Scomberomorus guttatus and *lineolatus* identified in Sri Lanka by DNA barcoding

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Abstract

The mitochondrial COI (Cytochrome c oxidase subunit I) region was sequenced for 8 fish samples of genus *Scomberomorus* in Sri Lanka. Comparison with the COI region sequences registered in NCBI Genbank identified 6 samples as *Scomberomorus guttatus* and 2 samples as *Scomberomorus lineolatus*. Comparison with COI region sequences registered with clear indication of sampling location also indicated the existence of a new species, possibly occurring in India, whose appearance is similar to *S. guttatus*.

Material and Methods

Sample collection

FIsh belonging to the genus *Scomberomorus* were collected on Dec.10 and 12 at Negombo fishing port area, Sri Lanka. Among the 8 samples, clear spot pattern differences were observed. Table 1 shows the size and spot pattern of the 8 samples.

Table 1 Size and spot pattern of the *Scomberomorus* genus fish collected at Negombo fishing port area, Sri Lanka.

Sample ID	Sampling date	FL(cm)	Spot pattern	Sampling location
No.1	10 Dec. 2024	45	Round shape spots	Negombo Fishery Harbour, Sri Lanka
No.2	10 Dec. 2024	48	Round shape spots	Negombo Fishery Harbour, Sri Lanka
No.3	12 Dec. 2024	37	Round shape spots	Negombo Fish Market, Sri Lanka
No.4	12 Dec. 2024	37	Round shape spots	Negombo Fish Market, Sri Lanka
No.5	12 Dec. 2024	39	Round shape spots	Negombo Fish Market, Sri Lanka
No.6	12 Dec. 2024	40	Round shape spots	Negombo Fish Market, Sri Lanka
No.7	12 Dec. 2024	53	Elongated oval shape spots	Negombo Fishery Harbour, Sri Lanka
No.8	12 Dec. 2024	54	Elongated oval shape spots	Negombo Fishery Harbour, Sri Lanka

DNA barcoding

In order to isolate DNA of the COI region, muscle tissue of each sample was taken and preserved in a freezer. DNA was extracted using a commercially available kit and Polymerase chain reaction (PCR) was conducted using FishF2/FishR2 primers at NARA, Sri Lanka. Then, PCR products were sent to Macrogen Inc., Korea to obtain the DNA sequences.

Sequence analysis

Obtained DNA sequences of the COI region of the samples collected were compared to the

international DNA sequence database, NCBI Genbank, using BLAST(Basic Local Alignment Search Tool, available online).
Further analysis was conducted using the MEGA (version 5.2) program, by comparing the obtained sequence with COI region sequence of *Scomberomorus guttatus* and *Scomberomorus koreanus* whose sampling location is clearly identified available in the NCBI database. During this process, 5 sequences of *S. guttatus* obtained earlier by NARA were included in the analysis (Sri Lanka 1-5, see Table 2). Table 2 shows the COI region sequence downloaded from the Genbank for comparison.

Table 2 Downloaded COI region sequence of *S. guttatus* and *S. koreanus* from the Genbank. 5 sequences of *S. guttatus* obtained earlier by NARA were included in the analysis (Sri Lanka 1-5).

Species	Sampling location and ID	Sequence ID in Genbank
S. guttatus	Bangladesh1	MF611621.1
S. guttatus	India1	MZ914679.1
S. guttatus	India2	MZ854050
S. guttatus	China2: South China Sea	EU871700.2
S. guttatus	Indonesia1	GU674022.1
S. guttatus	Indonesia2, Java and Bali Island	MH085912.1
S. guttatus	China4: Beibu gulf	KY372122.1
S. guttatus	Bangladesh3: Ganges Fan, Cox's Bazar,	MH230970.1
S. guttatus	China3: Northern South China Sea	KY372121.1
S. guttatus	Bangladesh2	MF611610.1
S. guttatus	Taiwan1	KU945031.1
S. guttatus	India6: Kakinada	EU541328.1
S. guttatus	China1	EF607535.1
S. guttatus	India7	ON261362.1
S. guttatus	India7	ON261363.1
S. guttatus	India7	ON261364.1
S. guttatus	India7	ON261365.1
S. guttatus	Sri Lanka 1	OM462843.1
S. guttatus	Sri Lanka 2	
S. guttatus	Sri Lanka 3	
S. guttatus	Sri Lanka 4	
S. guttatus	Sri Lanka 5	
S. koreanus	India3	MZ914657.1
S. koreanus	India4	MN833315.1
S. koreanus	India5	OM416538.1
S. koreanus	India7	ON261366.1
S. koreanus	India7	ON261367.1
S. koreanus	India7	ON261368.1
S. koreanus	India8	MZ854038.1

Results and Discussion

Table 3 shows the identifications in NCBI blast for *Scomberomorus* genus fish collected in the present study. Sample ID No.1-6 which had round shape spots indicated matches with

"*Scomberomorus cf. guttatus*" registered in the Genbank (Fig.1 shows example No.5). Sample ID No.7-8 which had elongated oval shape spots showed a match with "*Scomberomorus lineolatus*" registered in the Genbank (Fig.2 shows example No.8).

Meaning of "c*f*" in "*Scomberomorus cf. guttatus*" is that the person registered his/her data was not sure about identification of fish. *S. guttatus* is known to have a species similar in appearance such as *S. koreanus* (Roul et al. 2022). Furthermore, Abdussamad et al. (2023) claims the existence of new species in the *Scomberomorus* genus. Considering the uncertainty of species identification by the person registered data to Genbank, DNA sequence was compared with the data in Table 2.

Table 3 Species registered in Genbank which showed the highest match with Scomberomorus genus fish collected in Sri Lanka.

Sample ID	Species ID by sequencing
No.1	*Scomberomorus cf. guttatus* (99.23%)
No.2	*Scomberomorus cf. guttatus* (98.69%)
No.3	*Scomberomorus cf. guttatus* (99.86%)
No.4	*Scomberomorus cf. guttatus* (99.71%)
No.5	*Scomberomorus cf. guttatus* (99.86%)
No.6	*Scomberomorus cf. guttatus* (100%)
No.7	*Scomberomorus lineolatus* (99.68%)
No.8	*Scomberomorus lineolatus* (100%)

Fig.1 *Scomberomorus cf. guttatus* identified by DNA barcoding. No.5, FL 39cm, D XVI-19+8; A 19+8

Fig.2 *Scomberomorus lineolatus* identified by DNA barcoding. No.8, FL 54cm, D XIV-15+9; A 17+10

Fig. 3 shows the Neighbor Joining Tree (phylogenetic tree) created by bootstrap method (500 replications) with Kimura 2-parameter method, using the COI sequence of the present study and sequence in Table 2. The phylogenetic tree showed 4 groups divided with a discrepancy in a species level. The COI sequence of the present study was divided in two groups showing consistent results with Table 3, which identifies "*Scomberomorus cf. guttatus*" for No.1-6 and "*Scomberomorus lineolatus*" for No. 7-8. Sample ID No.1-6 was nested in the same group with *S. guttatus* registered sequence which sampling locations are 2 locations in Indonesia, 1 location in India, 1 location in Taiwan, 4 locations in China and 5 samples earlier sequenced by NARA, Sri Lanka. Other identified species consisted of 2 groups. One group was *S. koreanus* registered by 5 locations in India and *S. guttatus* registered by 2 locations in Bangladesh. The other was *S. guttatus* registered by 3 locations in India.

The results shown in Fig.3 may indicate two things. First, misidentification of species by the person who registered the data to Genbank. The group where *S. koreanus* was nested included registration by Bangladesh identifying the species as *S. guttatus*. This may have occurred by misidentification of Bangladesh data to the Genbank, which was *S. koreanus*. Second, the existence of new species whose appearance is similar to *S. guttatus.* Unfortunately the present study lacks detailed observation of morphology. Hence the difference of the two *S. guttatus* cannot be made clear. Identification of either group nested with *S. guttatus* in Fig.3 is the recognized *S. guttatus* is unclear. However, if we hypothesize that limited occurrence was the cause of the undiscovered new species, the new species would be the group registered in India, which is India 1, 2, 7 in Table 3 and Fig. 3. Further morphological and genetic data collection in India and adjacent areas may be required to conclude the existence of new species whose appearance is similar to *S. guttatus.*

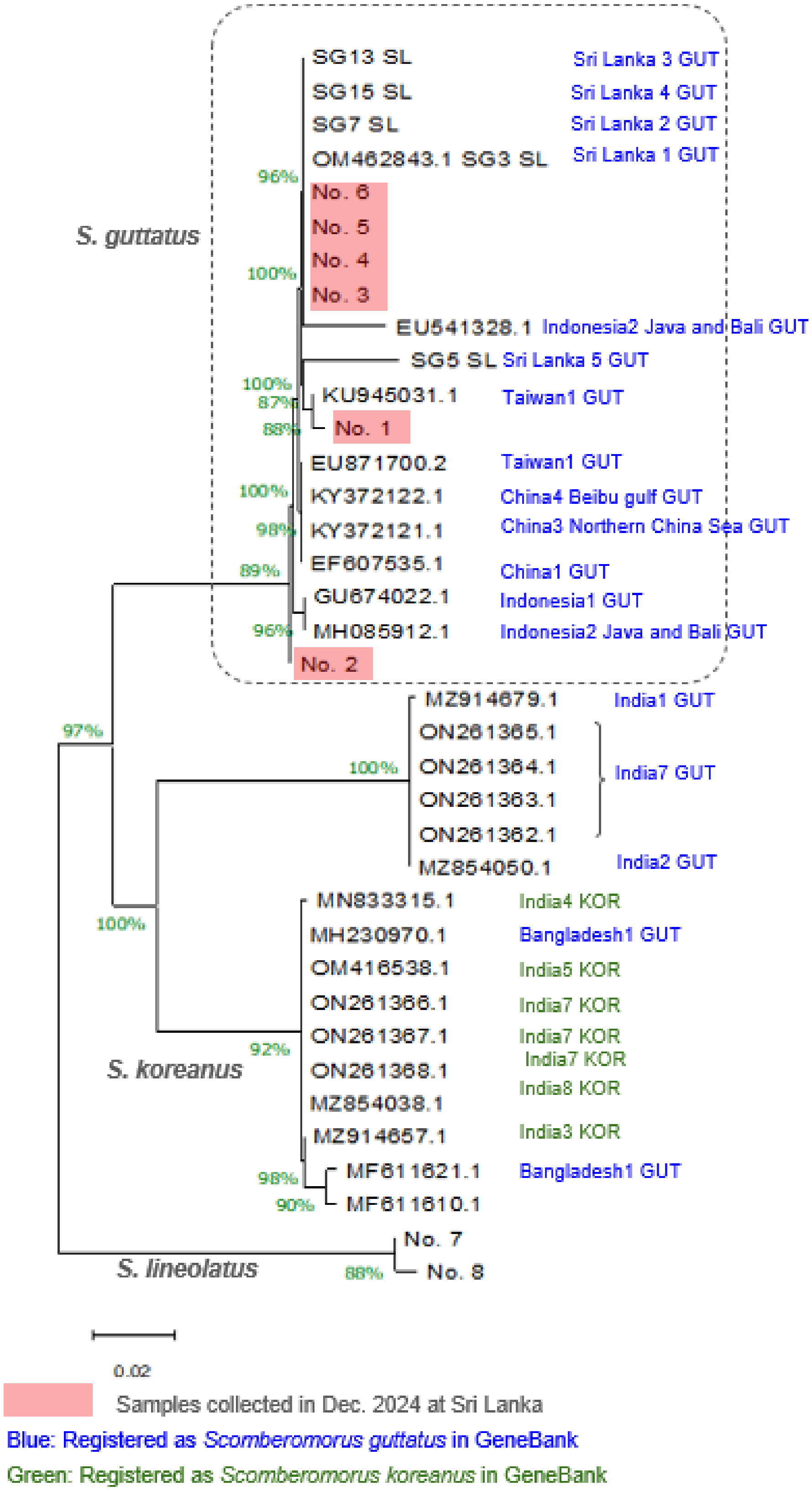

Fig. 3 Neighbor Jointing Tree (phylogenetic tree) created using the present study COI sequence and COI sequence in Table 2. Red highlights indicate samples of the present study. Blue character indicates data registered as *S. guttatus* in the Genbank. Green character indicates data registered as *S. koreanus* in the

Genbank.

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