

The Coelacanth and Its Genome

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This special issue of JEZ, Part B (Mol. Dev. Evol.)¹ contains 10 companion papers for the recently published landmark article on the African coelacanth genome (Amemiya et al., 2013). Here, we provide a brief background of the living coelacanth, a historical perspective of the coelacanth genome project, and highlight the major findings of the 10 reports.

The first living coelacanth was discovered 75 years ago on December 22, 1938 in South Africa by Marjorie Courtenay Latimer (1919–2004) as by catch off the Chalumna River near East London in 1938. She reports: "I picked away at the layers of slime to reveal the most beautiful fish I had ever seen. It was five foot long, a pale mauvy blue with faint flecks of whitish spots; it had an iridescent silver blue green sheen all over. It was covered in hard scales, and it had four limb like fins and a strange puppy dog tail" (Weinberg, 2000). This fish, often hailed as the zoological sensation of the 20th century, was described by J. L. B. Smith (the famous South African ichthyologist, after whom an institute would later be named), *Latimeria chalumnae*, in honor of its discoverer (Smith, '39). The discovery of this fish that belonged to an evolutionary lineage thought to have gone extinct more than 70 million years ago, was, as Smith exclaimed, like seeing a dinosaur walking through the street. This fish appeared overtly similar to its relatives and ancestors that lived from about 400 to about 66 million years ago. Smith went on a quest to locate a second specimen, which was finally found exactly 14 years later, on December 21st 1952 on the Comoron island of Anjouan (Smith, '53). In the gripping science thriller, *Old Fourlegs*, that hooked all of us into wanting to study the coelacanth, Smith tells the incredulous story describing the hunt for the second living specimen of a coelacanth (Smith, '56).

Less than 300 catches of coelacanths are known to science (Bruton and Coutouvidis, '91; unpublished). At least 150 additional coelacanths were observed off the Comoros by Hans Fricke and his team, using a submersible (personal communication). Moreover, additional catches made off Mozambique and

Madagascar (Heemstra et al., '96), prove the existence of coelacanth populations throughout the Western Indian Ocean. In another complete surprise, a serendipitous happenstance on a honeymoon to Indonesia in 1997, the first specimen, of what would become the second extant coelacanth species (*Latimeria menadoensis*), was sighted and photographed in a local fish market. This first specimen could not be secured, but a live specimen was captured off Manado, North Sulawesi in Indonesia (Erdmann et al., '98; Pouyaud et al., '99).

Ever since its rediscovery, the coelacanth has been the subject of intense interest and great fascination for both scientists and the general public worldwide (Forey, '88; Thomson, '91; Weinberg, 2000). However, the animal has also been a lightning rod for politics, exploitation, greed, intrigue, fraud, and intense rivalry (Smith, '56; Erdmann and Caldwell, 2000; Weinberg, 2000). Both the African and Indonesian coelacanths are listed as *critically endangered* and placed in Appendix I of the Convention on International Trade in Endangered Species (CITES).

The idea of an African coelacanth genome project was first proposed by one of us (Dorrington) and Greg Blatch, (Rhodes University) late in 2001 following the discovery of coelacanths off the northeast coast of South Africa at Sodwana Bay (Venter et al., 2000). What was remarkable about the Sodwana Bay coelacanths was that they were found at depths of 70–100 m, accessible to SCUBA divers, in contrast to the Comoran and Indonesian animals, which occur in deeper waters from 400 to 700 m (Fricke, '88; Fricke et al., 2000; Venter et al., 2000). The existence of a viable South African population once again captured the imagination of the scientific community and public at large, prompting Dorrington and Blatch along with researchers from SAIAB (South African Institute for Aquatic Biodiversity,

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formerly the J.L.B. Smith Institute) to propose the initiation of a flagship, multidisciplinary research program focusing on the coelacanth and its habitat. The proposal was enthusiastically received by the [then] South African National Department of Arts, Culture, Science and Technology, which provided the funding to launch the African Coelacanth Ecosystem Programme (ACEP) in 2002. One of the broad objectives of the ACEP was to build a coelacanth genome resource, including blood and other tissue samples for providing DNA and RNA of sufficient quality for molecular genetic studies and for constructing suitable libraries for genome sequencing.

While the scientific importance of a coelacanth genome project was widely supported, the overwhelming problem was (and still is) obtainment of tissue samples of sufficient quality for biological studies. The first problem is that *Latimeria* is critically endangered, which prohibits any capture of animals, even for research purposes. Another problem is their inaccessibility. With the exception of the Sodwana Bay population, coelacanths generally occur at depths below 100 m, which means that they can only be reached by submersible or using a remote operating vehicle (ROV). Animals that are brought to the surface do not survive the changes in temperature, pressure and reduced oxygen availability. Consequently, Hans Fricke and his team devised a non destructive method for collecting scales with their submersible, Jago, providing small amounts skin tissue for DNA phylogenetic analyses (Schartl et al., 2005), but not nearly enough for a large scale genome project.

In view of the restrictions on the capture of animals listed on the CITES Appendix 1, the only opportunity for obtaining enough coelacanth tissue for a genome project was to take advantage of a chance catch, which was most likely to occur in the Comoros. The Comoros are an archipelago of volcanic islands in the West Indian Ocean off the coast of Mozambique, northwest of Madagascar and they are home to the largest population of coelacanths (Fricke et al., '91). However, following its CITES listing in 1989 and due to the efforts of the Comoran *Association pour le protection du Gombessa* (APG), the number of chance coelacanth catches had dropped dramatically with the most recent prior catch being in 1997. Compounding this issue was the lack of infrastructure and the wherewithal to collect and preserve tissues on the islands, where access to unreliable electricity is confined to the capital, Moroni, and most of the villages along the coast where coelacanths had been captured in the past, had little access to telephones or transport. Nevertheless, it was decided that the opportunity presented by a chance catch was too important, and Dorrington, representing ACEP, traveled to Grande Comore to establish a team who would be able to respond quickly to a chance catch. The team was led by a local biologist conservationist, Sahid Ahamada, and included the APG and local fishermen.

On September 15, 2003, Comoran fishermen found a dead coelacanth with a swordfish in its mouth floating off Moheli Island. They froze it immediately after it was brought to shore and

shipped it across to Grand Comore Island, where tissues including blood, gills, liver, and heart were harvested by Ahamada and his team, and frozen at -20°C . Then, as luck would have it, 3 days later, on the evening of 18th of September, a coelacanth was caught by line off Hahaya Village on Grand Comore and towed to shore behind the fishing canoe. It was still alive, barely, the following day when Ahamada arrived to collect it. The fishermen had taken the trouble to keep the fish in the water, so Ahamada was able to take blood from the animal shortly after it expired. Serendipitously, an inaugural coelacanth conference organized by ACEP was being held in October of 2003 in East London (South Africa) and Ahamada was scheduled to attend. Thus, he was able to personally deliver the tissue samples to Dr. Dorrington, who then immediately analyzed the nucleic acids that she extracted from the tissues. Although the Moheli samples proved to be too degraded, the Hahaya sample showed good preservation of high molecular weight DNA. Upon recalling the series of events, Ahamada stated, "I was excited that this fish from the Comoros was going to be used for science, but at that time I had no idea how important it would be" (Weinberg, 2013).

At the conference, Dorrington announced that a suitable tissue sample had been procured by Ahamada, and the news was eagerly received by Marjorie Courtenay Latimer (then a spry 96 years old), an honored guest at the proceedings (Fig. 1). Several months prior to the conference, Dr. Courtenay Latimer sent a hand written letter to Rosie Dorrington lauding her efforts and expressing how pleased she was that there was still so much interest regarding the coelacanth (Fig. 2). Axel Meyer, who had long been studying the molecular phylogenetics and comparative genomics of



Figure 1. Marjorie Courtenay-Latimer at the inaugural coelacanth meeting in East London, South Africa, October 2003. On the left is Sahid Ahamada, the key field Comoran scientist who collected the tissue samples from chance catches of *Latimeria chalumnae* in September 2003. Marjorie is on the right and is speaking to the then President of the South African National Research Foundation, Dr. Khotso Mokhele. Sahid had just arrived with the blood sample that would later provide the genomic DNA for the genome project.

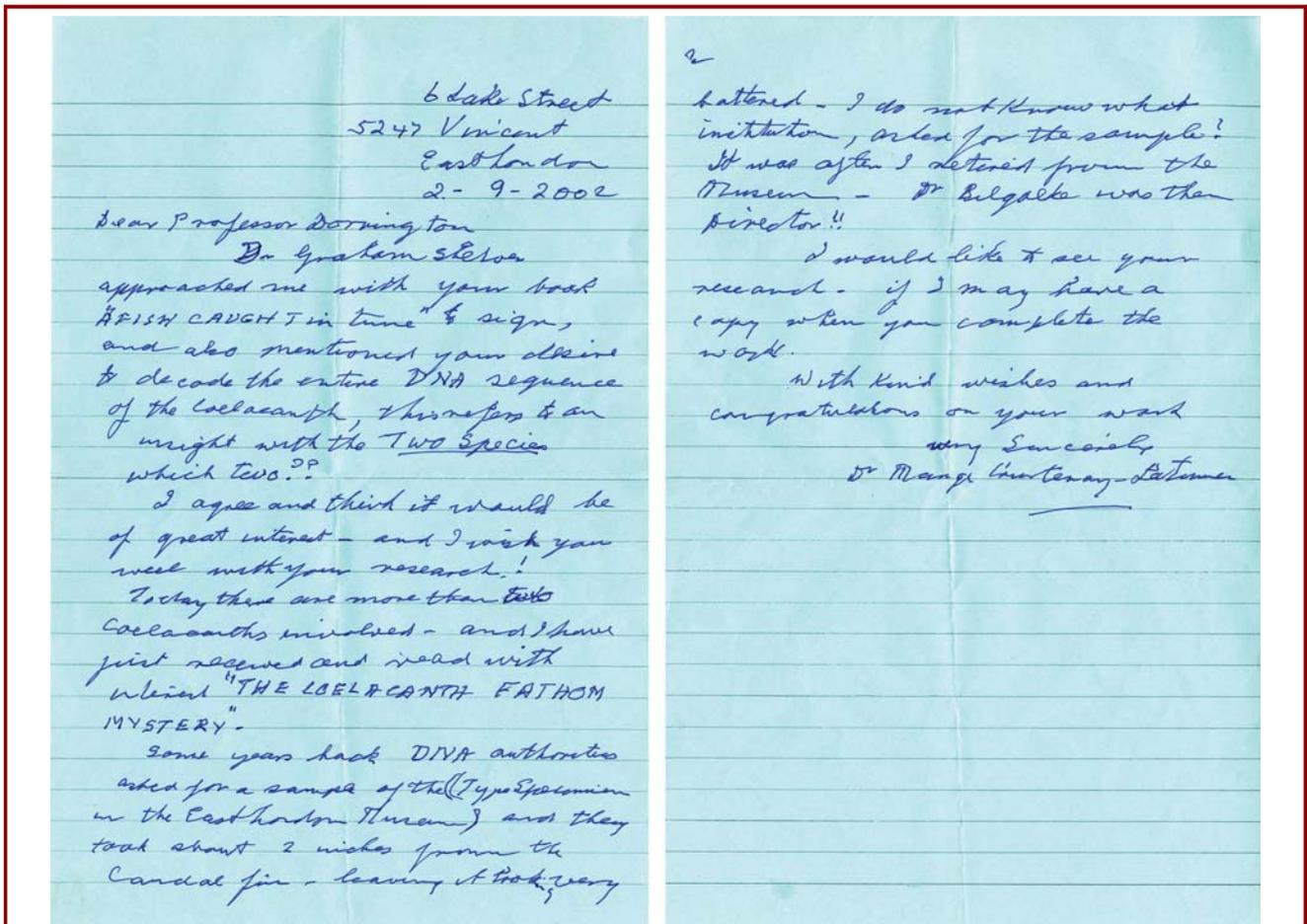


Figure 2. Handwritten letter from Marjorie Courtenay-Latimer to Rosie Dorrington lauding the coelacanth genome project. Marjorie was still excited by coelacanth research 60 years after her seminal discovery, and noted that there were now two species of coelacanths.

coelacanths, and lungfish (Meyer and Wilson, '90; Meyer, '95; Zardoya and Meyer, '96; Zardoya and Meyer, '97a; Zardoya and Meyer, '97b; Brinkmann et al., 2004) also was an invited participant at this historic meeting (Fig. 3). And while Ms. Courtenay Latimer never got a chance to read the genome paper (which was finally published 10 years later), her vigilance, keen eye, and importance to the entire coelacanth community cannot be understated.

Back in the United States, Chris Amemiya's lab had been independently working on coelacanth genomics for several years, and, as with Meyer, had a lifelong interest in the biology of the coelacanth. Importantly, his laboratory had generated a bacterial artificial chromosome (BAC) library from the Indonesian coelacanth (Danke et al., 2004) and this resource formed the basis for several initial investigations on the coelacanth genome (Noonan et al., 2004; Shashikant et al., 2004; Bejerano et al., 2006; Saha et al., 2006). Funds for a coelacanth genome project had not been procured in South Africa despite Ahamada's and Dorrington's

success in obtaining a usable tissue sample, and this Hahaya sample languished in the freezer in Dorrington's laboratory for several years. Eventually, largely through negotiations between Dorrington and Amemiya (Fig. 4), this blood sample was sent to Amemiya's laboratory in Seattle, where high molecular weight DNA was extracted and embedded in agarose blocks. The DNA obtained (roughly 400 μ g) was of good quality for a future genome project, if and when funding support could be mustered. To this end, Amemiya, along with Rick Myers (then of Stanford University) and Eric Lander (Broad Institute), submitted a "white paper" to the National Institutes of Health (NIH) requesting the sequencing of the genome of the Hahaya coelacanth (Amemiya et al., 2006). The request was approved: genome sequencing would be undertaken at the Broad Institute and NIH would cover the costs. Pilot genome shotgun experiments utilizing Sanger sequencing commenced in 2007-2008, however, it would not be until 2012 for full scale production sequencing to be carried out in earnest using the more cost effective Illumina platform.



Figure 3. Axel Meyer and Marjorie-Courtenay-Latimer at the inaugural coelacanth meeting in East London, South Africa, October 2003. Axel has been involved in understanding the relationships between coelacanths, lungfishes, and tetrapods using molecular evolutionary tools.

Additional transcriptome data from both African and Indonesian coelacanths as well as from an African lungfish were obtained and analyzed as part of the landmark coelacanth genome article (Amemiya et al., 2013).

Of the ten companion papers published here, eight were largely part of the original body of work but whose vignettes did not make



Figure 4. Chris Amemiya and Rosie Dorrington in the bush in South Africa, January 2008, discussing coelacanths and other wild species. By this time, pilot sequencing had been carried out on the DNA of the Hahaya specimen, though it would not be until 2012 when production sequencing could commence.

it into the main text, whereas two of the reports are new analyses. The manuscripts from Chalopin et al. (10.1002/jez.b.22521; pages 322–333) and Forconi, Chalopin et al. (10.1002/jez.b.22527; pages 379–389) focus on the transposable element (TE) content of *Latimeria*, and showed that some 25% of the genome encodes TEs and that a relatively large percentage (23%) show active transcription. These findings indicate that TEs are dynamic in the coelacanth genome, a finding that appears at odds with the slower rate of protein coding evolution observed in the species. The paper by Nitsche et al. (10.1002/jez.b.22542; pages 342–351) used computational methods to sift through transcriptome datasets from both species and screen for both normal and “atypical” messages, which had been identified recently in mammalian genomes. Several atypical messages were identified and included circular RNAs as well as those in which fusions were generated from nonlinear and nonadjacent coding sequences in the coelacanth genome. The biological implications from such atypical RNAs remain speculative.

The paper by Mulley and Holland (10.1002/jez.b.22513; pages 352–358) examined the content and organization of the ParaHox (homeodomain) genes in the coelacanth genome. Their analysis showed that the ParaHox genes are an example of coelacanths retaining the inferred ancestral gene content for gnathostome vertebrates, with no additional gene losses or gains. An analysis of the genes encoding chaperonins was carried out by Bishop et al. (10.1002/jez.b.22541; pages 359–378). This paper focused on Hsp90 and Hsp40 chaperones of the Indonesian coelacanth, and, as had been found for the African coelacanth (landmark paper), there are many similarities to the vertebrate homologs. However, there are number of key differences such as DnaJB13, which is predicted to be a non functional Hsp40 in humans, mouse, and zebrafish due to a corrupted histidine proline aspartic acid (HPD) motif, whereas the coelacanth homolog has an intact HPD. In the report by Forconi, Biscotti et al. (10.1002/jez.b.22515; pages 334–341), the purine catabolic pathway genes of coelacanths were characterized in light of the obvious biochemical changes that have occurred in excretory physiology during the aquatic to terrestrial transition, due presumably to a progressive shortening of the pathway in the sarcopterygian lineage. The presence of an intact ancestral gene set in the coelacanth indicates that the functional loss of some genes in the lineage leading to tetrapods occurred after the transition to terrestrial life. The paper by Kawasaki and Amemiya (10.1002/jez.b.22546; pages 390–402) focused on specific mineralization genes and showed that the repertoire of SPP genes is essentially the same in tetrapods as in the coelacanth, but clearly different than in teleosts. Notably, the five enamel genes known in mammals are all identified in the coelacanth genome. This result suggests that the tooth surface tissue in the coelacanth is true enamel that is equivalent to our own tooth surface but different from the tissue in sharks or teleosts (which possess enameloid), and genetically confirms tenets based on long held histological findings.

In the paper by Picone et al. (10.1002/jez.b.22531; pages 403–414), gene families encoding coelacanth G protein coupled receptors for olfaction and taste were identified and characterized. The intermediate sized repertoires for these chemosensory receptors are in keeping with the presumed expansions necessitated by aquatic to terrestrial transitions. The final two papers in this issue, by Boudinot et al. (10.1002/jez.b.22559; pages 415–437) and Saha et al. (10.1002/jez.b.22558; pages 438–463), describe the genes, respectively, of the innate and adaptive immune systems of the coelacanth. Both systems show remarkable conservation with those of tetrapods in terms of gene content and phylogenetic relatedness. The report by Saha et al., however, showed that coelacanth differs from other vertebrates in that it possesses two highly distinctive IgW loci but no IgM genes, which were previously thought to be a *conditio sine qua non* for vertebrates. The coelacanth also exhibits what might be an ancestral condition of overt interdigitation of T cell receptor components with Ig heavy chain variable region genes.

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