

Preliminary findings on Halmahera moony fish (amphidromous goby post larvae) of North Moluccas, Indonesia

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Abstract. Moony fish is the local name given by the coastal community of Halmahera, North Moluccas, for a group of small fishes that live in several river estuaries on Halmahera Island. This study goal was to describe the type of moony fishes found in estuaries of Lako Akelamo River at Sahu and of Tiabo River at Galela Bay on Halmahera Island, North Moluccas, Indonesia. Identification of the fish species was carried out based on morphological analysis approaches, variations in melanophore patterns, and molecular analysis using the cytochrome oxidase subunit I (COI) gene (mitochondrial gene). Sampling was carried out on new moon phase on 21 December 2022 at both study locations using fish caught by local fishers. The collected samples were sorted and grouped based on the melanophore pattern variations. Then, 5 individuals were selected from each group for molecular analysis. From the results of the morphological analysis, 7 groups of melanophore patterns were identified which were coded as L4.1, L4.2, L4.3, L5.1, L5.2, L5.3 and L5.4. Based on the results of genetic analysis, it showed that there were 5 identified fish species, namely L4.1 was *Sicyopterus lagocephalus*, L4.2 and L5.3 were *Sicyopterus cynocephalus*, L4.3 and L5.4 were *Sicyopterus microcephalus*, L5.1 was *Awaous grammepomus*, and L5.2 was *Stiphodon semoni*. Those five species are included in the amphidromous gobies family Gobiidae. The result of this study contributes new data and information on Halmahera fish diversity and development of knowledge in distribution or biogeography of amphidromous gobies in Indonesia.

Key Words: amphidromous, gobies, molecular genetics, nike fish.

Introduction. '*Ikan bulan-bulan*' (moony fish) is the local name given by coastal communities of Halmahera Island for a group of small fish with a size of about 2-4 cm that are usually found during new moon phase at estuary areas. The fish has not been so far scientifically studied. However, it has similarities to '*nike*' fish found in Gorontalo and other parts of Sulawesi Island (Olii et al 2017). Nike fish in Gorontalo are proven to be post larvae and juveniles of several goby species (Sahami et al 2020) included in amphidromous goby group as supported by the discovery of 6 species of the adult phase that make up the nike fish (Sahami & Habibie 2020). Information on Halmahera moony fish has never been published to date. Because the local people have used it for consumption, it is feared that it will disappear without any scientific information. The ecological and social value of gobies has been widely recognized, but many of them are at risk of extinction at regional and global levels (Arthington et al 2016).

Amphidromous gobies have important characteristics as they migrate in two directions, namely between freshwater and seawater habitats in the diadromous fish life cycle. The mother fish spawn in fresh water and shortly after hatching the larvae are washed away by river currents to the sea where they grow and develop for a period of time before return back to rivers to reproduce (Keith et al 2015a; Pezold et al 2016; Iida

et al 2017). Estuaries are essential habitat for amphidromous larvae of Gobiidae and Eleotridae groups, and when they have reached post-larvae they migrate back to fresh waters en masse (Simanjuntak et al 2021).

A number of studies have shown that juvenile amphidromous gobies migrate regularly from sea to river every month. Large assemblages of juvenile gobies were identified when entering estuary areas (Valade et al 2009; Vedra & Ocampo 2014; Ollie et al 2017; Pasingi & Abdulah 2018; Nurjirana et al 2019; Sahami et al 2019a, b; Pangemanan et al 2020; Pasingi et al 2020; Simanjuntak et al 2021). The species composition of an amphidromous gobi school that migrates in river estuaries varies seasonally, where only one species appears in January and 3 species in February (Salindeho et al 2022). A number of factors cause variations in post-larvae migration, namely spawning time, spawning and hatching locations, variations in body size and size of the yolk-sac before the larvae reach the sea, the length of time migration takes, the age at migration, and the condition of the post-larvae body (Egan et al 2019).

Although many studies have been reported on post-larvae of amphidromous gobies, there are no publications on composition of Halmahera moony fish (post larvae of amphidromous gobies). Lack of attention and management efforts can have impacts on the sustainability of this fish in the future. The study of various fish species is expected to contribute to development of knowledge about fish richness in Indonesia (Gani et al 2020). Morphological characteristics are one way to facilitate the identification of fish species, because the differentiation of morphological characters that occur in a species due to adaptation to its environment can lead to changes in morphology (Bhagawati et al 2012). Nike fish species in Gorontalo have morphological variations in the appearance of their melanophore patterns, where one species can have more than one pattern (Sahami et al 2019a, b, 2020).

Identifying the post-larval stage of fish is a major challenge due to their small size and incomplete body composition. Thus, genetic identification is needed, such as molecular and morphometric variations methods for more accurate identification to get more information about Halmahera gobies larvae diversity. Fish larvae are largely ecologically and morphologically different compared to their adult counterparts, along with their developmental processes (Rezagholinejad et al 2016). Genetic identification can be carried out to overcome the ineffectiveness of morphological identification of gobies that do not have a distinctive morphology (Shen et al 2016). Research on the genetic identification of amphidromous gobies post-larvae on Halmahera Island has never been carried out. So this research was carried out to reveal the amphidromous gobies species through molecular genetic analysis of their post-larvae caught in coastal waters and river estuaries.

Material and Method

Sample collection technique. Sampling was carried out at two different locations, namely at Lako Akelamo River estuary, Sahu (01°07'59.3" N - 127°25'40.6" E) and Tiabo River estuary, Galela Bay (00°46'08.5" N - 127°22'41.6" E), on the first new moon phase on December 21, 2022. Sampling locations are presented on map in Figure 1. Fishing was carried out by local fishers when fish appear in schools on both sampling locations.

Moony fish samples were collected using a shovel net fishing gear with a mesh size of 0.5 inches. As much as ±100 grams of sample was filled in a sample bottle and put inside a cool box container that had been given ice. Analysis of the melanophore pattern referred to Sahami et al (2019a, 2020) and was carried out in the laboratory of Fisheries and Marine Sciences Faculty, Sam Ratulangi University, Manado. Samples were separated into several groups, sorted and grouped based on their body melanophore patterns and coded L4 for samples from Lako Akelamo estuary and L5 for samples from Tiabo estuary. Then, 5 individuals were randomly from selected each group and put in sample containers and preserved with 95% alcohol for genetic identification/DNA testing at the BIONESIA (Indonesian Biodiversity) Laboratory in Denpasar, Bali, Indonesia.

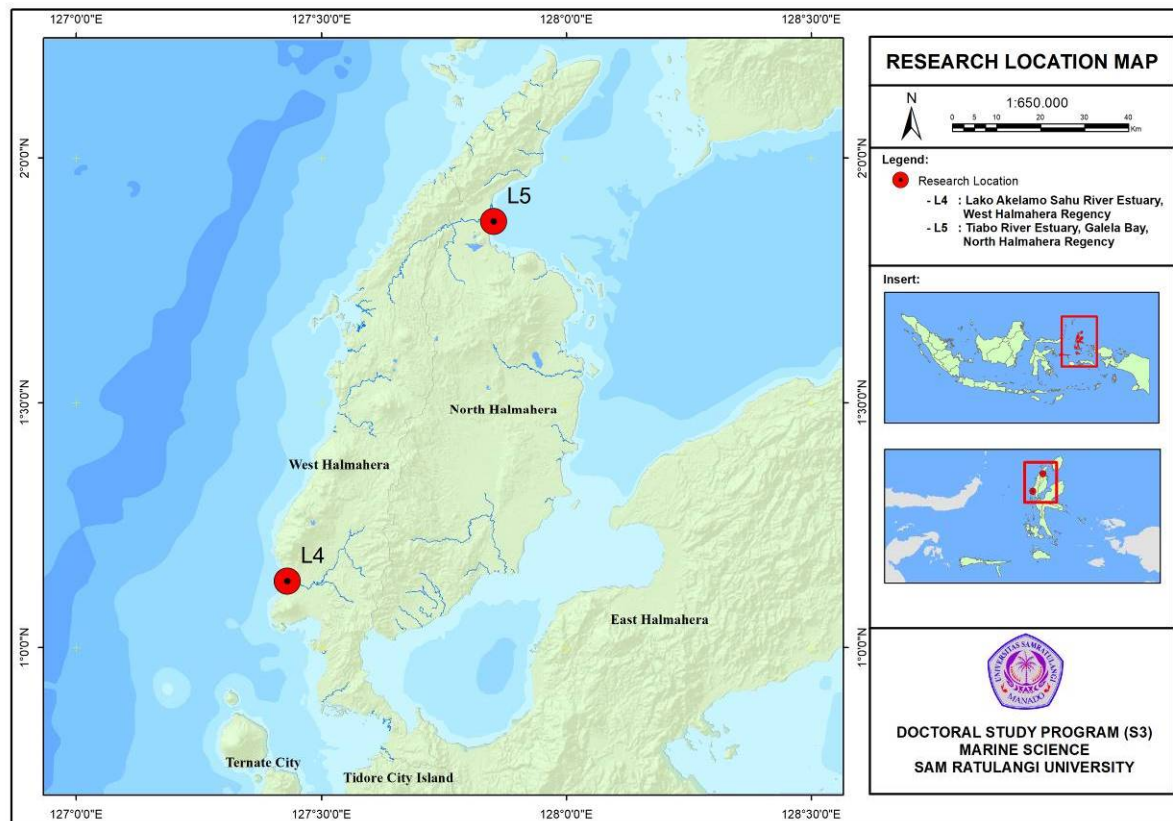


Figure 1. Sampling locations at Lako Akelamo River estuary at Sahu (L4) and Tiabo River estuary at Galela Bay (L5).

Molecular analysis. Approximately 10 grams of tissue sample was taken for the extraction process to isolate DNA. The extraction results are then analyzed to the next stage, namely polymerase chain reaction (PCR). The PCR process used the BIONESIA laboratory protocol using F1/R1 primers. The positive sample (sparkling DNA bands) was then processed for DNA reading (sequencing) using the Sanger dideoxy method at PT Genetics Science Jakarta, Indonesia.


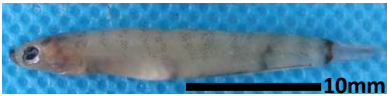





The results of the samples sequencing were then analyzed using computer program. Sequence data obtained was then edited and aligned using the MUSCLE method in the MEGA X program. Finally, the data resulted was then matched with the database in the data bank (Genbank NCBI) through the Basic Local Alignment Search Tools (BLAST) method on the NCBI website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Each data was then recorded based on their similarity and accuracy levels.

In addition to the BLAST method, the data was also analyzed using a phylogenetic tree to see the phylogenetic relationship between samples as well as to confirm the BLAST results in the identification process down to the species level. The kinship tree was created using the Neighbor-joining (NJ) method with bootstrap replication 1,000 times in MEGA X software. Genetic distance values were then analyzed using the p-distance method to compare one sample with another.

Results and Discussion. Based on the morphological characteristics of the samples, 7 different groups of fish were found based on their melanophore patterns, which were coded L4.1, L4.2, L4.3, L5.1, L5.2, L5.3 and L5.4 (as presented in Table 1). The grouping of melanophore patterns in samples L4.2 and L5.3 was different, but in genetic analysis it turned out that both were identical or were the same species as *Sicyopterus cynocephalus*. The same thing was also found in samples L4.3 and L5.4 which had different melanophore patterns but in the genetic analysis they were found as one species namely *Sicyopterus microcephalus*. Valade et al (2009) reported that the

melanophore changes in *S. lagocephalus* during the larval stage could become a problem in morphological identification process.

Table 1
Samples schematic morphological characteristics based on their melanophore patterns

Sample codes	Sample photos
L4.1	
L4.2	
L4.3	
L5.1	
L5.2	
L5.3	
L5.4	

Nike goby fish in the bay of Gorontalo City experienced an increase in melanophore patterns as they headed towards the river mouth (Sahami et al 2019a). Gobies develop various morphological specializations as adaptations to their environment, making it difficult to predict evolutionary scenarios using morphological information alone (Roesma et al 2020). The results of molecular analysis showed that based on nucleotide matching of the 7 sample groups it was confirmed that there were only 5 species with an average length of the nucleotide sequences of the samples obtained were 644-655bp (Table 2).

Table 2
Samples barcode resulted using BLAST using primer FISH-F1/FISH-R1

No	Sample codes	Species	bp	GenBank access number	Query coverage (%)	Identity index (%)
1	BIOSUB197.001 (L4.1)	<i>Sicyopterus lagocephalus</i>	655	HQ639043.1	100	99.85
2	BIOSUB197.002 (L4.2)	<i>Sicyopterus cynocephalus</i>	655	MT706721.1	100	99.54
3	BIOSUB197.003 (L4.3)	<i>Sicyopterus microcephalus</i>	655	KU693043.1	99	100
4	BIOSUB197.004 (L5.1)	<i>Awaous grammepomus</i>	655	KU692311.1	99	98.31
5	BIOSUB197.005 (L5.2)	<i>Stiphodon semoni</i>	655	MT706725.1	99	100
6	BIOSUB197.006 (L5.3)	<i>Sicyopterus cynocephalus</i>	655	MT706721.1	100	100
7	BIOSUB197.007 (L5.4)	<i>Sicyopterus microcephalus</i>	644	KU693055.1	100	99.84

The proofreading results of the forward and reserve sequences were combined with the sequences of 7 samples (L4.1, L4.2, L4.3, L5.1, L5.2, L5.3 and L5.4) and completed with

procedures detailing the nucleotide sequence cycles contained in DNA from 7 different samples. The analysis results showed that the average length of the seven nucleotide network samples was ± 644 -655 bp. Table 2 shows that the Halmahera moony fish school found in two sampling locations consisted of 3 genera and 5 species, namely *Sicyopterus lagocephalus*, *S. cynocephalus*, *S. microcephalus*, *Stiphodon semoni*, and *Awaous grammepomus*. At the Lako Akelamo (L4) estuary, 3 species were found, they were *S. lagocephalus*, *S. cynocephalus*, and *S. microcephalus*. Meanwhile, at the Tiabo estuary (L5), 4 species were found, namely, *Sicyopterus cynocephalus*, *S. microcephalus*, *Stiphodon semoni*, and *Awaous grammepomus*. The results of the study showed that there were similarities and differences in the species that make up the moony fish in both locations. At location L4, *A. grammepomus* and *S. semoni* were not found, while at location L5, *S. lagocephalus* was not found. The species *S. lagocephalus* is the genus *Sicyopterus* with the widest distribution area in the Indo-Pacific region (Keith et al 2015b; Lord et al 2019).

BLAST analysis results showed that all the seven samples collected with the codes L4.1, L4.2, L4.3, L5.1, L5.2, L5.3 and L5.4 had matching DNA nucleotide sequences with data available in GenBank (namely, HQ639043.1, MT706721.1, KU693043.1, KU69211.1, MT70625.1, MT706721.1, KU693055.1, respectively (NCBI2023)). The BLAST analysis result is shown in Table 3 below.

Table 3

The list of DNA nucleotide sequences of each sample based on BLAST analysis

<i>Sample codes</i>	<i>DNC nucleotide sequence</i>
BIOSUB197.001 (L4.1)	CCTGTACCTTGTTTTCGGTGCCTGAGCAGGAATGGTAGGAACTGCCCTAGCCTAC TTATCCGAGCTGAATTAAGTCAACCTGGGGCTCTTCTAGGAGACGACCAAATTTA CAATGTAATTGTTACTGCACATGCCTTTGTAATAATTTTTTATAGTAATACCAATC ATGATTGGAGGCTTTGGGAACTGACTCATCCCTATGATTGGTGCCCCAGATATAG CCTTCCCCGAATGAACAACATAAGCTTTTGACTCCTCCCCCTTCATTCTTCTC CTCCTAGCATCTTCAGGTGTTGAGGCAGGAGCTGGGACTGGCTGAACAGTCTAC CCCCCTTAGCAGGAAACCTTGCCCATGCAGGGGCTTCTGTTGATTTAACTATTTT CTCCCTTACCTAGCTGAAACCTCCTGCAATTTACAATATCAGACACCCCTTTTC GTCTGAGCTGTCCTTATCACAGCAGTTCTACTACTTCTTTCCCTCCCAGTTCTTGC AGCAGGCATTACAATGCTACTGACAGACCCGAAACCTCAACACAACCTTCTTTGAC CCGTCAGGGGGAGGTGACCCAATTCTCTACCAACATCTATTC
BIOSUB197.002 (L4.2)	CCTATACCTTGTTTTCGGTGCCTGAGCAGGAATGGTAGGAACTGCCCTAAGCCTA CTTATCCGAGCTGAACTAAGCCAACCTGGGGCTCTTCTAGGAGACGACCAGATTT ACAATGTAATTGTTACTGCACATGCCTTTGTAATAATTTTTTATAGTAATGCCAATC ATGATTGGAGGATTTGGAACTGACTCATCCCCCTAATGATCGGTGCCCCAGATA TGGCCTTCCCCGAATGAACAACATAAGCTTTTGACTCCTCCCCCTTCATTCTT CTCCTCTGGCATCTTCAGGTGTTGAAGCAGGGGCTGGGACTGGCTGAACAGTC TACCCCTCTAGCAGGAAACCTTGCCCATGCAGGGGCTTCTGTTGATCTGACTA TTTTCTCCCTCCACTAGCAGGTATTTTCATCAATTCTTGGTGCAATTAATTTTATTAC AACTATTCAAATATGAAACCTCCTGCAATTTACAATATCAGACACCTCTCTTCGT CTGAGCTGTTCTTATTACGGCAGTCTACTACTTCTTCTCTCCCAGTTCTTGCAG CTGGCATACAATGCTACTGACAGACCCGAAACCTCAACACAACCTTCTTTGACCC ATCAGGAGGAGGTGACCCAATTCTCTACCAACATCTATTC
BIOSUB197.003 (L4.3)	CCTATACCTTGTTTTCGGTGCCTGAGCAGGAATAGTAGGGACTGCCCTCAGC CTACTCATCCGAGCTGAATTAAGTCAACCTGGAGCTCTTCTAGGGGATGACC AAATTTACAATGTAATTGTTACGGCACATGCCTTTGTAATAATTTTTCTTTATAG TAATACCAATCATGATTGGAGGCTTTGGGAACTGACTTATCCCTAATGATC GGTGCCCCGATATGGCCTTCCCTCGAATGAACAACATAAGCTTTTGACTCC TTCCCCCTTCATTCTTCTTCTTAGCATCTTCGGGTGTTGAAGCAGGGGCT GGAAGTGGCTGAACAGTCTACCCCTCTAGCAGGAAACCTTGCCCATGCAG GGGCTTCTGTTGACTTAACTATTTTCTCCCTCCATCTAGCAGGTATTTTCATCA ATTCTGGGTGCAATTAATTTTATTACAACCATCCTAAACATGAAACCCCTGC AATTTACAATATCAGACACCTCTATTTGTATGAGCTGTTCTAATTACAGCAG TTCTCCTACTTCTCTCCCTCCCTGTTCTTGCAGCTGGAATTACAATGCTACTAA CAGACCCGAAACCTAAACACAACCTTCTTTGACCCATCAGGCGGAGGTGATCC CATTCTACCAACACCTATTC

BIOSUB197.004 (L5.1)	CCTATACCTGGTTTTCGGTGCCTGAGCTGGAATAGTAGGCACAGCTCTTAGC CTTCTCATCCGAGCTGAACTTAGCCAACCTGGGGCTCTTTTAGGAGACGACC AAATCTATAATGTCATTGTAACAGCACATGCATTTGTAATAATTTCTTTATAG TAATACCAATTATGATTGGTGGCTTTGGGAAGTACTAATCCCCCTAATGATT GGTGCCCTGACATGGCCTTCCCCGAATGAATAACATGAGCTTTTACTTC TCCCTCCTTATTCTTTCTTCTCCTAGCATCCTCAGGGGTTGAAGCTGGAGCA GGGACTGGTTGAACTGTTTACCCCCACTAGCAGGGAACCTTGCCCATGCTG
BIOSUB197.005 (L5.2)	CCTATACCTTGTTTTCGGTGCCTGAGCAGGAATGGTAGGCACAGCCCTTAGC CTACTCATCCGAGCTGAACTAAGCCAACCTGGGGCTCTTCTAGGTGACGACC AAATTTATAATGTAATTGTTACTGCACATGCCTTTGTAATAATTTCTTTATAGT AATACCAATCATGATTGGAGGCTTTGGGAAGTACTAATCCCCCTAATGATCG GTGCCCTGACATGGCCTTCCCCGAATAAATAACATGAGCTTCTGACTGCTT CCTCCCTCATTCTTCTTCTCCTAGCCTCCTCAGGAGTTGAAGCTGGAGCTG GGACTGGCTGAACAGTTTACCCCCACTAGCAGGAAACCTTGCCCATGCAGG AGCTTCTGTTGACCTTACAATTTCTCCCTACACTTAGCAGGAATTTCTTCAAT TTTAGGTGCAATTAATTTATTACAACCATTCTAAACATGAAACCCCTGCAAT CTCACAATACCAGACACCCCTGTTTGTCTGAGCTGTCTTATTACAGCAGTTC TACTGCTTCTTCTCTACCTGTTCTTGCAGCTGGCATTACAATGCTACTAACA GATCGAAATCTAAACACAACCTTCTTTGACCCCTCAGGAGGTGGTGACCCAA TTCTTTACCAACACCTATTC
BIOSUB197.006 (L5.3)	CCTATACCTTGTTTTCGGTGCCTGAGCAGGAATGGTAGGAACTGCCCTAAGC CTACTTATCCGAGCTGAACTAAGCCAACCTGGGGCTCTTCTAGGAGACGACC AGATTTACAATGTAATTGTTACTGCACATGCCTTTGTAATAATTTCTTTATAG TAATGCCAATCATGATTGGAGGATTTGGAAACTGACTCATCCCCCTAATGATC GGTGCCCCAGATATGGCCTTCCCCGAATGAACAACATAAGCTTTTACTCC TCCCCCTTATTCTTCTCCTCCTGGCATCTTCAGGTGTTGAAGCAGGGGCT GGGACTGGCTGAACAGTCTACCCCCCTTAGCAGGAAACCTTGCCCATGCAG GGGCTTCTGTTGACCTAACTATTTCTCCCTCCACCTAGCAGGTATTTTCACTA ATTCTTGGTGCAATTAACCTTCACTACAACCTTCTAAATATGAAACCTCCTGCA ATTTACAATATCAGACACCTCTCTTCGTCTGAGCTGTTCTTATTACGGCAGT CCTACTACTTCTTCTCTCCAGTCTTGCAGCTGGCATCACAATGCTACTGA CAGACCGAAACCTCAACACAACCTTCTTTGACCCATCAGGAGGAGGTGACCC AATTCTCTACCAACATCTATTC
BIOSUB197.007 (L5.4)	CCTATACCTTGTTTTCGGTGCCTGAGCAGGAATAGTAGGGACTGCCCTCAGC CTACTCATCCGAGCTGAATTAAGTCAACCTGGAGCTCTTCTAGGGGATGACC AAATTTACAATGTAATTGTTACGGCACATGCCTTTGTAATAATTTCTTTATAG TAATACCAATCATGATTGGAGGCTTTGGGAAGTACTAATCCCCCTAATGATC GGTGCCCCGATATGGCCTTCCCTCGAATGAACAACATAAGCTTTTACTCC TTCCCCCTTATTCTTCTTCTTCTGGCATCTTCGGGTGTTGAAGCAGGGGCT GGAAGTGGCTGAACAGTCTACCCCCCTTAGCAGGAAACCTTGCCCATGCAG GGGCTTCTGTTGACTTAACTATTTCTCCCTCCATCTAGCAGGTATTTTCACTA ATTCTGGGTGCAATTAATTTTATTACAACCCTAAACATGAAACCCCTGC AATTTACAATATCAGACACCTCTATTTGTATGAGCTGTTCTAATTACAGCAG TTCTCCTACTTCTCTCCCTCCCTGTTCTTGCAGCTGGAATTACAATGCTACTAA CAGACCGAAACCTAAACACAACCTTCTTTGACCCATCAGGCGGAGGTGATCC CATCTCTACCA

Genetic identification was carried out to ensure the species composition of the moony fish since post-larvae amphidromous gobies have melanophore patterns that vary in a single species, possibly influenced by their larval development (Sahami et al 2019a). So far, this study is the first scientific report on the species composition of the post-larvae and juvenile stages of the amphidromous gobies (moony fish) found on Halmahera Island.

The phylogenetic tree in Figure 3 shows clearly the kinship of the gobies with the Halmahera moony fish that have been reported and found in this preliminary study. The whole tree forms 3 monophyletic clades as a genus. The first monophyletic clades is the genus *Sicyopterus* which consists of 3 species, namely *S. lagocephalus*, *S. cynocephalus*, and *S. microcephalus*. The second clade is the genus *Stiphodon* with only one species, namely *Stiphodon semoni*. Finally, the third clades is the genus *Awaous* consisting of one species, namely *Awaous grammepomus*. The study finding of those 5 species of

amphidromus gobies in the study two locations, namely at Lako Akelamo estuary (L4) and Tiabo estuary (L5) on Halmahera Island contributes new additional biodiversity data of Halmahera moony fish which has not been disclosed so far.

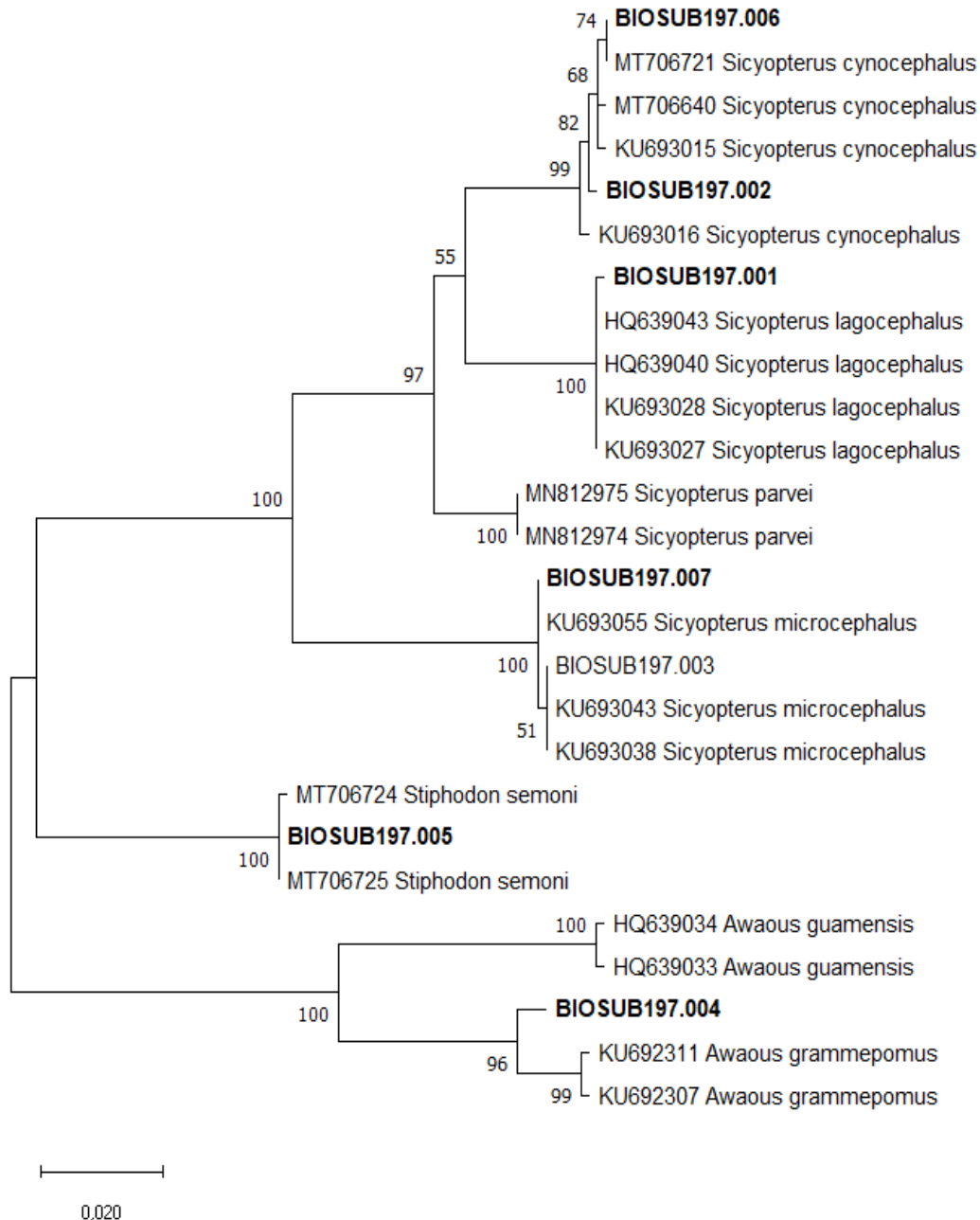


Figure 3. Phylogenetic tree of the five species identified from electrophoresis analysis using FISH-F1/FISH-R1 primers.

Several other studies have found the same 5 species as *S. cynocephalus* in Gorontalo Bay (Sahami et al 2019a, b), in Luwuk Banggai, Central Sulawesi Province (Gani et al 2020), and Poigar River estuary (Pangemanan et al 2020). *S. lagocephalus* was found in Gorontalo Bay (Sahami et al 2019a, b, 2020), in Leppangan estuary, West Sulawesi (Nurjirana et al 2019), Poigar estuary, North Sulawesi (Pangemanan et al 2020; Salindeho et al 2022), and at the Tondano River (Salindeho et al 2022). *A. grammepomus* was also found in the Luwuk Banggai area, Central Sulawesi (Gani et al 2020). *S. semoni* was found in Lampung, Sumatera (Watson 2008), Bengkulu, Sumatera (Maeda & Tan 2013), in Sukabumi, West Java (Dahrudin et al 2017), Gorontalo Bay (Sahami et al 2019a, b), in Leppangan estuary, West Sulawesi (Nurjirana et al 2019), in

Luwuk Banggai area, Central Sulawesi Province (Gani et al 2020), Poigar estuary (Pangemanan et al 2020).

To date, finding of post larvae of *S. microcephalus* was only reported from Lariang Rives estuary in West Sulawesi (Nurjirana et al 2022). Information on species identification and composition is an important part to develop effective fish resources management (Sahami & Habibie 2020). Anecdotal local information indicated that moony fish also occur at several other locations on Halmahera Island but not yet supported by proper scientific data.

Conclusions. This study has confirmed that moony fish, the Halmahera amphidromous gobies, is the same species of amphidromous gobies as Sulawesi nike fish (amphidromus goby post-larvae) based on morphological analysis, melanophore patterns, and DNA analysis. The study found that Halmahera moony fish composed of 5 species, namely *Sicyopterus lagocephalus*, *Sicyopterus cynocephalus*, *Sicyopterus microcephalus*, *Stiphodon semoni*, and *Awaous grammepomus*. The results of this study contribute to Halmahera fish biodiversity data and information and the development of knowledge regarding the biogeography of amphidromous gobies in Indonesia.

Conflict of interest. The authors declare that there is no conflict of interest.

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