

Short Communication:

Presence of the vulnerable freshwater goby *Sicyopus auxilimentus* (Gobiidae, Sicydiinae) on Sangihe Island, Indonesia

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Abstract. Hasan V, Valen FS, Islamy RA, Widodo MS, Saptadjaja AM, Islam I. 2021. Short Communication: Presence of the vulnerable freshwater goby *Sicyopus auxilimentus* (Gobiidae, Sicydiinae) on Sangihe Island, Indonesia. *Biodiversitas* 22: 571-579. A single specimen of freshwater goby *Sicyopus auxilimentus* was photographed and collected using fish traps between 8 and 15 September 2019 in the Laine waterfall, Sangihe island district, North Sulawesi Province, Indonesia. *S. auxilimentus* is amphidromous that live in both freshwater and marine environments. This species is currently listed as Vulnerable (VU) within the IUCN Red List Status. The specimen was identified as male *S. auxilimentus* based on the coloration of the preserved specimen: background yellowish; scale edges brown; posterior flanks and caudal peduncle orange; first dorsal fin black, second dorsal fin dusky black; pectoral fin slightly brown; ventral fin slightly dusky; anal fin blackish; caudal fin dusky brown. Specific morphological features were as follows: the base of the first dorsal fin was not connected to the second dorsal fin base; distance between the base of first and second dorsal fin was generally less than half of eye diameter; ventral fin rays were fused to belly only between fifth rays; posterior margin of caudal fins rays was rounded; scales were all ctenoid; scales appeared on the caudal peduncle, and between anal and second dorsal fins; anterior to which, scales became widely spaced and did not imbricate. Meristic characters were as follows: first dorsal fin rays VI; second dorsal fin rays I+9; ventral fin rays I+5; pectoral-fin rays 14; anal-fin rays I+9; caudal-fin rays 13; scales in lateral series 13; scales in zigzag series 7; scales in transverse series backward 7; scales in transverse series forward 4. This finding is considered the first record in Sulawesi and the fifth from Indonesian waters after findings in Halmahera, Java, Bali and Lombok. This record enhances the understanding of the distribution of *S. auxilimentus* in Indonesian waters. Monitoring is needed to assess the possibility of Sangihe Island being a growth ground, spawning ground, and/or on the migration route of *S. auxilimentus*. In the Laine waterfall, Sangihe island, freshwater conditions were as follows: salinity 3.5 psu, temperature 23-25°C, and dissolved oxygen 7.7-9.2 mg/l, which were ideal habitat for *S. auxilimentus*. *S. auxilimentus* from Sangihe Island, had 0.000 genetic distance than from *S. auxilimentus* from Bali, while the next closest genetic distance was *S. zosterophorus* at genetic distance of 0.090. In addition to onsite conservation, domestication programs are needed to increase commercial availability without depending on natural catches.

Keywords: Biogeography, distribution, freshwater goby, vulnerable

INTRODUCTION

The family Gobiidae (Gill and Mooi 2012; Taillebois et al. 2013; Taillebois et al. 2014) consists of more than 1700 goby species in more than 200 genera inhabiting brackish, marine, and freshwaters (Murdy 2011a; Murdy 2011b; Pezold 2011; Thacker 2011; Thacker and Roje 2011). Gobies are one of the most diverse groups of vertebrates in the world, second only to the Cyprinidae (Patzner 2011; Tornabene et al. 2013). They greatly contribute to the diversity seen in a number of aquatic habitats, including oceanic island streams (Keith et al. 2006; Keith et al. 2015). The Sicydiinae goby is associated with various river or nearshore habitats not only because of the physical habitat that may offer protection or favorable conditions for

their physiology but also because those habitats provide sources of nutrients such as microbenthos, zooplankton, periphyton, and phytoplankton (Hoareau et al. 2012; Boseto et al. 2016; Christoffersen et al. 2019). Estuarine, freshwaters, and bays have been considered important habitats for different life cycle stages of Sicydiinae. These areas often have high productivity, serving as a potential spawning ground and migration route (Taillebois et al. 2012; Iida et al. 2015; Cloern 2018; Islamy and Hasan 2020). Adult Sicydiinae goby can be found in tropical freshwaters in the Indo-Pacific, where they greatly contribute to the diversity of the freshwater fish communities (Lord et al. 2010; Teichert et al. 2013; Ellien et al. 2014). One of the genera from the family of Sicydiinae goby which is the amphidromous fish is

Sicyopus (Watson and Kottelat 1994; Keith et al. 2014a).

Sicyopus are distributed in tropical and subtropical freshwater streams from Fiji, the South Pacific region to the eastern coast of Madagascar, Western Indian Ocean (Watson et al. 2007; Keith et al. 2014b). *Sicyopus* spawn in freshwaters, where the free embryos drift downstream to the sea where they undergo a planktonic phase in the offshore, before returning to the rivers to grow and reproduce (Keith 2003a; McDowall 2007). This marine pelagic larval phase varies between 91 and 265 days, and it is believed to be essential in explaining the spatial and temporal patterns of dispersal (Keith 2003b; Yamasaki and Tachihara 2006; Taillebois et al. 2014). It is assumed to be amphidromous as the other members of the subfamily (Keith et al. 2009; Keith and Lord 2011). This assumption arises from their physiology where the pelvic fins are modified into sucker disks, the soft parts and teeth associated with the jaws and jaw's suspensorium modifications (Harrison 1989; Parenti and Maciolek 1993; Watson 1995; Keith and Lord 2011). These characteristics have facilitated the ability of amphidromous gobies to ascend waterfalls to exploit high island streams (Keith and Lord 2011; Keith et al. 2011b). Many Sicydiine gobies are considered to be rare or endemic to specific regions, but some are wide-spread (Keith et al. 2005; Keith et al. 2014a; Keith and Hadiaty 2014).

The amphidromous nature of *Sicyopus* also indicates that they are sensitive to environmental changes, even of slight impact (Yamasaki and Tachihara 2006; Keith et al. 2009; Thuesen et al. 2011). Overall, it is generally believed that many *Sicyopus* are highly vulnerable or in high risk of extinction in the wild (Keith 2003a; Walter et al. 2012). One of the vulnerable species of this genus is *S. auxilimentus* (Watson and Kottelat 1994). This species is categorized as Vulnerable (VU) by the IUCN because their habitats are located near human settlements (Kottelat 1996). *S. auxilimentus* was recorded in the Indo-Pacific regions, including the Ryuku Islands, Amami-Oshima and Okinawa in Japan (Suzuki et al. 2009), Taiwan (Lin 2007), Leyte island and Cebu in the Philippines (Watson and Kottelat 1994; Chen and Shao 1998), and Halmahera (Watson and Kottelat 2006), Java, Lombok and Bali in Indonesia (Keith et al. 2015). Although the knowledge about *S. auxilimentus* and its distribution has been increasing since the last decades, the full extent of its range in some regions remain unclear, especially in Indonesia. We report the presence of *S. auxilimentus* in the Sangihe island, North Sulawesi, Indonesia, which documents an extension of the known distribution range for this species. The movement and migration patterns of *S. auxilimentus* in time and space are fundamental to the study of its habitat ecology, interpreting of the influence of human activities on these species, and in design of effective resource management and conservation (Ebner et al. 2011; Keith et al. 2015; Boseto et al. 2016).

Currently, the studies and the confirmation of species are no longer only based solely on morphological data. Researchers increasingly combine morphological and molecular data as well as, environmental, geographical, and behavioral data to refine and improve species delineation

(Padial et al. 2010; Keith et al. 2011a). The molecular barcoding approach using Cytochrome c oxidase I (COI) has been classically used (Ratnasingham and Hebert 2007). Partial COI allows the genetic identification of species in numerous taxa. Divergence, which appeared by mutation over time, between different individual's sequences is low for individuals of the same species and high between individuals of a different species (Imtiaz et al. 2017; Md-Zain et al. 2018a; Md-Zain et al. 2018b; Roesma et al. 2019; Nuryanto et al. 2019). This paper also provides a description based on morphometric, meristic, and molecular data (COI) of *S. auxilimentus* from Sangihe Island, North Sulawesi, Indonesia.

MATERIALS AND METHODS

Sampling site

The site is in the Laine waterfall (Figure 1), Sangihe Island District, North Sulawesi Province, Indonesia (3°25'52"N; 125°35'55"E), about 0.5 km away from the mouth of the river. The site is located in an area used for tourism and nearby fishery activities. Sangihe Island is a remote island in the Sulawesi (Celebes) Sea and bordered by Philippines.

Fish collection and water quality

Fish collection could be freely carried out without special permits, but the locals prohibit the use of non-environmentally friendly fishing gear (Kolding and van Zwieten 2014; Hasan and Widodo 2020). Four fish traps with a size of 90x90x40 cm³, made of wire and mesh with dimension of 3 cm were used in this study. The study was conducted from 8 to 15 September 2019 with two traps deployed at each of two different depths, of 0.5 m and 0.8 m of the river bottom. The traps were left for one week, and checked daily for trapped fish. Fish caught were collected from all four traps and brought to the laboratory for the next step. The water quality parameters included salinity (psu), temperature (°C), and dissolved oxygen (mg/l), were determined in situ at each location immediately after capture events.



Figure 1. Laine river, location where *S. auxilimentus* found in Sangihe Island, North Sulawesi, Indonesia

Preserve fish and identification

Sicyopus auxilimentus were killed before the identification. Fish killing is considered an ethical treatment of a live fish that will be used for scientific purposes (Wall 2001; Metcalfe and Craig 2011). The pectoral fin on the right body was cut for DNA extraction. The right side was chosen for this and other similar operations, such as the removal of scale samples, because the left side was used to obtain morphometric data and is commonly shown in photographs. The collected specimen was labeled and fixed in 96% alcohol solution (Hasan and Tamam 2019). Materials examined were deposited at the zoology laboratory, Generasi Biologi Indonesia Foundation (GBI0044). Diagnostic morphological characters of the specimen were analyzed following Watson and Kottelat (1994) and Keith et al. (2015). The determined meristic characters include the first dorsal fin rays, second dorsal fin rays, ventral fin rays, pectoral fin rays, anal fin rays, caudal fin rays, scales in lateral series, scales in zigzag series, scales in transverse series backward, and scales in transverse series forward. Meristic characters of specimen were measured using digital calipers to the nearest 0.1 mm.

DNA extraction, isolation, and amplification

The sample extraction of DNA used the Genomic DNeasy Blood & Qiagen Tissue Kit. Amplification of cytochrome C oxidase subunit I (COI) gene was conducted with the AmplyTaq Red™ (Applied Biosystems) and the universal fish DNA-Barcoding primers Fish_F1 and Fish_R1 (Ward et al. 2005).

Fish F1-5'TCAACCAACCACAAAGACATTGGCAC3';
Fish R1-5'TAGACTTCTGGGTGGCCAAAGAATCA3'.

The temperature profile at the time of amplification included: initial denaturation of 94°C for three minutes, 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 60 seconds, with a final extension of 72°C for two minutes. The PCR results were then visualized in 1% agarose gel via electrophoresis by staining Nucleic Acid Gel Stain (GelRed®).

Species were identified by comparing the sequences to the GenBank NCBI (National Center for Biotechnology Information) via BLASTn (Basic Local Alignment Search Tool-nucleotide) method to analyze a sequence homology. The history of evolution was concluded using the Neighbor-Joining method (Saitou and Nei 1987; Ardura et al. 2010; Lakra et al. 2011) with a bootstrap test of 10000 replications (Felsenstein 1985) and the evolutionary distance was calculated using the p-distance method (Nei and Kumar 2000) in MEGA6 (Tamura et al. 2013).

RESULTS AND DISCUSSION

New record

Indonesia: Sulawesi: North Sulawesi Province: Sangihe Island: Tahuna District: Laine Waterfall: rapid and boulder-strewn streams with rocky bottoms, (3°25'52"N; 125°35'55"E), 15 October 2020, single male specimen 7.9 cm, adult male, photographed.



Figure 2. Fixed specimen of male *Sicyopus auxilimentus* (GBI0044) found in Laine river in Sangihe Island, North Sulawesi, Indonesia

Scientific classification

| | |
|------------|--|
| Kingdom | : Animalia |
| Phylum | : Chordata |
| Class | : Actinopterygii |
| Order | : Gobiiformes |
| Family | : Gobiidae |
| Subfamily | : Sicydiinae |
| Genus | : <i>Sicyopus</i> |
| Species | : <i>Sicyopus auxilimentus</i> (Watson and Kottelat 1994) (Figure 2) |
| Local name | : Ikan Gobi |

Meristic and morphological characters of *Sicyopus auxilimentus* collected from Sangihe Island

Meristic characters of *S. auxilimentus* are given in Table 1. Other specific morphological characters of male *S. auxilimentus* are as follows: the base of the first dorsal fin was not connected to the base second dorsal fin; the distance between the base of first dorsal fin and second dorsal fin was generally less than half of the eye diameter; ventral fin rays were fused to belly between fifth rays only; caudal fin rays posterior margin were rounded; scales were all ctenoid; scales presented on caudal peduncle and between anal and second dorsal fins; while anterior to this, scales became widely spaced and did not imbricate. Coloration in preserved specimen was as follows: background yellowish; scales edge in brown; posterior part of flanks and caudal peduncle orange; first dorsal fin black, second dorsal fin dusky black; pectoral fin slightly brown; ventral fin slightly dusky; anal fin blackish; and, caudal fin dusky brown.

Table 1. Meristic of male *Sicyopus auxilimentus* collected from the Laine river in Sangihe Island, North Sulawesi, Indonesia ($n = 1$ specimen)

| Meristics (counts) | Present study | Keith et al. (2015) |
|--------------------------------------|---------------|---------------------|
| First dorsal fin rays | VI | VI |
| Second dorsal fin rays | I+9 | I+9 |
| Ventral fin rays | I+5 | I+5 |
| Pectoral fin rays | 14 | 14-15 |
| Anal fin rays | I+9 | I+9 |
| Caudal fin rays | 13 | 13 |
| Scales in lateral series | 19 | 18-23 |
| Scales in zigzag series | 7 | 6-11 |
| Scales in transverse series backward | 7 | 4-12 |
| Scales in transerves series forward | 4 | 2-7 |

Contemporary records and their habitat

The discovery of *S. auxilimentus* in the Sangihe island is a new record of this species in addition to its previous records. Several studies on freshwater goby in Indonesian waters have sometimes been limited to a single island. As is the case for *S. auxilimentus* records in Indonesian waters, Watson and Kottelat (1994) have recorded *S. auxilimentus* in Halmahera, then Keith et al. (2015) recorded *S. auxilimentus* in East Java, Bali, and Lombok (Figure 3). Among other biological topics, new records of vulnerable fish contribute to understanding species diversity and biogeography (Hasan et al. 2019a,b; Saptadaja et al. 2020; Ihwan et al. 2020). As reported in this paper, the new record of *S. auxilimentus* has improved knowledge of the species, as it extends recorded distribution range of the species (Thuesen et al. 2011; Maeda and Palla 2015).

The presence of *S. auxilimentus* in Sangihe Island indicates that the freshwater ecosystems in Sangihe island are in a good state. Compared to Western Indonesia, inland waters in Sangihe island are protected from heavy pollution, and this island has many clear streams. In the future, data collection, i.e., assisted by local fishermen is

needed to assess the occurrence of *S. auxilimentus* and to evaluate the importance of small islands in Sulawesi waters as part of the migration route, nursery ground, and growth ground of this species.

The life history of *S. auxilimentus* is strongly dependent on freshwater. This species has the osmoregulatory plasticity to acclimate to the marine environment and prefers low salinity of inland river waters. A reason for this may be to avoid predators, and to take advantage of prey abundance in lower-salinity areas, rather than because of a physiological constraint. This species spawns in freshwaters, with the embryos drifting downstream to the sea were to undergo a planktonic phase, before returning to the rivers to grow and reproduce, and hence are referred to as amphidromous (McDowall 2007; McDowall 2009a). Details of their biological cycle and the parameters leading to such extreme evolution in amphidromous gobies are poorly known (Lord and Keith 2007; McDowall 2009b), despite the fact that these gobies contribute most to the diversity of fish communities in the Indo-Pacific, have the highest levels of endemism (Nelson et al. 1997; Keith and Lord 2011).

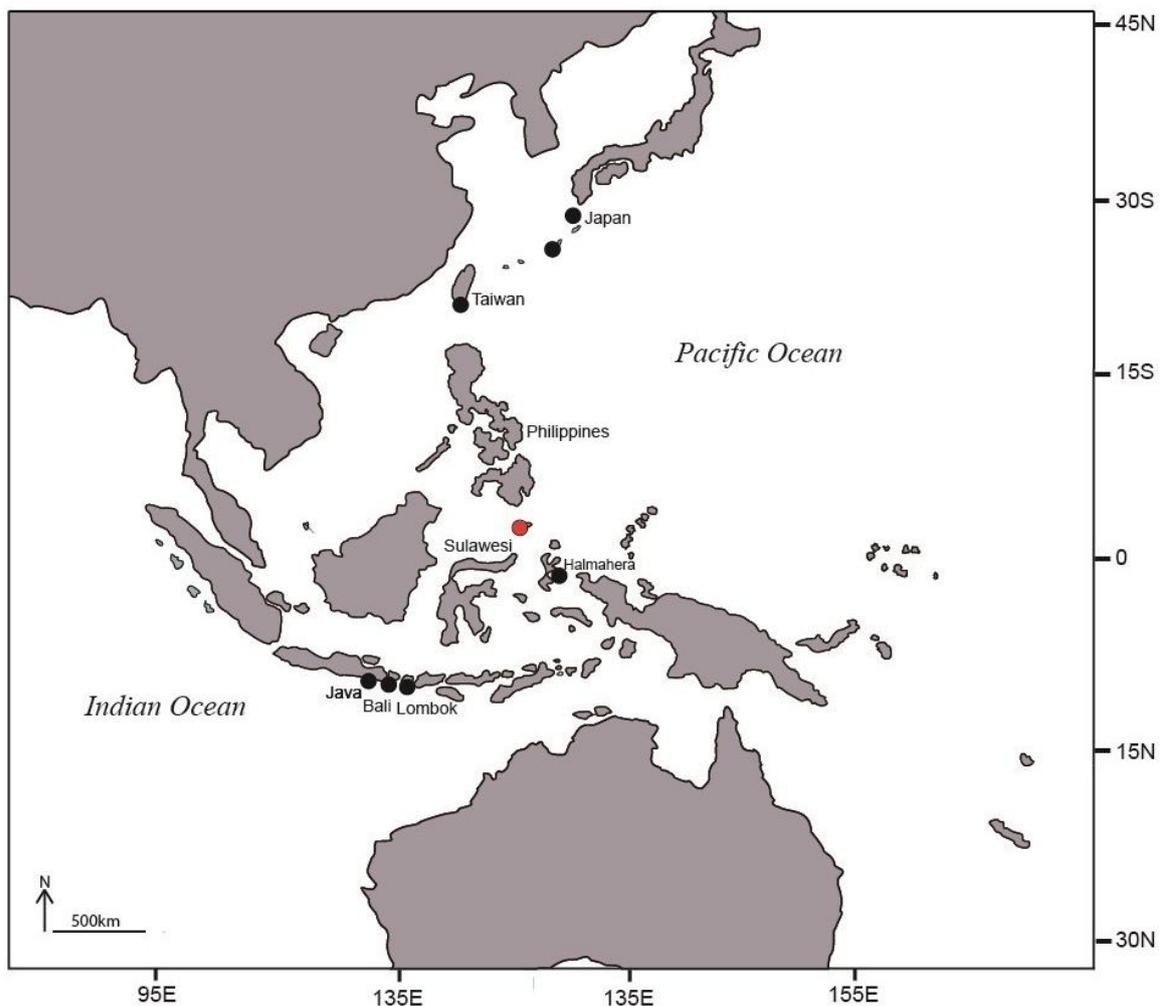


Figure 3. Distribution of *S. auxilimentus* in Indo-Pacific regions. Black circles are the previous record. Red circle is the recent record from Sangihe Island, North Sulawesi, Indonesia

Environmental factors influencing the movement of *S. auxilimentus* have received little attention, even though the study of these factors is important to help predict how these gobies will respond to increasing modifications of aquatic systems (Jenkins et al. 2009; Ebner and Thuesen 2011; Ebner et al. 2011). Several environmental factors may interact to influence *Sicydiinae* movement patterns, including water temperature, dissolved oxygen, and salinity. Water quality parameters may also play an indirect role in *S. auxilimentus* movement due to the influence of fluctuating conditions on the distribution of prey species. Water conditions of Laine waterfall, Sangihe island, such as salinity 3.5 psu, temperature 23-25°C, and dissolved oxygen 7.7-9.2 mg/l, were ideal for *S. auxilimentus* habitat (Bell and Brown 1995; Ellien et al. 2011; Keith et al. 2011a; Watanabe et al. 2014; Muthiadin et al. 2020). Future research, incorporating long-term trends with these short-term results, would help form a more complete understanding of the degree to which environmental parameters influence movement of the species. The role of temperature and salinity in presence of *S. auxilimentus*, when observed over a longer period, will reveal the part played by seasonal variation.

Utilization of *Sicyopus auxilimentus*

S. auxilimentus is not the main commodity of fisheries capture on Sangihe Island because their numbers are very low. Several other fish were caught during the sampling apart from *S. auxilimentus*, including Tank goby *Glossogobius giuris*, Throat-spine gudgeon *Belobranchius belobranchius*, Golden tank goby *Glossogobius aureus*, Scribbled goby *Awaous grammepomus*, Broadhead sleeper *Eleotris melanosome*, Sharptail goby *Oligolepis acutipennis*, Milkfish *Chanos chanos*, Sleepy goby *Psammogobius biocellatus*, Puntang goby *Exyrias puntang*, Barramundi *Lates calcalifer*, Northern mud gudgeon *Ophiocara porocephala*, Eyebrow goby *Oxyurichthys ophthalmonema*, Hasselt's goby *Callogobius hasseltii*, Jungle perch *Kuhlia rupestris*, Dark-margined flagtail *Kuhlia marginata*, Flathead grey mullet *Mugil cephalus*, Bearded roguefish *Tetraroge barbata*, Long tongue sole *Cynoglossus lingua*, Buffon's river-garfish *Zenarchopterus buffonis*, Snakehead gudgeon *Giuris margaritacea*, Dusky sleeper *Eleotris fusca*, and Nile tilapia *Oreochromis niloticus*. All the brackish and freshwater fishes caught were native fish of Indonesia, except for *O. niloticus* (Hasan et al. 2019c; Insani et al. 2020). There is no official record of how many *S. auxilimentus* are caught because they are not a targeted species for Indonesia commercial fisheries, even though *S. auxilimentus* is in great demand as an ornamental fish, i.e., by hobbyists from abroad (Delventhal 2003; Maeda et al. 2011; Mukai 2011; Hubert et al. 2015). Unique body shape and striking color patterns can make this species quite expensive. The Indonesian government needs to strictly prohibit the practice of catching *Sicydiinae* goby, especially *S. Auxilimentus*, Such that if the fish are caught as part of commercial catch, they can immediately release again, so that the population of *S. auxilimentus* remains stable in Indonesian waters.

The collection of only one male individual of *S. auxilimentus* in a timeframe of four days of sampling at Laine river, Sangihe island during our study, suggests that this species, like its subfamily, is rare. In addition, there is worry that the location where this species is found is very close to tourist areas. Cooperation among the government, researchers, and local people is needed to protect the waters in Sangihe island so that the *S. auxilimentus* population remains stable.

Conservation status

The range of *S. auxilimentus* can determine the species' potential interaction with fisheries and its vulnerability to human intervention. Because it lives in inshore, estuarine, and freshwater regions, close to human settlements, it is vulnerable to human activity. *S. auxilimentus* is not currently legally protected in any part of its distribution area. Further research is necessary to better characterize its ecological, biological, and fishery significance (Kottelat 1996; Keith 2003b; Palidoro et al. 2010; Walter et al. 2012).

Sicyopus auxilimentus is categorized as Vulnerable (VU) by the IUCN because their habitats are located near human settlements (Kottelat 1996). The consequences of human actions to freshwater and marine ecosystems have become apparent with the profound, recent global declines of fish population, including *S. auxilimentus*. However, despite their ecological significance, populations of many species of *Sicydiinae* are declining on a global scale, mainly due to unsustainable and illegal fishing practices (Devick et al. 1995; Keith 2003a; Walter et al. 2013). In addition, the entry of invasive species can cause the loss of local fish populations in Indonesia (Hasan et al. 2019c; Hasan et al. 2020a; Hasan et al. 2020b; Hasan et al. 2020d; Serdiati et al. 2020).

Freshwater gobies such as *S. auxilimentus* are potentially highly vulnerable to urban habitat modification. Freshwater habitats have more limited volume, a disadvantage in resisting changes in physicochemical properties (Ceccarelli et al. 2013; Potter et al. 2015). Because of their association with freshwaters, coastal, and estuarine environments, *S. auxilimentus* are also susceptible to both the habitat degradation and influence of climate change (Bell 2007; Pankhurst and Munday 2011; Chabarría and Pezold 2013). In the future, more complete data collection is needed to assess the occurrence of *S. auxilimentus* and to evaluate the importance of Sangihe waters as breeding areas, growth ground, and migration routes (Yamasaki and Tachihara 2006; Keith et al. 2011a; Walter et al. 2012).

DNA barcode and species confirm

DNA-barcoding of freshwater goby from Sangihe island was successfully sequenced with a base-pair length of 672 bp (Table 2). According to Ward et al. (2005), a total of 207 DNA barcoding fish species in Australia is identified using Fish_F1 and Fish_R1 primers obtain 655 bp of COI gene. Fragments that have more than 658 base pairs of COI genes can be used as a standard to distinguish

species (Hebert et al. 2003; Ivanova et al. 2007). The goby specimen from Sangihe island with a base pair length of 672 bp, therefore fulfilling the standard for species identification using COI genes, and the sequence results could be analyzed for similarity with species in GenBank to confirm the species.

The specimen of freshwater goby from Sangihe island was compared to the GenBank NCBI (National Center for Biotechnology Information) to match the similarity of the species via BLASTn (Basic Local Alignment Search Tool-nucleotide) method (Sutarno et al. 2017). The results showed that the specimen from Sangihe island had an identical sequence to the specimen from Bali (KU693082) and East Java (KU693086.1) named *S. auxilimentus* with a level of similarity of about 99-100% (Table 3), where species with 99-100% similarity level are identical (Hebert et al. 2003; Dahrudin et al. 2017).

Phylogenetics

The phylogenetic tree of the freshwater goby genus involved 10 nucleotide sequences. One (1) sequence from Sangihe Island, Indonesia, while nine (9) sequences were downloaded from the GenBank. Species from Sangihe island Indonesia (marked with a red circle) is in the same group with *S. auxilimentus*, which means that the species found in Sangihe island is *S. auxilimentus* (Figure 3).

Sicyopus auxilimentus from Sangihe island had 0.000 genetic distance to the *S. auxilimentus* from Bali island, with no mutation among them, which means that they are identical. The genetic similarity between the *S. auxilimentus* from Sangihe island and Bali island could happen because the two islands are in the same ocean currents, allowing *S. auxilimentus* larval movement from Sangihe to Bali. The closest genetic distance of *S. auxilimentus* from Sangihe island in the same genus is with *S. zosterophorum*, with a genetic distance of about 0.090 (Table 3).

Table 2. DNA barcoding of *Sicyopus auxilimentus*

| DNA barcoding | |
|---|--|
| CCTGNCTTGTTCGGTGCCTGAGCAGGAATGGTAGGCACAGCCCTTAGCCTGCTTATCCGAGCTGAATTAAGTCAACCTGGAGCTCTTCT | |
| AGGGGACGACCAAATTTACAATGTAATTGTTACTGCACATGCCTTGTAATAATTTCTTTATAGTAATGCCAATTATGATTGGGGGATTT | |
| GGAAACTGACTAATCCCTCTAATGATTGGCGCCCTGACATGGCCTTCCCTCGAATGAACAACATGAGCTTTTGACTTCTCCCCCATCAT | |
| TCCTTCTCCTACTAGCATCCTCGGGTGTGAAGCTGGAGCAGGAACCTGGCTGAACAGTTTACCCACCCCTAGCAGGAAACCTTGCACATGC | |
| CGGGCTTCTGTTGACCTAACAATTTCTCACTCCACTTAGCAGGTATTTTCATCAATTTTAGGTGCTATTAATTTTATTACAACAATCCCTA | |
| AACATGAAACCCCTGCAATCTCACAATACCAGACACCCTATTGTCTGAGCTGCTCATTACAGCAGTCCTTCTGCTTCTCTCCCTAC | |
| CAGTGCTTGCAGCTGGCATCACAATGCTACTGACAGACCGAAACCTAAACACAACCTTCTTTGACCCTT CAGGAGGAGGTGATCCAATTTCT | |
| TTACCAACATCTATTCTGATTTCTTGGCCACCAAAAAGTCTAA | |

Table 3. Species identification and similarity

| Specimen | Similarity GenBank | Species identified | Accession number (GenBank) |
|---------------------------|--------------------|------------------------------|----------------------------|
| <i>Sicydiinae</i> Sangihe | 100% | <i>Sicyopus auxilimentus</i> | KU693082.1 |
| | 99.84% | <i>Sicyopus auxilimentus</i> | KU693087.1 |

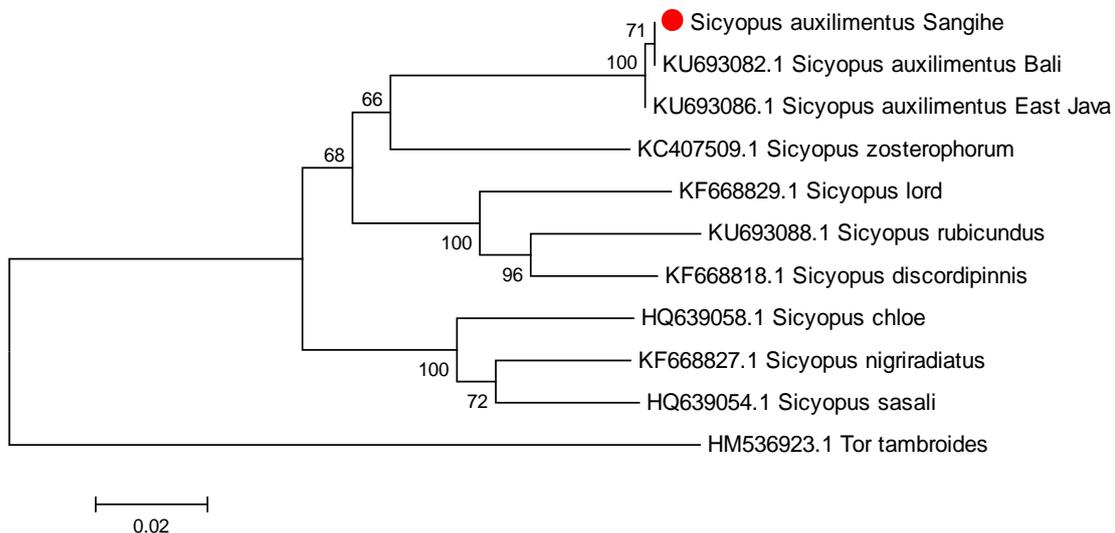


Figure 3. Phylogenetic tree of *Sicyopus auxilimentus* based on COI Gene

Domestication efforts

S. auxilimentus has an important economic value, especially for aquarium trade (Mukai 2011; Maeda and Tan 2013). They are rare in several regions in the Indo-Pacific island and are mostly wild catch, whether as juveniles or adults, without any aquaculture program. A comprehensive study of the biology of the fish is necessary before carrying out a domestication program (Hasan et al. 2019d; Widodo et al. 2020; Valen et al. 2020). Mapping potential on natural distribution is one of the necessary strategies (Hasan et al. 2019b; Hasan and Islam 2020), and aspects of the environment, reproduction, and food availability must be taken into account (Herawati et al. 2016; Hasan et al. 2020c; Saptadajaja et al. 2020). Until now, the study of *S. auxilimentus* remains very limited, so opportunities to study it are open to support *S. auxilimentus* domestication effort.

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