GENETIC DIVERSITY COMPARISON OF NATIVE AND NON NATIVE FRESHWATER FISH FROM MAMBERAMO RIVER

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Abstract

The Genetic diversity and architecture of native and non native of dominant freshwater fish were examined employing standard mitochondrial gen COI sequences from 500bp. The research was conducted in middle-upper Mamberamo River, Papua during the sampling campaign in 2016, there are four sampling selected purposively. Fish were caught using standard seine net comprises various sizes from 1 inch to 3 inch diameter and left overnight. DNA extracted using DNA extraction kits, PCR amplification and sequenced in Macrogen Company, South Korea. The result show a total of 669 bp mtDNA COI sequences of *Barbonymus gonionotus* were amplified successfully from 8 individuals and PCR produced a 668 bp mtDNA COI sequences of *Chilatherina_fasciata* from 26 individuals of four sampling sites resulting in identification of 2 common haplotype defined by one variable site. The native species of *Chilatherina_fasciata* exhibits the level of variability of h = 0.2123, π = 0.000318. Compared to native species, the introduced species (*Barbonymus gonionotus)* resulted in less variable sites and haplotypes and less informative character of the COI gene. To meet the challenge of reducing the rate of aquatic invasions, management strategies will be needed to control propagule supply, before and after establishment. Molecular studies might also be important in developing strategies for the post-invasion control of introductions in the context of fisheries management.

Key words: fish, Mamberamo, native and non native

Introduction

Effective conservation of regional biotas requires accurate information on the distribution, endemicity, local richness, and taxonomic composition of species assemblages across multiple geographic scales. This is especially true in the Melanesian region, which contains ten percent of the world's biota. Although the overall condition of freshwater ecosystems in the New Guinea region is excellent, there are still obvious threats to the biota, which tend to manifest themselves on local rather than regional scales, such as invasive species (Polhemus *et al*. 2004).

Allen (1991) reported the presence of 22 species representing 19 genera, 11 families and all six continents. Since then at least six more introductions have been noted, and more can be expected, especially on the Indonesian side of the island. Most of the introductions have had a negative impact, either by competing for space and limited food resources, or by feeding on natives species, including their eggs and fry. Species including carp appears to be undergoing rapid population increases and therefore pose a serious threat to native fishes (Polhemus *et al.* 2004). Allen *et al.* (2002) noted that the Mamberamo River in Papua Province had the highest percentage (17.1) of introduced fishes of any major river system in New Guinea. The appearance of species such as three species of cyprinids is particularly alarming, given the relative isolation of this system and lack of major population centers. Across New Guinea as a whole, particularly the Mamberamo and Sepik-Ramu basins, are badly contaminated (Polhemus *et al.* 2004).

Like many invasive fish species, carp modify their environment to conditions for which they are better suited to survive in than native fish species. Worldwide, carp are regarded as a pest fish because of their tendency to uproot and destroy aquatic vegetation that results in increased turbidity and deterioration in habitats used by native species (Fuller *et al.* 1999). Carp have been found to not only impact native fish species directly through egg predation, but also

negatively impact waterfowl by increasing turbidity causing a reduction in food availability needed by both birds and native fish (Fuller *et al.* 1999).

Small founding populations of introduced species are expected to have genetic variation that is lower than that of native populations as a result of bottlenecks (Allendorf & Lundquist, 2003). Here, we are comparing the genetic diversity of native and introduced populations in aquatic ecosystems. These systems are particularly interesting for several reasons according to Roman & Darling (2007). The rise of transoceanic shipping and globalization, and the subsequent increase in aquatic invasion rates (Cohen & Carlton, 1998), has enhanced motivation to understand invasion success, or establishment. The number of potential case studies has risen with increased research effort. Many of these studies reveal that multiple introductions play an important role in the expansion of invasive populations.

Material and methods

Ethics statement
A permit to collect fish was given to A. Wibowo from the Research Institute of Inland Fisheries, Ministry of Marine and Fisheries Affairs, Republic of Indonesia. No experimentation was conducted on live specimens during this study, because the permit granted does not extend to experimentation on animals and there are no experimental facilities at the Institute.

Area study, sample collection and preservation
Sampling collections were done at four sites (Kalimerah, 03°44′37.8″S, 140°18′55.5″E; Kerumi, 03°44′38.9″S, 140°17′88.2″E; Telaga, 03°43′98.4″S, 140°18′19.2″E; and Sungai Putus, 03°42′86.1″S, 140°16′79.9″E) (Figure. 1) and four times between March and October 2016 along the Mamberamo River, West New Guinea (Papua). Sampling and collection of representative samples of the adult fish community and fish larvae were performed using five sets of experimental gill nets (stretch mesh size 12.7, 25.4, 38.09, 50.8, 76.19 and 101.6 mm). All nets were 1.5 m deep and 15 m long comprising five randomly placed sections of different mesh size. Nets were placed in the water in the evening (17.00 hours) and were collected in the morning (07.00 hours).

All adult fish caught were identified to the species level or, alternatively, to the genus level when systematic knowledge was inadequate for reliable identification of the species following (Allen, 1991). An approximate 1-cm^2 piece of fin clip tissue was taken from every decease individual using a scalpel and tissue samples were stored in 1.5 mL absolute ethanol. Live adult fish were killed by an instantly knock on the head at the collection site, this *method* is much *faster*, less expensive, and does a better job of minimizing animal suffering.

DNA extraction, amplification and sequencing
Total genomic DNA was extracted from muscle tissue or whole larvae using a Geneaid extraction procedure as described by company protocol. A partial fragment (530 bp) of the mitochondrial *COI* was amplified using modified universal primers Fish-COI-F and COI-Fish-R, as described by Ivanova *et al.* (2007). The primer sequences were as follows: Fish-COI-F, 5′-TAA TAC GAC TCA CTA TAG GGT TCT CCA CCA ACC ACA ARG AYA TYGG-3′; COI-Fish-R, 5′-ATT AAC CCT CAC TAA AGG GCA CCT CAG GGT GTC CGA ARA AYC ARAA-3′.

Amplification of the *COI* fragment was performed in a 50-µL reaction volume consisting of 16 µL of ultrapure water, 2 µL of each primer (1 mM), 25 µL of PCR ready mixture solution (KAPPA). The polymerase chain reaction (PCR) cycling parameters included an initial DNA polymerase activation step of 15 min at 95°C, followed by 35 cycles of 30 s at 94°C, 90 s at 55°C and 30 s at 72°C, and ending with a final extension of 5 min at 72°C. The PCR products were visualised on a 1% agarose gel and purified using the PCR purification kit (Thermo Scientific). A sequencing reaction was performed by the EZ-Seq service (Macrogen) using the reverse primer (COI-Fish-R).

Data analysis

Sequence chromatograms were viewed and edited manually using BIOEDIT Applied Biosystem. Once edited, multiple alignments were performed using Clustal W (Thompson *et al.,* 1997). Haplotype diversity (*ĥ)* (Nei & Tajima, 1981) and nucleotide diversity (∏) (Nei, 1978) were calculated using MEGA (Version 4.0, Tamura *et al.* 2007). An analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) was performed to partition the total phenotypic variance into intra- and inter-population variance using ARLEQUIN 3.01 (Excoffier *et al.* 2005). The neighbor-joining (NJ) method was used to reconstruct the phylogenetic relationships among haplotypes with MEGA (Version 4.0, Tamura *at al.* 2007).

Figure 1. Sampling locations in River Mamberamo, Indonesia. Sampling sites of Kalimerah (1), Sungai Putus (2), Sungai Kromi (3) and Talaga (4)

Results and Discussion

As representative an introduced species, a total of 669 bp mtDNA COI sequences of *Barbonymus gonionotus* were amplified successfully from 8 individuals of four sampling sites resulting in identification of 1 common haplotype defined by non variable sites, Figure. 2. Thus the genetic variation of the introduced species was *h* = 0, π = 0. The mean total nucleotide composition for this species was T = 26.6%, C = 16.7%, A = 28.1% and G = 28.6%. The neighbour-joining tree (Figure. 3) was constructed with the complete data set of 1 haplotypes including one reference sequence reiterated from GenBank. One main clade was identified for *Barbonymus gonionotus.*

AAGAATCAGAACAGGTGTTGGTAGAGAATTGGGTCTCCTCCGCCTGCTGGGTCAAAGAAT
GTAGTATTAAGGTTTCGATCTGTTAGTAGTATTGTAATTCCGGCGGCTAAAACAGGTAGTGA
TAGGAGGAGTAGTACGGCGGTTACGAGCACAGATCAAACGAATAGTGGTGTTTGATATTG
GGAGATGGCTGGGGGTTTTATGTTAATAGTTGTGGTGATAAAATTAATTGCACCTAGAATTG
AGGATACACCTGCTAAGTGGAGTGAGAAAATTGTTAGGTCTACTGATGCTCCTGCGTGAGC
TAGGTTTCCTGCAAGAGGGGGATATACTGTTCATCCTGTCCCAGCTCCGGCTTCAACACCA
GAGGAGGCTAATAATAGCAGGAATGATGGGGGTAGTAATCAGAAGCTTATGTTGTTTATTC
GTGGGAATGCTATGTCGGGGGCTCCAATTATTAGAGGCACAAGTCAGTTTCCGAATCCTCC

GATGAGGATGGGCATTACTATAAAGAAGATTATTACGAAGGCATGGGCAGTAACGATAACA
TTATAAATTTGATCATCGCCTAGAAGTGACCCGGGTTGGCTAAGCTCAGCTCGAATAAGGA
GGCTTAGGGCGGTTCCCACTATTCCGGCTCAGGCACCAAATACAAGATAGAGGGT

Figure. 2. mtDNA COI sequences of *Barbonymus gonionotus*

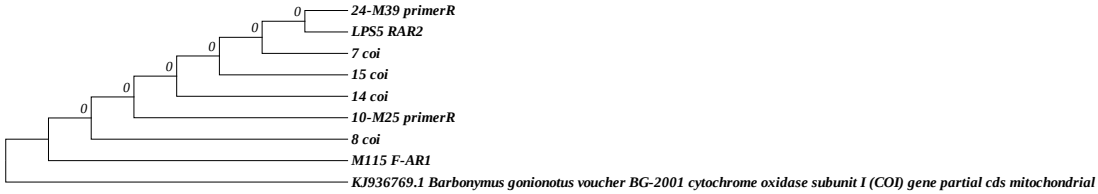

Figure 3. Neighbor-joining tree of COI haplotypes of *Barbonymus gonionotus*

Chilatherina_fasciata is a native species within the system, PCR produced a 668 bp mtDNA COI sequences of *Chilatherina_fasciata* from 26 individuals of four sampling sites resulting in identification of 2 common haplotype defined by one variable site, Figure. 4. Based on the analysis, the mean total nucleotide composition was T = 24%, C = 18.3%, A = 29.0% and G = 28.7%. In the line with *Barbonymus gonionotus,* the percentage of A + T base composition for *Chilatherina_fasciata* (55.3%) was much higher than C + G, which coincides with vertebrate protein-coding genes (Miller *et al*. 2005; Pin *et al.* 2007). The native species of *Chilatherina_fasciata* exhibits the level of variability of *h* = 0.2123, π = 0.000318. The constructed neighbour-joining tree (Figure. 5) from set of samples of *Chilatherina_fasciata comprised* two main clades.

AAGAATCAGAAAAGATGTTGGTAAAGAATTGGATCGCCCCCTCCTGCCGGGTCGAAGAAG
GTGGTATTTAGGTTCCGGTCTGTTAAAAGCATTGTGATCCCGGCAGCTAGCACGGGAAGA
GAGAGAAGAAGAAGAACTGCAGTAACTAGGACTGCTCAGACAAACAGAGGTGTTTGGTATT
GTGAAATTGCTGGGGGTTTTATATTAATAATTGTTGTGATAAAATTAATAGCACCTAGGATT
GAGGAAATACCTGCAAGGTG**c**AGGGAGAAAATGGTGAGGTCAACGGATGCGCCTGCATGG
GCTAAGTTTCCGGCCAGAGGGGGGTATACTGTCCAACCTGTCCCAGCTCCAGCTTCTACC
CCGGAGGATGCAAGTAGGAGGAGAAACGAAGGGGGGAGTAGTCAGAAACTTATGTTATTC
ATGCGAGGAAATGCTATGTCAGGGGCCCCGATTATCAAAGGGACTAGTCAATTCCCGAAG
CCTCCGATCATGATGGGTATTACTATAAAGAAAATTATTACGAAGGCATGGGCTGTTACGAT
TACATTATAGATTTGGTCGTCCCCTAGGAGAGAGCCCGGTTGGCTTAATTCTGCTCGAATT
AGAAGGCTTAGGGCGGTTCCGACTATTCCGGCTCAAGCACCAAATACTAGATAGAGGGTG
CC

Figure 4. mtDNA COI sequences of *Chilatherina_fasciata*

The present study sequencing an approximately 668-669 bp fragment of COI gene region revealed 1 haplotype for *Barbonymus gonionotus* (non variable site) and 2 haplotypes based on polymorphisms at 1 nucleotide sites for native species with considerably gene and haplotype diversity. Compared to native species, the introduced species (*Barbonymus gonionotus)* resulted in less variable sites and haplotypes and less informative character of the COI gene. Even though the genetic diversity of introduce species usually low, however, the introduced species has specific mechanism of adaptation, such as *T. thymallus* and *P. reticulata* possess sufficient additive genetic variance for local adaptation despite low diversity at neutral markers (Roman & Darling, 2007).

Diversity at presumably neutral markers (e.g. mtDNA) might correlate with diversity at quantitative trait loci (Merila¨ & Crnokrak, 2001). Furthermore Populations can successfully invade with no genetic variation, this absence of diversity parallels a lack of differentiation at neutral loci, suggesting that phenotypic plasticity, not adaptation, has allowed the species to colonize diverse habitats (Roman & Darling, 2007).

To meet the challenge of reducing the rate of aquatic invasions, management strategies will be needed to control propagule supply, before and after establishment. Molecular studies might also be important in developing strategies for the post-invasion control of introductions. An understanding of population genetics will be critical in assessing proposed control efforts using genetic engineering or biological control agents such as parasites and pathogens (Roman & Darling, 2007).

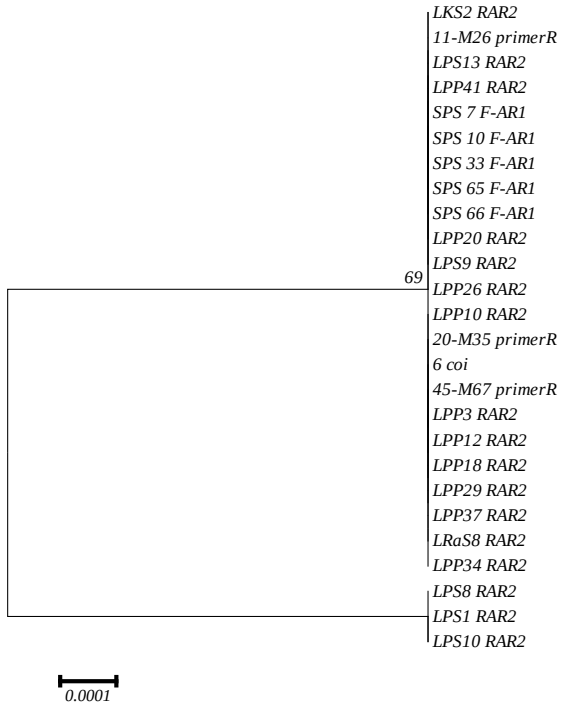

Figure 5. Neighbor-joining tree of COI haplotypes of *Chilatherina_fasciata*

Conclusions

A total of 669 bp mtDNA COI sequences of *Barbonymus gonionotus* were amplified successfully from 8 individuals and PCR produced a 668 bp mtDNA COI sequences of *Chilatherina_fasciata* from 26 individuals of four sampling sites resulting in identification of 2 common haplotype defined by one variable site. The native species of *Chilatherina_fasciata* exhibits the level of variability of h = 0.2123, π = 0.000318. Compared to native species, the introduced species (*Barbonymus gonionotus)* resulted in less variable sites and haplotypes and less informative character of the COI gene. To meet the challenge of reducing the rate of aquatic invasions, management strategies will be needed to control propagule supply, before and after establishment. Molecular studies might also be important in developing strategies for the post-invasion control of introductions in the context of fisheries management.

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