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Record of *Hemiramphus lutkei* Valenciennes, 1847 from Seram Sea, Maluku, Indonesia with its molecular characterization

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Abstract. *Talakua M, Suadi, Djumanto, Setyobudi E. 2022. Record of* Hemiramphus lutkei *Valenciennes, 1847 from Seram Sea, Maluku, Indonesia with its molecular characterization. Biodiversitas 23: 1560-1566. Hemiramphus* sp. is a fish species widely distributed throughout the world. However, the area distribution of each species differs between regions. *Hemiramphus archipelagicus* could be found in Seram Sea, Maluku and Ekas Bay, Lombok, *Hemiramphus sp.* from North Maluku Sea, and *H. brasiliensis* from the North Minahasa Sulawesi. The identification of *Hemiramphus* in Indonesia uses mainly the morphological approach. This study aims to determine the morphometric and meristic character and identify the halfbeak collected from the Seram Sea, Maluku, using a molecular approach. A total of 214 fish samples were used to determine the morphometric and meristic characters of halfbeak, following the measurement method that has been developed previously. All samples of halfbeak showed similar morphological characteristics. Identification by direct sequencing of the cytochrome c oxidase subunit I (COI) gene was conducted from selected samples. The halfbeak caught in the Seram Sea had an elongated flat body shape, the dorsal part of the body is blue-green, the tip of the lower beak is sharp, enlarged towards the base in the oral cavity; the lower beak is reddish-orange and longer than the upper beak. The fin formula of halfbeak from the Seram Sea is D.13-15, P.9-12, V.5-6, A.9-12, C.7/8, and the number of scales at the lateral line is 52-58. Based on molecular identification, the halfbeak species from the Seram Sea was confirmed as *Hemiramphus lutkei* with a genetic distance of 0.0055 compared to *H. lutkei* from India, and a genetic distance of 0.0540 with *H. balao.*

Keywords: COI, diversity, garfish, Maluku, taxonomy, zoogeography

INTRODUCTION

Halfbeak (*Hemiramphus* spp., Family: Hemiramphidae) is a fish species widely distributed throughout the world. Most halfbeak species are marine, but some inhabit freshwater (Collette 1999). The groups of halfbeak are found in the coastal waters of islands and continental coastlines, especially in rich vegetation areas. Halfbeak is an omnivorous fish (Tabassum et al. 2017); the adults consume mainly seaweed, while in the earlier stages, they consume zooplankton, green algae, and diatoms (Akyol and Ertosluk 2019). In a particular region, halfbeak has become a commercially important fish commodity. For example, the black-barred halfbeak is an important species in Pakistani waters (Tabassum et al. 2017). Ballyhoo halfbeak is commonly caught in the coastal waters of Rio Grande do Norte Brazil (Oliveira et al. 2015). This fish is also an important fish commodity in the Maluku Islands (Ahmad et al. 2019; Natan et al. 2019), mainly caught using small circular nets (mini purse seines) called "*soma roa* and *giop*".

The taxonomy and position of members of the genus *Hemiramphus* are still developing today. Of the dozens of species that are considered members of the genus *Hemiramphus*, only 11 species are recognized as valid species. Additionally, synonyms are also found in several species that were previously regarded as separate species. For example, following further identification of *H. fasciatus* and *H. japonicus*, these two species are recognized as synonyms of *H. lutkei*. Halfbeak shows the differences in the distribution of each species (Akça and Bilecenoglu 2010; Falautano et al. 2014; Hata et al. 2018; Zhu et al. 2018). Many references state that some species have a specific geographic distribution. *Hemiramphus archipelagicus* has been distributed in the Indo-West Pacific, East Africa, Madagascar east to the Cook Islands north to Taiwan. In contrast to *H. balao*, this species is widespread in the western and eastern Atlantic. *H. Hemiramphus robustus* is found mainly in the Eastern Indian Ocean, western Pacific, and tropical Australia south to Rottnest Island (Western Australia) and southern Papua New Guinea (Fricke et al. 2022).

The Indonesian archipelago extends from the west in the Indian Ocean to the east in the Pacific Ocean, which might be the habitat for various *Hemiramphus* species. However, the diversity and number of *Hemiramphus* species living in this area are still unknown. The presence of *Hemiramphus* in Indonesia has been reported by several studies (Kawimbang et al. 2012; Suryawan et al. 2016; Ahmad et al. 2019; Natan et al. 2019). Previously, fish identification was conducted mainly by a morphological approach based on morphometric and meristic characteristics. However, this method has challenges due to the similarity of shape, size, and color of some species (Allken et al. 2019). Nowadays, molecular approaches have been widely used to support the morphological method in fish identification (Milana et al. 2011). Taxonomic analysis based on morphological and molecular (DNA-based) approaches is needed to obtain an accurate identification (Srihari et al. 2020). The DNA approach is recognized as the most promising method to identify species levels appropriately and can be used for studying genetic diversity and the degree of variation between species (Imtiaz et al. 2016). This research aimed to determine the morphometric and meristic characteristics; and identify the halfbeak collected from the Seram Sea, Maluku, using a molecular approach. Precise taxonomic identification and species determination are essential for bio-ecological studies and sustainable halfbeak fisheries management.

MATERIALS AND METHODS

Location and fish collection

Fish samples were collected from two locations at Kaiely Bay (Ubung Village of Lilialy Sub-district and Waelapia Village of Kaiely Bay Sub-district), Buru District, Maluku Province, Indonesia (Figure 1). In total, 214 fish caught by fishermen were taken randomly for morphological, morphometric, and meristic characterization. Then, the operculum, pelvic fin, and scales of four selected samples were preserved in absolute ethanol (96%) for further molecular analysis.

Morphological identification

Morphological and meristic characterization was carried out using a modification of previously developed methods (Marčeta 2016; Gonzalez-Martinez et al. 2020). Data collections do not differentiate between male and female fish. The body structure parts were measured at their length using a digital caliper with an accuracy of 0.1 mm. Then the samples were weighed using a digital scale with an accuracy of 1.0 gr. The measurement of fish body parts for morphometric characterization is shown in Figure 2.

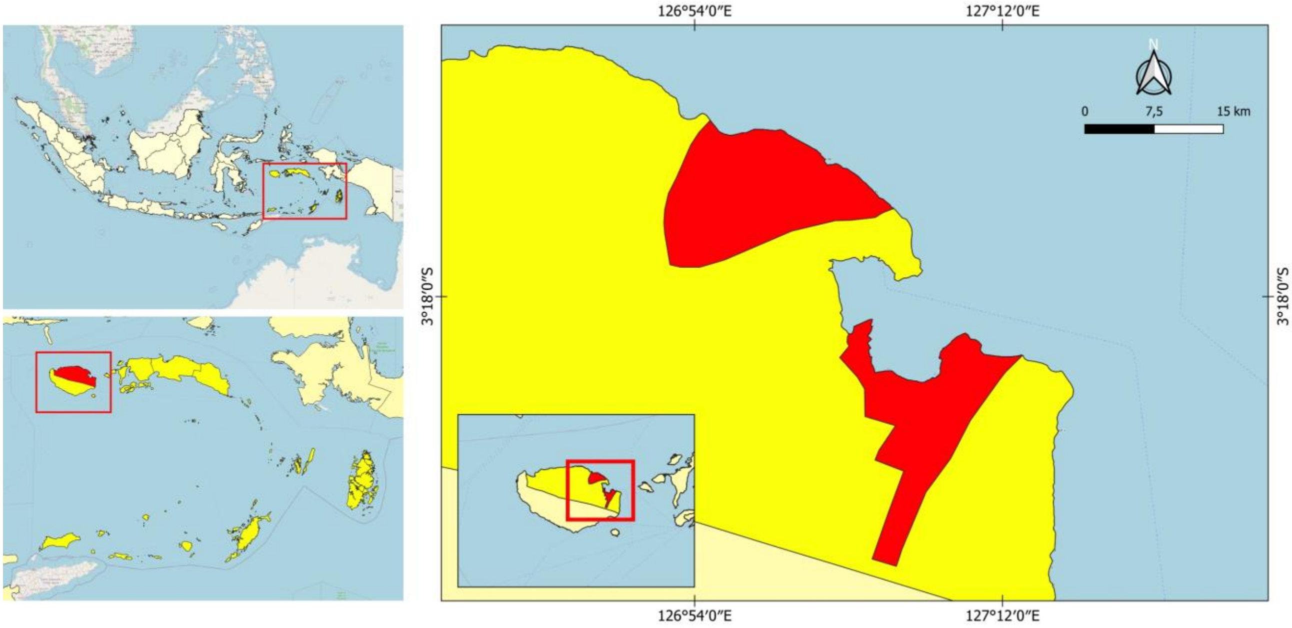

Figure 1. Sampling locations of halfbeak in the Seram Sea, Maluku, Indonesia

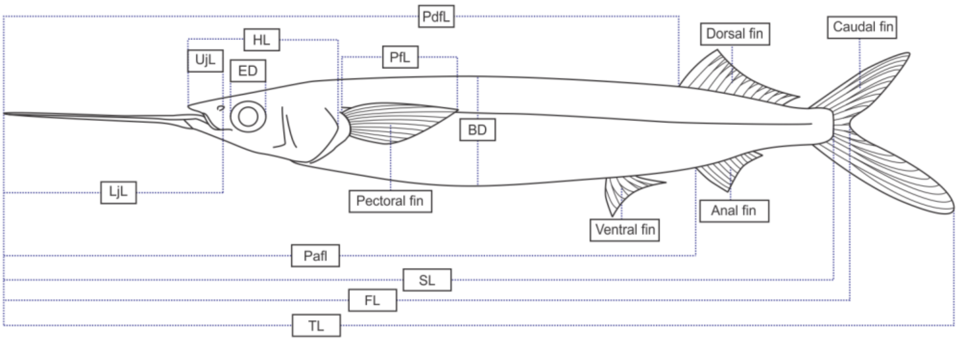

Figure 2. Measurement of the morphometric characters of halfbeak. Note: TL: total length; SL: standard length; FL: fork length; HL: head length; BD: body depth; BW: body width; PfL: pectoral fin length; pDfL: pre-dorsal fin length; LjL: lower jaw length; pAfL: pre-anal fin length; UjL: upper jaw length; ED: eye diameter

Molecular identification

Molecular identification was performed using four selected samples. DNA extraction was carried out using the Tissue Genomic DNA Extraction Mini Kit (Kit Favorgen) according to company instructions. DNA extraction results were amplified by a PCR method targeting the cytochrome c oxidase subunit I (COI) genes, using a forward primer: HCO2198 5' TAAACTTCAGGGTGACCAAAAAAATCA 3' and a reverse primer, LCO1490 5' GGTCAACAAATCA TAAAGATATTGG 3'. Amplification was conducted using Thermal cycler (Biorad, T100TM) under PCR conditions were set as follows: 94°C for initial denaturation (5 min), and followed by 34 cycles of 94°C (30 s), 46°C (30 s), 72°C (1 min), and the final extension at 72°C (12 min). Five microliter volumes of PCR product was run on 1% agarose gel supplemented with 0.75 µL Flourosave DNA stain (1st base) to visualize COI bands. Electrophoresis was carried out for 15 minutes, and the result was then photographed on a UV Transilluminator (Pacific Image, Electronic). The PCR product was sequenced using a DNA sequencing service company (Singapore's 1st Base Laboratory through PT Genetika Science Indonesia). The nucleotide sequences were verified using BioEdit software, then analyzed using the BLAST (Basic Local Alignment Search Tools) program under NCBI (ncbi.nlm.nih.gov) website to determine the species of the halfbeak. DNA sequence from fish samples was aligned with similar data published in GenBank, and the phylogenetic tree and genetic distance were arranged using Mega X software (Kumar et al. 2018).

RESULTS AND DISCUSSION

Description of halfbeak (*Hemiramphus* sp.)

Halfbeaks caught in the Seram Sea, Buru District, Maluku have single dorsal and anal fins; paired pectoral and ventral fins; no spines on dorsal, anal, pectoral and ventral fins; black spots on the weak rays of the ventral fin; the caudal fin is fork-shaped, the lower part is longer than the upper part and the fin rays are sometimes turquoise; very small teeth in the oral cavity, located at the bottom of the upper beak and at the top of the base of the lower beak; the lateral line (*linea lateralis*) is clearly visible, extending along the body starting from behind the base of the pectoral fin to the tail (posterior); the operculum is sometimes blue-green or sometimes there is a blue-green spot (Figure 3).

Morphometric and meristic characterization

The halfbeak found caught in the Seram Sea, Maluku has a total length (TL) ranged from 244.49-317.70 mm (274.68 ± 13.61 mm), standard length (SL) ranged from 212.35-279.07 mm (240.83 ± 11.91 mm), body height (BD) ranging from 24.09-35.49 mm (28.82 ± 1.80 mm), head length (HL) ranged from 16.79 to 65.66 mm (42.12 ± 2.90 mm), and eye diameter (ED) ranged from 9.38 to 13.52 mm (11.07 ± 0.72 mm). The detailed measurements of the morphometric characters are shown in Table 1.

Table 2 shows the comparison of certain body parts to total length and a head length of halfbeaks from Seram Sea Maluku. The halfbeak caught from the Seram Sea shows the variation of the meristic character. The detailed calculation of the meristic character of the halfbeak is shown in Table 3. Table 3 shows the fin formula of halfbeak from the Seram Sea was D.13-15, P.9-12, V.5-6, A.9-12, C.7 (upper lobes) /8 (lower lobes), and the number of scale at the lateral line was 52-58.

Molecular identification

The GenBank Accession numbers for the sequences from this study are OM927975, OM927976, and OM927977. Analysis of nucleotide sequences using the BLAST (Basic Local Alignment Search Tools) showed that the halfbeak from the Seram Sea, Maluku had high similarity with the *H. lutkei* species in the GenBank with Accession Number MN855097 (99.45%), compared to the other species, i.e., *H. balao* (Accession Number HQ991934: 98.53%) and *H. archipelagicus* (Accession Number HQ149857: 91.74%). The detailed estimates of evolutionary divergence between halfbeak's nucleotide sequences are shown in Table 4.

The phylogenetic tree analysis result showed that the samples of *Hemiramphus lutkei* from the Seram Sea, Maluku were closely related to *H. lutkei* accession number MN855097 from India (Figure 4).

Table 1. Morphometric character of halfbeak (*Hemiramphus* sp.)

Morphometric character	**Present study Seram Sea, Maluku**
	Length (mm) (Average+SD), (max-min)
Total length (TL)	274.68 ± 13.61, (244.49-317.70)
Standard length (SL)	240.83 ± 11.91,(212.35-279.07)
Fork length (FL)	245.39 ± 12.02, (217.71-283.77)
Body depth (BD)	28.82 ± 1.80, (24.09-35.49)
Body width (BW)	17.15 ± 1.22, (14.26-21.24)
Pectoral fin length (PfL)	34.53 ± 1.83, (30.25-40.37)
Pre-dorsal fin length (pDfL)	195.31 ± 10.09, (168.56-227.54)
Pre-anal fin length (pAfL)	205.59 ± 10.42, (179.21-238.87)
Upper jaw length (UjL)	6.21 ± 0.62, (4.81-7.95)
Lower jaw length (LjL)	60.27 ± 3.60, (40.36-70.29)
Head length (HL)	42.12 ± 2.90, (16.79-65.66)
Eye diameter (ED)	11.07 ± 0.72, (9.38-13.52)
Interorbital width (IoW)	11.87 ± 0.96, (9.63-15.53)

Note: the data do not differentiate between ♂ dan ♀ fish

Figure 3. Halfbeak (*Hemiramphus* sp.) from Seram Sea, Maluku, Indonesia

Table 2. Size comparison of halfbeak (*Hemiramphus* sp.) in the Seram Sea, Maluku, Indonesia

Ratio of morphometric character	**Present study Seram Sea Maluku (in TL)**	***Hemiramphus lutkei* Odisha Coast (in SL) Mohanty et al. (2020)**
Head Length Index (HL/TL)	6.52 : 1	4.1-4.3 : 1
Height Index (BD/TL)	9.53 : 1	N/A
Dorsal Fin Index (pDL /TL)	1.41 : 1	1.3 : 1
Anal Fin Index (pAfL/TL)	1.34 : 1	N/A
Pectoral Fin Index (pfL/TL)	7.89 : 1	5.3-5.5 : 1
Eye Width Index (ED/ HL)	3.81 : 1	3.5-4.6 : 1
Upper Half Index (UjL/HL)	6.78 : 1	N/A

Note: the data do not differentiate between ♂ dan ♀ fish, N/A: data not available

Table 3. Meristic character of halfbeak (*Hemiramphus* sp.) from the Seram Sea, Maluku, Indonesia

Meristic character	**Present study Seram Sea, Maluku**	***Hemiramphus lutkei* Odisha Coast (Mohanty et al. 2020)**	***Hemiramphus lutkei* (Collette 1999)**
Dorsal Fin (D)	13-15	13	12-15
Anal Fin (A)	9-12	11-12	10-13
Pectoral Fin (P)	9-12	11	N/A
Ventral Fin (V)	5-6	N/A	N/A
Caudal Fin (C) upper/lower lobe	7/8	N/A	N/A
Scales on lateral line	52-58	N/A	N/A
First Arch of Gill raker	35-44	N/A	33-46

Note: the data do not differentiate between ♂ dan ♀ fish, N/A: data not available

Table 4. Genetic distance of *Hemiramphus* sp. caught in the Seram Sea, Maluku, Indonesia

No.	**Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	OM927975 (this_study)	-	0.0037	0.0037	0.0055	0.0540	0.0561	0.0680	0.0893	0.1565	0.1744
2	OM927976 (this_study)	0.0037	-	0.0037	0.0055	0.0541	0.0562	0.0681	0.0894	0.1567	0.1746
3	OM927977 (this_study)	0.0037	0.0037	-	0.0055	0.0541	0.0562	0.0681	0.0894	0.1567	0.1746
4	MN855097 *Hemiramphus lutkei*	0.0055	0.0055	0.0055	-	0.0480	0.0500	0.0619	0.0829	0.1567	0.1722
5	GU672680 *Hemiramphus balao*	0.0540	0.0541	0.0541	0.0480	-	0.0018	0.0722	0.1002	0.1589	0.1699
6	JQ841599 *Hemiramphus brasiliensis*	0.0561	0.0562	0.0562	0.0500	0.0018	-	0.0701	0.1024	0.1565	0.1723
7	KF714951 *Hemiramphus far*	0.0680	0.0681	0.0681	0.0619	0.0722	0.0701	-	0.0581	0.1404	0.1842
8	HQ149857 *Hemiramphus archipelagicus*	0.0893	0.0894	0.0894	0.0829	0.1002	0.1024	0.0581	-	0.1474	0.1772
9	MZ576070 *Hyporhamphus australis*	0.1565	0.1567	0.1567	0.1567	0.1589	0.1565	0.1404	0.1474	-	0.1541
10	KJ641744 *Rhynchorhamphus malabaricus*	0.1744	0.1746	0.1746	0.1722	0.1699	0.1723	0.1842	0.1772	0.1541	-

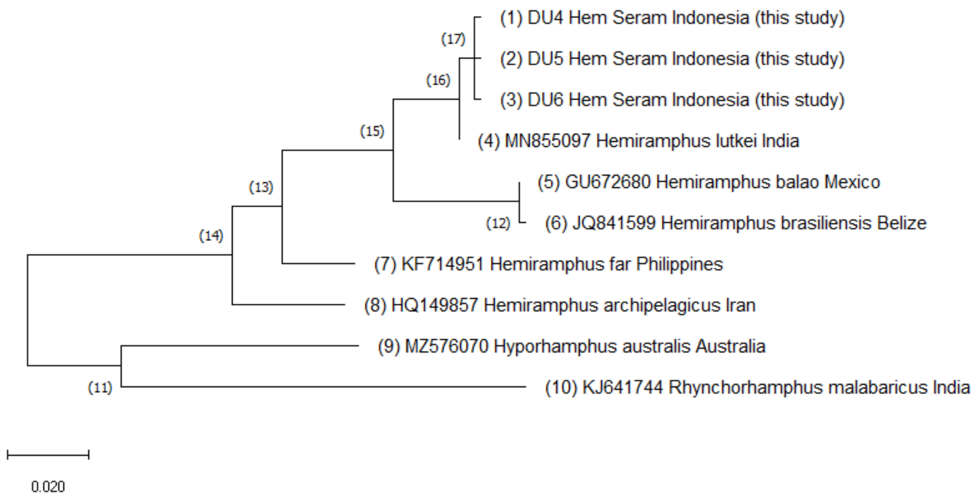

Figure 4. The genetic relationship between halfbeak. Reconstructed based on the Maximum Likelihood method and Kimura 2-parameter model. Evolutionary analysis was carried out with the help of MEGA X software

Discussion

Hemiramphus, commonly called halfbeaks, is a genus of schooling marine fish widely distributed throughout the world, although a certain halfbeak species might have a specific distribution and differ from the others. Currently, 11 members of the genus *Hemiramphus* are considered as valid species, i.e., *Hemiramphus archipelagicus*, *H. balao*, *H. bermudensis*, *H. brasiliensis*, *H. convexus*, *H. depauperatus*, *H. far*, *H. lutkei*, *H. robustus*, and *H. saltator* (Fricke et al. 2022). Based on the morphological approach, this study recorded Lutke's halfbeak, *H. lutkei* at Kaiely Bay, Maluku. The halfbeak has a turquoise color on the dorsal side and a silvery color on the ventral body. The lower beak is longer than the top and has a reddish-orange color. This characteristic was similar to the *H. lutkei* originating from the Gulf of Odisha, India, which has a dark blue body on the dorsal side, silvery-white on the ventral side, the lower jaw is bright red, and the upper lobe of the caudal fin is bluish (Mohanty et al. 2020).

Hemiramphus lutkei showed morphological similarities with *H. archipelagicus* and *H. far*. *Hemiramphus lutkei* is characterized by scales absent on the upper jaw; preorbital ridge absent, and pectoral fins relatively long, 4.5 to 5.4 times in standard length (Collette 1999). *Hemiramphus archipelagicus* can be distinguished from *H. lutkei* by the differences in length of the pectoral fin, *H. archipelagicus* has a relatively shorter pectoral fin and does not reach the anterior margin of the nostrils when folded forward. *Hemiramphus lutkei* also has more gill rakers and pre-dorsal scales than *H. archipelagicus* (Behera et al. 2020).

The morphometric character of halfbeak (*H. lutkei*) found in the Seram Sea, Maluku had a total length (TL) ranging from 244.49-317.70 mm, and standard length (SL) ranged from 212.35-279.07 mm. As a comparison, *H. lutkei* found in Karachi Bay, Pakistan, has a total length (TL) ranging from 140.0-278.0 mm (Tabassum et al. 2017). Based on Fishbase, the standard length of halfbeak can reach 400.0 mm but is generally as long as 250.0 mm. The halfbeak (*H. lutkei*) caught in the Seram Sea has a greater total length than the halfbeak (*H. lutkei*) caught at the Karachi Bay of Pakistan and is thought to have entered the mature stage. Mehanna et al. (2019) reported the size at the first maturity of halfbeak (*H. lutkei*) from Egypt was 231.0 mm. Each fish species has various strategies used to maximize reproduction and ensure the survival of their offspring to adulthood, such as gonadal maturity size, fecundity, and spawning period, which are considered essential information in regulating fishing and fish stock conservation (Oliveira and Chellappa 2014). The availability of food and the conditions of the aquatic environment also plays an important role in determining the growth rate and condition of fish (Tabassum et al. 2014).

The fin formula of a halfbeak from Seram Sea, Maluku was D.13-15, P.9-12, V.5-6, A.9-12, and C.7/8, with 52-58 scales at the lateral line (Table 3). The number of dorsal fins, anal fins, and pectoral fins of *H. lutkei* from the Seram Sea was similar to that of *H. lutkei* from the Odhisa Coast (Mohanty et al. 2020), but this study did not present the number of ventral fins, caudal fins (upper/lower lobe, scales on lateral line, and first arch of gill raker. Collette (1999) described the number of dorsal-fin rays of *H. lutkei* as ranging from 12 to 15 (usually 13 or 14) and anal-fin rays as ranging from 10-13 (usually 12). Morphometric and meristic analysis are essential tools for determining species of organisms that have a number of characteristics. Morphometric characteristics of fish can indicate a fish's ability to adapt to different environmental conditions, such as temperature and food availability (Fagbuaro et al. 2015). Additionally, morphometric analysis can be used to classify and understand taxonomic relationships. Meristic characteristics of fish are influenced in part by environmental factors during egg and larval development. Therefore, meristic variations might indicate the geographic origins of populations throughout their early life stages, which is valuable information for stock identification (Cadrin et al. 2014).

Morphological characteristics are still frequently used to differentiate species functionally and to measure fish diversity (Villéger et al. 2017). In many cases, individuals with a few morphological differences are very likely to be misidentified by traditional taxonomic procedures (Torres et al. 2015); therefore, a molecular approach has been developed for fish identification. Molecular analysis of the cytochrome c oxidase subunit I genes revealed that the halfbeak caught in the Seram Sea of Maluku was *Hemiramphus lutkei* species. *Hemiramphus lutkei* from Maluku waters was closely related to *H. lutkei* from India with a genetic distance of 0.0055. The species of the genus *Hemiramphus* with the closest genetic distance from *H. lutkei* are *H. balao* and *H. brasiliensis*, i.e., 0.0540 and 0.0561, respectively (Table 4). The study of kinship based on the phylogenetic relationship between the taxons is an essential aspect of animal taxonomy (Betancur-R et al. 2017). Based on the results of phylogenetic analysis (Figure 4), halfbeak (*H. lutkei*) from the Seram Sea of Maluku is closely related to *H. lutkei*.

The presence of *H. lutkei* in Maluku waters has never been reported before. The members of the genus *Hemiramphus* reported previously in this area are *Hemiramphus* sp. (Ahmad et al. 2019) and *H. archipelagicus* (Natan et al. 2019) around Maluku waters. *Hemiramphus lutkei* is found in the Indo-West Pacific region, from East Africa to the Philippines and Samoa, north to Japan, and south to northern Australia (Fricke et al. 2022). *Hemiramphus lutkei* is native to the northwest Pacific (Hata et al. 2018). This fish was also found in the coastal waters of India (Mohanty et al. 2020). The presence of *H. lutkei* in Indonesian water has been reported at Rote Island, East Nusa Tenggara (Balukh et al. 2021), although the identification method was not clearly described. This finding provides additional information that various members of the genus *Hemiramphus* are found in Indonesian waters. Other halfbeak species found in Indonesian waters include *H. far* in Siau Tagulandang Biaro, North Sulawesi (Kawimbang et al. 2012) and *H. archipelagicus* in Ekas Bay Lombok, West Nusa Tenggara (Suryawan et al. 2016).

Various *Hemiramphus* have been reported to be found in Maluku as well as Indonesian waters. However, there is still limited information regarding their morphological and molecular characteristics. Mistakes in identifying fish species can lead to serious problems in managing and exploiting fish stocks (Carvalho and Hauser 1994; Garcia-Vazquez et al. 2012). Bariche et al. (2015) explain that the reliability of biological identification is essential for the effective management of fisheries resources. Increased fishing efforts without proper management will decrease stocks and disrupt the sustainability of fish resources. Effective fisheries management requires comprehensive data to support the sustainability and conservation of fish species (Stat et al. 2017). As a result, it is necessary to determine the members of the genus *Hemiramphus* using morphological and molecular approaches and their distribution in the Maluku waters. Adequate data and information on fish biology and fishing activities could be used as a basis for the regulation and fisheries management actions of halfbeak fisheries.

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