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# Validation of occurrence of tropical shads, *Tenualosa ilisha* (Hamilton, 1822) and *T. toli* (Valenciennes, 1847) (Teleostei, Clupeidae), in Malaysian waters

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**Abstract**
The tropical shads of the genus *Tenualosa* are important and high-value commercial clupeid fishes in the Indo-Pacific region. Although there is difficulty in accurately identifying tropical shads based solely on morphology, we successfully identified *T. ilisha* and *T. toli* from Peninsular Malaysia and Borneo, which were confirmed by an analysis using 16S ribosomal RNA gene sequences. We propose that accurate identifications of tropical shad species should always include molecular genetic analysis for precise species confirmation. There is an urgent need for studies on the ecology, population dynamics, and conservation of these species in Malaysia.

**Keywords**
Distribution, genetic identification, Indian Ocean, Malaysia, *Tenualosa*, tropical species.

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## Introduction

Shads are the most common group of clupeid fishes and are mainly found in coastal and estuarine waters. Some of them are migratory, and they ascend upstream for spawning, while others complete their life history in coastal waters or estuaries (McDowall 1988). Five species of tropical shads, *Tenualosa ilisha* (Hamilton, 1822), *T. toli* (Valenciennes, 1847), *T. macrura* (Bleeker 1852), *T.* reevesii (Richardson, 1846), and *T. thibaudeaui* (Durand, 1940), live in the Indo-Pacific region (Preston 2004). All species of *Tenualosa* are currently under heavy fishing pressure and in decline (Brian 1997, Preston 2004).

In Malaysia, 2 species of *Tenualosa*, *T. toli* and *T. macrura*, have been recorded in East Malaysia on Borneo (Blaber et al. 1997). These 2 species are locally known as "Terubok" and are the most commercially important species in Malaysia (Blaber et al. 1996). Moshin and Ambak (1996) and Arai and Amalina (2014) reported *T. ilisha* in West Malaysia (Peninsular Malaysia). Malaysia imports species of *Tenualosa* from Bangladesh and India (Hossain et al. 2018), and this genus has some of the most important tropical fish species in the Indo-Pacific region; they occupy a top position among the edible species of fishes (Nowsad et al. 2012). Only a few studies are available on the distribution, life history and habitat use of *Tenualosa* species in Peninsular Malaysia (e.g. Blaber

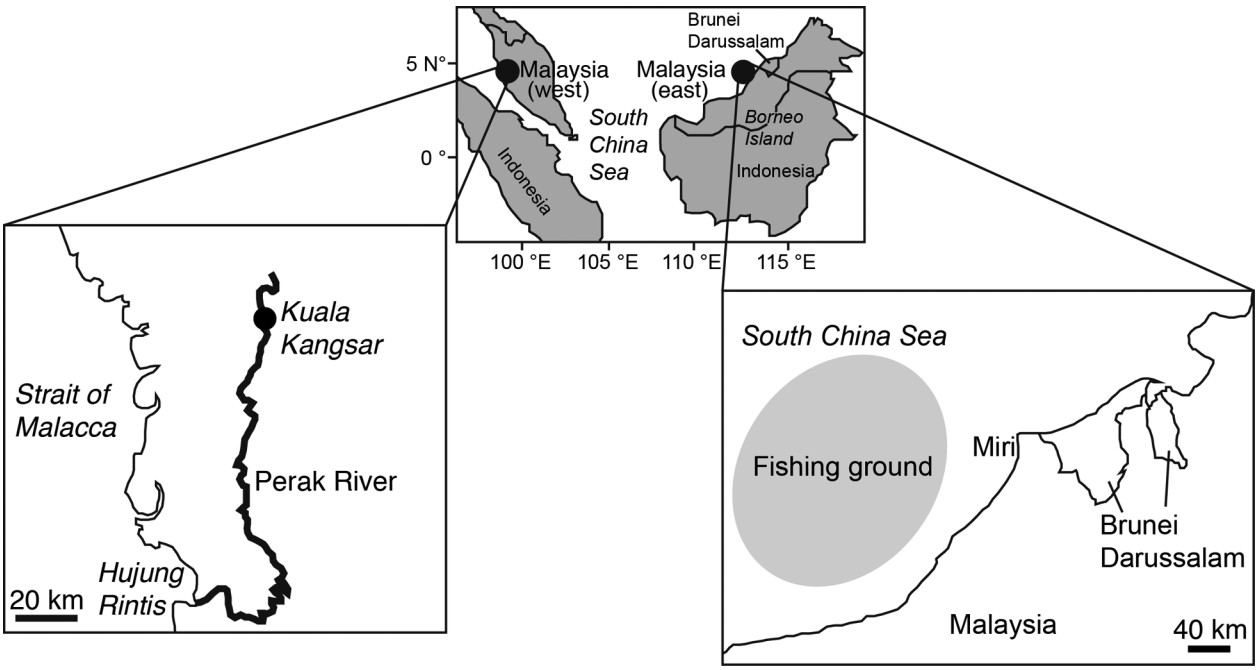


Figure 1. Map showing the collection sites of the tropical shads *Tenualosa* in the Perak River in western Peninsular Malaysia (West Malaysia) of Perak State on 30 July 2013 and off Miri in the South China Sea of the Sarawak State in the Borneo Island (East Malaysia) on 23 April 2017. Black circles on the map indicate the sampling location.

et al. 1996, 1997, Arai and Amalina 2014, Rahim et al. 2014, Arjunaidi et al. 2016, Puvanasundram et al. 2018).

The identification of tropical shads to species solely on morphological characters is difficult because of similarities and overlapping characteristics which can lead to misidentifications (FAO 2012, Arai and Amalina 2014). Recently, several authors have suggested that to be accurate, species identifications need to be validated by molecular genetic analyses (Arai et al. 2015, Arai and Wong 2016, Abdul Kadir et al. 2017, Wong et al. 2017). Thus, identification of tropical shad species require the use of both traditional morphological characters and molecular genetic analyses, which we do here for *T. ilisha* and *T. toli* from Malaysian waters.

## Methods

Fish samples and morphological analysis. Ten specimens of *Tenualosa* were collected for this study. Five specimens were collected by gill nets in the Perak River, Perak State (western Peninsular Malaysia) (Fig. 1), and 5 specimens were collected by trawling nets off Miri in the South China Sea of the Sarawak State (Borneo) (Table 1). Specimens are vouchered at the Faculty of Science, Universiti Brunei Darussalam (UBDTB).

For each specimen, 23 morphometric characters were measured and 8 meristic counts were conducted following the methods of Arai and Amalina (2014). We could only roughly identify species using morphometric and meristic characters following (FAO 2012). The 5 species of *Tenualosa* are not clearly differentiated by their morphological characters due to overlapping and out of range characters (Table 2) (FAO 2012, Arai and Amalina 2014). We were unable to determine the sex our specimens due to their undeveloped gonads.

Molecular genetic analysis. We analysed our 10 samples (Table 1) using the mitochondrial 16S ribosomal RNA (16S rRNA) gene. Genomic DNA was extracted from the dorsal fin clip of each specimen using the QIAGEN DNeasy Blood and Tissue Kit, according

**Table 1.** Biological information of the tropical shads *Tenualosa* used in this study. TL = total length, BW = body weight, NGR = number of gill rakers, CFL % of SL = ratio of caudal fin length to standard length.

| Sampling site | TL (mm) | BW (g) | NGR | CFL % of SL | Species by morphology | Species by molecular genetics |
|---|---|---|---|---|---|---|
| Peninsular Malaysia | 308 | 450 | 178 | 32 | *T. ilisha* | *T. ilisha* |
| | 267 | 281 | 219 | 29 | *T. ilisha* | *T. ilisha* |
| | 262 | 284 | 209 | 31 | *T. ilisha* | *T. ilisha* |
| | 282 | 255 | 215 | 31 | *T. ilisha* | *T. ilisha* |
| | 263 | 203 | 228 | 31 | *T. ilisha* | *T. ilisha* |
| Borneo Island | 409 | 589 | 137 | 33 | *T. toli* | *T. toli* |
| | 266 | 135 | 113 | 35 | unknown | *T. toli* |
| | 277 | 151 | 146 | 36 | unknown | *T. toli* |
| | 410 | 665 | 125 | 37 | unknown | *T. toli* |
| | 467 | 924 | 132 | 36 | unknown | *T. toli* |

**Table 2.** Distinctive biological characters and geographical distribution of five tropical shads found in Indo-Pacific region (FAO 2012). CFL = % of SL: ratio of caudal fin length to standard length.

| | *T. macrura* | *T. toli* | *T. ilisha* | *T. reevesii* | *T.thibaudeaui* |
|---|---|---|---|---|---|
| Caudal fin | Long, 40 to 42% of CFL % of SL | Relatively short, 31 to 34% of CFL % of SL | Moderately long, 25 to 31% of CFL % of SL | Moderately long, 25 to 31% of CFL % of SL | Moderately long, about 25 to 30% of CFL % of SL |
| Gill rakers | Gill rackers fine but not numerous, 60 to 75 | Fine but not numerous, 60 to 100 | Fine and numerous, about 100 to 250 | Fine and numerous, 80 to 250 | Fine and very numerous, 204 to 316 |
| Distribution | E Malaysia (Borneo) and Indonesia (Sumatra) | India to Java Sea and South China Sea but now found only in Sarawak (Borneo) | N Indian Ocean from N Sumatra of Indonesia in E to Kuwait in the W | China (to about 30 °N) and possibly S into South China Sea | Lower and middle Mekong system |

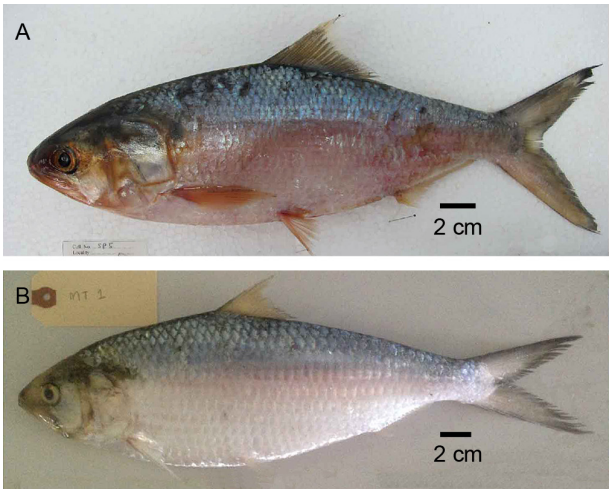


Figure 2. Photographs showing a tropical shad *Tenualosa ilisha* (top) and *T. toli* (bottom) collected in Peninsular Malaysia and Borneo Island of Malaysia, respectively.

to the manufacturer's instructions. An approximately 500 bp section of the 16S rRNA gene was amplified using the primer pair 16SarL (5′-CGCCTGTTTATCAAAAACAT-3′) and 16SbrH (5′-CCGGTCTGAACTCAGATCACGT-3′) (Palumbi 1996). Each PCR reaction contained 2 µl of DNA sample, 2.5 µl of each 10 µM of each primer, 50 µl of the QIAGEN Taq PCR Master Mix, and 43 µl of distilled water. The PCR temperature reaction was 94 °C for 2 min, 30 cycles at 93 °C for 30 s, 55 °C for 40 s, and 72 °C for 1 min, with a final extension at 72 °C for 5 min. All PCR amplicons were purified using the QIAGEN QIAquick PCR Purification Kit, and sequenced bidirectionally with the same primers (First Base Laboratories). Generated sequence trace files were manually edited and assembled using MEGA version 7 (Kumar et al. 2016). The contig sequences were compared for percentage similarity with the reference sequences in the GenBank using BLAST. The ribosomal 16S sequences were deposited in GenBank.

## Results

**Materials examined.** Malaysia: Perak: Kuala Kangsar: Perak River (04°405′13″ N to 04°46′17″ N, 100°56′44″ E to 100°57′35″ E), collected by local fishermen in gill nets, 30 July 2013 (Fig. 1), 5 specimens, sexes indeterminate (UBDTB 1–5, Fig. 2A). Temperature and salinity were ranged from 31.4–31.7 °C and 0.0–0.1 ppt, respectively. GenBank accession no. MH177261 to MH177265.

Malaysia: Borneo: Sarawak: South China Sea: Zone C (12–30 nautical miles off of Miri) (04°26′7″ N to 04°43′55″ N, 112°55′5″ E to 113°46′26″ E), 5 specimens, sexes indeterminate specimens (UBDTB 6–10, Fig. 2B). GenBank accession no. MH177256 to MH177260.

**Identification.** The total lengths and body weights of our specimens were 294–350 mm and 203–450 g for those specimens from Perak River and 266–467 mm and 135–924 g for specimens from off Miri (Table 1). The ratio of caudal fin length to standard length (CFL % of SL) and number of gill rakers (NGR) on the lower arch of the first gill are used as key morphological characteristics to identify *Tenualosa* species. CFL % of SL and NGR of the Perak River and Borneo specimens ranged from 29–32% and 178–228, and from 33–37% and 113–146, respectively (Table 1).

These species can be identified using morphological characters due to their clear morphological differences. *Tenualosa macrura* has a higher CFL % of SL (40–42%) than *T. toli* (31–34%) (Table 2). However, 4 of the 5 specimens and all the specimens from off Miri were out of range for CFL % of SL and NGR of both species (Table 1). Although the specimens from the Perak River showed similar external morphologies to those of *T. toli*, NGR was much more than in *T. toli* (60–100) (Table 2). Based on the morphological key characters and geographical distribution, the 5 specimens from Perak River were identified as *T. ilisha*; 1 specimen from off Miri were identified as *T. toli* and the other 4 specimens from off Miri were of unknown identity (Table 1).

We were able to successfully amplify and sequence the ribosomal 16S region for all 10 samples, revealing definitive identity matches in the range of 99–100% and an alignment E-value of 0.0 for all samples and indicating highly significant similarities. As the samples agreed with the GenBank reference sequences, we were able to identify the 5 samples from the Perak River as *T. ilisha* and the 5 samples from off Miri as *T. toli* (Fig. 2).

## Discussion

Using molecular genetic analysis, we are able to verify the identity of 2 *Tenualosa* species. Initially we could not identify 4 of the 5 specimens from off Miri by morphological characters alone because the key morphological characters were out of range for the species. The

most widespread and well-studied species is *T. ilisha*, which is found from northern Sumatra (Indonesia) west to Kuwait; it is the basis of the most important fishery in Bangladesh, India, Myanmar, Pakistan, and Kuwait (Whitehead 1985, Al-Baz and Grove 1995, Blaber 1997). The closely related *T.* reevesii occurs intermittently along the South China coast and far up the Yangtze, Pearl, and Qiantang rivers in China (Wang 1996). Once widespread, *T. toli* is now found only in the estuaries and adjacent coastal areas of Sarawak (Borneo) (Blaber et al. 1996). *Tenualosa thibaudeaui* lives only in the lower and middle Mekong river system and is believed to be close to extinction (Roberts 1993). *Tenualosa macrura* lives in the coastal waters of Sumatra and Borneo (FAO 2012).

There are few reports on the occurrence of *Tenualosa* species in Peninsular Malaysia. For example, Arai and Amalina (2014) reported *T. ilisha* in Malaysian waters but based their identification only on external morphological characters. However, misidentifications are known to result from damaged or poor specimens and ambiguous morphological features (Arai and Wong 2016). Morphological characters are ineffective to distinguish species at some stages of development (Ward et al. 2009). Several authors have suggested that molecular genetic analyses are necessary for precise species confirmation (Arai et al. 2015, Arai and Wong 2016, Abdul Kadir et al. 2017).

*Tenualosa toli* is in high demand in local markets on Borneo, and it is sometimes exported due to its popular meat and roe. Rahim et al. (2014) reported that the population of *T. toli* has been heavily exploited and is in drastic decline. We recommend that additional studies are needed on species of *Tenualosa* to understand their life history, distribution, and population structure and dynamics for the sustainable use and conservation of *Tenualosa* in Malaysia.

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## Authors' Contributions

All authors conceived the study; TA performed the morphological comparison; RAA and TH performed the molecura genetic analyses. All authors discussed the results and contributed to the final manuscript.

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