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Population structure of commercially important groupers in the coral triangle Gorontalo, Indonesia to support conservation

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Abstract. *Achmad DS, Nurdin MS, Taslim I, Moore AM. 2023. Population structure of commercially important groupers in the coral triangle Gorontalo, Indonesia to support conservation. Biodiversitas 24: 6592-6601.* Appropriate management units are fundamental to the conservation of species and ecosystems. In the case of exploited fishes, sustainable approaches to fisheries management are generally based on population units (stocks). This study aimed to address the lack of data to define the population structure of the stocks of two commercially important grouper species (*Variola albimarginata* (Baissac, 1953) and *Variola louti* (Forsskål, 1775)) in the Sulawesi Sea and Tomini Bay waters of Gorontalo Province, Sulawesi, Indonesia, in the Wallacea biodiversity hotspot. We used a combined morphometric and molecular (DNA barcoding, COI marker). We used truss network analysis with 14 characters to evaluate inter and intraspecies morphometric differences. DNA was extracted (Qiagen protocols) from tissue samples from individuals of both species in both sea areas, followed by polymerase chain reaction (PCR) and Sanger sequencing. Sequence processing and phylogenetic analyses conducted in MEGA X. Both approaches show clear separation between *V. albimarginata* and *V. louti* but indicate close kinship between the north coast (Sulawesi Sea) and south coast (Tomini Bay) populations of these groupers. Under the authority of the Gorontalo Province Marine and Fisheries Service, these two populations could be managed as one stock without compromising fisheries sustainability or the conservation of these ecologically and economically valuable species.

Keywords: DNA barcoding, management and conservation, stock units, truss morphometrics, *Variola*

INTRODUCTION

Groupers are marine fishes of the order Perciformes, Family Epinephelidae, and Subfamily Epinephelinae (Félix-Hackradt et al. 2022). Distributed and fished throughout the Indonesian seas, these mostly coral reef-associated top predators are a prime Indonesian fisheries export commodity (Halim et al. 2020; Dimarchopoulou et al. 2021). Indonesia is one of the world's largest producers of high-value groupers (Khasanah et al. 2020), and Indonesia contributed nearly a quarter (24.27%) of global grouper production volume over the period 2016-2020 according to the Food and Aquaculture Organisation of the United Nations (FAO) statistics (Food and Agriculture Organization 2022). Indonesian government statistics indicate that grouper export volume and value were quite volatile over this period, increasing from 7,112 tons (USD 39,630,162) in 2016 to 7,692 tons (USD 42,370,051) in 2019 and decreasing to 6,056 tons (USD 32,308,460) in 2021 (Ministry of Maritime Affairs and Fisheries. 2022).

The high demand for groupers and their high value in the export-oriented trade encourage widespread and mostly uncontrolled fishing, which has resulted in overfishing of several grouper species (Khasanah et al. 2019; Mehanna et al. 2019; Efendi et al. 2020; Nadiarti et al. 2021; Pane et al. 2021). This is a matter of concern from ecological, conservation, social, and economic perspectives. As high trophic-level predators, groupers are thought to play important roles in coastal marine ecosystems (Frisch et al. 2016; Condini et al. 2018). Indo-Pacific grouper species generally have large distribution areas but are generally relatively sedentary except when congregating at spawning aggregation sites, which can serve several species and attract individuals normally dispersed over large areas (Sadovy de Mitcheson et al. 2008).

Therefore, connectivity between populations is still poorly understood but is thought to rely mostly on larval dispersal and settlement processes rather than migration of juvenile or adult life stages. Such traits can lead to populations with limited genetic or demographic connectivity, meaning that they should be regarded as separate stocks or management units in fisheries and conservation contexts (Timm and Kochzius 2008; von der Heyden 2017).

In the northern arm of Sulawesi, the largest island in the Wallacea biogeographic region, the Indonesian province of Gorontalo faces two seas: the Sulawesi Sea and the Tomini Bay (Ambo-Rappe and Moore 2019). There are reports of grouper overfishing in Gorontalo waters, especially in the Sulawesi Sea (Achmad et al. 2022), with indications of

reduced intraspecies genetic diversity of the flagship species *Plectropomus leopardus* (Lacepède, 1802) (Hidayani et al. 2022). Therefore, management units should consider the stock structure of grouper populations in the area to conserve groupers and ensure their sustainable use. An understanding of management units is fundamental in fisheries management (Begg et al. 1999), especially as fisheries management is implemented in Indonesia is currently based on sub-sets of territorial waters known as Fisheries Management Areas (FMA) (Afifah et al. 2020; Muawanah et al. 2021). The Sulawesi Sea off the north coast of Sulawesi is included in FMA 716, while Tomini Bay is included in FMA 715. However, the number and extent of grouper stocks has not been identified.

Several approaches can be adopted to determining fish stocks as management units, including but not limited to morphometric approaches and the increasingly common molecular biology approaches (Cadrin et al. 2014; Valenzuela-Quiñonez 2016). One of the former is the truss network morphometric approach, which can provide relatively high accuracy and additional insights compared to older, classic morphometric methods (Ndobe and Moore 2013; Abinawanto et al. 2018). Molecular methods are increasingly popular, particularly the use of standard molecular markers, such as the mitochondrial DNA (mtDNA) cytochrome oxidase I (COI), to identify species and examine evolutionary relationships and relatedness between and within taxa (Madduppa et al. 2021). Combined morphometric and molecular approaches are increasingly recommended and used in fisheries stock delineation (Hawkins et al. 2016; Pertiwi et al. 2019).

The lack of data on the structure of grouper stocks in the Tomini Bay and Sulawesi Sea areas under the jurisdiction of Gorontalo Province is an obstacle to designing sustainable management strategies for these ecologically and economically important fishes. Groupers found in Gorontalo waters comprise at least 26 species belonging to 8 genera (Achmad et al. 2023). Groupers of the genus *Variola* are valuable fisheries commodities in this area. Like other groupers, they are also of conservation concern, as there are signs of grouper overfishing in the waters of Gorontalo Province, including the white-edged lyretail *Variola albimarginata* and the yellow-edged lyretail *V. louti* (Achmad et al. 2023). Therefore, there is a need to examine the population structure of *Variola* spp. groupers in the Sulawesi Sea and Tomini Bay of Gorontalo Province to determine the number of stocks as appropriate management units.

MATERIALS AND METHODS

Study area

Field research was conducted from August to October 2022 at sites in the Sulawesi Sea and Tomini Bay, Gorontalo Province, Indonesia (Figure 1). Samples from the Sulawesi Sea fishing area were collected from the fish landing site in Kwandang fishing port, which is the main grouper fishing base on this coast (Achmad et al. 2022). Samples from the Tomini Bay fishing area were collected from the fish landing site in the Tenda fishing port, a major fishing base for grouper fishers operating in Tomini Bay (Nurkhozin et al. 2022). The samples were groupers identified as *V. albimarginata* and *V. louti* based on external morphology (Heemstra and Randall 1993).

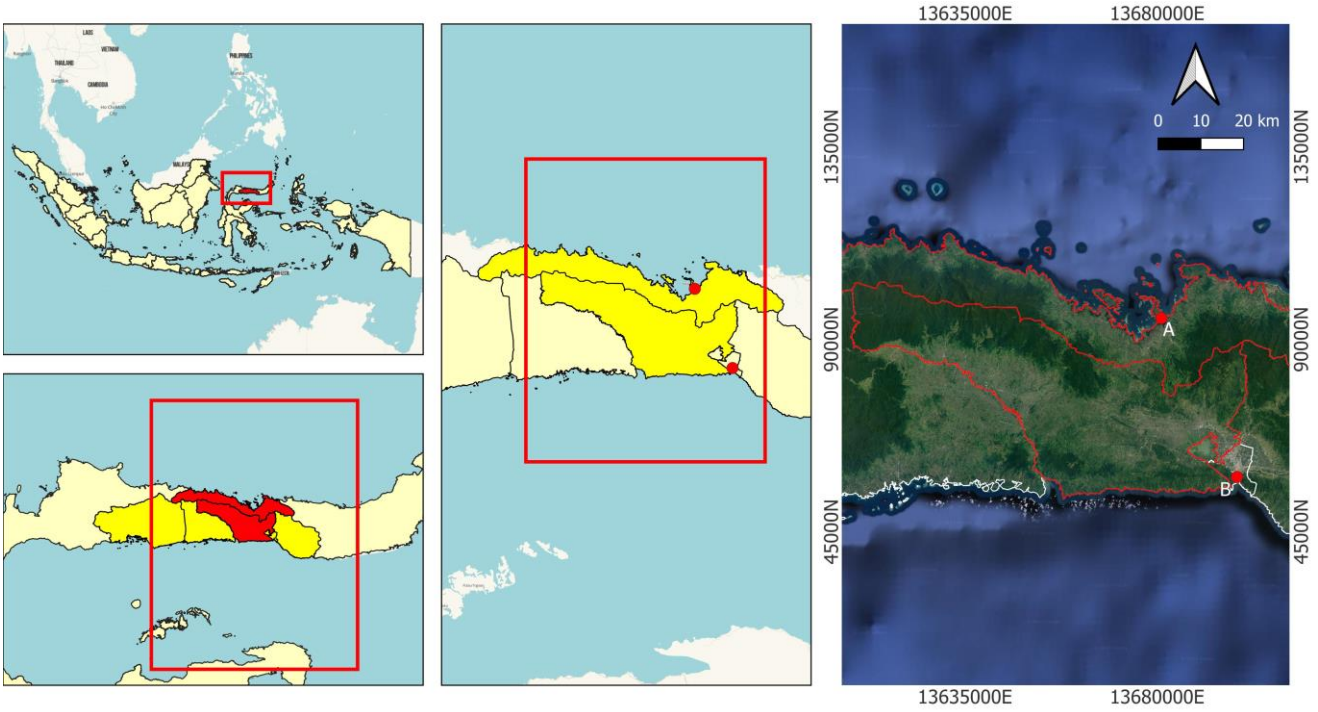

Figure 1. Specimen collection sites in Gorontalo Province, Indonesia: A) Kwandang fishing port in North Gorontalo District (Sulawesi Sea) and; B) Tenda fishing port in Gorontalo District (Tomini Bay)

All specimens were taken to the Biology Laboratory of the Fish Quarantine Station, Quality Control and Safety of Fishery Products in Gorontalo, for morphometric analysis. For molecular analysis, fin clippings and tissue samples were taken from the right side of three specimens of each species (two from Kwandang and one from Tenda). These samples were placed in cryotubes containing 96% absolute ethanol.

Procedures

Truss network morphometric characters

Truss network morphometrics observations were conducted at the Biology Laboratory of the Fish Quarantine Station, Quality Control and Safety of Fishery Products in Gorontalo. The truss network landmarks developed by Ariyanto et al. (2011) for tilapia were adapted to the body shape of V*ariola* groupers. These landmarks divide the grouper body into three truss sections. The 14 truss network characters measured were: (A) the distance from the anterior tip of the snout to the beginning of the dorsal fin-base; (B) the distance from the start of the dorsal-fin base to the end of the dorsal-fin base; (C) the distance from the end of the dorsal fin base to the end of the caudal peduncle; (D) the distance from the top end of the tail bar to the bottom end of the tail bar; (E) the distance from the start of the caudal peduncle to the end of the anal fin base; (F) the distance from the end of the anal-fin base to the end of the pelvic-fin base; (G) the distance from the end of the pelvic fin base to the origin of the pectoral fin; (H) the distance from the origin of the pectoral fins to the tip of the snout; (I) the distance from the start of the dorsal-fin base to the end of the pelvic-fin base; (J) the distance from the start of the dorsal-fin base to the end of the anal-fin base; (K) the distance from the end of the dorsal fin base to the end of the pelvic fin base; (L) the distance from the end of the dorsal fin to the origin of the anal fin; (M) the distance from the end of the dorsal fin to the end of the lower caudal peduncle; and (N) the distance from the end of the caudal peduncle to the end of the anal fin base.

DNA extraction, PCR, and sequencing

The DNA extraction and polymerase chain reaction (PCR) amplification of the target molecular marker were performed at the Bionesia Laboratory in Bali. DNA was extracted from a tissue sample (approximately 10 g) following the Qiagen Blood and Tissue Kit protocols. The PCR process to amplify the mtDNA COI marker followed the Bionesia laboratory protocol on an Applied Biosystems™ 2720 Thermal Cycler. The primer pair used was forward FISH-BCL(5'-TCAACYAATCAYAAAGATATYGGCAC), reverse FISH-BCH(5'TAAACTTCAGGGTGACCAAAAAATCA) (Baldwin et al. 2009). The PCR reaction volume was 25 µL, comprising 2 µL extracted DNA template; 1.25 µL of each primer (10 mM concentration); 9 µL ddH2O; and 12.5 µL Ready Mix. The PCR profile was: initial denaturation at 94°C for 3 min, then 38 cycles of denaturation at 94°C for 30 s; annealing at 50-55°C for 30 s; extension at 72°C for 60 s, followed by final extension at 72°C for 2 min. The PCR product was then visualized on 1% agarose gel with nucleic acid gel stain (GelRed®). If the PCR product showed a clear DNA band, it was sent to PT. Genetica Science, Jakarta, for sequencing using the Sanger dideoxy method. Sequence data were received in the form of .abi chromatogram trace files.

Data analysis

Truss network morphometric measurement data for each species were transformed to remove size-dependent variation through an allometric approach using the equation (Palma and Andrade 2002):

$$\text{Mtrans} = \log \text{M} - \beta\,(\log \text{L} - \log \text{Lmean})$$

Mtrans is the transformed measurement, M is the original measurement of the character, β is the within-group slope regression of log M vs. log L, L is the standard length of the fish, and Lmean is the overall mean of the standard length.

The magnitude of phenotypic diversity was analyzed descriptively and compared between populations and species using each character's average coefficient of variation (CV). Kinship relationships between groups were inferred based on the transformed morphometric truss characters using canonical and discriminant analyses (Safira et al. 2019; Afifah et al. 2020) and the percentage of shared component values (Muchlisin 2013).

The nucleotide base assignment in the chromatogram files was checked to ensure that the data used were of good quality. The sequence data were trimmed and aligned using the MEGA X MUSCLE option (Kumar et al. 2018). The barcode data obtained were then compared to homologous sequences in the NCBI GenBank database using the online Basic Local Alignment Search Tools (BLAST-n) facility (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (National Center for Biotechnology Information 2016). Homologous sequences obtained were downloaded in FASTA format and aligned with the *Variola* sequences using the MUSCLE option in MEGA X (Kumar et al. 2018).

A phylogenetic tree was constructed from the combined aligned sequence dataset using the Neighbor-joining (NJ) method (1000 bootstrap replicates) in MEGA X (Kumar et al. 2018). Genetic distances were then computed using the *p*-distance method, and Analysis of Molecular Variant analysis (AMOVA) was applied to assess genetic differentiation (F_{st}) between and within populations of *V. albimarginata* and *V. louti* in the Sulawesi Sea and Tomini Bay using Arlequin (version 3.0) (Excoffier et al. 2005). The F_{st} was used to evaluate whether *V. albimarginata* and *V. louti* populations in the Sulawesi Sea and Tomini Bay are genetically different (different genetic stocks) (Madduppa et al. 2021).

RESULTS AND DISCUSSION

Truss network morphometric analysis

The discriminant analysis between *V. louti* and *V. albimarginata* showed that 11 of the 14 truss network morphometric characters measured (A, B, D, E, G, H, I, K,

L, M, and N) differed significantly between the two sampling sites and could be used as markers or identifiers to differentiate between *V. louti* and *V. albimarginata* populations in the Sulawesi Sea and Tomini Bay (Table 1). The maximum values of the coefficient of variation were low for both species (7.7983 and 18.3824% for *V. louti*; and 7.8585 and 8.2233% for *V. albimarginata*), indicating that the fish sampled from the Sulawesi Sea and Tomini Bay populations had, on average, similar but not identical body shapes.

The Chi-Square test showed that centroids for *V. louti* and *V. albimarginata* collected from the Sulawesi Sea and Tomini Bay differed significantly between the two species and for each species between the two sites ($\chi 2 = 255.677$, $p<0.01$; $\chi 2 = 121.093$, $p<0.01$; and $\chi 2 = 28.747$, $p<0.01$ respectively). This indicates that the Tomini Bay and Sulawesi Sea samples represent distinct sub-populations for each species. The distribution of the *V. louti* and *V. albimarginata* groups at their respective centroids can be seen in the Canonical Discriminant Functions histogram (Figure 2).

The percentage of the interspecies truss network morphometric phenotype shared between *V. louti* and *V. albimarginata* ranged from 6.9-7.9% for specimens collected from the Sulawesi Sea and 12.9-21.4% for specimens collected from Tomini Bay. The intraspecies shared phenotype percentage was highest (92.1%) for *V. louti* from the Sulawesi Sea and lowest for *V. albimarginata* from Tomini Bay (58.1%) (Table 2). This indicates that *V. louti* in the Sulawesi Sea has comparatively high within-population phenotypic similarity. Meanwhile, the *V. albimarginata* specimens from Tomini Bay were more variable, with 41.9% of individuals closely resembling other *Variola* species/site groups.

DNA molecular analysis

The nucleotide sequences obtained from PCR amplification of the target COI-mtDNA barcode region were 600-700 bp (base pairs) long. The BLAST-n results (Table 3) and phylogenetic analysis (Figure 3) confirm the identifications made based on the six specimens' morphological traits and the truss network analysis. Two specimens from Kwandang Bay in the Sulawesi Sea (BIOSUB.181.001; BIOSUB.181.002) and one from Tenda in Tomini Bay (BIOSUB.181.005) were identified as *Variola albimarginata* with over 99% similarity to GenBank database (NCBI) reference accessions, while two Sulawesi Sea specimens (BIOSUB.181.003; BIOSUB181.004) and one from Tomini Bay (BIOSUB181.006) were identified as *V. louti* with 100% identity. The high query cover and identity values for *V. albimarginata* and *V. louti* species indicate a high confidence level. They are supported by the structure of a phylogenetic tree where the sequences were assigned to the species clades with a 100% bootstrap value (Figure 3).

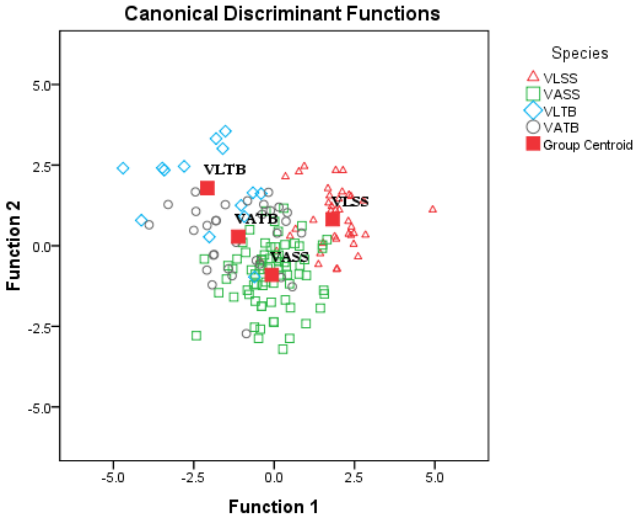

Figure 2. The *Variola* canonical discriminant function. Legend: VASS = *V. albimarginata* from the Sulawesi Sea; VLSS = *V. louti* from the Sulawesi Sea; VATB = *V. albimarginata* from Tomini Bay; and VLTB = *V. louti* from Tomini Bay

Table 1. Mean values and coefficient of variation of 14 truss network characters for two species of the genus *Variola* collected from two sites (statistical significance at the 99% confidence level)

Landmark	Sulawesi Sea				Tomini Bay				Significance (p-value)
	V. louti		*V. albimarginata*		*V. louti*		*V. albimarginata*		
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	
A	1.0068	2.1555	0.9707	2.3759	0.9979	2.2539	0.9843	2.3830	p<0.01
B	1.0944	1.7383	1.0702	1.8178	1.0887	2.0541	1.0733	2.0109	p<0.01
C	0.7096	6.4283	0.6991	7.8585	0.7044	4.6830	0.7011	6.6943	p>0.01
D	0.6566	5.2595	0.6179	6.1772	0.6941	8.9856	0.6416	8.2233	p<0.01
E	0.7560	4.6650	0.7116	6.7400	0.7460	18.3824	0.7212	5.1211	p<0.01
F	1.0699	3.7061	1.0636	2.6494	1.0500	3.1961	1.0464	3.5194	p>0.01
G	0.6503	7.7983	0.6491	5.8610	0.6946	9.5878	0.6676	7.5539	p<0.01
H	0.9706	3.0516	0.9113	5.2047	0.8771	8.7419	0.9164	5.9393	p<0.01
I	1.0103	4.6743	0.9748	2.5438	0.9574	6.0188	0.9633	2.5901	p<0.01
J	1.1469	3.6998	1.1415	3.9287	1.1506	3.6771	1.1628	3.7948	p>0.01
K	1.1558	2.5322	1.1357	2.1290	1.1179	3.0818	1.0948	3.7096	p<0.01
L	0.7687	5.7776	0.7133	5.7340	0.7197	7.5343	0.7218	6.4265	p<0.01
M	0.8067	4.5678	0.7993	4.4413	0.7661	4.6737	0.7949	4.1034	p<0.01
N	0.8484	3.8063	0.8246	3.4402	0.7882	4.7378	0.8121	2.6026	p<0.01

The AMOVA results (Table 4) show minimal genetic structure, indicating genetic mixing between the Sulawesi Sea and Tomini Bay populations. Although the F_{st} for *V. louti* was higher than for *V. albimarginata*, the F_{st} values for both *V. albimarginata* and *V. louti* were very low, with *P*-values substantially greater than 0.05. The genetic distance between the six *Variola* specimens (Table 5) ranged from 0.06441 to 0.06766 between specimens from different species but was very low (zero to 5 decimal places) between specimens of the same species from different sites.

Table 2. Percentage sharing component of the truss network morphometric phenotype of the genus *Variola*

Species	VLSS	VASS	VLTB	VATB	Total
VLSS	92.1	7.9	0	0	100
VASS	6.9	79.2	1.4	12.5	100
VLTB	0	7.1	71.4	21.4	100
VATB	6.5	22.6	12.9	58.1	100

Notes: VASS = *V. albimarginata* from Sulawesi Sea; VLSS = *V. louti* from Sulawesi Sea; VATB = *V. albimarginata* from Tomini Bay; and VLTB = *V. louti* from Tomini Bay

Table 3. Selected closest NCBI GenBank reference accession matches to six *Variola* sequences

Specimen reference	Truss network assignment	BLAST-n validation	Accession number	Query cover (%)	Identity (%)
BIOSUB.181.001	*V. albimarginata*	*V. albimarginata*	KJ130977.1	100	99.69
BIOSUB.181.002	*V. albimarginata*	*V. albimarginata*	KJ130977.1	100	99.69
BIOSUB.181.003	*V. louti*	*V. louti*	MN475883.1	97	100
BIOSUB.181.004	*V. louti*	*V. louti*	MN475883.1	97	100
BIOSUB.181.005	*V. albimarginata*	*V. albimarginata*	KJ130977.1	100	99.69
BIOSUB.181.006	*V. louti*	*V. louti*	MN475883.1	96	100

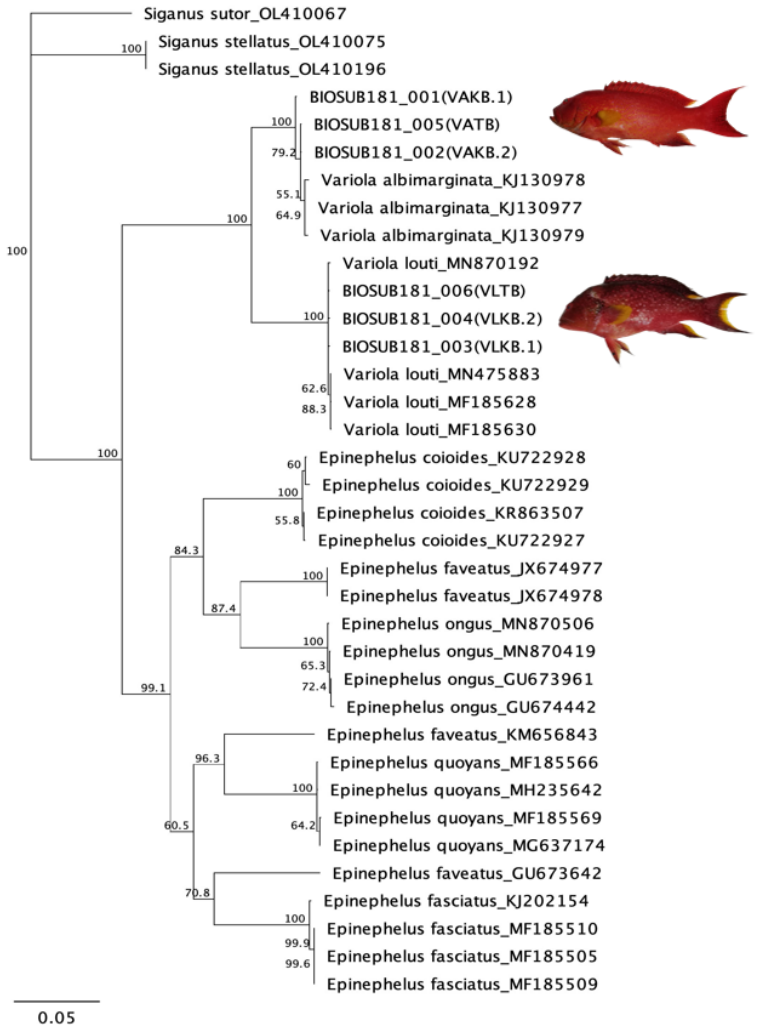

Figure 3. Neighbor-Joining phylogenetic tree (1000 × bootstrap) of the six *Variola* sequences from Gorontalo with selected NCBI GenBank grouper accessions and *Siganus* as an outgroup

Table 4. AMOVA results for two species of the genus *Variola*

Species	Source of variation	d.f	F_{st} Value	P-value
V. albimarginata	Between populations	2	0.06110	0.51808
	Within populations	7		
V. louti	Between populations	2	0.15789	0.57380
	Within populations	4		

Table 5. Genetic distance between groupers of the genus *Variola* identified in this study (COI mtDNA)

Sample Sequence	BIOSUB181_001	BIOSUB181_003	BIOSUB181_005
BIOSUB181_001	-		
BIOSUB181_003	0.06441	-	
BIOSUB181_005	0.00000	0.06766	-
BIOSUB181_006	0.06500	0.00000	0.06766

Notes: BIOSUB181_001 = *V. albimarginata* from the Sulawesi Sea; BIOSUB181_003 = *V. louti* from the Sulawesi Sea; BIOSUB181_005 = *V. albimarginata* from Tomini Bay; and BIOSUB181_006 = *V. louti* from Tomini Bay. Identical sequences from the same site are not shown

Discussion

Population structure

The truss network morphometric and genetic analyses identified the *Variola* grouper species in the Sulawesi Sea and Tomini Bay as *V. albimarginata* and *V. louti*, with identical identification results for the six specimens included in the genetic analysis. Both methods showed a greater separation between the two congeneric species than between conspecific individuals. However, the two methods differed in terms of the population structure indicated. The truss network morphometric approach found significant differences between the Sulawesi Sea and Tomini Bay populations of both *V. louti* and *V. albimarginata*. While the discriminant power of each character was quite low, eleven morphometric truss network characters differed significantly. They could potentially be used to differentiate between *V. louti* and *V. albimarginata* species and between the populations of each species.

Conversely, the genetic distance within each species for the COI mtDNA barcode gene fragment targeted in this study was zero or extremely low. The smaller the genetic distance between species, the higher the degree of kinship, and vice versa, with genetic distances in the range 0.01000-0.09999 categorized as low (Nei 1972). The interspecies distance between *V. albimarginata* and *V. louti* (0.06441-0.06766) was within this range. Furthermore, these closely related species appear to have low genetic diversity. This closely observed kinship indicates that genetic mixing has occurred between the Sulawesi Sea and Tomini Bay populations of the two *Variola* species studied; however, genetic connectivity does not necessarily entail effective demographic connectivity between populations (Selkoe et al. 2016). The phylogenetic tree reconstruction in Figure 3 shows the *Variola* genus as well separated from other grouper clades (100% bootstrap), with two well-defined sub-clades (also 100% bootstrap) representing the two species *V. albimarginata* and *V. louti*, neither of which shows between-site structure.

The apparent contradiction between the results from the two methods used can be at least partially explained by phenotypic plasticity (Rodriguez-Dominguez et al. 2022), which is known to be quite high in groupers (Basith et al. 2021). Identification errors can occur in distinguishing between *V. louti* and *V. albimarginata* (Abdullah and Rehbein 2016) because they are morphologically similar and generally only distinguished by the color of the tail. The margin of the caudal fin is white in *V. albimarginata* (Damora et al. 2021) and yellow in *V. louti* (Michailidis et al. 2020). This close resemblance is typical of groupers (Epinephelidae), with high morphological similarity between several species and the presence of cryptic species (Razi et al. 2021). Although some grouper species are quite readily recognized by their size and morphological characteristics, molecular identification is increasingly used to validate the identification of cryptic species such as some groupers (Jefri et al. 2015; Basith et al. 2021; Fadli et al. 2021; Razi et al. 2021). Identification of species with a molecular approach can uncover cryptic species and can thereby enable greater accuracy in species identification; however, this approach is intrinsically dependent on the completeness and accuracy of the sequence data stored in public databases, such as the NCBI GenBank and Barcode of Life Database (BoLD) (Durand et al. 2020; Moore et al. 2021; Bemis et al. 2023). Therefore, a combination of morphometric (e.g., truss network) and DNA molecular approaches should make grouper identification more precise and accurate (Kocovsky et al. 2013). The overlap between the two species in terms of truss characters revealed by the centroid mapping (Figure 2), albeit low, could reflect not only the close genetic kinship within this genus (Basheer et al. 2017) but also the similar ecological niches of these two grouper species (Froese and Pauly 2023). This overlap could also indicate hybridization between the two species. This is known to occur and might

even be relatively common between closely related groupers (von der Heyden et al. 2014) but cannot be detected through mitochondrial DNA, which is only passed down through the female line.

Based on genetic distance and the phylogenetic analysis, the COI-mtDNA data indicate that *V. albimarginata* from the Sulawesi Sea and Tomini Bay belong to an evolutionary group with sufficient genetic connectivity between the populations to maintain common alleles for this genetic marker, and the same applies to *V. louti*. This low within-species diversity and apparent connectivity over large distances are not limited to the Sulawesi Sea and Tomini Bay *Variola* grouper populations. A similar low intraspecies diversity has been reported in India (Basheer et al. 2017) and Aceh, Indonesia (Fadli et al. 2021) for both species and in the Philippines for *V. albimarginata* (Alcantara and Yambot 2016). Furthermore, a more detailed analysis of GenBank accessions for the genus *Variola* indicates very low intraspecies diversity for the COI-mtDNA marker with no geographically related structure in an area comprising Indonesia (Aceh to Ambon) and the Philippines (Davao to Manilla).

Ocean current connectivity can lead to genetic connectivity because currents carry the planktonic larval stages of many marine organisms (Almany et al. 2009; Almany et al. 2013; Abesamis et al. 2017). Although grouper life-history traits such as spawning aggregations and planktonic larval dispersal are conducive to at least short-range population connectivity, the dispersal radius for the majority of larvae, including groupers, is typically on the order of dozens of kilometers (Almany et al. 2013; Abesamis et al. 2017). Thus, the extent of this area with low intraspecies diversity would indicate that the high inferred genetic connectivity does not reflect direct demographic connectivity. It is more likely to reflect many stochastic larval or adult-phase movements between relatively close populations, influenced by factors including variability in past and present currents. While direct contact between the populations of *V. louti* and *V. albimarginata* in the Sulawesi Sea and Tomini Bay could occur because these two sea areas meet at the eastern extremity of the northern arm of Sulawesi Island, it is more likely that both areas could receive at least some grouper recruits from the same source populations further north in the Sulawesi Sea, for example in the Philippines. Surface water currents flow from the north branch in the Sulawesi Sea; one main branch flows west and then south through the Makassar Strait, while another flows east and then south down the east coast of northern Sulawesi, with branches entering Tomini Bay and flowing towards Morotai and Halmahera (Masumoto et al. 2001; Han et al. 2016; Santoso et al. 2022). Seasonal and interannual changes in this overall pattern and eddies can result in highly variable current flow along the north coast of Sulawesi. Hence, the currents in the Sulawesi Sea north of Gorontalo could flow into the Makassar Strait or to the east and possibly enter Tomini Bay (Masumoto et al. 2001). It is, therefore, logical to expect close kinship relationships between grouper populations in eastern Indonesia, and in particular, from around Sulawesi (including Tomini Bay and the Sulawesi Sea), the Moluccas, Nusa Tenggara, and Papua, as has been found for other fish such as mackerels (*Rastrelliger* spp.) (Akbar et al. 2022).

Management and conservation

Based on the IUCN (International Union for Conservation of Nature and Natural Resources) Red List conservation status category, *V. albimarginata* (Sadovy de Mitcheson et al. 2018) and *V. louti* (Nair et al. 2018) are both categorized as Least Concern. The main difference is in the population trend, which is decreasing for *V. albimarginata* but stable for *V. louti*, although both are likely experiencing localized declines due to overexploitation (Sadovy de Mitcheson et al. 2018; Nair et al. 2018). Overexploitation of *V. albimarginata* is reported from western to eastern Indonesia seas, including Sibolga in North Sumatra (Hargiyatno and Faizah 2021), North Maluku (Ernawati et al. 2021), and Saleh Bay, West Nusa Tenggara, where the spawning ratio is reported to be below 20% (Halim et al. 2020). Information on *V. louti* is more limited, with little information on the status of populations (stocks) of either *Variola* species in the Sulawesi Sea and Tomini Bay, although other groupers such as *Plectropomus leopardus* and *Epinephelus coioides* are heavily or over-exploited in these seas (Achmad et al. 2020, 2022).

The genetic population structure of *V. louti* and *V. albimarginata* shows no indication of stock separation. The populations of *V. louti* and *V. albimarginata* in the Sulawesi Sea and Tomini Bay could be considered as belonging to one genetic stock unit, which could reasonably be considered as one management unit spread across more than one FMA, in particular FMA 716, which comprises the Sulawesi Sea north of three provinces (Central Sulawesi, Gorontalo, and North Sulawesi), and FMA 715, which includes Tomini Bay as well as waters east to Papua (more than one FMA). In the context of the management and conservation of *V. louti* and *V. albimarginata* fisheries in Gorontalo Province (Sulawesi Sea and Tomini Bay), there is some justification for the suggestion that they be managed jointly under the authority of the Gorontalo Province Maritime Affairs and Fisheries Service. This is especially so for any interventions involving fish movement between the two areas or the development of captive breeding and subsequent juvenile/fingerling release for commercial or conservation purposes. However, it might be wise to validate the genetic homogeneity with one or more other genetic markers.

However, several aspects should be further investigated before adopting a fully single-stock approach. Firstly, although direct or indirect (e.g., through stepping-stone populations) connectivity events almost certainly occur with sufficient frequency and volume to maintain the genetic homogeneity observed in this and other studies, it appears unlikely that there is direct demographic connectivity between the *Variola* populations in the Sulawesi Sea north of Gorontalo Province and those in the Tomoni Bay coastal waters south of this province. This would entail larvae or adults moving considerable distances, first east and north along the coasts of North Sulawesi and then turning south and moving westwards

around the full extent of the North Sulawesi coast to reach Gorontalo provincial waters. While the distance is likely unrealistic for any life stage, juveniles or adults would also be at risk from fisheries targeting groupers throughout this journey. Therefore, if a *Variola* grouper population is depleted in one of these areas, the probability of natural replenishment from the other (i.e., meaningful demographic connectivity) is vanishingly small.

The data on *Variola* grouper population genetics indicate a need to work across administrative boundaries, both in terms of FMAs (715 and 716) and in terms of provincial fisheries management units in the three provinces bordering both the Sulawesi Sea and Tomini Bay (Central Sulawesi, Gorontalo, and North Sulawesi). This is especially important as most groupers in Indonesia are caught by small-scale fishers (Halim et al. 2019; Halim et al. 2020; Kadir et al. 2023), and therefore, under the aegis of local government line agencies rather than national or FMA agencies and management structures. Furthermore, the need to formulate plans for the sustainable management of the groupers *V. louti* and *V. albimarginata* in the Sulawesi Sea and Tomini Bay requires further research to fill the gaps in current knowledge on stock assessment and population dynamics parameters such as reproductive biology, recruitment patterns, population size, and trends, as well as their ecology and behavior; all of which are important for both conservation and fisheries-oriented management.

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