POPULATION GENETIC ANALYSIS OF WESTERN INDIAN COELACANTHS BASED ON MITOCHONDRIAL DNA

ミトコンドリアDNA配列解析に基づく西インド洋産シーラカンスの遺伝学的分析



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In resent years, a large number of individuals of coelacanths have been landed off the coast of Tanzania. Although coelacanth specimens have also been landed at other localities in the western Indian Ocean, so far, viable populations of this species have been identified only at two localities, Comoros and South Africa. Therefore, the recent active catch off Tanzania suggests a new habitat for coelacanth. Based on the current situation surrounding the Tanzanian coelacanth, to examine the genetic background of the fish, we determined complete mitochondrial sequences of Tanzanian coelacanths collected from the north and south coasts of Tanzania. Using the criteria for six haplotypes established in a population genetic study for coelacanths living in the western Indian Ocean (Schartl et al., Nature 2005; 435:901), we analyzed mitochondrial haplotypes of Tanzanian coelacanths. As a result of haplotype characterization, we suggest that the southern specimen is a member of the Comoran group, but were swept away by the South Equatorial current. On the other hand, some of the northern specimens formed a haplotype group that contains Kenyan coelacanth. We assumed that the north Tanzanian specimens may be a member of an undiscovered population that exists near the boundary between Tanzania and Kenya.

近年タンザニア沿岸では多数のシーラカンスが捕獲されている。これまでに西インド洋沿岸海域ではさまざまな場所でシーラカンスの捕獲が記録されているのだが、そのうち実際繁殖集団が確認されているのはコモロ諸島沿岸と南アフリカ沿岸の2箇所に過ぎない。それゆえ近年のタンザニア沿岸におけるシーラカンスの頻繁な捕獲記録はその周辺において新たな繁殖集団が存在していることを期待させる。このような現状を踏まえて我々は発見されて間もないタンザニア産シーラカンスの遺伝的背景を分析するためミトコンドリアDNAの全長配列を決定した。標本はタンザニア北部と南部から得られたシーラカンスである。また、西インド洋沿岸のシーラカンス多数個体から同定されたミトコンドリアDNAハプロタイプ分析結果（Schartl 他、Nature 2005; 435:901）に基づきタンザニア産シーラカンスのミトコンドリアDNAハプロタイプを分析した。その結果タンザニア南部で捕獲された個体はコモロ諸島沿岸の集団から南赤道海流によって大陸沿岸に流されてきた個体である可能性が考えられた。一方、北部の数個体はケニア産シーラカンスとともに一つのハプロタイプグループを形成した。この結果から我々はタンザニア北部で捕獲された個体はタンザニア北部沿岸に生息する未知の集団に由来するのではないかと推測している。

1. Research background

1-1. Donation of Tanzanian coelacanth to Tokyo Institute of Technology

In December 2004 in Tanzania, one individuals of coelacanth (*Latimeria chalumnae*) was donated from Tanzania Fisheries Research Institute (TAFIRI) to Tokyo Institute of Technology (Tokyo Inst. Tech.) commemorating to start collaborative research between those two institutes (Fig. 1). Then Tokyo Inst. Tech. exchanged memorandum of agreement regarding the collaborative research of Tokyo Inst. Tech. and TAFIRI, and transported a frozen specimen of coelacanth from Tanzania to Japan under the Washington Convention in November 2005.

Based on the donation and the transportation of coelacanth, coelacanth donation memorial ceremony was performed in Tokyo Inst. Tech. Suzukakedai campus in November 2005. In the ceremony, Tanzania ambassador to Japan and South Africa ambassador to Japan attended as the guests, and Dr. Philip Bwathondi (director of TAFIRI) gave a lecture. After the ceremony, the coelacanth was opened to the public, and over 500 people visited to Tokyo Inst. Tech. to see the rare animal.



Fig. 1. Coelacanth donation memorial ceremony (December 2004 in Dar es Salaam, Tanzania).

The following year our research group organized memorial lecture meeting "the living fossil coelacanth" in Tokyo Inst. Tech. Ookayama campus in April 2006. We invited Anthony Ribbink who is a central figure of African Coelacanth Ecosystem Program (ACEP) as a speaker (Fig. 2). After that we opened the coelacanth to the public in the National Museum of Nature and Science of Japan and annual meeting of Society of Evolutionary Studies, Japan. Growing need for environmental protection on a global scale, a living fossil coelacanth, which is designated as an endangered species, is taken as a symbolic animal. Through the public event, we feel that news regarding a living fossil coelacanth will also attract the interest of general people.



Fig. 2. Pictures of memorial lecture meeting "the living fossil coelacanth" in Tokyo Inst. Tech. (Ookayama campus in April 2006)

In October 2007, 2nd coelacanth specimen was transported to Tokyo Inst. Tech. This individual was the largest individual (total length: approx 160cm) captured in Tanzania (Fig. 3). In addition, this individual was pregnant female, which contained 36 fertilized eggs in its body (Fig. 4). As shown in figure 4, the fertilized eggs are apparently early developmental stage. That kind of coelacanth embryos is quite rare and considerably important specimens for evolutionary developmental biology. In this time of coelacanth donation from TAFIRI to Tokyo Inst. Tech., our research group obtained six fertilized eggs.



Fig. 3. Second coelacanth specimen transported to Tokyo Inst. Tech.



Fig. 4. Fertilized eggs discovered from the specimen shown in Fig. 3.

1-2. Establishment of coelacanth consortium

Our research group (Okada laboratory, Tokyo Inst. Tech.) invited public participation to establish coelacanth research group that was composed of the front-line researcher of Japan. In consequence of strict examination, we established the research team of which was composed of anatomical research group and biochemical research group. In accordance with the establishment, we organized first meeting for coelacanth consortium in November 2007 and discussed about future plan of investigation. The coelacanth consortium presented four-year scheme from December 2007 to December 2011.

2. Research progress in Okada laboratory (Tokyo Inst. Tech)

2-1. Anatomical study using advanced technology

By obtaining cooperation with GE Yokogawa Medical System company, we performed image analysis using computed tomography scan (CT scan, see Fig. 5) and magnetic resonance imaging (MRI). In consequence, we could succeed to take distinct images of skeletal structure, muscle and internal organs without damaging the body. These images will be valuable data in the future anatomical study for coelacanth. In addition, this research contains the viewpoint of proposal of research strategy for anatomical study of rare creatures utilizing advanced technology, namely CT and MRI (Fig. 5).

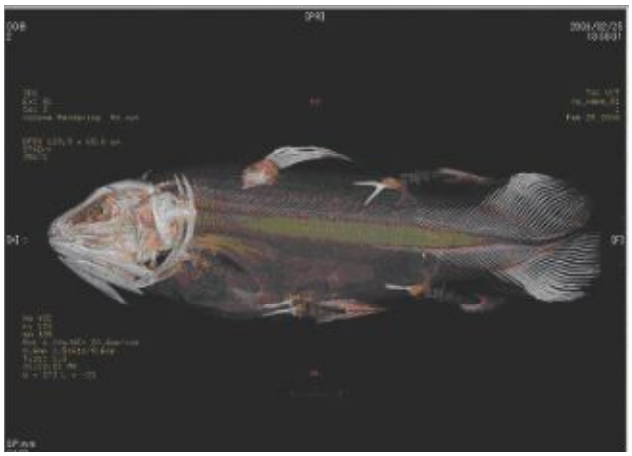


Fig. 5. Lateral view of coelacanth CT scanning image. Bone tissue was observed as white color image.

2-2. Population genetic study using mitochondrial DNA

Our research group determined complete mitochondrial DNA sequence from two Tanzanian individuals (Fig. 6). One individual (Kigombe-9) was caught off Kigombe village, in the Tanga region in north Tanzania in November 2004. The other individual (Songo Mnara-1) was caught off Songa Mnara Island in south Tanzania in September 2004.

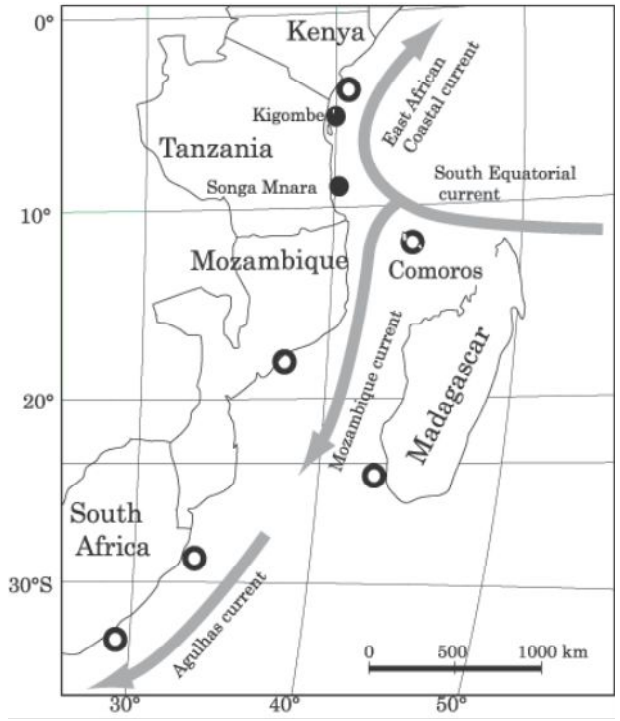


Fig. 6. Map showing the locations of *L. chalumnae* capture. Capture location of Tanzanian individuals used in this study is indicated by filled circles, and the capture locations for the other specimens are indicated by open circles.

Previously, Schartl et al. (2005) reported six mitochondrial haplotypes based on six variable sites in the control region among 47 *L. chalumnae* individuals. In the present study, two variable sites between the two Tanzanian individuals were observed in the control region, each of which corresponds to one of the six informative sites defined by Schartl et al. (2005). Based on the Schartl et al. haplotype classification, Tanzanian coelacanths Songa Mnara-1 and Kigombe-9 were assigned to haplotype 1 and the haplotype 5, respectively (Table 1).

	Nucleotide positions						Locality (number of individuals)	Reference
	15,770	15,772	16,176	16,220	16,241	16,286		
Haplotype 1	A	A	A	A	G	A	Comoros (9) Songa Mnara (1)	Schartl et al. (2005) Sasaki et al. (2007)
Haplotype 2	A	A	A	A	A	A	Comoros (12)	Schartl et al. (2005)
Haplotype 3	A	G	G	G	G	A	Comoros (15) South Africa (4) Madagascar (3)	Schartl et al. (2005) Schartl et al. (2005) Schartl et al. (2005)
Haplotype 4	A	G	G	G	G	G	Mozambique (1)	Schartl et al. (2005)
Haplotype 5	A	G	G	A	G	A	Kenya (1) Kigombe (1)	Schartl et al. (2005) Sasaki et al. (2007)
Haplotype 6	T	G	G	G	G	A	South Africa (2)	Schartl et al. (2005)

*Numbers of nucleotide positions designated in the complete mitochondrial genome sequence determined by Sasaki et al. (2007).

Table 1. Nucleotide variation corresponding to mitochondrial haplotypes of *L. chalumnae* from Tanzania and other localities.

We constructed genealogical relationships among western Indian Ocean coelacanth individuals using the TCS software package (Clement et al., 2000) (Fig. 7). In the resulting relationships, haplotypes 1 and 5, each of which includes one of the two Tanzanian individuals, were directly connected by two nucleotide substitutions. Comoran individuals were largely divided into two genealogical groups, haplotype 1 + 2 and haplotype 3, and these two groups were connected by three nucleotide substitutions. Haplotype 5 was located at an intermediate position between the two genealogical groups of Comoran individuals. Songa Mnara-1 was categorized in the haplotype 1 + 2 group. If haplotype 1 represents a population localized specifically at Comoros, Songa Mnara-1 may be a stray that was swept away from Comoros. Songa Mnara is located nearly due west of the Comoros Islands and is exposed directly to the South Equatorial current (Fig. 6), and thus it is likely that this individual drifted from Comoros to Songa Mnara on this current. On the other hand, Kigombe-9, which was captured off the north coast of Tanzania, was categorized as haplotype 5 together with an individual from Malindi, Kenya. Nearly 40 *L. chalumnae* specimens have been captured off the north coast of Tanzania in recent years. In the present study, we analyzed the sequence of only one of these individuals from north Tanzania (Kigombe-9). Sequence analysis of the other coelacanth individuals from north Tanzania may reveal additional examples of haplotype 5. Our population genetic study of Tanzanian coelacanth mentioned above was recently published in the journal "Gene" (Sasaki et al., 2007).

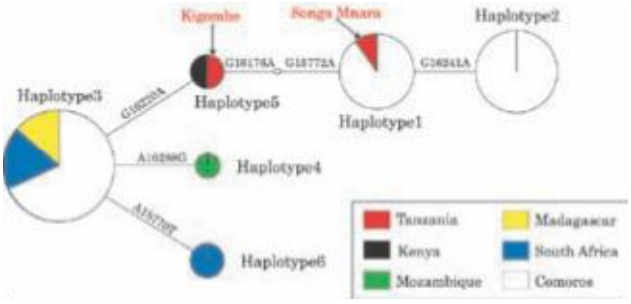


Fig. 7. Haplotype network of *L. chalumnae* living in the western Indian Ocean. The size of circle corresponds to the haplotype frequency. Nucleotide substitution between haplotypes is indicated in each branch

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