

Utilization of DNA barcodes for the identification of tropical larval fishes in Klang Strait, Straits of Malacca

By Cecilia Chu

16 November 2022

SEAFDEC/TD, Samut Prakan, Thailand



Sample collection and preservation
using denatured ethanol

DNA extraction

PCR (Polymerase Chain Reaction)

Sequencing of DNA (Barcode)

Phylogenetic analysis

SPECIES IDENTITY

Work flow



(outsourced to
commercial lab)

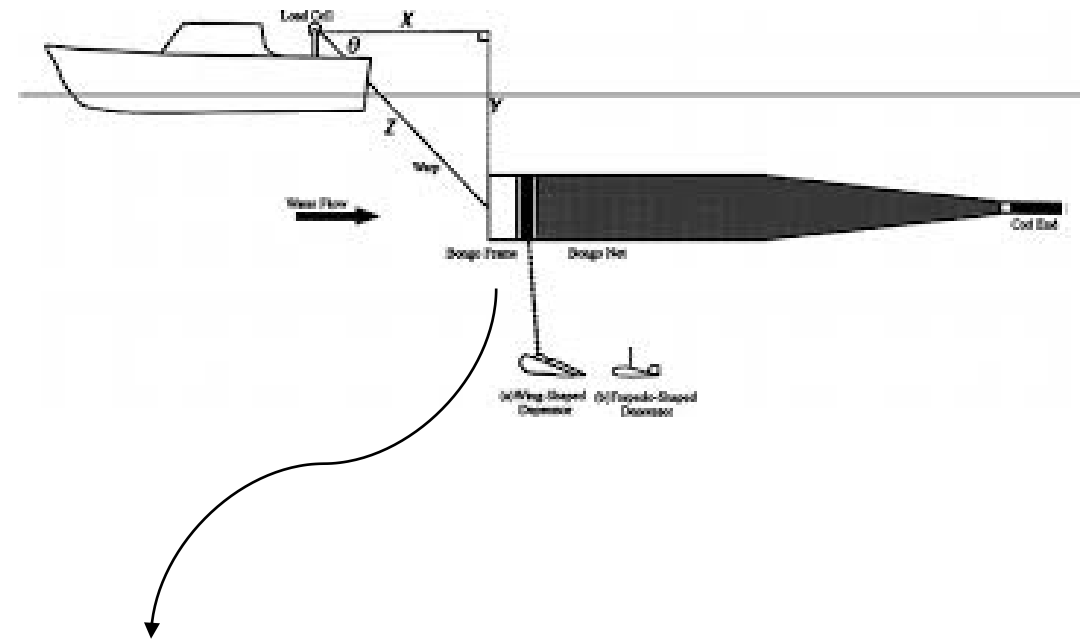


Sample collection



Image source: © 2020 Google

oblique tow
10 minutes
1.5 knots



45 cm diameter
180 & 363 μm



Left: Twin bongo nets (363 & 180 μ m mesh)

Right: Plankton net



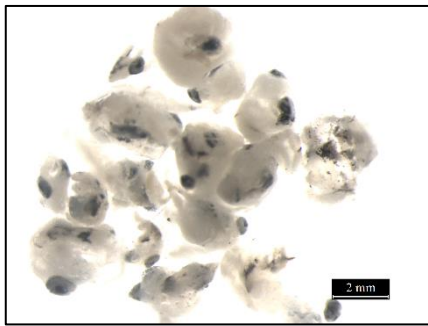


Sampling of adult to create a database for local fish species

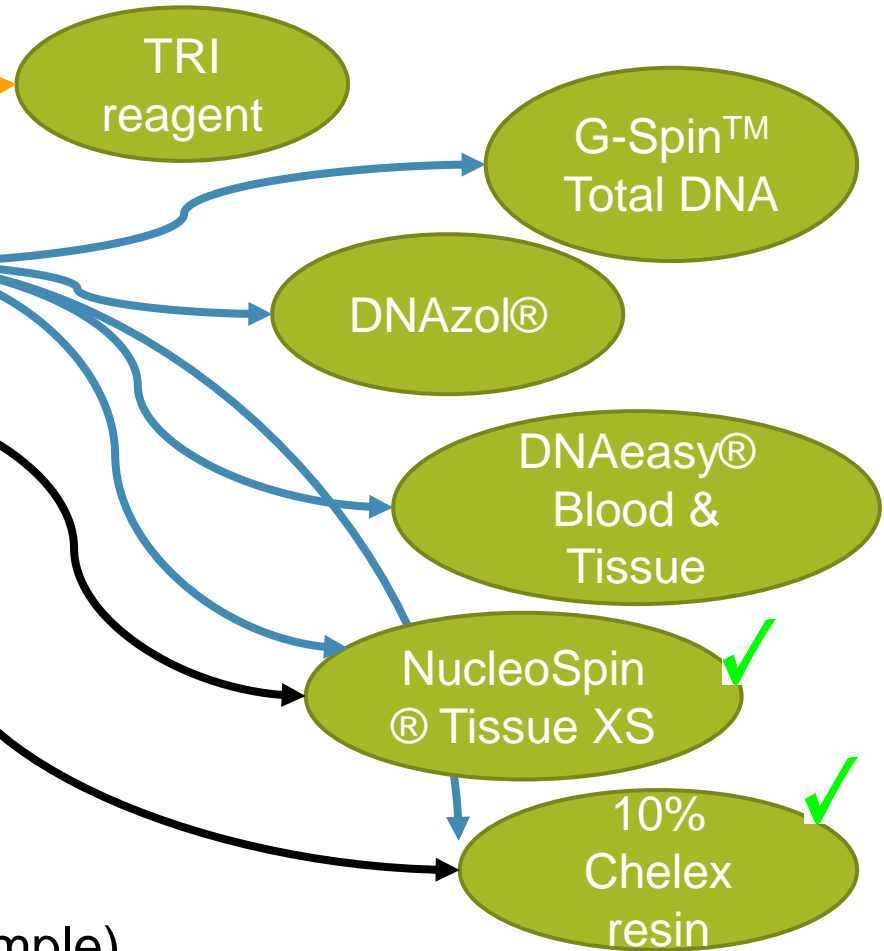
Sample preservation and DNA extraction

Tissue preservation method

DNA Extraction method



- 1 RNAlater
- 2 100% un-denatured ethanol
- ✓ 3 50% un-denatured ethanol
- ✓ 4 80% un-denatured ethanol
- 5 Ice cubes
- 6 10% borax-buffered formaldehyde in seawater



Chelex extraction is much less expensive (USD0.07/sample) compared to the extraction kits (USD1.28–3.26/sample).

Vol. 286: 269–277, 2005

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published February 2

**Shipboard identification of fish eggs and larvae
by multiplex PCR, and description of fertilized eggs
of blue marlin, shortbill spearfish, and wahoo**

J. R. Hyde^{1,2,*}, E. Lynn², R. Humphreys Jr.³, M. Musyl⁴, A. P. West⁵, R. Vetter²

- ✓ Cheap
- ✓ Rapid
- ✓ Easy
- ✓ Whole
body of
larva can
be kept for
future
reference

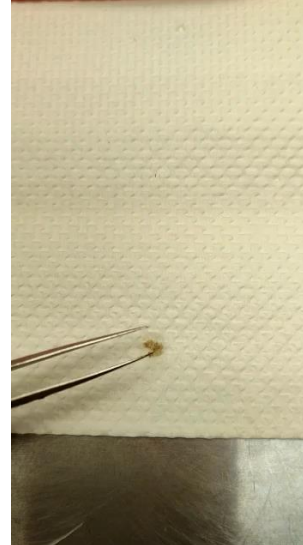
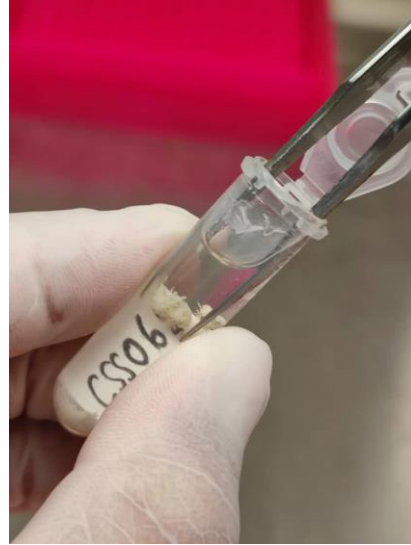
DNA extraction using 10% Chelex resin

1

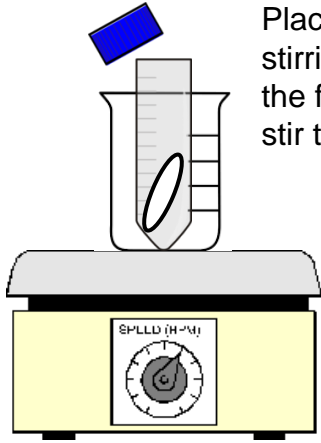


5g Chelex resin +
dH₂O

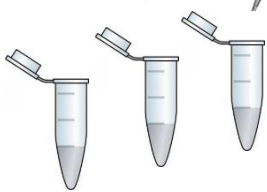
2



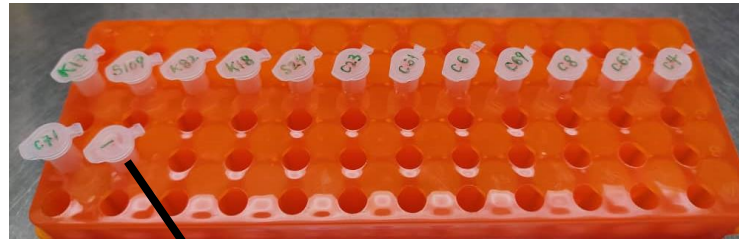
Place magnetic
stirring bar inside
the falcon tube and
stir the solution



While mixing, aliquot
150µl 10% Chelex resin
into 0.2ml centrifuge tube



Store at -20°C



No tissue (NT)

3



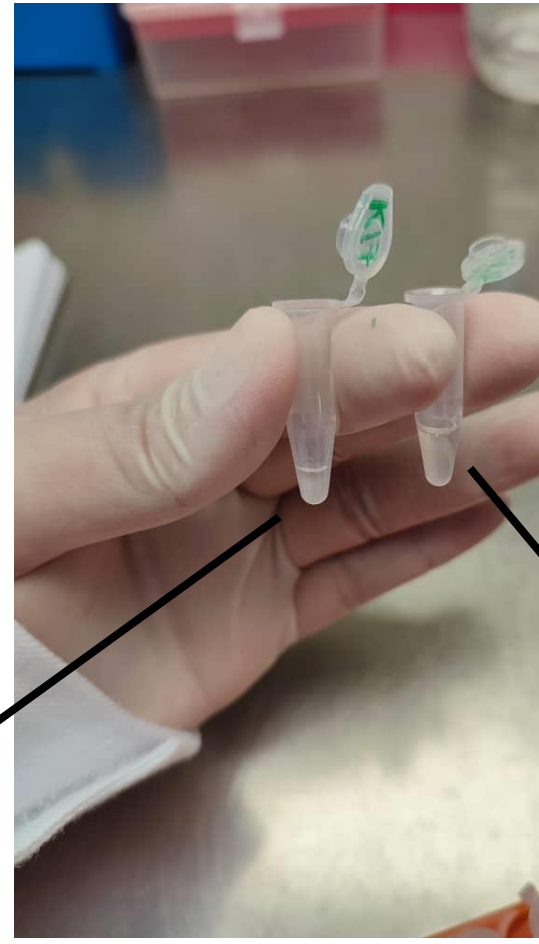
1. 60°C, 2 min
2. 103°C, 25 min
3. Leave at room temperature

4



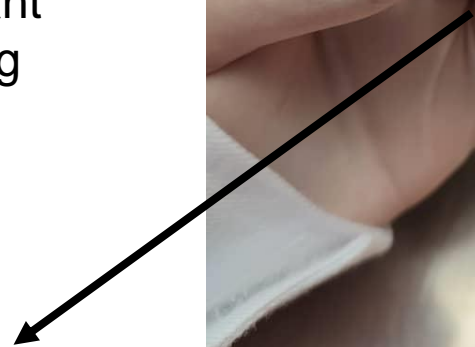
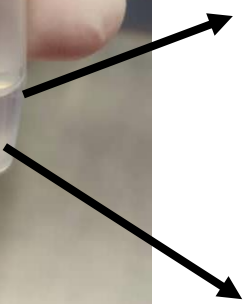
Supernatant
containing
DNA

Chelex
resin



Supernatant
containing
DNA

Keep at 20°C
until PCR



PCR amplification

Barcoding primers (Ward et al., 2005)

F2 5' – TCG ACT AAT CAT AA GAT ATC GGC AC – 3'

R2 5' – ACT TCA GGG TGA CCG AAG AAT CAG AA – 3'

COI
(Cytochrome oxidase subunit I)

exTen 2x PCR master mix	12.5 µl
forward primer	0.5 µl
reverse primer	0.5 µl
DNA template	1.0 µl
dH ₂ O	10.5 µl
Total	25.0 µl

Initial denaturation	95°C	10 minutes	x 1
	94°C	1 minute	x 35
	55°C	1 minute	
	72°C	1.5 minutes	
Final extension	72°C	7 minutes	∞

~ 3 hours



Thermal cycler



DNA sequencing
(outsourcing)

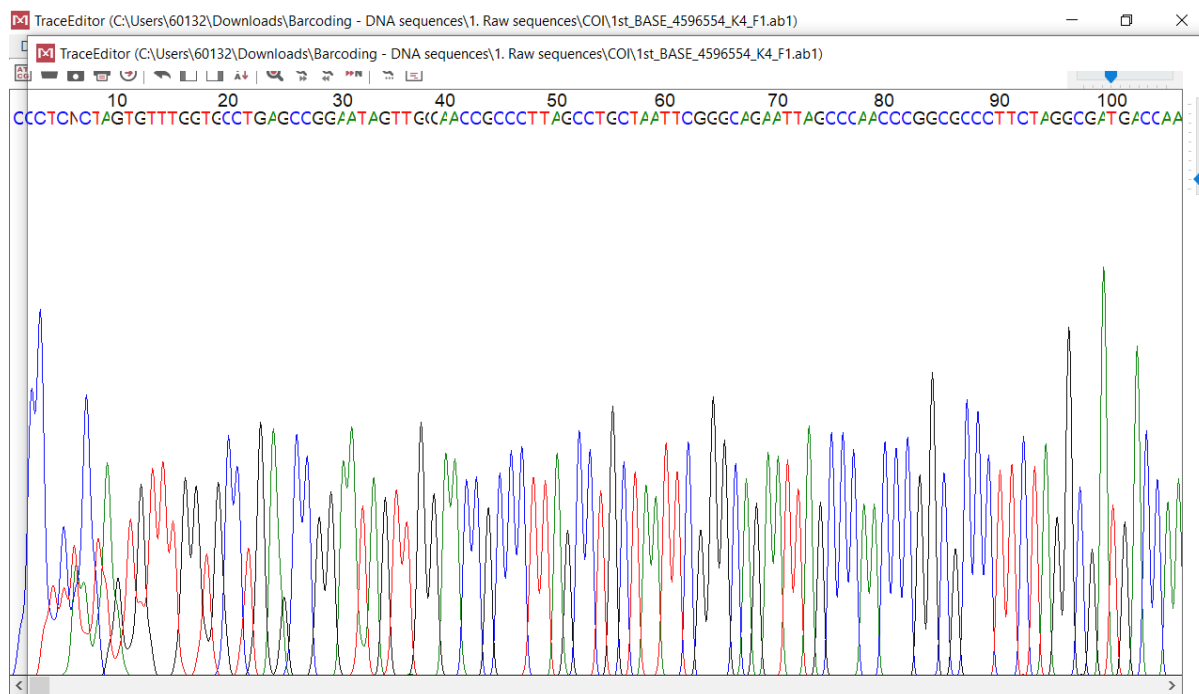
Data analysis

Sequence Scanner 2.0:

http://resource.thermofisher.com/page/WE28396_2/

MEGA software:

<https://www.megasoftware.net/>



```
1st_BASE_4596554_K4_F1 - Notepad
File Edit Format View Help
>1st_BASE_4596554_K4_F1
CCCTCNCTAGTGTTCCTGAGCCGGAATAGTTGCGCCAGCAATTAGCCCAACCCGCGCCCTTCTAGGCGATGACCAA
CCTGCTAATTCGGGCAGAATTAGCCCAACCCGCGCCCTTCTAGGCGATGACCAA
CCAAATCTATAACGTTATTGTTACCGCCCACGCTTTTGTAAATATTTCTT
TATAGTAATACCAATCATAATTGGAGGCTTTGGAAATTGACTTGTTCCCTT
AATAATTGGAGCCCCGACATAGCATTCCCCGAATGAATAATATGAGTTT
CTGACTCCTCCCCCATCCTTCCTACTTCTCCTTGCTTCATCAGGAGTTGA
AGCAGGAGCAGGGACAGGATGAACTGTATACCCACCCCTTGCTGGAAATCT
TGCACACGCAGGAGCTCCGTTAGACCTTACTATTTTTTCCCTCCACTTAGC
AGGAGTCTCATCAATTCTAGGGGCCATCACTTCATCACAATATCATTAA
CATGAAACCTCCAGCTATCTCACAATATCAAACACCTTTATTTGTTGAGC
TATTCTAATACTGCTGTACTTCTACTTCTTTCCCTCCCCGTCCTTGCTGC
CGGTATTACAATACTATTAACAGACCGAAACCTTAATACTACTTTCTTTGA
TCCCGCAGGAGGGGGAGATCCAATCCTTTACCAACATCTTCTGATTCTT
TGGCCACCCAGAAAGTCTAA
```

Website:

https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome

BLAST[®] » blastn suite Home Recent Results Saved Strategies Help

Standard Nucleotide BLAST

blastn | blastp | blastx | tblastn | tblastx

BLASTN programs search nucleotide databases using a nucleotide query. [more...](#) Reset page
Bookmark

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) [?](#) [Clear](#)

```
CCCTCNCTAGTGTGGTGCCTGAGCCGGAATAGTTGGGAACCGCCCTT
AGCCTGCTAATTCGGGCAGAATTAGCCAACCCGGCGCCCTTCTAGGCG
ATGACCAAATCTATAACGTTATTGTTACCGCCCACGCTTTTGTAATAATTT
CTTTATAGTAATACCAATCATAATTGGAGGCTTTGGAAATTGACTTGTCC
```

Or, upload file No file chosen [?](#)

Job Title


Enter a descriptive title for your BLAST search [?](#)

Align two or more sequences [?](#)

Query subrange [?](#)

From

To



1. Paste DNA sequence

Choose Search Set

Database

Standard databases (nr etc.): rRNA/ITS databases Genomic + transcript databases Betacoronavirus

Nucleotide collection (nr/nt)

2. Select nucleotide

Organism

Optional

Enter organism name or id—completions will be suggested

exclude

[Add organism](#)

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown [?](#)

Exclude

Optional

Models (XM/XP) Uncultured/environmental sample sequences

Limit to

Optional

Sequences from type material

Entrez Query

Optional

Enter an Entrez query to limit search [?](#)

[YouTube](#) [Create custom database](#)

Program Selection

Optimize for

Highly similar sequences (megablast)
 More dissimilar sequences (discontiguous megablast)
 Somewhat similar sequences (blastn)

Choose a BLAST algorithm [?](#)

3. Select "Highly similar sequences"

BLAST

Search database **Nucleotide collection (nr/nt)** using **Megablast (Optimize for highly similar sequences)**

Show results in a new window

4. Click on BLAST

[← Edit Search](#)

[Save Search](#)

[Search Summary ▾](#)

[? How to read this report?](#)

[▶ BLAST Help Videos](#)

[↶ Back to Traditional Results Page](#)

Job Title	Nucleotide Sequence
RID	R90AJV4P013 <small>Search expires on 11-17 13:21 pm</small> Download All ▾
Program	BLASTN ? Citation ▾
Database	nt See details ▾
Query ID	lcl Query_19163
Description	None
Molecule type	dna
Query Length	683
Other reports	Distance tree of results MSA viewer ?

Filter Results

Organism only top 20 will appear exclude

Type common name, binomial, taxid or group name

[+ Add organism](#)

Percent Identity to **E value** to **Query Coverage** to

[Filter](#) [Reset](#)

Descriptions

[Graphic Summary](#)

[Alignments](#)

[Taxonomy](#)

Sequences producing significant alignments

[Download ▾](#)

[Select columns ▾](#)

Show [?](#)

select all 100 sequences selected

[GenBank](#)

[Graphics](#)

[Distance tree of results](#)

[MSA Viewer](#)

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	Arius dispar mitochondrion, complete genome	Arius dispar	1020	1020	98%	0.0	94.18%	16792	NC_048969.1
<input checked="" type="checkbox"/>	Arius maculatus mitochondrion, complete genome	Arius maculatus	1020	1020	98%	0.0	94.18%	16710	NC_045222.1
<input checked="" type="checkbox"/>	Netuma thalassina mitochondrion, complete genome	Netuma thalassina	1020	1020	98%	0.0	94.18%	16709	MG587041.1
<input checked="" type="checkbox"/>	Arius arius mitochondrion, complete genome	Arius arius	1020	1020	98%	0.0	94.18%	16711	KX211965.1
<input checked="" type="checkbox"/>	Netuma thalassina mitochondrion, complete genome	Netuma thalassina	1020	1020	98%	0.0	94.18%	16711	KU986659.1

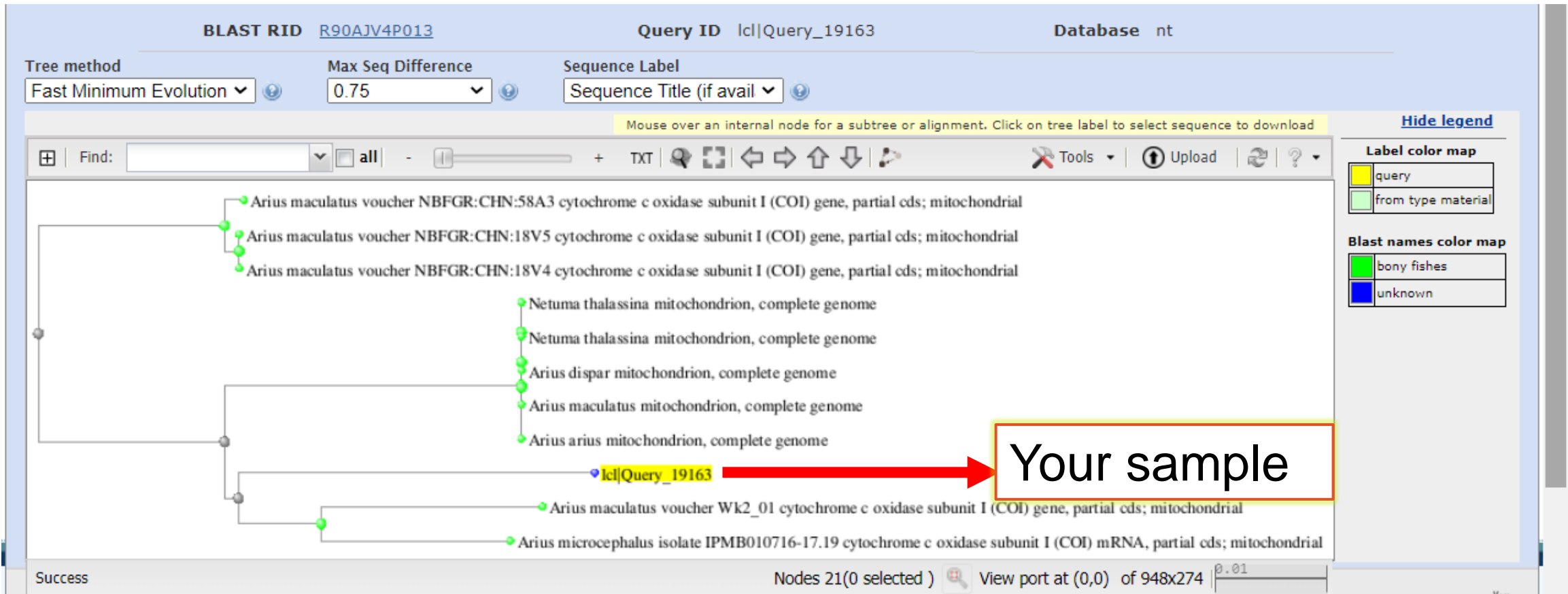
Distance tree

Show 10

MSA viewer

[Feedback](#)

Distance Tree



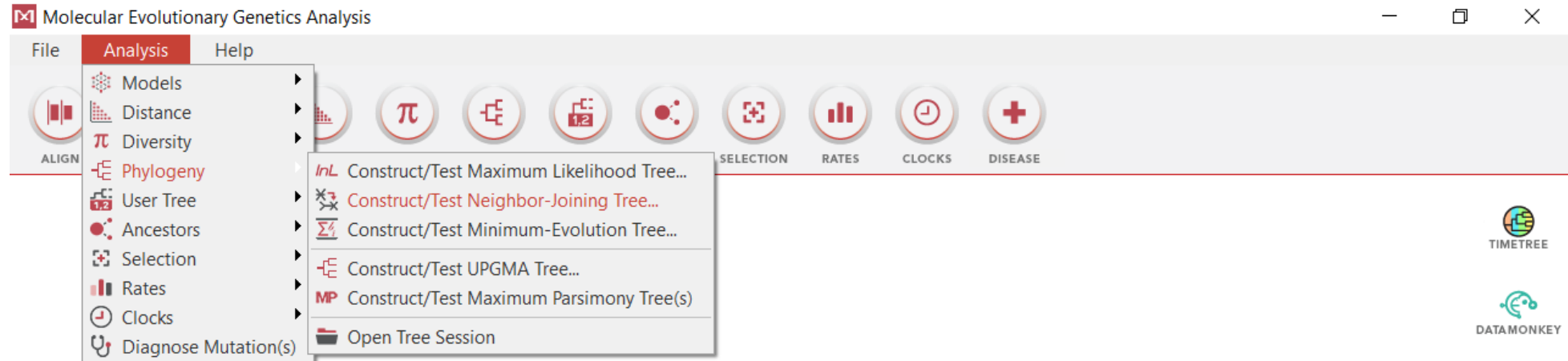
MSA viewer

Alignment

[Link To View](#) | [Feedback](#)



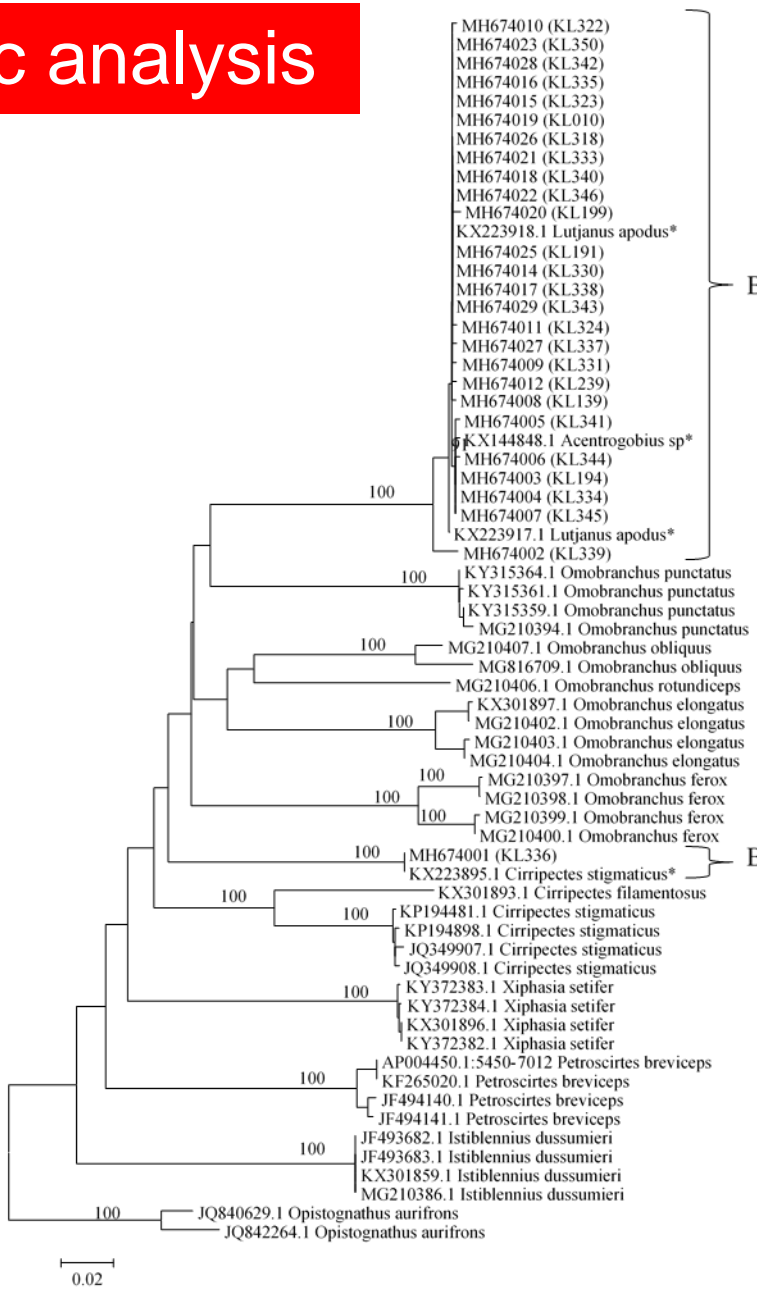
MEGA software



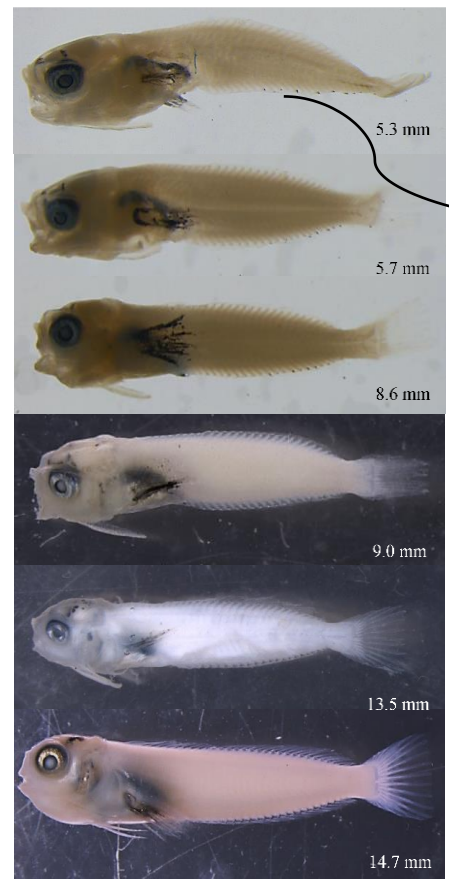
RECENT PUBLICATIONS



Phylogenetic analysis



Blenniidae sp. 2

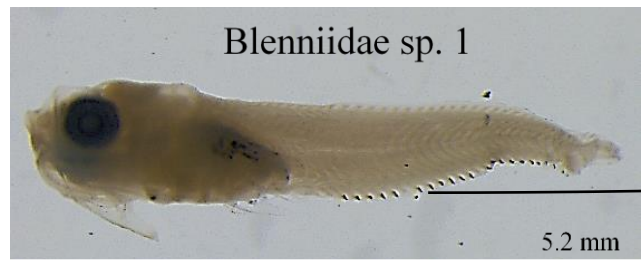


Scarce pigments along anal fin base

Blenniidae sp. 2

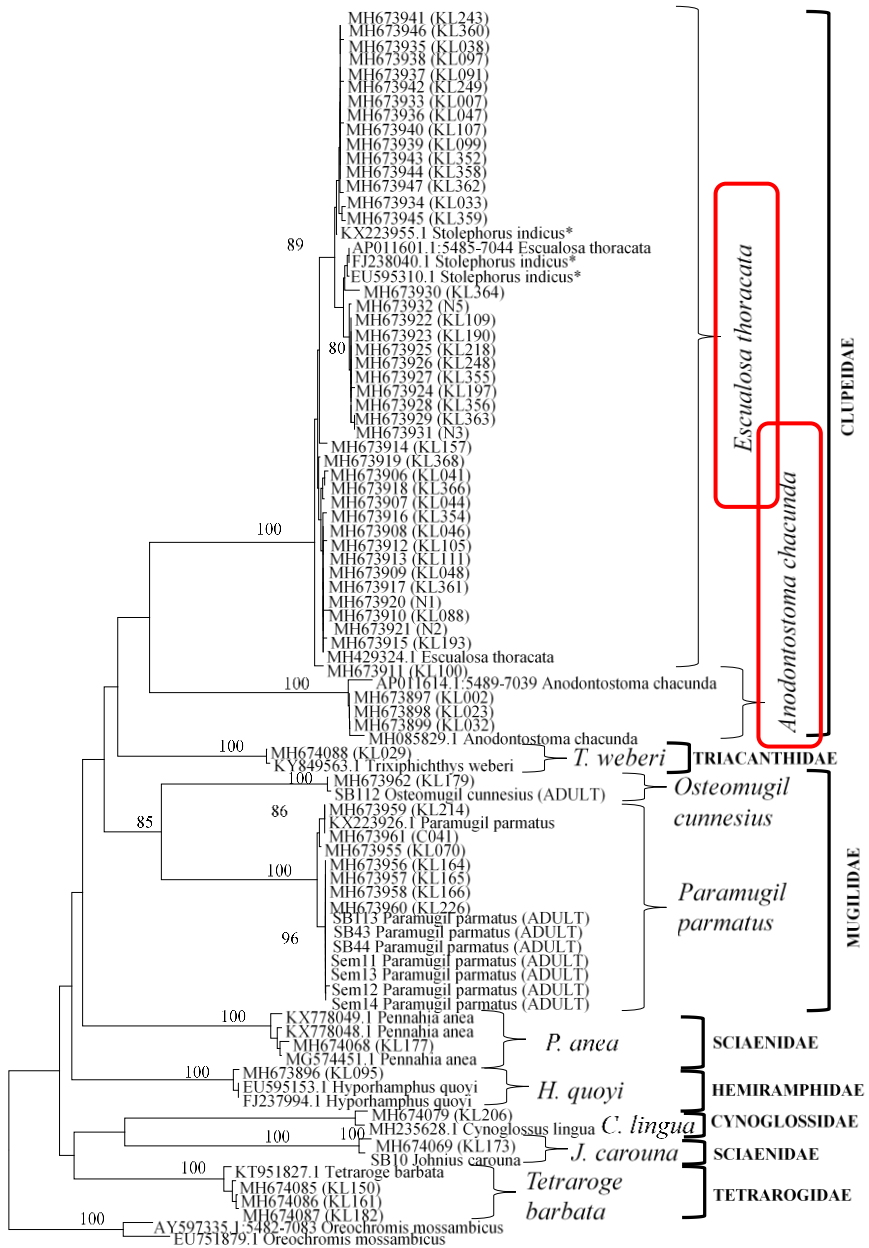
Blenniidae sp. 1

Blenniidae sp. 1



Pigments along anal fin base

5.2 mm



CLUPEIDAE

Dorsal



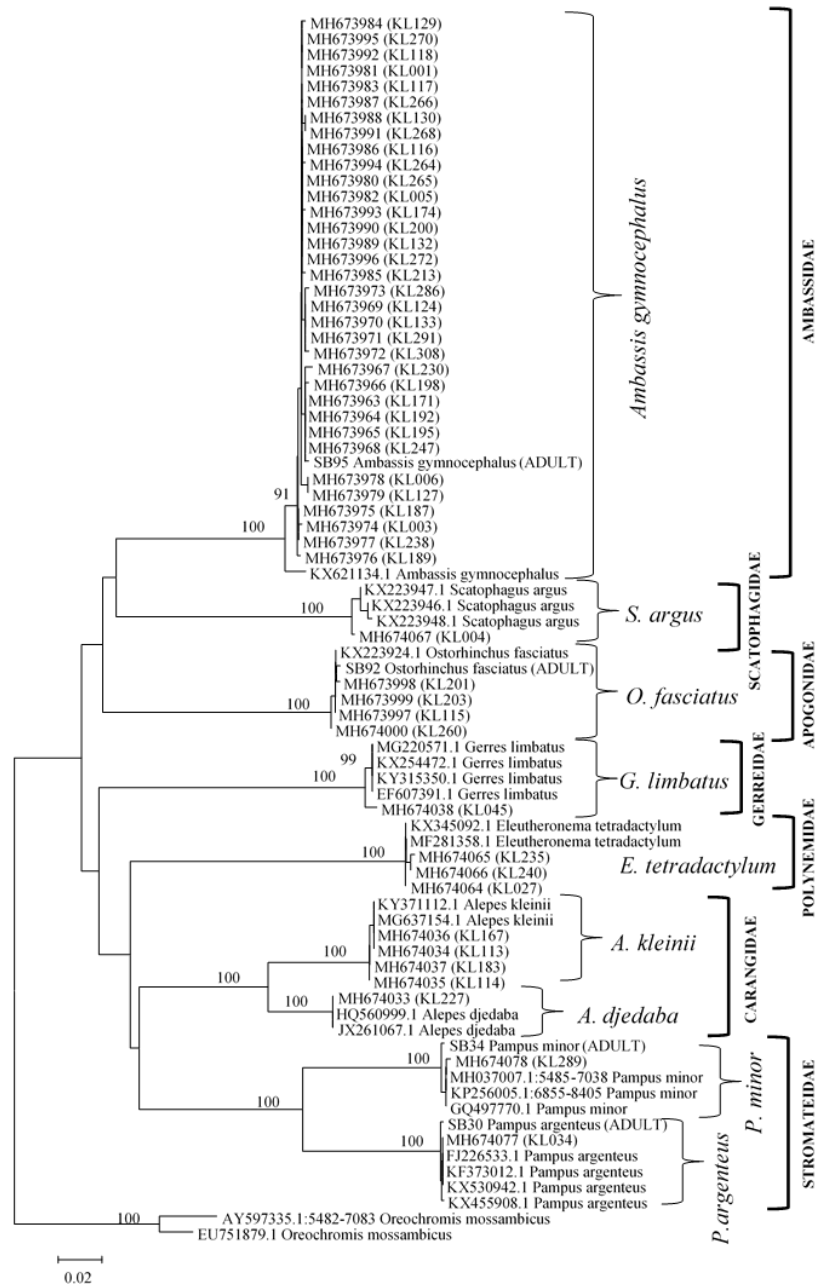
Anal

ENGRAULIDAE

Dorsal



Anal

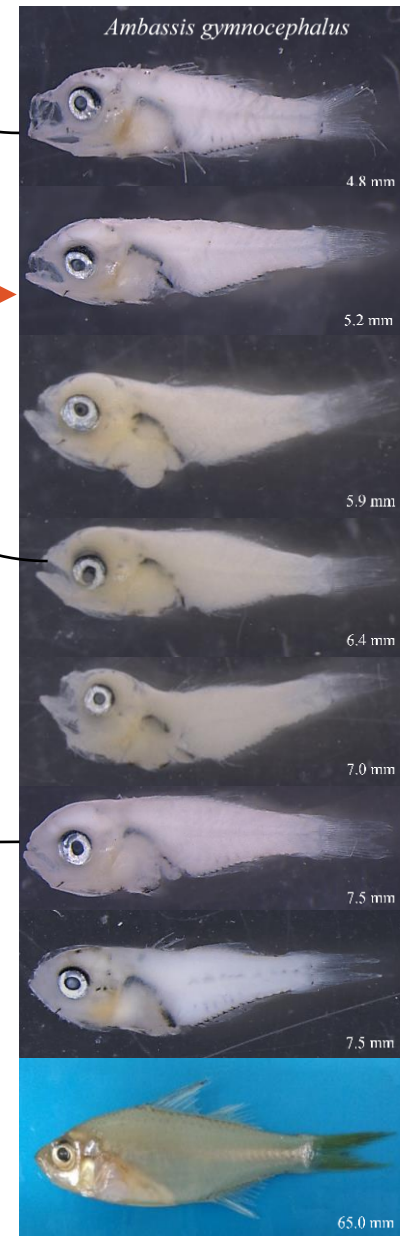


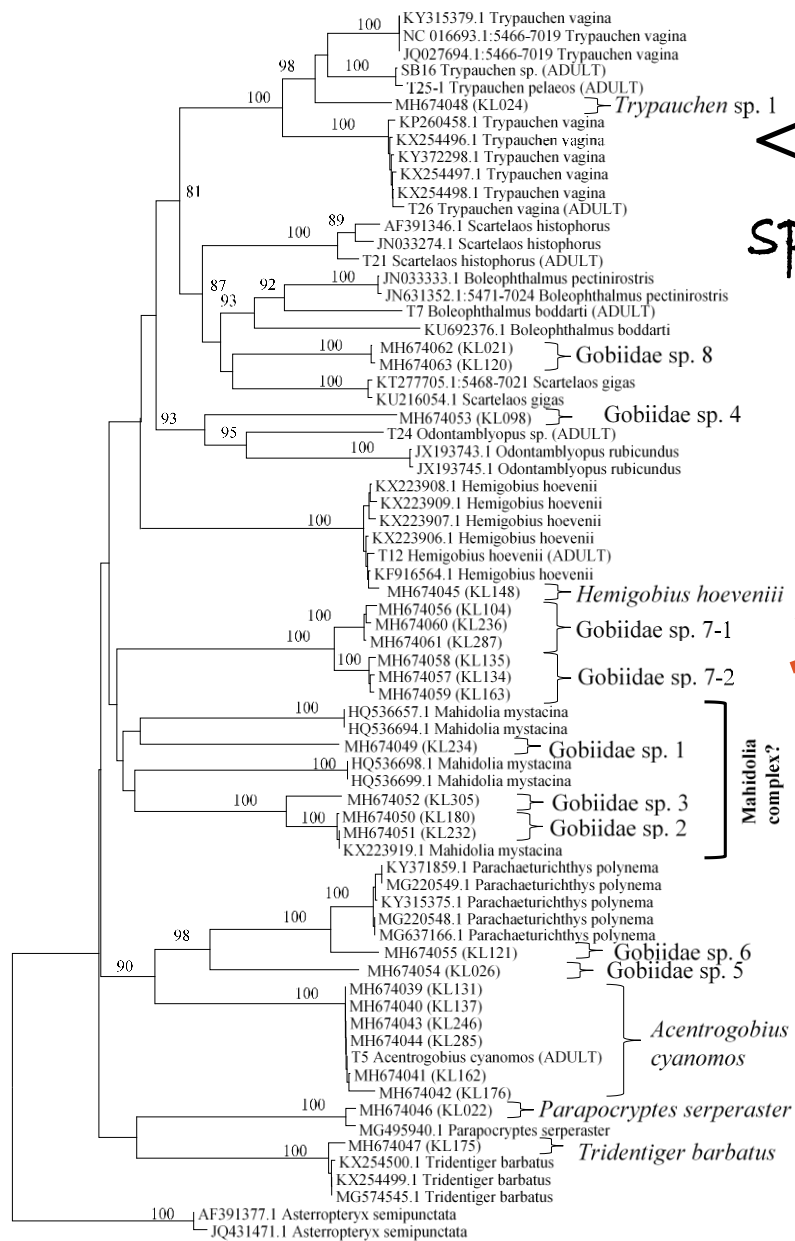
Heavy pigments
on top of head

No pigments
on top of head

Moderate pigments
on top of head

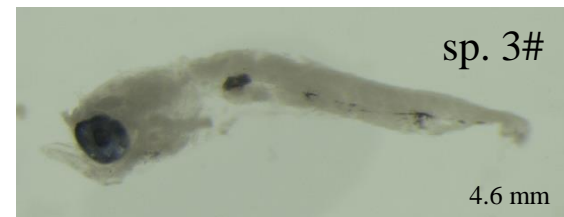
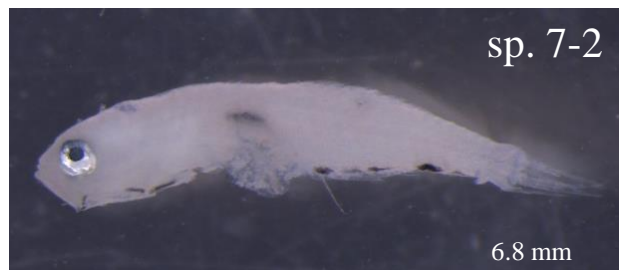
AMBASSIDAE

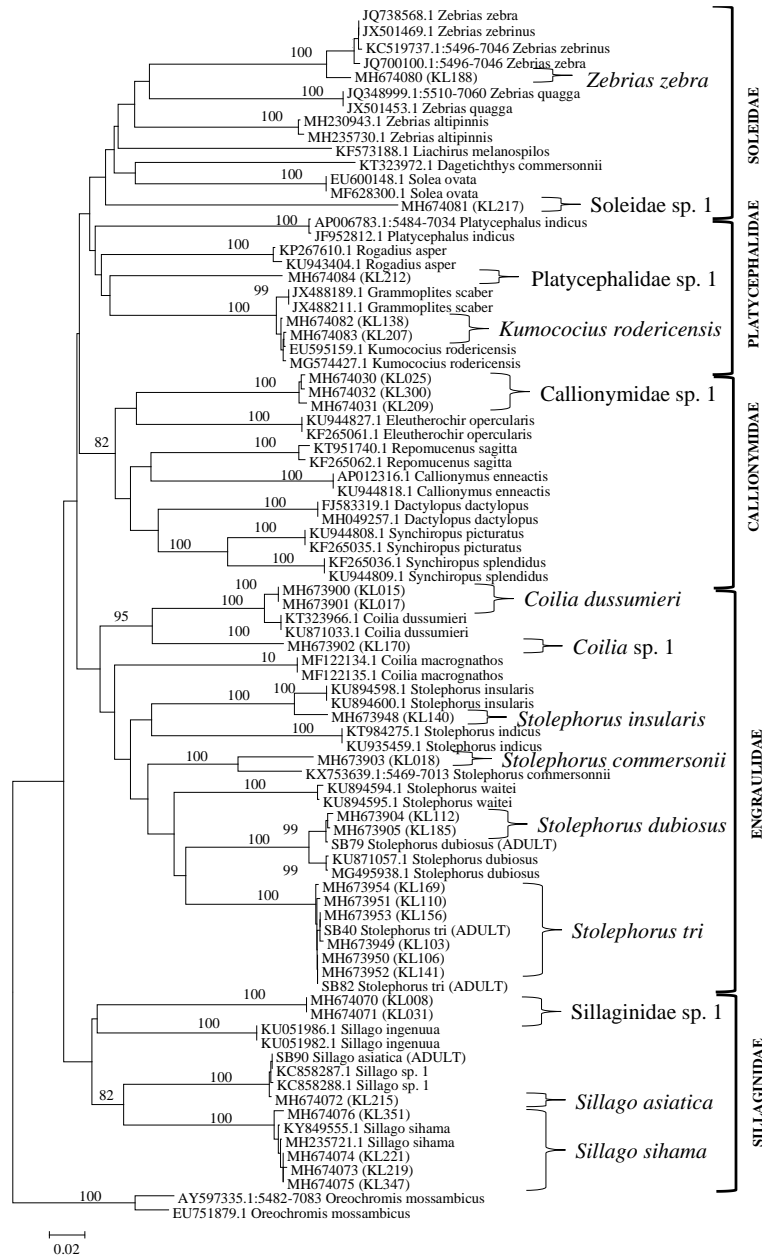




<3.5% inter-species divergence

>3.5% inter-species divergence





Q: Can DNA barcode differentiate between the species of larvae and used for routine identification of the larval fishes?

A: Yes, for most species

But...

- Species assignment is not straightforward because of **ambiguous** matches and wrong identification
- General **lack of reference sequences** especially for speciose and non-commercial fish families such as Gobiidae, Blenniidae, and Callionymidae
- Other gene markers** may be needed to elucidate the identity and phylogenetic relationships of cryptic species such as Ambassidae and Gobiidae

Species of larvae identified using DNA barcode

- 48 taxa from 21 families

Hemiramphidae (*Hyporhamphus quoyi*)

Clupeidae (*Anodontostoma chacunda*,
Escualosa thoracata)

Engraulidae (*Coilia dussumieri*, *Coilia* sp. 1,
Stolephorus commersonii, *Stolephorus*
dubiosus, *Stolephorus insularis*, *Stolephorus tri*)

Mugilidae (*Paramugil parmatus* and *Osteomugil*
cunnesius)

Ambassidae (*Ambassis gymnocephalus*)

Apogonidae (*Ostorhinchus fasciatus*)

Blenniidae (Sp. 1, Sp. 2)

Callionymidae (Sp. 1)

Carangidae (*Alepes djedaba* and *Alepes kleinii*)

Gerreidae (*Gerres limbatus*)

Gobiidae (*Acentrogobius cyanomos*,
Hemigobius hoevenii, *Parapocryptes*
serperaster, *Trypauchen* sp. 1, *Tridentiger*
barbatus, Sp. 1, Sp. 2, Sp. 3, Sp. 4, Sp. 5, Sp. 6,
Sp. 7, Sp. 8)

Polynemidae (*Eleutheronema*
tetradactylum)

Scatophagidae (*Scatophagus argus*)

Sciaenidae (*Pennahia anea* and *Johnius*
carouna)

Sillaginidae (*Sillago asiatica* and *Sillago*
sihama, Sp. 1)

Stromateidae (*Pampus argenteus* and
Pampus minor)

Cynoglossidae (*Cynoglossus lingua*)

Soleidae (*Zebrias zebra*, Sp. 1)

Platycephalidae (*Kumococius*
rodericensis, Sp. 1)

Tetrarogidae (*Tetraroge barbatus*)

Triacanthidae (*Trixiphichthys weberi*)

Using DNA Barcodes to Aid the Identification of Larval Fishes in Tropical Estuarine Waters (Malacca Straits, Malaysia)

Cecilia Chu¹, Kar Hoe Loh¹, Ching Ching Ng², Ai Lin Ooi³, Yoshinobu Konishi⁴, Shih-Pin Huang⁵, and Ving Ching Chong^{2,*}

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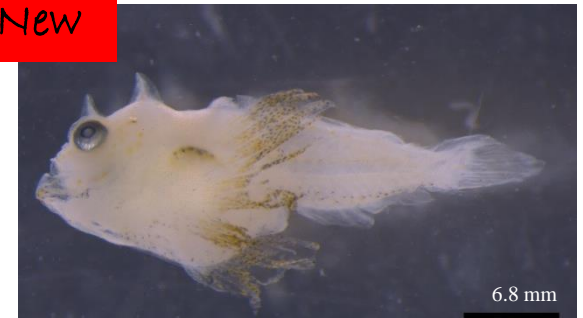
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New



Tetraroge barbata
TETRAROGIDAE

New



Tridentiger barbatus
GOBIIDAE

THANK YOU~



July 2016, SEAFDEC/TD, Samut Prakan, Thailand