and tetrapods (the axolotl) (**Paper V**). Despite disparate adult morphologies, sequences of appearance of pelvic elements and pelvic muscles were found to be very similar. I observed that development in both the lungfish and the axolotl begins with small cartilaginous condensations of the pubis where the acetabula will later form. From this, I proposed that the acetabulum does not “move” during the fish-tetrapod transition but is rather a fixed landmark around which other structures develop (as observed in lungfish and axolotl) and evolved. In lungfish, cartilaginous condensations develop anterior to the acetabula, forming the pubis, but in salamanders, they develop both anteriorly and posteriorly, the latter forming the ischium. From this chondrogenesis data as well as evidence from adult mus-

cle homologies and comparative muscle development, I suggest that the ischium evolved from pubic pre-chondrogenetic cells being allowed to migrate both anteriorly and posteriorly. If this hypothesis is true, the “shift” in acetabulum position and the evolution of an ischium are coupled events and would only have necessitated a small change in molecular signaling to occur. As for the evolution of the ilium, I suggest that it evolved from an elongation of the iliac process or ridge present in most tetrapodomorph fish. This is based both on the mode of development of the ilium in salamanders, on adult muscle homologies as well as on muscle development patterns. In salamanders, the ilium and the sacral rib elongate toward one another and eventually join this way. This suggests the presence of molecular signaling between the ilium and the sacral rib, or their precursors. *Neoceratodus* and *Ambystoma* have very similar pelvic muscles and modes of development despite their long evolutionary separation (see Fig 2 for phylogeny). This suggests that the morphology and development of taxa immediately on either side of the transition must have been even more similar, making this seemingly large evolutionary jump a smaller step.

As seen in **Paper II and III**, the pectoral fin of *Panderichthys* is derived, often more than that of *Tiktaalik*. However, this is not true for its pelvic fin, which is much more primitive than the pectoral fin (**Paper IV**). This indicates that the fin to limb transition started in the pectoral appendages and that the transformations of the pelvic appendages occurred in the relatively short time period of the node between *Panderichthys* and *Acanthostega*. The transition from “front wheel drive” fish locomotion to “back wheel drive” tetrapod locomotion must also have occurred during that time period. If *Panderichthys* were to have made excursions on land, it would most likely have used its fins as anchors to move along through body flexion. This mode of locomotion is very different from the limb-propelled lateral movements of *Acanthostega* or the hypothesized caterpillar up-and-down motion of *Ichthyostega* (Ahlberg et al., 2005). As with unique features in the morphology of limb bones of tetrapodomorph fish and tetrapods (**Paper III**), this shows that they were already specialized in their own right, each of them trying their own ways of exploiting the new environments at their disposal.

Svensk sammanfattning

Livet under Devon (375 miljoner år sedan)

Föreställ dig en varm sommarnatt utan ljud av syrsor, lukten av blommor, och, utan myggor! Så var det under Devon-tidsperioden, för ungefär 375 miljoner år sedan. Om du kunde besöka jorden vid denna tidsperiod är det tveksamt om du skulle känna igen dig. Då var kontinenterna helt annorlunda i form och placering (bild 1). Det fanns två stora kontinenter i stället för fem och Sverige låg under vatten. Delar av våra nutida kontinenter existerade redan men de var placerade annorlunda. Till exempel så skulle solresorna ha tagit dig till Grönland eftersom det låg vid ekvatorn och hade ett tropiskt klimat.

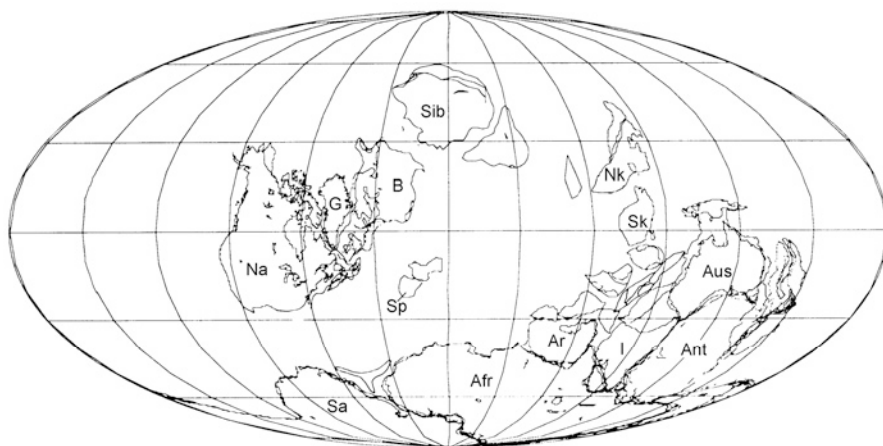


Bild 1: Jorden under devon. Östra Nordamerika, Grönland och Baltikum (bl. a.) låg nära ekvatorn och hade tropiskt klimat. Förkortningar: Afr: Afrika, Ant: Antarktis, Ar: Arabiska halvön, Aus: Australien, B: Baltikum, G: Grönland, I: Indien, Na: Nordamerika, Nk: Nordkina, Sa: Sydamerika, Sib: Sibirien, Sk: Sydkina, Sp: Spanien.

Vid kusten av dessa två underliga kontinenter växte flera meter höga lummer och fräken, och förfäder till ormbunkar och barrträd. Det fanns inget gräs, inga blommor och inga riktiga löv. Det måste ha varit en väldigt tyst värld. Det fanns skorpioner, spindlar, kvalster och tusenfotingar som levde på marken i dessa märkliga skogsvåningar men det fanns inga flygande insekter i

luften. På land fanns det inga ryggradsdjur, hela den diversiteten levde fortfarande i vattnet.

Runt om i världens hav, floder och kuster, fanns en mångfald av fisk. Några käklösa fiskar (Bild 2) som hade varit mycket framgångsrika under silurperioden (445-415 miljoner år sedan), eftersom de var så väl skyddade av sina beniga pansar, var emellertid på väg att utrotas under devonperioden. De blev utkonkurrerade av fiskar som hade utvecklat käkar. Bland fiskarna med käkar fanns pansarhajar som blev mycket framgångsrika under den här tiden. De blev senare utrotade för runt 360 miljoner år sedan, men under devon var de stora, ibland upp till 6 meter långa skräckinjagande rovdjur. Det är ganska lustigt att tänka sig att de hajar som levde då, inte olika våra dagars hajar, troligtvis blev jagade av stora pansarhajar.

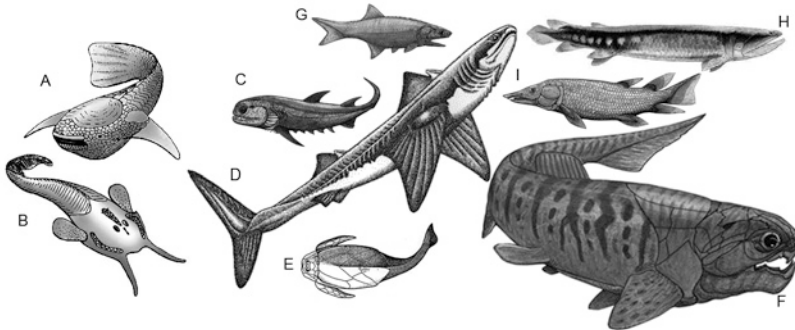


Bild 2: Några av devons märkliga fiskar. Bilderna ger dig ett intryck av hur stora fiskarna var men är inte skalensligna. Till vänster, käklösa fiskar: A och B är pansar-rundmunnar som var mellan 15 och 40cm långa. Till höger, gnathostomer (djur med käke): C) *Climatius*, en "taggig haj" som var mindre än 15cm D) en tidig haj, *Cladoselachus* (1,8m), E och F) Pansarhaj *Remigolepis* (E) är en antiarch som är små pansarhajar (35cm) medan *Gorgonichthys* (F), en arthrodir, kunde bli så lång som 6m! G-I) Beniga fiskar: G) *Mimia* var en liten (ca. 20cm) strålfenig fisk och en förfader till bl. a. torsk, lax och sjöhästar, H och I) är kvastfeniga fiskar, gruppen ger senare upphov till fyrbenta djur som grodor och kaniner I) *Griphognathus* var en 60cm långnosad lungfisk och *Gooloogongia* (H) en 90cm rhizodont, av vilka några blev 6m långa rovdjur 30 miljoner år senare.

Av alla konstiga fiskar som levde under devon är benfiskar viktigast för mina studier. Till skillnad från hajar har benfiskar ett skelett bestående av ben som utvecklats från brosk. Det finns två grupper av benfiskar: strålfeniga fiskar som lax och sjöhästar och kvastfeniga fiskar som lungfiskar. Om man tänker "fisk" idag tänker man oftast på strålfeniga fiskar, som utgör stora delar av ryggradsdjurens nuvarande mångfald. Däremot var det kvastfeniga fiskar, med fenor med ett långt inre skelett, som var den mest omfattande och mångskiftande gruppen av benfiskar under devon. I denna grupp ingår lungfiskar och tofsstjärtfiskar, som fortfarande lever i dag, liksom stora rovdjur som rhizodonter. Det är också ur denna grupp de fyrbenta ryggradsdjuret (fyrfotingar) utvecklades för ungefär 375 miljoner år sedan. Fyrfo-

tingarna är förfäder till alla amfibier, kräddjur, fåglar och däggdjur som lever idag (Bild 3). Detta gör att det är viktigt att studera fyrfotingarna för att begripa hur livet på jorden, i luften och även tillbaks till vattnet igen, utvecklades.

Vi kommer aldrig veta varför fyrfotingar tog steget upp på land men vi hittar dem i grunt vatten, i tropiska miljöer nära ekvatorn (alltså bl. a. i Grönland, Baltikum och Nordamerika). Kanske blev det lite trångt i vattnet, och att gå upp på land under mindre eller längre tid var ett bra sätt att undkomma rovdjur. Eller så var det skönt att bara ligga i solen ett tag och höja kroppstemperaturen, så att när djuren var tillbaka i vattnet kunde de röra sig snabbare. I vilket fall som helst var det de djur som var bäst på att kräla eller att röra sig på land som överlevde. Det var deras anpassningar som behölls, och sedan ledde till djur som var helt anpassade för ett liv på land. Övergången från fisk till fyrfotingar tog åtminstone 10 miljoner år och det verkar som varje art testade ett eget sätt att anpassa sig till sin nya miljö.

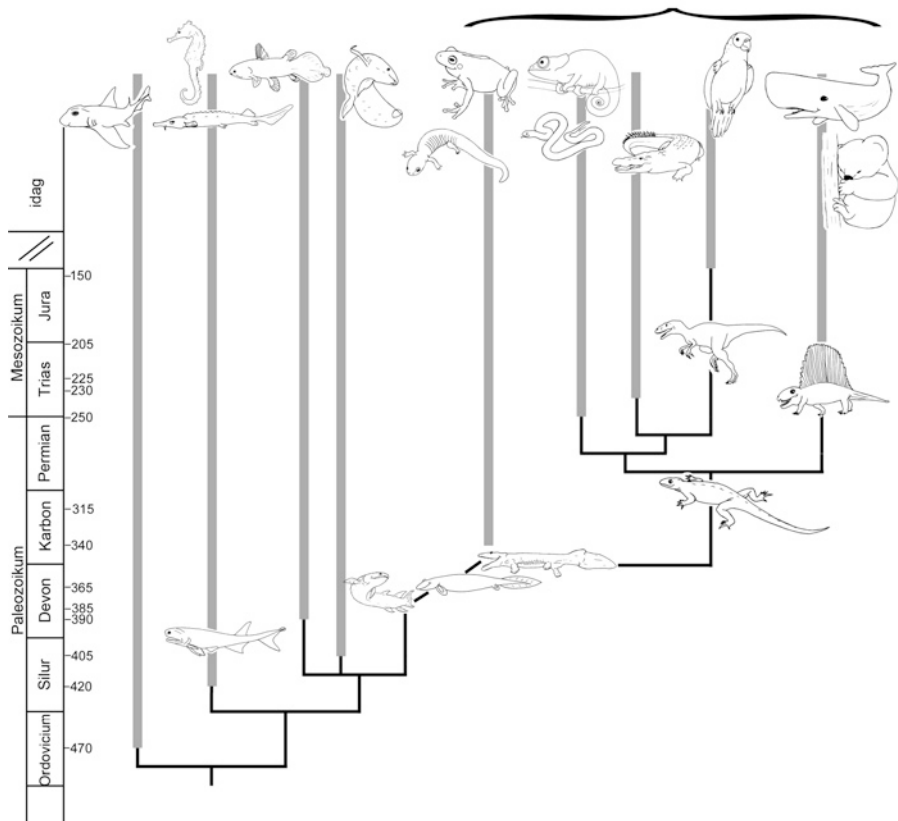


Bild 3: Familjeträd för käkfiskar. Till vänster är en tidskala med ungefärliga datum för den första förekomsten av olika djur. Grå linjer visar grupper som lever idag. Från vänster till höger: Hajar; *Cheirolepis*, en strålfenig fisk från Devon, och moderna strålfeniga fiskar; Kvastfeniga fiskar: havstofsstjärt (tofsstjärtfiskar), lungfiskar, *Eusthenopteron* och *Panderichthys*, som är utdöda; Fyrfotingar (inom parentes): *Acanthostega*, en tidig fyrfoting som är utdöd, amfibier, reptiler, krokodiler, dinosaurier, fåglar och däggdjur.

Övergången från fisk till fyrfoting

Om man tittar på ett familjeträd över kvastfeniga fiskar och fyrfotingar märker man tre djur som är nära släkt med varandra. Alla tre är kända från stort sett komplett material och de är mycket bra exempel på hur fiskar, övergångsdjur och tidiga fyrfotingar såg ut (bild 4). Den första är *Eusthenopteron*, en kvastfenig fisk som hittats i mängder i Miguasha, Kanada. De flesta exemplar är välbevarade i tre dimensioner och de blev studerade i detalj av två forskare, Erik Jarvik (svensk) och Mahala Andrews (engelska). Precis som andra kvastfeniga fiskar har *Eusthenopteron* lungor och likaså gälar. Skelettet i deras fenor består av ben motsvarande de i våra egna armar och ben. Samtidigt som deras inre skelett och skalle liknade de tidiga fyrfotingar.

tingarnas, så var de yttre delarna av deras fenor täckta av fenstrålar. Fisken var frisimmande, och fenstrålar samt rygg- och analfenor hjälpte *Eusthenopteron* att röra sig i flodmynningar.

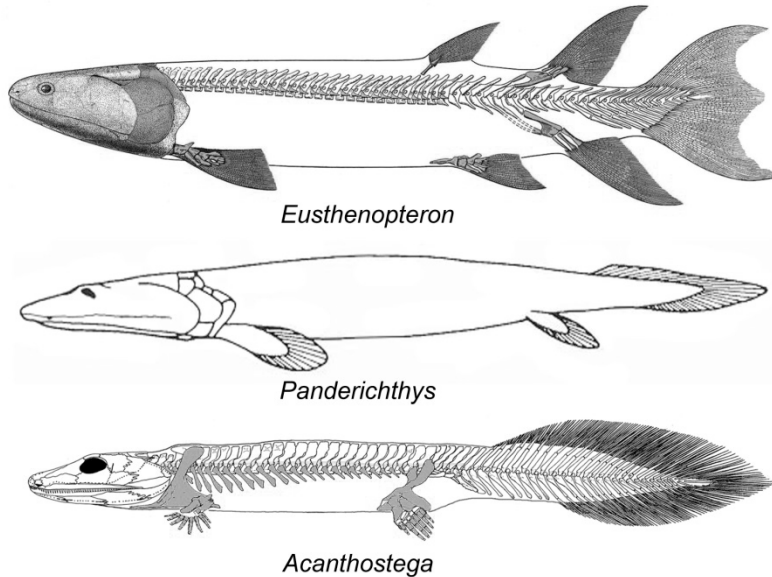


Bild 4. Tre djur som föreställer övergången från fisk till fyrfoting. *Eusthenopteron* är en kvastfenig fisk som levde för ungefär 385 miljoner år sedan och kunde bli upp till 1,5m. *Acanthostega* är en tidig fyrfoting som levde för ungefär 365 miljoner år sedan. Den var lite mindre än *Eusthenopteron*, ca. 80 cm. I mitten ses *Panderichthys*, en övergångsart mellan fisk och fyrfotingar. *Panderichthys* levde för ungefär 385 miljoner år sedan i Baltikum och var mellan 90 och 130 cm. De röda inslagen förlo- rades under övergången, de gröna inslagen förändrades och fälten markerade i gult är en ny struktur i fyrfotingar.

Övergångsdjuret *Panderichthys* var en fisk som levde för ungefär 385 miljo- ner år sedan i Baltikum. Den var lika stor som *Eusthenopteron* men levde i floder och strömmar, i grundare vatten. Djuret var anpassat till att leva nära vattenytan med en lång spetsig nos, ögonen på övre delen av skallen och utan ryggfenor. *Panderichthys* hade fortfarande kvar fenstrålarna trots att rygg- och analfenor saknades. Skelettet med bröst- och bukfenor, liksom många andra egenskaper i dess anatomi, är ett mellanting mellan fiskar och tidiga fyrfotingar. Det är således lärorikt att studera *Panderichthys* anatomi för att förstår hur fiskar förvandlades till fyrfotingar.

Ett bra exempel på en tidig fyrfoting är *Acanthostega* som levde på Grön- land för ungefär 365 miljoner år sedan. *Acanthostega* liknade *Panderichthys* mycket i kroppsformen men hade ben med fingrar och tår istället för fenor. Även om *Acanthostega* kunde röra sig på land tillbringade de säkert merpar- ten av sina liv i grunda, varma vattendrag. *Acanthostega* hade fortfarande gälar täckta av skallben men några av benen som var närvarande i *Eusthe- nopteron* är nu frånvarande (röd, Bild 4). Detta bidrog till att huvudet fri-

gjordes från kroppen och det tillät djuret att röra huvudet oberoende från bålen. En annan stor förändring hos fyrfotingar är att de använder bakbenen mycket mer än frambenen för att röra sig. Fiskar simmar med sin bålmuskulatur med hjälp av sina bröstfenor. Deras bukfenor är därför mycket mindre än deras bröstfenor och de är inte sammankopplade med ryggraden. Hos fyrfotingar är det tvärtom: bakbenen är stora och muskulösa och bäckenet är sammanlänkat med ryggraden genom ett speciellt revben (Bild 4 i grönt). Detta gör att de fyrfota djuren rörde sig på ett helt annat sätt än sina fiskförfäder. Det är dessa aspekter av fisk- till fyrfotingövergången jag har studerat i denna avhandling. I samband med ny forskning och nyligen upptäckta fossiler börjar vi förstå bättre hur övergången gick till. Emedan det för ett femtiotal år sedan ansågs att dessa förändringar i anatomin var ett resultat av mycket stora evolutionära språng, så det blir alltmer uppenbart att de flesta av dem är gradvisa förändringar.

Hur studerar vi evolution?

Det är givetvis inte lika enkelt att studera utdöda djur som det är att studera de som fortfarande lever. Inom paleontologin studerar man fossil, bevarade djur, delar av skelett eller växtdelar som blivit förstenade. Man måste spendera lång tid med att preparera fram ben från sten med hjälp av nål eller andra tryckluftsverktyg. Det finns dock nya sätt att studera fossil på förutom med mikroskop. I denna avhandling har jag använt medicinsk röntgen för att titta inuti kroppen av ett exemplar av *Panderichthys* utan att behöva ta bort överliggande ben och sten. Man tar sitt fossil till sjukhuset och producerar en serie bilder med fasta intervall. Resultatet analyseras med hjälp av mjukvaror som skapar tredimensionella modeller av de strukturer man vill studera. Det finns även möjligheter att skapa animerade bilder och på så sätt dissekera fossil utan att skada dem.

Fossil är oerhört upplysande om livet i det förflutna och utan dem skulle vi kanske inte ens veta att livet utvecklades. Däremot är det inte alla djur som en gång levt som blir bevarade och upphittade. Det krävs mycket speciella omständigheter för att man skall bli förstenad, bevarad i miljoner år och sen upptäckt av paleontologer. Ibland finner vi mycket få djur från en viss tidsperiod och ibland är det bara små delar av dessa djur som vi hittar. För att hjälpa till med detta har ett nytt fält av forskning inom evolution utvecklats under de senaste tio åren. Det kallas för Evo Devo (evolution och utveckling) och kombinerar data från bland annat utvecklingsbiologi, genetik, molekylär biologi och paleontologi, för att bättre förstå hur djurgrupper eller kroppsdelar utvecklades. Jag har jämfört hur en nutida kvastfenig fisk (lungfisk) och en fyrfoting (salamander) utvecklar sina fenor/ben och bäcken. Jag kombinerade dessa uppgifter med data från fossilregistret och fick en bättre

förståelse för hur fenor förvandlades till ben för ungefär 380 miljoner år sedan.

Det finns olika sätt att studera en organism som utvecklas. Det enklaste är att färga in strukturer man är intresserad av i djur vid olika tillväxtstadier (i tillväxtserier). Med hjälp av mikroskop kan man då jämföra olika stadier och se exempelvis vilka ben som framträder först och hur en struktur utvecklas. En av dessa tekniker kallas för ”clearing and staining”; genom en serie av kemiska bad blir ben infärgat i rött och brosk i blått (se artiklar ett och fem för bilder). Vid immunofärgning kan man färga in vävnader i fosforescenta färger med hjälp av antikroppar. I forskningsdjur som mus eller kanin induceras en immunreaktion med vävnaden vi är intresserade av att studera. Antikroppar samlas in, modifieras och sedan används de för att visualisera vävnader, till exempel muskelvävnad, i de djur man vill studera (se artikel fem för bilder). Med samma princip kan man synliggöra genuttryck under försöksdjurens utveckling. Man måste först få fram sekvenser av de gener vi är intresserade av att studera och göra en motsvarande kopia. När djuret inkuberas med denna kopia hittas, bland alla andra gensekvenser, den sekvens som den är kopierad ifrån. De två sekvenserna fäster sedan vid varandra. Vi kan därefter synliggöra detta band med hjälp av immunofärgning eftersom kopian lätt känns igen med hjälp av antikroppar (se bild 3 i artikel ett).

Fenor till ben, hur gick det till?

Under lång tid trodde vi att fingrar och tår var en evolutionär nyhet hos fyrfotingar. Fiskar verkade inte ha några ben i fenorna som liknade fingrar och dessutom var det fläktformade arrangemanget av fingrar helt unikt hos fyrfotingarna. År 1992 beskrevs bröstfenan hos övergångsdjuret *Panderichthys* men det visade sig ha stora benplattor där fingrar eller fingrarnas motsvarighet skulle ha suttit. Bara några år senare upptäcktes det att en strålfenig fisk, zebrafiskan, saknade genuttryck som framträder under utvecklingen av fingrarna hos en mus. På grund av det drog man slutsatsen att fingrar uppstod hos fyrfotingar till följd av detta nya genuttryck. Men även om den här teorin blev vedertagen så tog det inte lång tid innan bevis för att detta inte var sant dök upp. År 2006 hittade man ny art av övergångsfisk, *Tiktaalik*, med små ben i spetsen av sina fenor (radialer). Bara ett år senare avslöjades genuttryck i fenor av hajar, en primitiv strålfenig fisk och lungfisk (artikel ett i denna avhandling) som visade att de hade samma mönster som i mus, fast resultatet framträder som radialer i stället för som fingrar. Den sista pusselbiten var när vi visade att *Panderichthys* hade radialer i sina fenor vilka var ännu mer fyrfotingslika än hos *Tiktaalik* (Bild 5 och artikel 2 i denna avhandling).

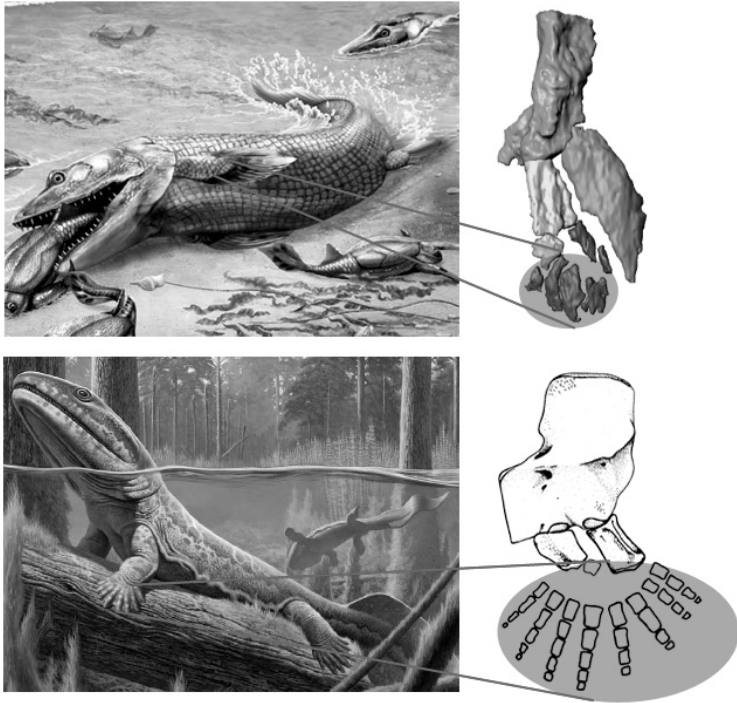


Bild 5: Fingrarnas ursprung. *Panderichthys* (översta bilden) är en övergångsfisk med små ben på spetsen av sina fenor (rosa cirkel). Dessa är föregångare till de fingrar man hittar i fyrfotingar som *Acanthostega* (nedre bild). *Acanthostega* hade 8 fingrar men ingen handflata. Fingrarna var först att utvecklas och en handflata utvecklades senare genom en utökning av utrymmet mellan strålben, armbågsben och fingrar.

Tänk om dina fingrar var fästa direkt på din arm. Ingen handled, ingen handflata. Så var det hos de tidigaste fyrfotingarna som *Acanthostega* (bild 5). I denna avhandling (artikel 1) föreslår vi att handflatan utvecklades genom en utökning av utrymmet mellan strålbenet, armbågsbenet och fingrarna. Små ben utvecklades i detta utrymme och vi kan se bevis för detta hos mer utvecklade fyrfotingar än *Acanthostega* (bild 1 i artikel 1).

En annan stor skillnad mellan fiskar och fyrfotingar är deras bäcken. Hos fiskar är bäckenet en liten halvmåne av ben utan anknytning till ryggraden, men hos fyrfotingarna är det en stor vikt bärande struktur i tre delar: det främre blygdbenet, sittbenet på baksidan och tarmbenet vid sidan, som sammanlänkar gördeln till ryggraden (Bild 6). När jag började studera bäckenet hos *Panderichthys* hade jag stora förväntningar på att det skulle förklara hur denna övergång skett. Dessvärre var strukturen mycket enkel och ganska primitiv även hos fiskar (artikel fyra) och det betydde att jag behövde hitta informationen någon annanstans. Jag fann den med hjälp av utvecklingsbiologin. Jag jämförde utvecklingen hos australiensiska lungfiskar, som är vår

närmaste levande fisksläkting, och hos primitiva fyrfotingar, salamandrar. Jag kom fram till att sittbenen måste ha utvecklats från blygdbenet genom en liten ändring i molekylär signal som tillät blygdbenscellerna att vandra bakåt och framåt (Bild 6). Jag konstaterade också att den struktur som de flesta kvastfeniga fiskar har på sina bäcken, som senare blir tarmben i de fyrfota djuren, måste ha växt mot ryggraden och genom en serie av molekylära signaler fäste ihop med revbenen (bild 6).

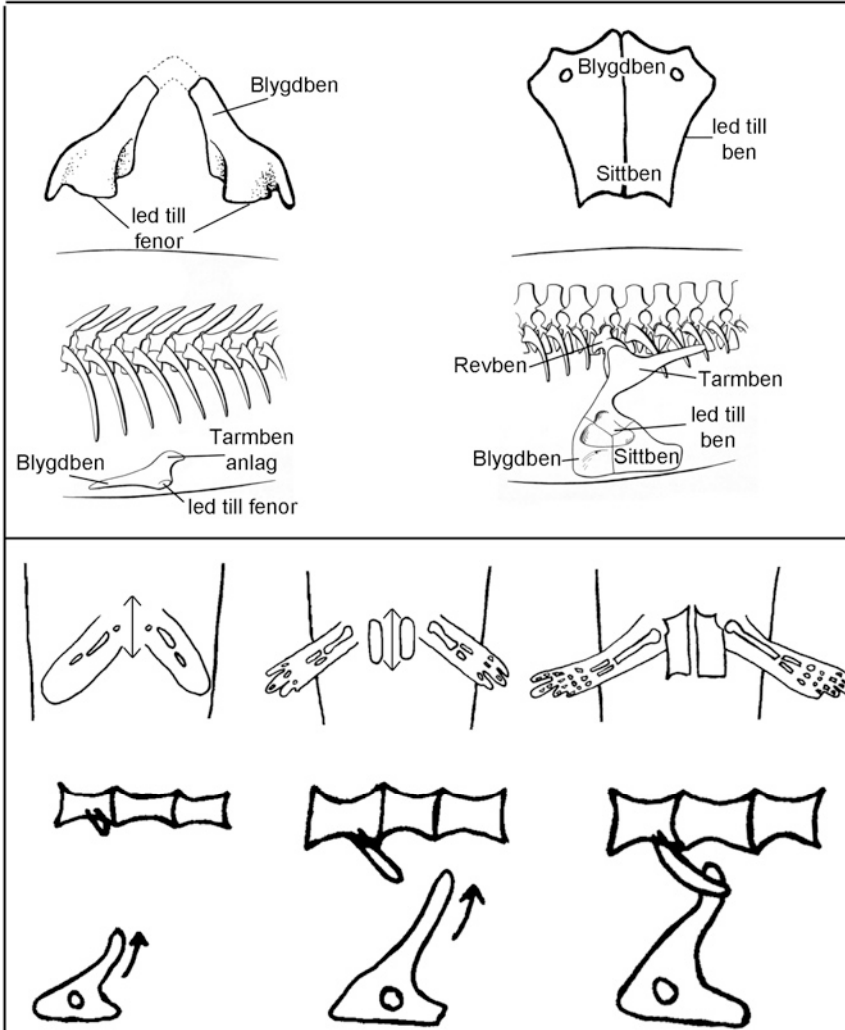


Bild 6. Bäckens övergång från fiskar till fyrfotingar. Övre delen: Hos fiskar (till vänster) är bäckenet mycket enkelt utan koppling till ryggraden, men hos fyrfotingar (som oss) är bäckenet större med en ny del, sittbenet, och en anslutning till ryggraden genom tarmbenet, samt ett speciellt revben (till höger). Nedre delen: högre rad visar hur sittbenet utvecklades från blygdbenet (pilar) och nedre raden visar hur tarmbenet utvecklades genom att växa mot ryggraden.

Genom dessa pusselbitar har vi nu en mycket bättre bild av hur övergången från fisk till fyrfoting och från fena till ben gick till. Det finns fortfarande en rad frågor om hur och varför fyrfotingar utvecklades men det finns också många upptäckta fossil att studera...

Résumé en Français

La vie au Dévonien (il y a 375 millions d'années)

Imaginez une belle soirée d'été sans le son des grillons, sans l'odeur des fleurs ou du gazon fraîchement coupé, sans chant d'oiseaux. Si vous aviez visité la terre durant le Dévonien (il y a environ 375 millions d'années), c'est cet environnement un peu bizarre que vous auriez observé. Lors de cette période, nos continents actuels étaient arrangés différemment et certaines parties étaient alors sous l'eau. L'Amérique du Nord, incluant l'Arctique canadien était alors située à l'équateur. La Gaspésie et le Groenland auraient donc à cette époque été de grandes destinations soleil avec leur climat tropical (Image 1).

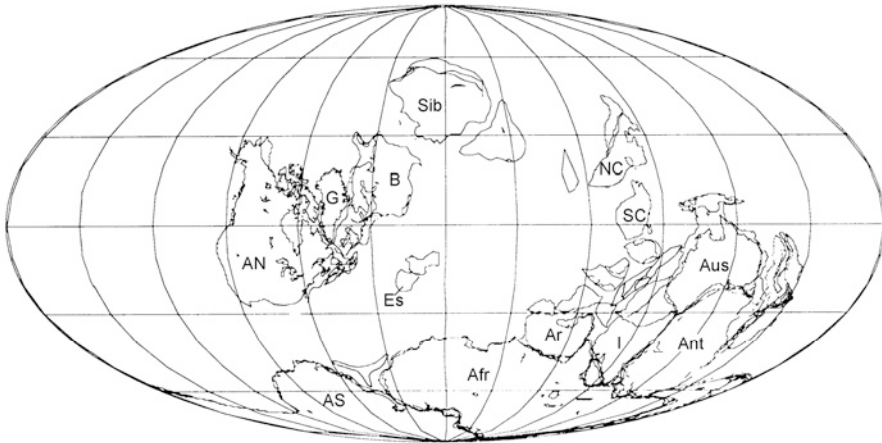


Image 1: La Terre au Dévonien. L'Amérique du Nord, le Groenland et les pays baltes (entre autres) étaient alors à l'équateur et bénéficiaient d'un climat tropical. Abréviations: Afr: Afrique, AN: Amérique du Nord, Ant: Antarctique, Ar: péninsule arabe, AS: Amérique du Sud, Aus: Australie, B: pays baltes, Es: Espagne, G: Groenland, I: Inde, NC: Nord de la Chine, Sib: Sibérie, SC: Sud de la Chine.

Sur les côtes de ces deux grands continents, poussaient des prêles et lycopodes de plusieurs mètres de haut ainsi que les ancêtres des fougères et des conifères. Ces forêts étaient très différentes de celles que l'on connaît aujourd'hui, puisqu'il n'y avait ni de fleur, ni herbe et les arbres n'avaient pas de vraie feuille. Ce monde du sous-bois un peu bizarre devait aussi être très silencieux. Des scorpions, araignées, acariens et mille-pattes peuplaient

le sol, mais il n'y avait pas encore d'insecte et seuls les collemboles primitifs s'essayaient à conquérir les airs. À cette époque, aucun vertébré n'habitait la Terre et toute leur diversité se trouvait dans l'eau.

Dans les océans, rivières et fleuves de cette Terre étrange, vivaient une grande diversité de poissons. Le règne des agnathes, poissons sans mâchoire, si prospère lors du Silurien (445 à 415 millions d'années) tira à sa fin. Malgré leurs fortes carapaces osseuses, ils se firent rapidement remplacer au cours du Dévonien (Image 2) par des poissons ayant évolué des mâchoires. Leurs prédateurs principaux étaient probablement les placodermes, des grands poissons à carapace osseuse pouvant atteindre jusqu'à 6 mètres de long. Ce groupe, un des plus riches et divers de toute l'histoire des vertébrés, comprenait aussi des espèces plus petites. Malgré leur succès, les placodermes disparurent à la fin du Dévonien (il y a 360 millions d'années). Les requins étaient aussi présents à cette époque mais ils étaient beaucoup plus petits et le groupe n'était pas aussi divers qu'aujourd'hui. Leurs cousins, les requins épineux (acanthodiens), aujourd'hui éteint, constituait un groupe de tout petits poissons très riche lors du Dévonien, munis de grandes épines à la base de leurs nageoires et sur leur ventre.

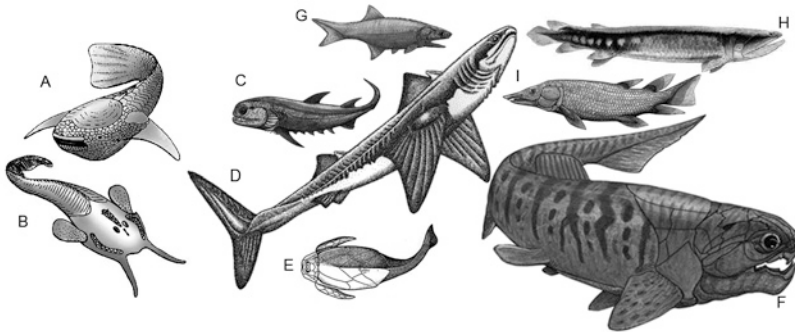


Image 2: Quelques poissons du Dévonien. À gauche, des poissons à carapace osseuse sans mâchoire (A et B) qui mesuraient entre 15 et 40 cm. À droite, tous les groupes de vertébrés avec mâchoire sont déjà présents au Dévonien: C) Les requins épineux comme *Climatus* qui mesurait moins de 15 cm, D) les requins primitifs comme *Cladoseiachus* mesurant 1,8 m E et F) Les deux classes de placodermes : les antiarches (E) étaient petits comme celui-ci de 35 cm alors que les arthrodières (F) pouvaient atteindre 6 m de long ! G-I) les poissons osseux : G) *Mimia* était un petit (environ 20 cm), poisson à nageoires rayonnées et l'ancêtre des truites, morues et hippocampes actuels, H et I) sont des poissons à nageoires charnues, ancêtres de tous les tétrapodes comme les grenouilles et les souris : *Griphognatus* (I) était un dipneuste de 60 cm et *Gooloogongia* (H) un rhizodonte de 90 cm, ancêtre des monstres marins de 6 m au Carbonifère (30 millions d'années plus tard).

Tous ces poissons avaient un squelette cartilagineux, semblable à celui des requins modernes alors que le reste de la diversité des poissons du Dévonien était composé de deux groupes de poissons osseux. Le groupe des poissons à nageoires rayonnées commençait à se diversifier à cette époque

mais comportait très peu d'espèces, la plupart très petites. Malgré tout, c'est à ce groupe que l'on pense aujourd'hui quand on dit « poisson » puisqu'il comprend maintenant plus de 30 000 espèces dont toutes celles dont on se nourrit (truite, saumon, espadon, même l'esturgeon donnant le caviar). Au Dévonien, le groupe de poissons osseux le plus divers était celui des poissons à nageoires charnues. Au lieu de posséder de grands rayons osseux, leurs nageoires étaient composées d'un squelette semblable aux os de nos bras et jambes, et ce sont les muscles attachés à ces os qui donnèrent le nom de « nageoires charnues » à ce groupe. Aujourd'hui, on ne compte que les coelacanthes et les dipneustes (poissons à poumons) parmi eux, mais c'était un groupe très diversifié lors du Dévonien, comprenant des poissons de taille moyenne ainsi que des prédateurs de plusieurs mètres comme les rhizodontes. C'est aussi ce groupe qui fait l'objet de cette thèse puisque c'est parmi celui-ci que les tétrapodes (animaux à quatre pattes) évoluèrent, il y a environ 375 millions d'années. Ces premiers tétrapodes sont les ancêtres de tous les amphibiens, reptiles, oiseaux et mammifères peuplant et ayant peuplé la Terre (Image 3). C'est donc en étudiant les individus éteints et actuels de poissons à nageoires charnues que l'on peut comprendre comment les tétrapodes réussirent à conquérir avec tant de succès la terre, les airs et même à retourner dans les mers.

On retrouve les premiers tétrapodes dans des endroits tropicaux autour de l'équateur, surtout au Groenland, dans les pays baltes et en Amérique du Nord. On ne saura jamais exactement pourquoi cette transition de poissons à tétrapodes se produisit mais c'est certainement une combinaison de facteurs environnementaux. Les poissons ancêtres des tétrapodes étaient tous des prédateurs d'environ un mètre, incapable de se nourrir de plantes poussant sur la terre. Ce n'est donc pas l'attrait de nourriture qui les mena à se déplacer sur terre, mais c'est peut-être pour éviter de devenir le dîner d'autres poissons qu'ils s'enfuirent sur terre pour une période de temps indéterminée. La compétition pour la nourriture et les endroits pour la reproduction se faisant plus intense vers la fin du Dévonien, cette dernière a aussi pu pousser les ancêtres des tétrapodes vers des environnements d'eau de moins en moins profonde. Les conditions différentes de ces milieux, telle que la rareté d'oxygène, ont certainement contribué à certaines adaptations visibles chez les ancêtres des tétrapodes. Puisqu'ils dérivent leur chaleur corporelle de leur environnement extérieur, il était certainement avantageux de prendre un peu de soleil sur la terre pour ensuite être plus rapides de retour dans l'eau et ainsi échapper aux prédateurs et attraper des proies. Peu important les raisons, les animaux les mieux adaptés pour se déplacer sur terre et retourner dans l'eau sont ceux qui ont le mieux survécu et se sont reproduits. Ce sont donc ces adaptations qui ont perduré et sont à l'origine des générations futures de tétrapodes de plus en plus terrestres. Cette sortie des eaux s'est étalée sur au moins 10 millions d'années et on aperçoit

plusieurs façons de s'adapter à cet environnement : certaines encore utilisées aujourd'hui, certaines appartenant au domaine de la vie fossile.

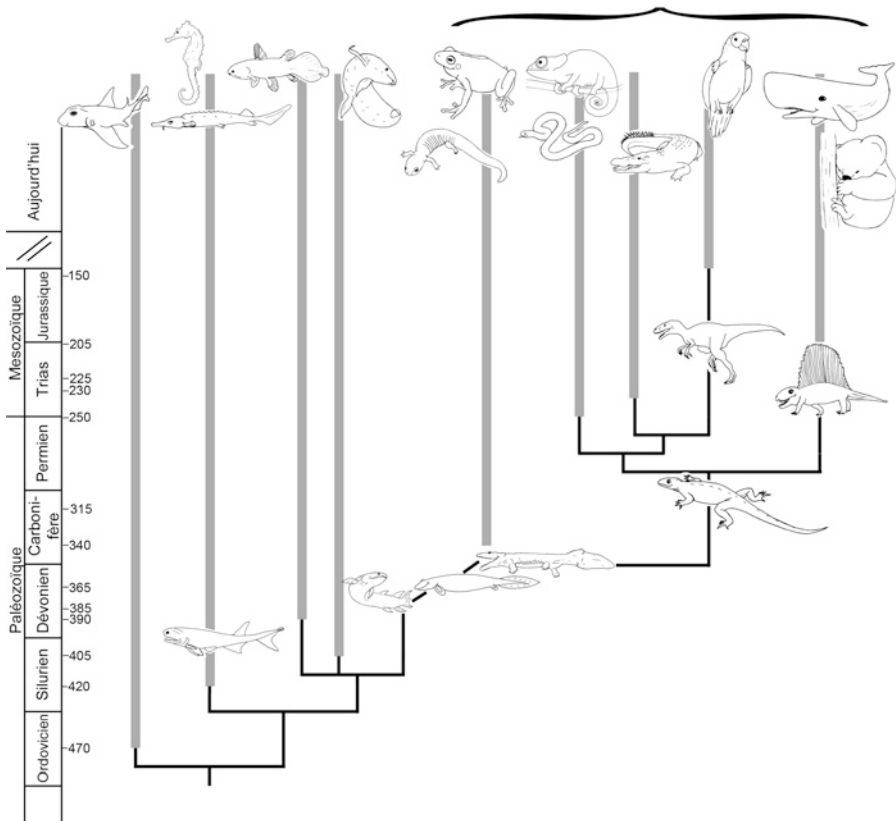


Image 3: Arbre généalogique (phylogénie) des animaux à mâchoire. À gauche, l'échelle des temps géologiques marquant la date approximative de l'évolution des groupes représentés par la base des barres grises ou des images. De gauche à droite : requins, poissons à nageoires rayonnées, poissons à nageoires charnues (cœlacanthes, dipneustes, *Eusthenopteron*, *Panderichthys*) et tétrapodes (regroupés par une accolade). Sur ce diagramme, la lignée des tétrapodes commence avec *Acanthostega*, un amphibien primitif. Certains des descendants d'*Acanthostega* deviennent des amphibiens modernes alors que d'autres se transforment en amniotes ayant la capacité de se reproduire hors de l'eau. De ce groupe, représenté à la base par *Hylonomus*, certains donnent lieu à nos reptiles modernes alors que d'autres donnent naissance à un groupe comprenant les crocodiles et les dinosaures (d'où évoluent les oiseaux) et un autre d'où évoluent les mammifères.

La sortie des eaux

Si on regarde l'arbre généalogique des poissons à nageoires charnues et des premiers tétrapodes, on remarque trois animaux étroitement liés les uns aux autres (les trois images à la base des tétrapodes dans l'image 3). Ils sont tous trois décrits à partir de spécimens presque complets et représentent bien les étapes dans la transition poisson-tétrapode (Image 4). Le premier, *Eusthenopteron* est un poisson à nageoires charnues très commun à Miguasha, un site fossilifère en Gaspésie (Canada). La plupart des spécimens sont préservés en trois dimensions et leur anatomie est connue en détail grâce au travail d'Erik Jarvik (suédois) et Mahala Andrews (britannique). Comme les autres poissons à nageoires charnues, *Eusthenopteron* avait des poumons en plus de ses branchies et pouvait respirer de l'air comme nous. Le squelette de ses nageoires est composé d'os équivalents à ceux de nos bras et jambes et son crâne ressemblait à celui des premiers tétrapodes. Malgré ces ressemblances, c'était un poisson pélagique possédant des nageoires dorsales et anales recouvertes, comme l'extrémité de ses nageoires pectorales et pelviennes, de rayons dermiques.

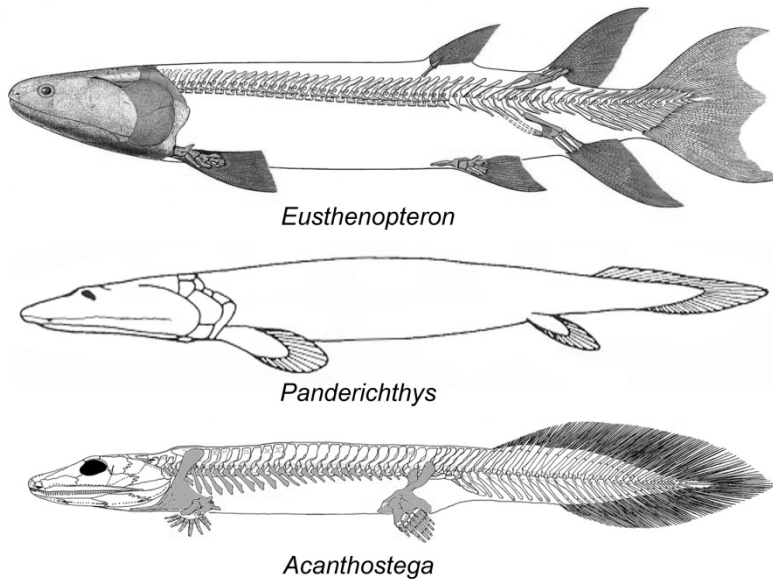


Image 4. Ces trois animaux représentent la transition des poissons aux tétrapodes. *Eusthenopteron* est un poisson à nageoires charnues qui vivait en Gaspésie il y a 385 million d'années et pouvait atteindre 1,5m ! *Acanthostega* est un tétrapode primitif un peu plus petit qu'*Eusthenopteron* (80 cm) qui vivait il y a 365 millions d'années au Groenland. *Panderichthys*, au milieu, est un intermédiaire entre les poissons et les tétrapodes. Il vivait dans les pays baltes il y a 385 millions d'années et mesurait entre 90 et 130 cm. Les éléments perdus lors de la transition sont en rouge, les éléments transformés sont en vert et les nouveautés chez les tétrapodes sont en jaune.

Panderichthys est un poisson transitoire qui vivait dans les pays baltes à la même époque qu'*Eusthenopteron*. Ils étaient à peu près de la même taille, mais *Panderichthys* vivait dans des habitats d'eau peu profonde. Avec son long nez et les yeux placés au-dessus de sa tête, il était adapté à une vie à la surface de l'eau. Par exemple, il ne possédait ni nageoire dorsale, ni nageoire anales, une des caractéristiques transitoires de *Panderichthys*. Le squelette de ses nageoires pectorales et pelviennes est aussi transitoire : leurs extrémités sont couvertes de rayons dermiques, perdus chez les tétrapodes, mais des petits os, précurseurs des doigts sont déjà présents (voir les articles II et IV de cette thèse).

Acanthostega est un tétrapode primitif ayant donc des pattes et des doigts, et pas seulement cinq par patte, mais huit ! Vivant il y a environ 365 millions d'années aux tropiques du Groenland, il était apte à se promener sur terre mais vivait principalement dans les fleuves et les environnements côtiers. Tout comme *Panderichthys* et *Eusthenopteron*, il possédait une grande queue et avait des branchies recouvertes d'un capuchon osseux. Par contre, *Acanthostega* a beaucoup moins d'os recouvrant cette région que ses cousins les poissons (en rouge, Image 4), ce qui lui permettait de bouger sa tête indépendamment de son corps. Un autre grand changement chez les tétrapodes est l'importance relative des pattes avant et arrière pour la locomotion. Les poissons utilisent principalement leurs nageoires avant pour se déplacer alors que chez les tétrapodes, c'est l'inverse. Les nageoires arrière des poissons sont petites et ne sont pas attachées à la colonne vertébrale alors que les pattes arrière des tétrapodes sont plus grosses que les pattes avant. La ceinture pelvienne des tétrapodes pouvait supporter leur poids, étant composée d'un ischion à l'arrière et d'un ilion joignant la ceinture à la colonne vertébrale (en vert sur l'image 4). L'articulation des pattes est sur le côté au lieu d'être à l'arrière, ce qui veut dire que les tétrapodes se déplaçaient très différemment de leurs ancêtres les poissons. Ce sont ces aspects de la sortie des eaux que j'ai étudiés dans cette thèse. Il y a environ cinquante ans, ces transformations anatomiques semblaient être des sauts évolutifs énormes, mais il est de plus en plus clair que la plupart d'entre elles étaient en fait très graduelles. Les résultats de cette thèse, combinés à la découverte de nouvelles espèces fossiles et de nouvelles analyses, nous permettent de comprendre beaucoup mieux comment la sortie des eaux s'est produite.

Comment peut-on étudier l'évolution?

La paléontologie est l'étude de la vie ancienne à partir des restes fossilisés d'organismes ayant autrefois peuplé la Terre. Il faut beaucoup de temps et d'efforts pour trouver et ensuite libérer ces restes de la pierre qui les entourent. Ceci se fait à l'aide d'une aiguille et d'outils pneumatiques

spécialisés ou parfois dans des bains d'acide pour dissoudre la pierre calcaire. Ces méthodes révèlent l'anatomie d'animaux anciens, nous permettant de comprendre comment ils ont évolué. Grâce à la technologie moderne d'imagerie médicale, il est maintenant possible d'observer l'intérieur d'un fossile sans l'altérer. C'est cette technique que j'ai utilisée pour deux des articles de cette thèse (articles deux et trois). Dans ce cas, on a tout simplement emmené un spécimen de *Panderichthys* à l'hôpital pour le radiographier et l'analyser par tomographie (CT-scan). Ceci nous donne une série d'images à intervalles réguliers qu'on peut ensuite analyser à l'aide de programmes spécialisés. Ces programmes nous permettent de construire un modèle en trois dimensions qui peut être manipulé, animé et même disséqué. Cette technique permet d'étudier des parties d'un fossile préalablement inaccessible par l'étude traditionnelle.

L'étude des fossiles est donc essentielle à la compréhension de la vie ancienne, sans eux, on saurait très peu de choses à propos de notre passé. Malgré tout, le registre fossile n'est pas parfait. La probabilité qu'un animal se fossilise est très faible et celle que ce fossile soit trouvé par un paléontologue l'est encore plus. Le registre fossile est donc imparfait, de plus en plus parsemé plus on remonte dans le temps et souvent ponctué d'épisodes lors desquels très peu de fossiles sont retrouvés. Pour mieux comprendre l'évolution et les procédés qui la dirigent, un nouveau domaine d'étude a vu le jour durant les dix dernières années. Le domaine de l'évo-dévo (évolution et développement) cherche à intégrer des domaines parfois disparates tels que la biologie du développement, la génétique, la biologie moléculaire et la paléontologie pour mieux comprendre comment des organismes, groupes ou morphologies ont évolué. Pour cette thèse, j'ai comparé le développement d'un poisson à nageoires charnues (le dipneuste australien) avec celui d'un tétrapode (l'axolotl) pour mieux comprendre comment les nageoires se sont transformées en pattes. J'ai combiné ces données avec celles provenant du registre fossile et réussi à suggérer des procédés pour l'évolution de morphologies, il y a de ça 380 millions d'années.

Il y a plusieurs façons d'étudier le développement d'un organisme. Une des techniques les plus simples consiste à colorer les tissus qu'on désire étudier chez des animaux d'âges différents (une série de croissance). Par exemple, en passant les spécimens dans plusieurs bains chimiques, on peut colorer le cartilage en bleu et les os, en rouge (images des articles un et cinq). En observant les différences à l'intérieur d'une série de croissance, on peut donc comprendre comment, quand et dans quel ordre les éléments squelettiques se développent. Une méthode similaire peut être utilisée pour les tissus mous. Avec la technique de coloration immunologique, on utilise une couleur fluorescente associée à un anticorps pour visualiser les nerfs ou les muscles (par exemple). Pour ce faire, on induit une réaction immunitaire chez une souris contre le tissu qu'on veut étudier. La souris produit donc des

anticorps spécifiques à ce tissu qu'on peut ensuite prélever et modifier pour lui associer une couleur fluorescente. On utilise ensuite ces anticorps pour incuber des séries de croissance pour étudier comment le tissu se développe (voir les images de l'article cinq). Le même principe est utilisé pour étudier l'expression de gènes chez un animal en développement. On commence par trouver la séquence du gène qui nous intéresse et on en fait une copie complémentaire. Lorsque l'on incube l'animal avec cette copie, cette dernière trouve sa jumelle parmi toutes les autres séquences et se joint à elle fermement. On peut ensuite visualiser ce lien à l'aide de coloration immunologique.

Évolution des pattes

Pour les premiers tétrapodes comme pour nous, les doigts sont une caractéristique marquante. C'est un peu pourquoi on a cru pendant longtemps qu'ils étaient des nouveautés chez les premiers tétrapodes. Une des raisons était le manque apparent d'os ressemblant aux doigts dans les nageoires des poissons. La première description de la nageoire pectorale du poisson transitoire *Panderichthys* en 1992 confirma cette hypothèse puisque l'extrémité de celle-ci semblait être composée de grandes plaques osseuses. Quelques années plus tard, on découvrait que les gènes nécessaires au développement des doigts chez les souris n'étaient pas exprimés chez le poisson zèbre, un poisson dérivé à nageoires rayonnées. On conclut donc que les doigts étaient des nouveautés chez les premiers tétrapodes. La découverte de *Tiktaalik*, un cousin de *Panderichthys*, en 2006 ébranla cette idée puisque ce poisson possédait de petits éléments au bout de ses nageoires (des radiaux distaux). Un an plus tard, on découvrait que les mécanismes génétiques nécessaires à la formation des radiaux chez les requins, le poisson spatule (un poisson primitif à nageoires rayonnées) ainsi que le dipneuste (article un) étaient identiques à ceux responsables de la formation des doigts chez les tétrapodes. Les doigts semblaient alors avoir évolué des radiaux de leurs ancêtres les poissons. Nous avons donc réétudié la nageoire de *Panderichthys* (article deux) par tomographie et avons découvert que les extrémités de celles-ci sont composées de petits éléments osseux arrangés en parallèle (Image 5). Cette dernière pièce de casse-tête confirme donc que les doigts ont évolué à partir d'éléments déjà présents chez leurs ancêtres.

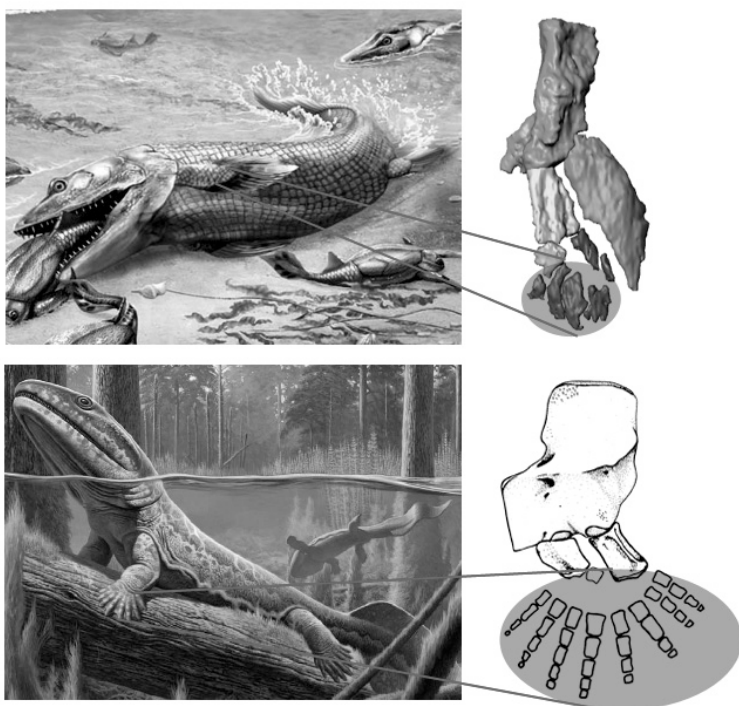


Image 5: L'évolution des doigts. *Panderichthys* (image du haut) est un poisson transitoire possédant de petits os au bout de ses nageoires (ovale rose). Ceux-ci sont les précurseurs des doigts présents chez les tétrapodes tels qu'*Acanthostega* (image du bas). *Acanthostega* avait huit doigts mais pas de paume. Les doigts ont évolué en premier et la paume et le poignet ont évolué plus tard dans l'espace entre l'avant-bras et les doigts.

Imaginez maintenant que vos doigts soient attachés directement à votre avant-bras : pas de poignet, pas de paume, qu'un bras et des doigts. C'était exactement le cas chez les premiers tétrapodes (Image 5). Dans le premier article de cette thèse, nous proposons que les paumes et poignets des tétrapodes un peu plus avancés aient évolué grâce à l'élargissement de l'espace entre les doigts et l'avant bras. De petits os se seraient donc formés dans cet espace, augmentant graduellement en nombre pour finalement créer un poignet et une paume complète (Image 1 du premier article).

L'apparition des doigts a certainement contribué à la conquête de la terre par les tétrapodes, mais sans doute pas autant que l'évolution d'une ceinture pelvienne capable de soutenir le poids du corps hors de l'eau. Chez les poissons, la ceinture pelvienne est une toute petite structure en demi-lune dissociée de la colonne vertébrale, alors que chez les tétrapodes, elle est en trois parties : le pubis en avant, l'ischion en arrière et l'ilion sur le côté, joignant la ceinture pelvienne à la colonne vertébrale (Image 6). Pour étudier cette transformation, j'ai commencé par étudier la ceinture pelvienne de

Panderichthys (article quatre) mais me suis malheureusement aperçue que celle-ci était très primitive, même pour un poisson. Je me suis donc tournée vers la biologie du développement pour m'aider, comparant le développement chez le dipneuste australien, notre cousin poisson le plus proche, et l'axolotl (salamandre), un tétrapode primitif. Grâce à cette étude, je suis arrivée à la conclusion que l'ischion a dû évoluer grâce à un changement de signalisation moléculaire permettant aux cellules du pubis de se déplacer vers l'arrière. Un procédé iliaque étant présent chez tous les poissons à nageoires charnues, je propose que celui-ci se soit progressivement allongé vers la colonne vertébrale pour finalement s'y joindre (Image 6).

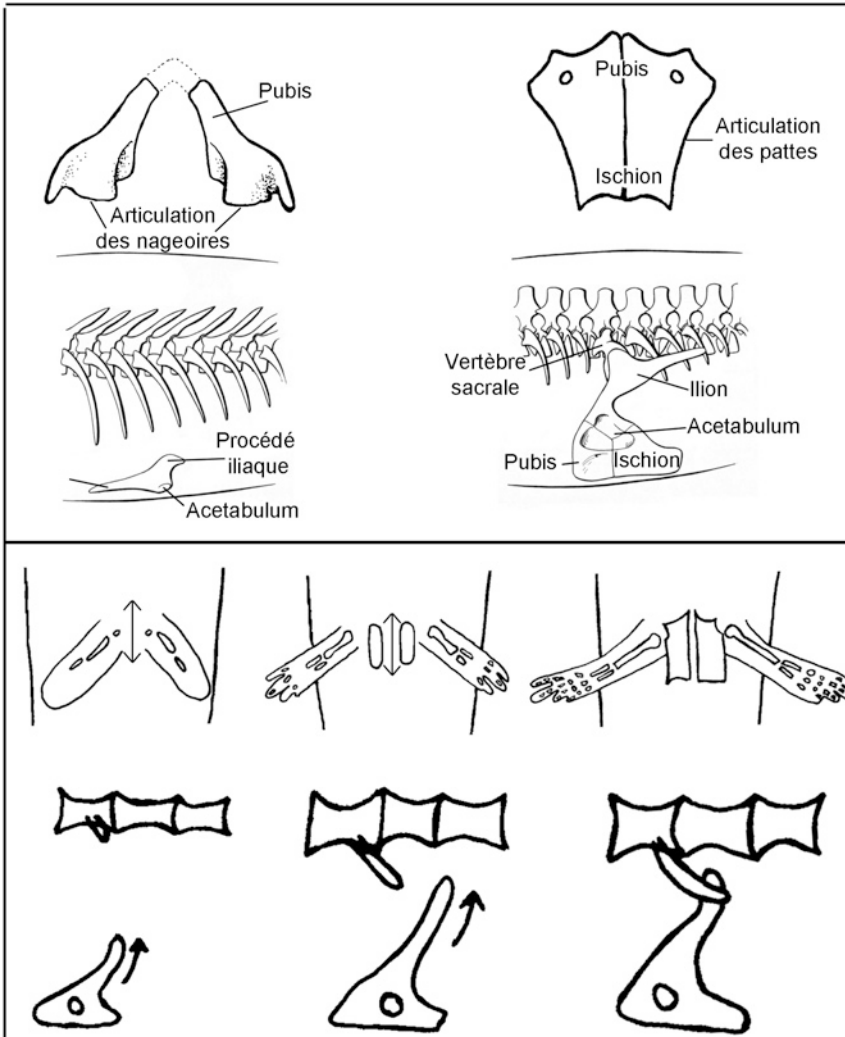


Image 6. Transformation de la ceinture pelvienne lors de la sortie des eaux. Chez les poissons (à droite, section du haut), la ceinture pelvienne est petite, sans association avec la colonne vertébrale alors que chez les tétrapodes (à gauche, section du haut), c'est une structure permettant de soutenir le poids de l'animal en reliant les pattes et la colonne vertébrale. L'ischion a évolué grâce à une élaboration du pubis (première ligne de la section du bas) alors que l'ilion s'est allongé pour finalement joindre la colonne vertébrale (deuxième ligne de la section du bas). Les premières lignes de chaque section sont des vues ventrales et les dernières de chaque section, des vues latérales.

Grâce à ces nouvelles pièces de casse-tête, nous avons maintenant une meilleure idée des événements menant à la sortie des eaux et la conquête de la terre. Il reste encore beaucoup de questions sans réponse, mais aussi beaucoup de nouveaux fossiles à découvrir et de nouvelles façons de les étudier !

Thank you / Tack / Merci !

Tack, **Per**, för att du gav mig denna fantastiska möjlighet att forska på ett spännande och utmanande projekt i ett kreativ och stödande miljö. Du alltid tog tid för mig, även när det handlade inte om forskning och detta generositet tog mig längre än jag kunde ha drömt om. **Henning**, du var inte officiellt min handledare, men jag visste jag kunde alltid fråga dig om jag behövde hjälp. Tack också för att du frågade hur det gick och om allt var under kontroll (om det var nån gång det!). Kära **Daniel**, hur kan jag tacka dig tillräcklig mycket? Du hjälpte mig att lära mig svenska, att anpassa mig till mitt liv här och du visste alltid hur vi kunde hitta informationen jag behövde. Det var inte heller alltid uppenbart hur jag skulle sköta allt den administrativa delen av min doktorand men **Rose-Marie**, du gjorde allt så enkelt och smidigt! Tack för att du finns min vän. **Barbro**, det är så rolig att ha dig i avdelningen, du gör varje dag en färggladare dag! **Johan**, tack för dina råd i labbet, jag ska fortsätta använda dina tekniker i framtiden ;-). **Martin**, not only were you my patriotic presence here in Sweden, but you helped me learn how to draw, thanks! **Anna**, it is really good to have you around, to enjoy your enthusiasm and drive. Tu me manques **Gaël**! C'était si bien de bénéficier de ta présence ici. Au moins, maintenant il y a **Sophie** pour me faire pratiquer mon français ! Tu es charmante Sophie et ton entrain et ton sourire égaient tous les jours au labo.

During my PhD, I had the chance to work in several labs. Thank you **Jean** for welcoming me to Macquarie and allowing me to work with those beautiful lungfish! **Zerina**, you took me under your wing when I first arrived to Australia and you made that visit and every one after that, a wonderful one. **Rolf**, livet vid labbet var så trist innan du blev postdoc hos Jean! Tack för din sällskap, din vänskap och för att du aldrig ger upp. **Peter, Nic, Jackie, Silke** and the crowd at the Victor Chang, thank you so much for your help, it was great working with you and I am looking forward to continuing to do so. **Georgy** welcomed me to his lab at the Wolfson Institute of Biomedical research and it was great to work in this environment. **Bettina**, you showed me so much in so little time, you are wonderful!

A palaeontologist's PhD is never complete with a little field work: thanks to Gaël, Martin, Zerina, **Alex, Edith, Kate** and **John** for taking me on wonderful adventures and thanks to the crew for making it a great experience. Det skulle också ha varit mycket svårt att forska inom utvecklingsbiologi

utan ett lab. Tack **Stefan** för att du sköte konstruktion av mitt lab med mig och att du och **Gary** alltid kunde hjälpa med mikroskopin.

Min tid i Sverige blev mycket bra tack vare fantastiska vänner. **Sofia**, du är en ängel. Du alltid har den rätta sak att säga när jag är stressad, trött eller ledsen. Våra spontana biokvällar gjorde att jag alltid visste jag var välkommen hos dig och katterna. Och ni **paletjejer**, det är så kul att umgås med er, jag blev så förvånad att det fanns så många av oss, ye! När jag började min doktorand fanns folk som välkomnade mig hit och visade mig runt Uppsala. Tack **Piero, Rosita, Rolf, Kerstin** och **Johanne** för det! och du, **Emil**, vad kul att vi kan träffa över lunch då och då.

Mitt liv utan musik är som en sommar dag utan sol: det går, men det finns ingen värme. Tack vare **Akademiska Kapellet**, fick jag 80 vänner direkt när jag blev medlem, drygt två veckor efter jag landade i Sverige. Ni spela så fint musik och ni är så bra människor! Varje gång livet blev lite svårt, forskning frustrerande eller vädret trist var ni där för att pigga upp mig! Det finns ingen orkester i världen som är lik er. **Stefan**, det är du som är vår drivkraft. Jag trodde aldrig att jag skulle hinna utveckla som musiker under min forskarutbildning men det gjorde jag tack vare dig. Nu vet jag att det är nyttigt att öva när man är helt slut! Tack också för alla fester vi ordnade tillsammans, gå KGB! **Petra**, du gör så mycket bakom kulisserna för oss och det gör att kappellister kan bara bryr sig om att spela fint och ha kul. Tack! And **Tom**, what would the orchestra do without you? I am honored to be your friend and am always so glad to play beautiful music with you. Till alla mina **kappellet vänner**, livet är mycket skönare och roligare med er.

Je veux finalement remercier ma **famille. Maman** et **Papa**, vous avez toujours nourri ma curiosité et m'avez toujours encouragé. Vous m'avez laissé partir dans un pays lointain malgré votre peine, parce que vous saviez que c'était mon rêve. Vous avez partagé mes voyages et vous vous êtes contentés de mes brèves visites. **Geneviève**, ma grande sœur, même quand on ne se parle par souvent, je sais que tu es là, que tu es fière de moi et de mes succès. **Dan** et **Léanore**, on ne se voit pas assez, mais vous êtes toujours dans mon cœur. **Simon**, mon petit frère, tu m'a toujours laissé la place dont j'avais besoin et a toujours partagé mes découvertes. Je suis si heureuse chaque fois que j'ai l'occasion de te parler. **David**, you make the hard times easier, the mundane, wonderful and the best even better. You encouraged me every step of the way and you always believed in me even when I did not, thank you.

To all of you, this thesis would not be this thesis without you; it is yours as much as it is mine.

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