# DNA Barcoding the Himalayan Torrent Ichthyofauna of Bhutan

**Zach Zbinden[1*], Marlis R. Douglas[1], Tyler K. Chafin[1], Karma Wangchuk[2], Chang Lu[2], Gopal Prasad Khanal[2], Pema Norbu[2], Sangay Norbu[2], Sonam Dorji[2], Singye Tshering[2], Michael E. Douglas[1]**

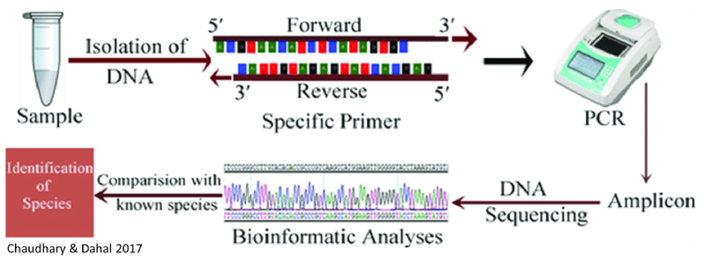
[1]Department of Biological Sciences| University of Arkansas | Fayetteville, AR | U.S.A.
[2]National Research Centre For Riverine and Lake Fisheries | Ministry of Agriculture & Forests | Royal Government of Bhutan
*Contact: zdzbinde@uark.edu

## INTRODUCTION

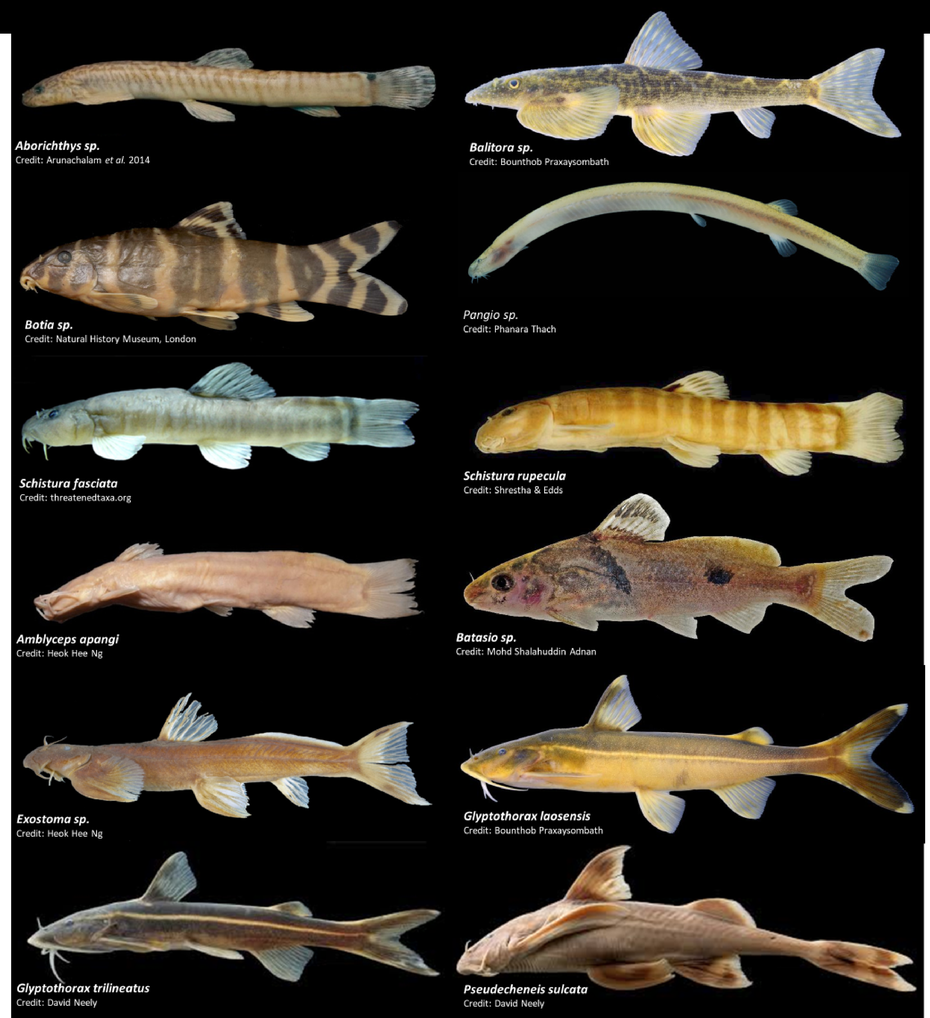
- **Rheophilic fishes** prefer fast flowing water and rocky bottoms (e.g. torrents, rapids, & chutes), and are adapted, with regards to morphology and behavior, to withstand these violently shifting habitats and avoid being swept downstream.[1]
- Steep torrent habitats in headwater streams are isolated from one another by long distances and unsuitable habitat for most rheophilic fish. Therefore, populations diverge genetically, and over evolutionary history this has led to much vicariant speciation in these fishes.[1]
- Headwater stream reaches in high elevation zones, in which rheophilic fishes are found, contain low species richness (α-diversity), but changes in species from stream to stream (turnover) results in variation in species present (β-diversity) among torrent reaches. Consequently, the torrent reaches of headwater streams as a whole are largely responsible for the diversity of regional species assemblages (γ-diversity).[1]
- Although species of rheophilic fishes diverge genetically and speciate, they tend to resemble each other closely due to the specificity of their habitat. **Morphological similarities** among even divergent taxa combined with high diversity of species makes identifying individuals with certainty difficult.
- **DNA barcoding** is used to improve species identification by sequencing short sequences (barcodes) from individuals and comparing them to standard barcodes from a genetic database (e.g. GenBank, EMBL, DDBJ, BOLD). Ideal barcode sequences will contain little variation within a species, while varying considerably among species. Consequently, the barcode sampled from an individual can be used to determine to which species it belongs.[2]

## METHODS

- Loach (Cobitoidea) & catfish (Siluriformes) were collected at 16 locations across tributaries of the Brahmaputra River in Bhutan during 2016-2018.
- DNA was extracted from fin clips using Qiagen Fast Kits.
- Mitochondrial gene cytochrome c oxidase subunit I (COI) was amplified with polymerase chain reaction (PCR) to serve as the barcode.
- Genetic barcodes were sequenced using dideoxynucleotide chain termination.
- Sequences were assessed for accuracy by eye and contigs of forward & reverse strands were assembled using Sequencher v.5.4.6. Sequences were aligned using MUSCLE and again checked for accuracy by cross-referencing the alignment with the chromatogram.
- FASTA files containing the sequences were compared to GenBank samples via National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST).
- Lists of possible matches for each sample were assessed. For match to be considered: match (Ident) value >93%, Coverage > 90%, and the top match must have been at least 1% better than the next taxa. If these criteria were not met, the sample was unassignable.



Chaudhary & Dahal 2017




SAMPLING LOCALITIES - Basins from West to East

| | | TOTAL | SITES | Amo Chhu: dort | pach | sing | Wang Chhu: pipp | toeb | Puna Tsang: kali | labr | Brahmaputra: burk | kalg | sarp | takl | bibi | Mangde Chhu: kiri | klat | lung | rind |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LOACH | *Aborichthys elongatus* | 11 | 3 | | | | 6 | | 3 | 2 | | | | | | | | | |
| | *Balitora brucei* | 4 | 1 | | | | | | | | | | 4 | | | | | | |
| | *Botia rostrata* | 3 | 2 | | | 1 | | | | | | | 2 | | | | | | |
| | *Lepidocephalichthys guntea* | 1 | 1 | | | | | | 1 | | | | | | | | | | |
| | *Pangio pangia* | 1 | 1 | 1 | | | | | | | | | | | | | | | |
| | *Paracanthocobitis botia* | 10 | 1 | | | | | | | | | | | | | | | | |
| | *Schistura fasciata* | 2 | 1 | 2 | | | | | | | | | | | | | | | |
| | *Schistura rupecula* | 40 | 7 | | 1 | 2 | 3 | | 12 | | 3 | | 11 | 8 | | | | | |
| | *Schistura scaturgina* | 4 | 2 | | | | | | | | | | | | 2 | | | | 2 |
| | *Schistura tirapensis* | 16 | 4 | 4 | | 6 | | | 3 | 3 | | | | | | | | | |
| | **UNASSIGNABLE** | **6** | **2** | | | | | | | | | | | | | **2** | **4** | | |
| | | **TOTAL** | **SITES** | **dort** | **pach** | **sing** | **pipp** | **toeb** | **kali** | **labr** | **burk** | **kalg** | **sarp** | **takl** | **bibi** | **kiri** | **klat** | **lung** | **rind** |
| CATFISH | *Amblyceps apangi* | 2 | 1 | | | | | | | | | | | | | 2 | | | |
| | *Amblyceps laticeps* | 1 | 1 | | 1 | | | | | | | | | | | | | | |
| | *Batasio sp.* | 6 | 1 | | | | | | | | | | | 6 | | | | | |
| | *Clarias gariepinus* | 1 | 1 | | | | | | 1 | | | | | | | | | | |
| | *Exostoma labiatum* | 1 | 1 | | | | | | | | | | | | | 1 | | | |
| | *Glyptothorax dakpathari* | 2 | 2 | | | | | | 1 | | | | | | | | | 1 | |
| | *Glyptothorax sp.* | 2 | 2 | | | 1 | | | | | | | 1 | | | | | | |
| | *Pseudecheneis sulcata* | 16 | 2 | | | | | 11 | | | | | | | | | | | 5 |
| | **UNASSIGNABLE** | **7** | **3** | | | | | | **1** | | | | **2** | | | | | | **4** |

## RESULTS

### *Loach*

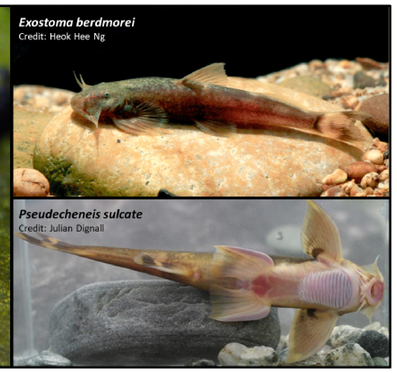
- 98 loaches were collected representing 10 species & 7 genera according to BLAST results.
- 94% of individuals could be assigned to species ( > 93% Identical match) while 6% of individuals could not be assigned with confidence.
- Assigned matches had an average sequence match of 96.33%.

### *Catfish*

- 38 catfish were collected representing 8 species & 6 genera according to BLAST results.
- 80% of individuals could be assigned to species, while 18% of individuals could not be assigned with confidence.
- Assigned matches had an average sequence match of 98.78%.

## CONCLUSION

- DNA barcoding is effective for confirming species identification or identifying unknown species.
- BLAST results should be interpreted with caution, because GenBank samples can be GenBank have been misidentified.
- Most specimens (90.4%) were assigned a species identification using COI barcodes.
- Unassignable specimens (9.6%) likely represent species not yet added to GenBank.
- The applicability of DNA barcoding will increase as more individuals are identified, barcoded, and added to public databases.


NCBI

Ministry of Agriculture and Forests
Royal Government of Bhutan

UNIVERSITY OF ARKANSAS

## LITERATURE CITED

1. Lujan & Conway. 2015. Life in the Fast Lane: A review of Rheophily in Freshwater Fishes. *Extremophile Fishes*
2. Hajibabaei *et al.* 2007. DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics*
3. Chaudhary & Dahal. 2017. DNA Bar-Code for identification of microbial communities: A Mini-Review. *EC Microbiology*