論文題　**Phylogenetic and phylogeographic diversity of tilapia, and their gut-bacterial microbiota**（ティラピア類の系統学的および生物系統地理学的多様性とその腸内細菌叢）

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Chapter 1: Background information

The introduction and distribution of tilapia to the Asian-Pacific region begun the wider distribution events for the well-adapted reproductive populations of tilapia across the region. The introduction of tilapia to Asia was celebrated as the solution to the short supply of animal protein in the region. The year 1954 marked the initial introduction of the first tilapia species to Japan from Thailand, which subsequently grew up to a total of nine species, from different parts of the world, by the year 1984. However, since tilapia was introduced to Japan, very little or nothing is known about the existing tilapia fauna in the freshwater systems of Japan. This study was conducted to establish the degree of phylogenetic and phylogeographic diversity of tilapia species found in Japan, and also to understand the quantitative diversity of the gut bacterial microflora.

Chapter 2: Population genetics and taxonomic signatures of wild tilapia in japan, based on mitochondrial DNA control region analysis.

Non-native tilapia species were repeatedly introduced to Asian and South American countries because of their adaptiveness to new environments and new environmental conditions. Such founder populations rapidly adapted by evolving outstanding ecological and physiological features, making them highly successful invaders throughout the tropical and subtropical

regions. In Japan, nine tilapia species were introduced. In recent years, several established populations were introgressed by the accidental release of aquaculture strains of *Oreochromis niloticus*, causing reductions in population sizes, genetic diversity, and genetic perturbation upon introgressive hybridization. This study aimed to assess the levels of genetic integrity, genetic population structure, and biogeographic relationships of wild tilapia species found in Japan. Phylogenetic and phylogeographic analysis based on mtDNA control region sequences yielded 52 distinct haplotypes, indicating significant differences in genetic variability among the analyzed populations. Analysis of molecular variance (AMOVA) showed high levels of variation within populations, except for tilapia species representing reproductively incompatible taxonomic units to *O. niloticus* aquaculture stains. Overall, a significant genetic structure emerged among the 11 populations analyzed. Our results indicated that the accidental release of *O. niloticus* via aquaculture contributed significantly to the current genetic differentiation among populations within a few generations, showing clear signals of mtDNA introgression across species boundaries.

Chapter 3: Morphological disparity between the largest genetically monophyletic taxon of tilapia in the Kyushu and Okinawa ecogeographical regions of Japan.

Post-introduction of non-native tilapia into Japan about 6.5 decades ago, saw the distribution and adaptation leading to the subsequent increase in the freshwater systems across the country. In this study, we used two multivariate statistical methods to investigate the morphological disparities relative to phenotypic plasticity and/or adaptive evolution for the most prominent genetically monophyletic taxon of tilapia species retrieved from five populations in Kyushu and Okinawa ecogeographical regions. Even though conductivity and salinity levels greatly vary for Kyushu than Okinawa populations, the PCA and LDA largely indicated significant morphological differentiation between all Okinawa than Kyushu

populations, whereas showing a higher degree of convergence between the Kyushu and Ishigakijima populations contrary to the other populations of Miyakojima and Naha. Additionally, allometric growth varies significantly in Okinawa than Kyushu, attesting morphological variations in Okinawa to adaptive or phenotypic plasticity than Kyushu. The statistical relationship between the ANOSIM and PERMANOVA also indicated a similar degree of differentiation between all populations. However, these observed variations are either due to genetic and/or environmental events influencing the morphology of tilapia populations in the wild, we also strongly believed the equal opposite whereby morphological variations are imported with different aquaculture strains of tilapia.

Chapter 4: Characterization of environmental and GIT bacteria microbiome of tilapia.

The study of GI microbiome is the ultimate scientific viewpoint for constructive development, prediction, and manipulation schemes aimed at successful prescription and adoption of prominent core microbiome as prebiotics/probiotics candidates. This investigation focused on the impact of niche bacterial microbiome on GIT colonization of resident teleost fauna by characterizing the environmental and gut microbiota of resident tilapia fauna. The microbiome of the environment and gut of tilapia was profiled using a culture-dependent method and identified by 16S rRNA sequencing. The 16S rRNA sequences generated OTUs dominated by *Bacillus* and *Pseudomonas* respectively, in terms of relative abundance. Comparatively, *Proteobacteria* dominated other representative phyla. Nevertheless, pathogenic bacteria such as *Vibrio*, *Enterobacter*, *Plesiomonas*, and *Klebsiella* were only recorded from the gut, but *Aeromonas* and *Pseudomonas* were found common between the environment and gut microbiota. The coexistence and/or cohabitation of opportunistic and/or pathogenic bacteria amidst fermentative/biodegradative/bioconversion, bioremediation, and

antimicrobial synthesizing bacteria (example, *Rhodococcus* and *Acinetobacter*), suggested a complex relationship that could competitively, sequentially, or symbiotically reduce disease incidences. This study indicated that the gut microbiome contained selected bacterial flora some of which could be adopted as functional prebiotic/probiotics in tilapia aquaculture.

Chapter 5: General Discussion

Phylogenetic and phylogeographic analysis based on mtDNA control region sequences indicated that the introduction of *O. niloticus* via aquaculture contributed significantly to the current genetic differentiation among populations within a few generations, showing clear signals of mtDNA introgression across species boundaries. Successively, the two multivariate statistical methods used to investigate morphological disparities relative to phenotypic plasticity and/or adaptive evolution for the most prominent genetically monophyletic taxon of tilapia species retrieved from five populations in Kyushu and Okinawa ecogeographical regions, largely indicated significant morphological differentiation between all Okinawa than Kyushu populations, whereas the degree of convergence between the Kyushu and Ishigakijima populations contrary to Miyakojima and Naha suggested that allometric growth varies significantly in Okinawa than Kyushu. Additionally, the statistical relationship between the ANOSIM and PERMANOVA also indicated a similar degree of differentiation between all populations. Finally, the total cultivable bacteria associated with the gut microbiota yielded 30 predominant pure culture isolates differentiated by colony morphologies, and taxonomically composed of *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Overall, our result indicated that the tilapia gut microbiome seemed respectively dominated by *Proteobacteria* and *Firmicutes* totaling about 90 % of the gut bacteria microflora, whereas *Actinobacteria* and *Bacteroidetes* represented a lesser

percentage.   The findings from our study suggested that the gut of tilapia and other fishes such as carp, salmon, zebrafish, and rainbow trout are selective of the bacteria phyla of *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* with similar marginal abundance for *Proteobacteria* and *Firmicutes* as the predominant phyla respective of rearing or growth environmental conditions.