DNA BARCODING OF ORNAMENTAL FISHES OF NORTHEAST INDIA

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Ornamental fishes

- Ornamental fishes usually mean attractive colorful fishes of various characteristics, which are kept as pets in confined space of an aquarium or a garden pool for fun and fancy.

- China is the pioneer in adopting aquarium fish as a hobby.

- The hobby of keeping fish in aquarium has become the second most popular hobby after photography in the developed countries such as USA.

- The World’s first public aquarium was established in England in 1853

Common ornamental fishes

- Angel fish
- Anthias
- Goat fish
- Dragonets
- Goby
- Hawk fish

Few indigenous ornamental fishes from India

- Puntius filamentosus
- Puntius denisonii
- Nemacheilus triangularis
- Horabagrus brachysoma
- Macrograthus aculeatus
- Channa barca
Ornamental fish trade: Economic potential, rise in the sector & % share of importing & exporting countries

- Annual international exports in ornamental fish are around US$ 250 million, with imports valued at US$300 million (FAO, 2006).

- The total value of wholesale ornamental trade is estimated at close to US$ 1 billion, and retail trade about US$ 6 billion.

- The entire industry, including accessories and fish feed, is estimated to be worth more than US $14 Billion.

- Since 1985 the value of international trade in exports of ornamentals has increased at an average growth rate of approximately 14% per year.

- The FAO estimates that nearly 60% of the international trade in ornamental fish originates from developing countries mainly from Asia.

- In Asia the top exporting country is Singapore followed by Hong Kong, Malaysia, Thailand, Philippines, Sri Lanka, Taiwan, Indonesia and India.

- The largest importer of ornamental fish is USA followed by Europe and Japan. China and South Africa are the emerging markets of ornamental fish trade.
Ornamental fish trade: Indian perspective

- The Indian ornamental fish sector is a small but vibrant segment with 20% rise per year and an annual earning of US$ 1.2 million.
- India is endowed with 500 species of freshwater and marine ornamental fishes.
- The ornamental fish hub mainly includes the northeastern states (80%) and southern states (20%) in India.
- About 80% of ornamental fishes from India to International market are exported via Kolkata Airport, of which the lion’s share (more than 80%) is contributed from North Eastern Region.
- Already 267 fish species belonging to 136 genera has been identified in Northeast India, of which about 250 species have been reported to be of ornamental value and in case of more than 50 species, overseas demand has been established.

![India's Ornamental fish Exports 1997 to 2009](chart.png)
Ornamental Fishery resource faces a range of challenges. WHY?

1) The growth in the sector paved great pressure on the wild stocks.  
   Effect: Overexploitation and disappearance of wild stocks from natural habitat

2) Trade regulation.  
   Effect: Does not have scope to differentiate the captive breeds from wild stocks

3) Nomenclature of traded fishes  
   Effect: Certain cryptic group of fishes shares the same common name

4) Fishes transport at different life stages.  
   Effect: Lagging of traditional taxonomy to mark the species

5) The need to ensure that benefits are equitably shared.  
   Effect: country of origin of a species is not getting the revenue and share.

6) Poaching of ornamental fishes.  
   Effect: The poachers in this sector takes the advantage of lagging of morpho-taxonomy and when they speculate any chance of getting caught they tempers or destroy the specimen as morpho-taxonomy fails to tag those.

7) Introduction of exotic vertebrates.  
   Effect: Destroy or imbalance of ecology of the introduced country

Big Solution
DNA barcode: A molecular marker for ornamental fish species identification

- A method of species identification based on DNA sequencing a single gene.

- The gene chosen is the mitochondrial DNA gene Cytochrome oxidase I (COI).

- The hypothesis is that, for that gene, every species will have a unique sequence or a unique assemblage of closely related sequences.

- This sequence is termed a ‘barcode’. For example:

  Species A: CCTAAGCTTACGTTCCTCC
  Species B: CCTAGGCTTACGTTCACC
Our initiative: DNA barcoding of ornamental fishes of Northeast India

- The northeast India (Latitude- 21°34’ N to 29°50’ N, Longitude- 87°32’ E to 97°52’ E, Area- ca 262060 sq km) is one of the 12 mega biodiversity rich zones of the world and forms a distinctive part of the Indo-Burma Hotspot that ranks 8th among the 34 biodiversity.

- Harbors about 267 species of fishes.

**Ornamental relevance:**
- Assam 187
- Arunachal Pradesh 165
- Meghalaya 146
- Manipur 139

<table>
<thead>
<tr>
<th>Fish species</th>
<th>Price in Rs.</th>
<th>Fish species</th>
<th>Price in Rs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambylopharyngodon mola</td>
<td>13</td>
<td>Osteobrama ticto</td>
<td>17.5</td>
</tr>
<tr>
<td>Ailia coila</td>
<td>13</td>
<td>Puntius chola</td>
<td>17.5</td>
</tr>
<tr>
<td>Badis badis</td>
<td>32</td>
<td>Rasbora daniconius</td>
<td>17.5</td>
</tr>
<tr>
<td>Botia dario</td>
<td>32</td>
<td>Balitora brucei</td>
<td>32</td>
</tr>
<tr>
<td>Channa striatus</td>
<td>32</td>
<td>Ompok bimaculates</td>
<td>32</td>
</tr>
<tr>
<td>C. orientalis</td>
<td>17.5</td>
<td>Nangra viridescens</td>
<td>32</td>
</tr>
<tr>
<td>Chanda nama</td>
<td>17.5</td>
<td>Sisor rhabhophorus</td>
<td>225</td>
</tr>
<tr>
<td>Mystus tengara</td>
<td>11.5</td>
<td>Chaca chaca</td>
<td>57.5</td>
</tr>
<tr>
<td>M. vittatus</td>
<td>11.5</td>
<td>Channa marulius</td>
<td>32</td>
</tr>
<tr>
<td>M. cavasius</td>
<td>17.5</td>
<td>Neolissichilus hexagonolepis</td>
<td>17.5</td>
</tr>
<tr>
<td>Ompok pabo</td>
<td>32</td>
<td>Anabas testudinues</td>
<td>17.5</td>
</tr>
<tr>
<td>Salmostoma bacila</td>
<td>13</td>
<td>Amblyceps mangois</td>
<td>17.5</td>
</tr>
<tr>
<td>Lepidocephalus guntea</td>
<td>32</td>
<td>Eutropiichthys vacha</td>
<td>17.5</td>
</tr>
<tr>
<td>Labeo calbasu</td>
<td>17.5</td>
<td>Exostoma labiatum</td>
<td>32</td>
</tr>
<tr>
<td>Gagata gagata</td>
<td>28</td>
<td>Glyphothorax telchitta</td>
<td>32</td>
</tr>
<tr>
<td>Gagata cenia</td>
<td>28</td>
<td>Tetrodon cutcutia</td>
<td>32</td>
</tr>
<tr>
<td>Xenotodon cancila</td>
<td>32</td>
<td>Barilius barila</td>
<td>17.5</td>
</tr>
</tbody>
</table>

Table-Species wise price of some freshwater ornamental fishes of northeast India exported to global market.
Sample collection

- The collection site mainly includes so far southern Assam and parts of Mizoram, Manipur, Tripura and Arunachal Pradesh.

- Manipur records the highest number of endemic fishes.

- A total of 175 species are collected so far of which 38 species are barcoded for their partial COI gene.
Valued ornamental fishes of Northeast India

- Acanthocobitis botia
- Aspidoparia morar
- Devario aequipinnatus
- Barilius bendelisis
- Esomus danricus
- Tetraodon cutcutia
- Devario assamensis
- Lepidocephalichthys guntea
- Barilius bendelisis
- Glyptothorax spp
- Xenetodon cancila
- Devario assamensis
- Amblypharyngodon mola
- Barilius dogarsinghi
- Monopterus cuchia
- Botia Dario
- Lepidocephalichthys annandalei
- Colisa fasciata
- Chela cachius
- Schistura vinciguerrae
- Nandus nandus
- Sisor spp
- Botia rostrata
- Barilius barila
- Pseudambassis baculis
- Puntius titco
Analysis

NJ-tree revealing species differentiation efficacy of DNA barcode sequences

Table. Summary of genetic divergence (K2P) for increasing taxonomic levels.

<table>
<thead>
<tr>
<th>Average K2P distance</th>
<th>Min</th>
<th>Mean</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within species</td>
<td>0.00</td>
<td>0.004</td>
<td>0.022</td>
</tr>
<tr>
<td>Between species</td>
<td>0.118</td>
<td>0.157</td>
<td>0.224</td>
</tr>
<tr>
<td>Between genus</td>
<td>0.177</td>
<td>0.193</td>
<td>0.224</td>
</tr>
</tbody>
</table>

The congener shows a 39.25 fold more K2P divergence than the conspecies which confirms they are separate species.
Design of mini-barcode

Kimura distance, \( d = \frac{-1}{2} \ln (1-2P-Q) - \frac{1}{4} \ln (1-2Q) \)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Average K2P distance between species</th>
<th>Transition (Avg. no of difference between species)</th>
<th>Transversion (Avg. no of difference between species)</th>
<th>Correlation coefficient</th>
<th>K2P vs. Transition</th>
<th>K2P vs. Transversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. vacha vs. E. murius</td>
<td>0.119 ± 0.015</td>
<td>52 ± 6.895</td>
<td>13.5 ± 3.632</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. vittatus vs. E. vacha</td>
<td>0.205 ± 0.021</td>
<td>62.4 ± 7.482</td>
<td>45.367 ± 6.487</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. vittatus vs. E. murius</td>
<td>0.22 ± 0.020</td>
<td>67.667 ± 7.753</td>
<td>46.533 ± 6.555</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. bleekeri vs. E. vacha</td>
<td>0.178 ± 0.038</td>
<td>60 ± 7.353</td>
<td>35.167 ± 5.756</td>
<td>0.682</td>
<td>0.908</td>
<td></td>
</tr>
<tr>
<td>M. bleekeri vs. E. murius</td>
<td>0.178 ± 0.019</td>
<td>53 ± 6.955</td>
<td>42.333 ± 6.276</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. bleekeri vs. M. vittatus</td>
<td>0.152 ± 0.018</td>
<td>59.6 ± 7.330</td>
<td>22.2 ± 4.625</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. cavasius vs. E. vacha</td>
<td>0.179 ± 0.019</td>
<td>61 ± 7.407</td>
<td>34.167 ± 5.678</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. cavasius vs. E. murius</td>
<td>0.187 ± 0.020</td>
<td>58 ± 7.243</td>
<td>41.333 ± 6.206</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. cavasius vs. M. vittatus</td>
<td>0.185 ± 0.020</td>
<td>72 ± 7.966</td>
<td>25.2 ± 4.915</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. cavasius vs. M. bleekeri</td>
<td>0.13 ± 0.016</td>
<td>54 ± 7.014</td>
<td>17 ± 4.065</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table: Comparison of the average K2P distance between species with the average number of Transition and Transversion changes between species of the barcode segment of \( cox1 \) gene

Figure: The distribution of the variable site (both Transition and Transversion) along the whole length barcode sequence (655 bp):
- Transversion
- Transition
<table>
<thead>
<tr>
<th>Site</th>
<th>Length (bp)</th>
<th>Conserved site%</th>
<th>Variable site%</th>
<th>Transition/Transversion between species</th>
<th>Avg. K2P distance between species</th>
<th>Avg. K2P distance within species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole barcode</td>
<td>655</td>
<td>71.77</td>
<td>28.23</td>
<td>Mystus 2.88 Eutropiichthys 3.85 Mystus and Eutropiichthys 1.48</td>
<td>0.156 0.119 0.191</td>
<td>0.007 0.0008</td>
</tr>
<tr>
<td>258 to 312</td>
<td>55</td>
<td>56.36</td>
<td>43.64</td>
<td>Mystus 2.20 Eutropiichthys 1.80 Mystus and Eutropiichthys 0.86</td>
<td>0.240 0.321 0.413</td>
<td>0.009 0.0000</td>
</tr>
<tr>
<td>324 to 366</td>
<td>43</td>
<td>65.12</td>
<td>34.88</td>
<td>Mystus 3.97 Eutropiichthys 1.00 Mystus and Eutropiichthys 1.07</td>
<td>0.297 0.155 0.259</td>
<td>0.006 0.0000</td>
</tr>
<tr>
<td>519 to 555</td>
<td>37</td>
<td>62.16</td>
<td>37.84</td>
<td>Mystus 1.24 Eutropiichthys 5.00 Mystus and Eutropiichthys 0.52</td>
<td>0.239 0.190 0.269</td>
<td>0.014 0.0000</td>
</tr>
</tbody>
</table>

Table: The properties of the whole length barcode sequence in comparison to the three sites (55 bp, 43 bp and 37 bp) in terms of Transition/Transversion and average intraspecific and interspecific K2P distance for the species of genus *Mystus* and *Eutropiichthys*.

Figure: Graphical representation of the comparison of mini-barcodes and full-length barcode.
Nucleotide site position in the mini barcode region (258 to 312)

<table>
<thead>
<tr>
<th></th>
<th>261</th>
<th>262</th>
<th>267</th>
<th>270</th>
<th>273</th>
<th>276</th>
<th>279</th>
<th>291</th>
<th>294</th>
<th>300</th>
<th>303</th>
<th>306</th>
<th>309</th>
<th>312</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. vacha</td>
<td>G</td>
<td>C</td>
<td>T</td>
<td>G</td>
<td>C</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>G</td>
<td>A</td>
<td>T</td>
<td>A</td>
<td>G</td>
<td>G</td>
</tr>
<tr>
<td>E. murius</td>
<td>T</td>
<td>C</td>
<td>A</td>
<td>A</td>
<td>T</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>G</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>M. vittatus</td>
<td>A</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>T</td>
<td>A</td>
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<td>C</td>
<td>C</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>G</td>
</tr>
<tr>
<td>M. bleekeri</td>
<td>A</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
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<td>T</td>
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<td></td>
</tr>
<tr>
<td>M. cavasius</td>
<td>A</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>A</td>
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<td>C</td>
<td>T</td>
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<td>T</td>
</tr>
</tbody>
</table>

Table: Nucleotide species specific variable sites along the 55 bp length of mini barcode
Summary

- The study brings into focus the ornamental fishes of northeast India.

- DNA barcode sequence generated as tag or label of the species traded into international market.

- Crypticism has been found among *Mystus vittatus* individuals while a possible new species of genus *Sperata* been deciphered.

- *Psudomonas sp.* and *Shawenella baltica* are found as parasites in some fishes collected from isolated waterbody of Manipur.

- Transversion mutation are found to be more effective in species differentiation and a transversion rich 55bp mini barcode and nucleotide character based barcode has been found within the whole length barcode.
A CHALLENGE FOR DEVELOPMENT OF DNA BARCODING OF LIFE IS OUR GOAL

Fish Group:
Joyraj Bhattacharjee (DBT-JRF)
Bijoya Khomdram
Mohua Chakraborty (DBT-JRF)
Bishal Dhar (DBT-JRF)
Dr. B A Laskar (DBT-Postdoc)

Turtle and Tortoise Group:
Shantanu Kundu
Kulendra Ch Das (DBT-post-doc)

Plant Group:
Pradosh Mahadani (Medicinal plants)
Mridul Mohan Das (Bamboo)
Prabal Ranjan Ghosh (Bird)
Monika Ahanthem (Endemic animals)
Miranda Ksh. Devi (Endangered species/wildlife)
THANK YOU!

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Presented by: Maloyjo Joyraj Bhattacharjee
Organization: Assam University, Silchar, India