DNA-barcoding of *Oreochromis* species from hatchery, introduced and wild stocks: Evidence for misidentification, hybridisation and cryptic species

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**Abstract**

The three most widely distributed *Oreochromis* (tilapia) species, *O. niloticus*, *O. mossambicus* and *O. aureus*, were introduced globally from the 19th century for aquaculture. Most knowledge gathered on these fishes comes from naturalised Asian and American populations of unknown wild source. Little is known of genetic diversity in Africa, their native range. Another challenge is that these species hybridise readily resulting in introgression, particularly among introduced populations. In order to better characterize diversity in *Oreochromis*, we generated mtDNA COI barcode sequences (N=91) from African wild populations and hatchery stocks, which we compare with existing sequences (N=636; 532 bp) from the BOLD and NCBI databases. Results: Phylogenetic trees showed robust bootstrap support for 34 divergent lineages, largely corresponding to species and river systems. However, the three widely introduced species *O. niloticus*, *O. mossambicus* and *O. aureus* occur in multiple lineages across the tree. Within their native range, different river systems yielded divergent lineages. These analyses reveal multiple cases of mismatch between phenotypic identification and DNA barcodes, in wild, hatchery and introduced stocks, which can be explained by misidentification, introgression or taxonomic uncertainty. Significance: These results highlight the limits of a single gene approach to species delimitation in *Oreochromis*. We need a genome-wide approach to understand the natural genetic resource in *Oreochromis*, which has global importance to aquaculture and food security.

**Introduction**

- *Oreochromis*: Cichlidae. > 30 species, endemic to Africa & Middle East, 8 in southern Africa (Zengeya et al. 2015)
- Global production in 2015 was 5.3 Mt, US$ 8.8 × 10^9, (D’Amato et al. 2007). Potential loss of drought, cold and salt tolerance
- Exceptions are *D’Amato ME, Esterhuyse MM, van der Waal BCW, Brink D, Volckaert FAM (2007)*
- *O. mossambicus*. "with additional hybrid strains.
- Few data on genetic diversity within the native range – exceptions are (D’Amato et al. 2007, Firmat et al. 2013).

**Aim:**
Analysis of mtDNA divergence among native populations; with a particular focus on *O. mossambicus*.

**Research Questions:**
1. What is the natural genetic structure of wild populations?
   - Are there multiple lineages within species?
   - Do these represent cryptic species?
2. How do these relate to farmed and introduced stocks?
   - Possible hybridization of aquaculture stocks?

**Methods**

- **Sampling locations:**
  - *Oreochromis moscovus* & *O. niloticus*.
- **Approach:**
  - COI from BOLD & NCBI (332 & 289 seqs)
  - Sequencing (91 COI seq x 683bp)
  - Model selection, phylogenetic analyses

**Results**

- > 90% Support
- ? = Cryptic lineages

- *O. niloticus* (Nile, Tanzania)
- *O. aureus* (Limpopo, Kafue, Kafue, Congo)
- *O. mossambicus* (Zambesi, LMK)

**Conclusion**

- **Taxonomic challenges:**
  - Cryptic species, possible misidentifications & mtDNA introgression
- Limitations of the barcoding data base
- Multilocus genotype approach is required to resolve hybrid origins

**References**