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学位論文全文に代わる要約 Extended Summary in Lieu of Dissertation

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A study on the genetic structure and diversities of tilapia from Ghana and Japan

学位論文題目: (ガーナと日本におけるティラピアの遺伝的集団構造と遺伝的多様性)

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Dissertation Summary

Tilapia is native to Africa. However, it has been introduced to many tropical and subtropical regions in the world. They belong to the family Cichlidae which has at least 1650 species. Three genera are commonly referred to as tilapia; *Oreochromis*, *Sarotherodon* and *Tilapia*. *Oreochromis* spp and *Sarotherodon* spp comprise the mouthbrooders and *Tilapia* spp are substrate brooder. However, the distinguishing feature between *Oreochromis* spp and *Sarotherodon* spp is for the former, the

females incubate the eggs whiles in the latter both male and female incubate the eggs.

Tilapias are the third most farmed fish resource after carps and salmonids. In 1978, Nile tilapia (*Oreochromis niloticus*) was introduced to China which currently leads in world production of tilapia and has consistently produced more than half the global production every year from 1992 to 2008. Tilapia introduced to Japan in 1962 was distributed from Hokkaido to Kyushu and Okinawa. Nile tilapia from Cote D'Ivoire was introduced to Brazil in 1971 and from Brazil it was sent to the USA in 1974. Due to certain characteristics, they are easily bred which makes them an excellent choice for aquaculture. These include, ease of reproduction, adaptability to intensive culture, acceptability of low input sustainable feeds, resistance to impaired water quality, resistance to diseases, fast growth, efficient use of natural aquatic food, tendency to consume a variety of supplementary feeds, herbivorous nature and widespread consumer acceptance (Balarin *et al.*, 1979).

From the 1980s, tilapia was introduced as aquaculture species that are often farmed in cages in open bodies of water. The fish can escape if the cage becomes damaged due to environmental forces, such as hurricanes, storms, or human actions. Throughout the world, documented cases of tilapia introductions are frequently due to both release and escape (ISSG, 2006). Blue tilapia (*Oreochrmomis* aureus) were introduced to Gulf states for weed control, in other cases it was for weed and insect control. They also have been released from aquariums and fish farms (GSMFC, 2003). Tilapia that have escaped from aquaculture facilities may interbreed and form hybrids (Costa-Pierce, 2003). Some species such as the Mozambique tilapia (Oreochromis mossambicus) have outcompeted native fish species and preyed on native larval fish in areas where it was introduced. Some species of tilapia can displace other species an example is, in Singapore, the problem with tilapia as an invasive species may be on its way of solving itself. During World War II, Oreochromis mossambicus was introduced to Singapore by the Japanese (this species has been named among the world's 100 most invasive species (Lowe et al., 2000)) soon, it became a common fish in both fresh and brackish water, as well as in the sea off the northern coast. Since the late 1980s, these feral population have been declining dramatically and this is believed to be caused by the introduction of other tilapias, probably the hybrid between Oreochromis mossambicus and Oreochromis niloticus and possibly also between O. mossambicus and O. urolepis hornorum or O. aureus. When Oreochromis mossambicus mates with certain species and hybrids, the resulting batches tend to have a much skewed sex ratio where almost all fishes are male. It is also common for hybrids to produce a lower number of fry per spawning. In addition to this, it is not unreasonable to assume that offspring containing genetic material from Oreochromis niloticus risk losing the high salinity tolerance of *Oreochromis mossambicus* and instead get the low salinity tolerance of Oreochromis niloticus.

The characteristics that make it suitable for aquaculture also enable it to wreak damage in places where their growth is not monitored especially where they are introduced as a form of pest control. They have become invasive species in many areas notably Australia, where their culture is strongly prohibited. In other places, they have managed to render other species within the same trophic level extinct or reduced their populations drastically due to completion and their ability to survive on many kinds of feed.

Historically, identification of tilapias has been based mainly on morphological and behavioural characteristics. In recent times, molecular markers are employed to give more conclusive results and to identify subpopulations within a species. The most commonly used markers are allozymes, mitochondrial DNA (mtDNA), Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPD)s, Amplified Fragment Length Polymorphisms (AFLP)s, microsatellites (SSR), Single Nucleotide Polymorphisms (SNPs), and Expressed Sequence Tags (ESTs).

In this study, mtDNA control region was used to identify and determine nucleotide and haplotype diversities within populations and between populations. It has been reported to better differentiate subpopulations due to the high variation observed.

Samples were collected from six locations in Ghana, the Avu Lagoon in the Volta region, Weija Lake, Densu Estuary and Kpeshie Lagoon in the Greater Accra region, the Fosu Lagoon in the Central region and the Barekese dam in the Ashanti region. Genetic material was extracted by the standard phenol chloroform method. Polymerase chain reaction (PCR) was done with the use of primers L19 and 98H. This was followed by sequencing. Sequencing data analysis resulted in the

identification of six species; *Oreochromis niloticus, Sarotherodon melanotheron, S. galilaeus, Tilapia zillii, T. rendalli* and *Hemichromis elongates* (Sowah *et al.*, 2016a).

Sequence data from Ghana was compared to sequence data from Japan and Thailand to trace the roots of some unknown species that were observed in Kochi, Japan. This showed no similarities, therefore, more locations need to be investigated to get a definite identification for Kochi's population.

Many tilapia species have been introduced to Japan over the years. According to the Food and Agricultural Organization (FAO) of the United Nations (UN), the introduction of tilapia to Japan began in the 1950's with the initial introduction in 1954. The first species introduced to Japanese freshwaters was the Mozambique tilapia (*Oreochromis mossambicus*) from Thailand. In all, nine species were introduced from various locations around the world, but two of those species *Tilapia zillii* and *Oreochromis niloticus* directly introduced from Africa (Egypt) in 1962 (De Silva et al., 2004). However, they were not monitored over the years so there is little or no information on their genetic structure and the impact of their introduction to the local population of freshwater fish. It is uncertain whether the tilapia in Japan were intentionally released or accidentally escaped from culture farms. Their prolific reproduction led to overpopulation which usually resulted in competition with co-occurring species (Noto Mihardio, 1986). Tilapias are considered invasive species in Australia where their sale or breeding, even on a subsistence basis is considered a crime (http://www.dpi.nsw.gov.au/fishing/pests-diseases/freshwater-pests/species/tilapia.).

On this premise, samples were collected from Nagoya, Yamaguchi, Kochi, Oita and Kagoshima (2 locations) and these were transported to the laboratory for further analysis. Extraction of DNA was done with the phenol chloroform method. This was followed by PCR with the ORMT-F and ORMT-R primers (Wu et al., 2012). Sequencing was done for these samples.

Samples collected were identified as; *O. niloticus*, *O. mossambicus* and *T. zillii*. Oita was recorded to have only *O. mossambicus*, while *T. zillii* was recorded in one location in Kagoshima (Kagoshima-T). The other locations had both *O. mossambicus* and *O. niloticus* (Sowah *et al.*, 2016b)

However, our study was unable to distinguish hybrids from pure strains, as mtDNA is maternally inherited. Therefore, the use of other molecular markers such as microsatellite DNA is required in near future works to determine the level of hybridization.

