VOL.67 NO.01 JANUARY-JUNE 2025 • PRINT ISSN 0025-3146 • ONLINE ISSN 2321-7898

JMBAI

JOURNAL OF THE MARINE BIOLOGICAL ASSOCIATION OF INDIA

MBAI
Marine Biological Association of India
ESTD.1958

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Available online at: www.mbai.org.in doi:10.6024/jmbai.2025.67.1.2558-07

High throughput development of microsatellite markers for yellowfin tuna (*Thunnus albacares*) and silver pompano (*Trachinotus blochii*)

P. R. Divya[1], Linu Joy[1], P. Sunitha[1], Divya Merin Jose[1], V. S. Basheer[1], Rajeshkumar[2], Rajeev Kumar Singh[2], Vindhya Mohindra[2] and Kuldeep K. Lal[2,3]

[1]PAGR Centre, ICAR-National Bureau of Fish Genetic Resources, Kochi-682 018, Kerala, India.
[2]ICAR- National Bureau of Fish Genetic Resources, Lucknow–226 002, Uttar Pradesh, India.
[3]ICAR-Central Institute for Brackishwater Aquaculture, Chennai- 600 028, Tamilnadu, India.

*Correspondence e-mail: divyanbfgr@gmail.com
ORCiD: https://orcid.org/0000-0003-1492-8005

Received: 11 Mar 2025 Revised: 15 Apr 2025
Accepted: 15 Apr 2025 Published: 13 Jun 2025

Original Article

Abstract

Yellowfin tuna (*Thunnus albacares*) constitutes approximately a quarter of all tuna catches, positioning it as the second-largest fishery among commercially significant tunas. Additionally, the Silver pompano (*Trachinotus blochii*) is another commercially important species in marine aquaculture. This study used PacBio RSII to develop well-characterized microsatellite markers for *T. albacares* and *T. blochii*, facilitating genetic stock identification and conservation management. A total of 2082 Mb and 2157 Mb polymerase read data was generated upon sequencing the 1.5 kb library of *T. albacares* and *T. blochii*, respectively. Marker panels were developed with 26 well-characterised polymorphic microsatellite loci in *T. albacares* and 36 loci in *T. blochii.* The Polymorphic Information Content (PIC) proved highly informative for both species, with values ranging from 0.623 to 0.924 in *T. albacares* and 0.31 to 0.89 in *T. blochii.* The average expected and observed heterozygosities were 0.77 and 0.73 for *T. albacares*, and 0.725 and 0.756 for *T. blochii*, respectively. Multiplex marker panels with 26 loci for *T. albacres* and 36 loci for *T. blochii* are developed as species-specific nuclear microsatellite markers, which allow the assessment of different population structures of the species across its distribution range, with more specificity. These loci in tuna have also been validated for cross-transferability in another widely distributed tuna species, *Euthynnus affinis.*

Keywords: *Conservation genetics, Fishery resources, Microsatellites, Silver pompano, Yellowfin tuna*

Introduction

Tunas belong to the Scombridae family and are valuable marine fish species in high demand worldwide. The Yellowfin tuna (*Thunnus albacares*) is one of the most commercially significant tuna species, widely distributed across the Pacific, Atlantic, and Indian Oceans in regions where surface temperatures are above 18 °C. Globally, YFT contributes approximately 27% of all tuna catches, making it the second-largest fishery among principal market tunas (FAO, 2020). The Maximum Sustainable Yield (MSY) estimate for the Indian Ocean stock is 349,000 t with a range between 286,000–412,000 t and the catch for 2023 was around 4,10,332 t. Although the catch of tuna and tuna-like species remained high, it decreased from 8.2 million t in 2019 to 7.8 million t in 2020 (FAO, 2020). This was due to COVID-19 restrictions which impacted fresh tuna exports and the sashimi market. Tuna is an important fish species due to its high economic value, extensive international trade, and large catch volumes, which lead to increased fishing volume. However, managing tuna stocks can be quite challenging because of their migratory behaviour and wide distribution across different regions. It is necessary to increase efforts related to data collection, reporting, and assessment of tuna and other allied species. According to the IOTC Report (2019), the stock status of Tuna has declined below the MSY reference level. The commission recommended ending overfishing and allowing the spawning stock biomass to recover as a protective measure. Despite all efforts by the

Regional Fisheries Management Organizations to introduce limited entry and catch quotas, tuna fleets have continued expanding, leading to increased tuna fishing mortality, biological overfishing, and stock declines (Heidrich *et al.,* 2023). Accurate identification of genetic stocks is necessary for making stock-specific regional management plans and further conservation steps. Stocks of YFT from the Indian Ocean region have received limited attention compared to those in the Pacific and Atlantic Oceans (Nishida, 1994).

The Silver pompano, *Trachinotus blochii* is another pelagic species belonging to the family Carangidae in the order Perciformes. They mainly inhabit the Indo-Pacific from the Red Sea and East Africa to the Marshall Islands and Samoa, north to southern Japan, and south to Australia. They are recognised as a popular cultured species in the genus *Trachinotus* (Ransangan *et al.,* 2011) due to their tremendous potential in biological characteristics such as rapid growth, fast adaptability to controlled conditions, and acceptability to formulated diet (Jory *et al.,* 1985). The aquaculture of silver pompano has been successfully established in many Asia-Pacific countries, especially China, Vietnam, Malaysia, India, and the Philippines. In India, several studies on broodstock development, induced breeding, larval production, and growth performance of hatchery-produced fingerlings have been conducted (Gopakumar *et al.,* 2012; Jayakumar *et al*., 2014; Pathak *et al*., 2019). However, few scientific studies have focused on understanding the genetic diversity of wild stocks and their population genetics. Therefore, generating genetic data for this species is fundamental for advanced breeding and genetic management studies.

Advances in next-generation sequencing technology and platforms have led to the fast development of several markers that can be used to address fish species' management and conservation issues. The neutral markers can provide insight into genetic diversity, local adaptation, and effective population size (Kirk and Freeland, 2011). Here, we used genome sequencing and simple sequence repeats (SSRs) to develop the genome-wide microsatellite loci for these commercially important species. We also developed multiplex panels using labelled M13-tailed polymorphic microsatellite loci, which will reduce the time and cost of genotyping. These panels will help understand the extent of individual dispersal and population structure within and between wild *T. albacares* and *T. blochii* populations, determine effective population sizes for the conservation and management of wild stocks and investigate the evolutionary processes underlying genetic divergence among populations along the Indian waters and across its distribution range.

Material and methods

Sample collection and DNA isolation

For the primer validation study, *T. albacares* were collected from Kochi, Arabian Sea (9.97°N, 76.28°E) and Visakhapatnam (17.69°N, 83.30°E), and Port Blair, Andamans (11.62°N, 92.73° E) in the Bay of Bengal (n=20 each). *T. blochii* samples were collected from Kochi, Kerala (9.97°N, 76.28°E) in the Arabian Sea; Tuticorin and Mandapam (9.2770° N, 79.1252°E) (n=20 each), along the Bay of Bengal. Muscle tissue from the caudal region of fish samples was excised and preserved in 95% ethanol at the sampling site before being transported to the laboratory. Total genomic DNA was extracted from the tissues of thesamples by the salting out method.

Screening and selection of microsatellite primers

This study identified a set of microsatellite loci for *T. albacares* using Single-Molecule Real-Time Sequencing technology. The sequencing of the 1.5 kb SMRT library generated 2081 Mb polymerase reads. Out of 298 reads examined, 151 contigs with repeat motifs were analysed and screened for sequences containing 187 SSR, resulting in 109 positive matches. Out of all the sequences that were analysed, we identified 137 di-repeats, 37 tri-repeats, eight tetra-repeats, and five penta-repeats. Among these, there were 100 perfect repeats, including 69 dinucleotide repeats (69%), 25 trinucleotide repeats (25%), and six tetra-nucleotide repeats (6%).

During the sequencing of the 1.5 kb library of *T. blochii*, 2157 Mb of polymerase read data was generated. In the sequenced data, 613 SSR-containing sequences were identified with 983 SSR. Out of 983 repetitive motifs, 216 were perfect repeats, with 75 being dinucleotides, 96 being trinucleotides, 33 being tetranucleotides, and 4 being hexanucleotides. Primer pairs specific to the flanking regions of putative SSRs were designed using PRIMER 3 input software (Version 4.1.0) for each SSR locus (Untergasser *et al*., 2012). Procedures for performing PCR and identifying polymorphic primers were followed by Joy *et al.* (2020). Seventy-five SSRs for *T. albacares* and 100 SSRs for *T. blochii* were initially screened using 12 individuals from three different locations *viz*: Kochi, Visakhapatnam, and Andamans for *T. albacares* and Kochi, Tuticorin and Mandapam for *T. blochii* to identify high-quality SSRs. Loci that exhibited no amplification, multiband patterns, or monomorphic bands were excluded, and selected polymorphic SSR loci were validated with a larger sample size. After validation, the identified regions were submitted to the NCBI for further utilization.

Genotyping and data analysis

Fragment analysis was done on Applied Biosystems ABI 3730 DNA analyser using the LIZ-500 size standard. GENE MAPPER 4.0 (Applied Biosystems) software was used for allele sizing. CERVUS 3.0.3 was used to determine the Polymorphic Information Content (PIC) (Kalinowski *et al.*, 2007). MICROCHECKER 2.2.3 (Van Oosterhout, 2004) was used to detect nonspecific amplification, null alleles, and significant large allele dropouts. Genetix 4.02 (Belkhir, 2004) software was used to assess the genetic variability parameters *viz.* expected (He) and observed heterozygosity (Ho). The number of alleles (Na), allele frequency, etc. were estimated through Population-wise linkage disequilibrium between pairs of loci. The deviations from Hardy-Weinberg expectations (HWE) were tested through Genepop v 4.2 (Rousset, 2008). The significance levels were adjusted using Bonferroni correction through multiple tests.STRUCTURE v.2.3.4 (Pritchard, 2000) assessed the distribution of genetic variance/ admixture among localities with the default parameters. Fifty thousand burn-in steps and 2, 00,000 Markov Chain Monte Carlo repetitions. Ten iterations were done for each number of populations, K, ranging from 0 to 7, to know if the number of populations identified was consistent across runs.

Cross-species transferability of identified primers

The cross-species transferability of 26 microsatellite markers developed in yellowfin tuna was tested in the most common tuna of Indian waters', the Kawakawa / Eastern little tunny *E. affinis.*

Results

Table 1 provides information on marker panels, types of repeats, and NCBI accession details for 26 polymorphic SSRs in *T. albacares*, out of the 75 screened. Similarly, 36 SSR primers were polymorphic in *T. blochii*, out of 100 different numbers of primers screened. The details related to marker panels, types of repeats for each primer, and NCBI Accession details for *T. blochii* can be found in Table 2.

The mean number of alleles per locus in *T. albacares* was 8.7 (7–16), and the mean observed and expected heterozygosity were 0.73 and 0.77, respectively. Among the 26 microsatellite loci, all loci had PIC values higher than 0.50, ranging from 0.623 to 0.924, with an average of 0.773. Neither significant linkage disequilibrium nor evidence of stuttering or large allele dropout at any of the loci was observed among the loci after the Bonferroni correction p = 0.001. Estimated null allele frequencies assessed with MICROCHECKER were insignificant ($P > 0.01$). Out of 78 tests, only five loci showed deviations from Hardy-Weinberg equilibrium ($p<0.001$) after applying Bonferroni corrections. The loci showing deviations from the HW equilibrium were TA3C-4 in samples from Kochi, TA2C-65 from Visakhapatnam, and TA2C-55, TA2C-67, TA2C-83 from Andaman populations, and the deviations were observed may be due to heterozygote deficiency, which are supported by significant p values. The validated microsatellite primers can be used for the analysis of stock structure.

The number of alleles per locus and the adequate number of alleles in *T. blochii* ranged from 2 (TB3C-10) to 16 (TB3C- 53) and from 1.36 (TB2C-230) to 7.45 (TB3C-53), respectively. Observed (H_O) and expected heterozygosity (H_E) were 0.18 to 0.75 (mean $H_O = 0.759$) and 0.19 to 0.77 (mean $H_E = 0.725$), respectively. The polymorphism information content (PIC) per locus ranged from 0.310 (TB3C-10) to 0.896 (TB2C-360), with most of the loci having PIC value > 0.75. The higher PIC further indicated the utility of these markers for population assignment (MacHugh *et al.*, 1997). Almost all the microsatellite markers selected for our analysis were characterised by a high polymorphism of heterozygosity or by high values of the PIC. The inbreeding coefficient (F_{IS}) ranged from -0.369 (TB3C-22) to 0.253 (TB3C-8, TB3C-32) with an overall -0.52. An overall negative F_{IS} might be an indication of an increase in heterozygosity level, which could be the result of a random mating system leading to high genetic variability. Tests on Hardy–Weinberg equilibrium for each locus revealed no deviation for all loci except two loci (TB3C-74 and TB3C-22). The data generated during primer panel validation were subjected to stock structure analysis. Bayesian STRUCTURE analysis of lesser microsatellite genotypes identified K = 2 as the most likely population number in *T. albacares* and K = 3 in *T. blochi* (Fig. 1a and b).

Discussion

This study aimed to generate species-specific polymorphic microsatellite markers in two commercially important marine species, *T. albacares* and *T. blochii.* Generally, specific markers are considered more beneficial than heterologous primers due to increased observed heterozygosities, larger alleles per locus, and the extent of polymorphism. Previously, several studies (Takagi, 1999; McDowell *et al.*, 2002; Clark *et al.*, 2004; Nikolic *et al.*, 2015; Lopes *et al.,* 2010) have developed microsatellite markers in *T. albacares* through a cross-priming approach. Lopes *et al.* (2010) emphasised that, if possible, population studies should be undertaken by using species-specific microsatellite primers. Antoni *et al.* (2014) identified and characterised 16 SSR loci in *T. albacares*, most of which comprised dinucleotide repeats. However, we developed and characterised 26 SSR loci in the present study, a combination of di, tri, and tetranucleotide repeats.

Table 1. Characteristics of the 26 microsatellite loci marker panel developed for *T. albacares*

Multiplex Panel No.	Loci Name	Primer Sequences (5'-3')	Fluorescent label	Repeat motifs	Tm (°C)	N_A	Allele size	PIC value	GenBank Accession Number
Panel 1	TA2C-5	F:CTA CGT GTG TGT GCG TTT R:CTG CAG AAA GAT GAT TAT GG	FAM	(TG)14	56	9	150-200bp	0.734	MT920678
	TA2C-8	F:CAG GAA AAG AAA AGA CAA GG R:CAG ACA GAA CTC TGG TTG CT	HEX	(TG)14	56	12	140-180bp	0.713	MT920679
	TA3C-16	F: GAG CTG GAC CGG GTA ACT A R: GCA CAC ACA AAC CAC AAC AA	TET	(TTA)7	56	11	140-180bp	0.718	MT920680
	TA2C-12	F: ATG GTG GAG GTG GAG CAA R: TTG GCG TCT GTG TAA TGA GG	FAM	(GT)11	54	10	130-170bp	0.910	MT920681
Panel 2	TA2C-38	F: CCG TGT CAG TCT CAT TTT ATG G R: GGT GGA GGC AAT TTT CAG AG	HEX	(CA)6	54	15	120-170 bp	0.731	MT920682
	TA2C-74	F:GCC CAG CAG CTA GTA TAT GT R:AAA AGA GGA GAG GAT GTT CA	TET	(AC)11	54	9	120-170 bp	0.924	MT920683
	TA2C-16	F- GAG CTG GAC CGG GTA ACT A R- GCA CAC ACA AAC CAC AAC AA	FAM	(AC)7	55	7	130-180bp	0.793	MT920684
Panel 3	TA2C-37	F- GGG GTC TGA CCT GGA TAC AA R- ACC AAC CTG TGA GGC TGA AG	HEX	(TA)6	55	9	120-180bp	0.755	MT920685
	TA2C-40	F- ACA GAG TGA TGG GTG CG TTA R- GCA GGA CAA AGG TGA AGG AG	TET	(AC)9	55	11	120-180bp	0.661	MT920686
Panel 4	TA2C-35	F- ACC TTG TCA GCC TCA TGC TC R- CCC CAA TAA ATT ATC TCA CTG TCC	FAM	(CA)11	55	13	130-180bp	0.852	MT920687
	TA2C-82	F:GGA GGT CAA ACA GCG TCT R:CCC TTT CTT CTTCTT CTC TTG	HEX	(GT)6	55	15	120-180bp	0.850	MT920688
Panel 5	TA2C-39	F- TTC CTT ATC CCA TCC TGT GC R- CAG CCA TTT ATC TCC CCT GA	FAM	(TG)8	55	14	120-180bp	0.881	MT920689
	TA2C-50	F:CGA GGA GAC AAC TTG TTG CTA A R:GCG GGA TAT GTG TGT GAG TG	HEX	(CA)10	55	9	120-180 bp	0.817	MT920690
Panel 6	TA2C-55	F: ATT GTC AAT GAC CAG TCT GT R:GTC TGT TTG TCT GTC TGC TG	FAM	(AG)14	55	8	90-140bp	0.634	MT920691
	TA2C-67	F:TGC CGG TAA TTA CTG TCG AG R:TTA TCT GAG CGG GTC TGC AT	HEX	(CA)11	55	11	120-180bp	0.852	MT920692
Panel 7	TA2C-76	F:AAA GCT CAG GTG AAC AGA GT R:CAG ACG AGA CAG ATG ATC C	FAM	(GT)8	53	14	110-200 bp	0.907	MT920693
	TA2C-77	F:TTG TCC CTC AAA GAC ACT TC R:TAA GAC GAT CAA AGC CAT GT	HEX	(TG)13	53	16	120-200bp	0.884	MT920694
	TA3C4	F: CAG CTC TGC TTA GAT TCT CCA R: CGT GTG CGC CTT TTA TTT T	TET	(ATA)7	53	8	120-200bp	0.817	MT920695
Panel 9	TA2C-53	F:CCC AAA CAA TAT GTT ACA GC R:ACA AGC AGA CTT GCA GTG	TET	(AC)15	54	15	120-200 bp	0.854	MT920696
	TA2C-81	F: CCT TCA TTC TCT GCT CTC AG R:GCG GTG TGT ATT AGT CAC TTT	FAM	(TG)10	54	9	100-180bp	0.892	MT920697
Panel10	TA3C-15	F:CCG ATC GAG CCA AAC AAA R:CGG ACT CAG AGG AGG AAG AG	HEX	(TCC)10	56	10	110-180bp	0.768	MT920698
	TA4C-2	F: GCC GCC AAA CAC AGT TTA GT R:TCA GCA GAA TTG AGC ACC TG	TET	(TTAT)5	56	12	120-180 bp	0.768	MT920699
Panel 11	TA3C-2	F:TGCTCTGATGATGATGGTTA R:AGTGAATGTGGTGTTGATTG	FAM	(CTC)11	54	13	180-250bp	0.832	MT920700
	TA3C-3	F: TTCCACTGTTCCATGTATCC R:GGACTGCTGAGTTTTGTTGT	HEX	(ATT)5	54	12	180-25bp	0.853	MT920701
Panel 12	TA2C-83	F:GGGAAAACAGTGGAGGAC R:TCAGCGGTGGAGTAGAGATA	FAM	(TG)11	54	11	180-250bp	0.881	MT920702
	TA2C-65	F:GTCTTGTGGATAGTCAGCAT R:CTGGAGCTTTTGTTGTTTC	HEX	(TA)6	54	8	180-250bp	0.623	MT920703

Table 2. Characteristics of the 36 microsatellite loci marker panel developed for *T. blochi*

Multiplex Panel No	Loci name	Primer sequence 5′-3′	Fluorescent label	Repeat motif	Tm (°C)	N_A	Allele size (bp)	PIC	Accession no.
Panel 1	TB2C26	AAACATCTTTGGTTCTGCTC GTTGACTGGTGAAATGGAAA	FAM	(TA)20	54-56	9	146-172	0.823	MW086735
	TB2C168	AACAGGCGAACGTGTAAA CTCAGTCATGGCTCTGAATA	HEX	(GT)22	54-56	11	136-176	0.867	MW086736
	TB2C401	AAAGAGTCAAGGGAGGAAAA TGTGTGTGTGCTCATCTGTA	TET	(TC)18	54-56	10	136-172	0.840	MW086737
Panel 2	TB2C150	TGAATAGTTCACTGGCCTTT AGCGATGATCAGACACAGA	FAM	(GT)26	54-56	8	124-178	0.771	MW086738
	TB2C230	TACGAACCTCATATGCCACA TTAAGTGTCCACACAGCTCA	HEX	(CA)16	54-56	5	124-146	0.263	MW086739
	TB2C236	TGTGGTAACAGCATTACACTCT ACTCAGGTGAGCTTCCATC	FAM	(TG)30	54-56	8	134-156	0.760	MW086740
Panel 3	TB2C249	GTGTTTTGTCTTCAGTCACAGT GTTCCTCTAATTCCCTCCAT	HEX	(CA)18	54-56	9	134-156	0.782	MW086741
	TB3C-59	GGGCCATTTATGAGATCC GCACCACACCTGTGTTATT	TET	(GAT)9	54-56	8	135-159	0.731	MW086742
	TB2C449	TCTGAACCACCTTCTGAACT AACAACTATTGCCTCTTTGC	FAM	(AC)16	54-56	11	118-158	0.813	MW086743
Panel 4	TB2C43	ATGAGTGAGAACACACACGA AGGACAATAACGGAGCTGA	HEX	(AC)18	54-56	9	118-158	0.810	MW086744
	TB3C-54	CAGACCCTGTTATTGCTCTT AGGATTGCGGCTCATTAC	TET	(GTT)10	54-56	14	117-159	0.853	MW086745
	TB2C239	TCTTTGTTAGAGCACTTACCG GACTTTGTTTTTGCCAACTC	FAM	(AC)16	54-56	15	188-222	0.869	MW086746
Panel 5	TB3C-48	CGTGGCAGCTATCTTTTT TCTATGGTCAGGACCAACTT	HEX	(TGT)7	54-56	12	186-225	0.878	MW086747
	TB2C368	TGACTTTACAGCAAACTGGA ATGAGACCTCGAAGCAGAC	TET	(AC)25	54-56	14	188-224	0.885	MW086748
Panel 6	TB2C498	AGTTTTGCAGAAGGTTGTGT TGTATGTGAGTGGGTGAATG	FAM	(AC)15	54-56	12	152-214	0.771	MW086749
	TB4C23	AGTGTGTGTGTGGCAAGA GGGAAAAAGGGAGAAGTAGTA	HEX	(TTTC)10	54-56	11	172-224	0.842	MW086750
	TB4C11	TATGCGACAAGCCCTAGA TAGTAACGGACCCTGATAGC	FAM	(AAAT)5	54-56	7	152-184	0.644	MW086751
Panel 7	TB3C-81	GAACATTACATGCAGGAGA TTCATTCACTCCCCCTAAC	HEX	(AAG)5	54-56	7	153-180	0.714	MW086752
	TB3C-74	CGGTGCACTAAACAAACAT GGTGTCATTGAGAATCGAGA	TET	(TAA)13	54-56	6	165-180	0.650	MW086753
	TB3C-77	TCTCCCGAGTGATTATGCT GTAAAATTGTGCCCTCTCC	FAM	(GAA)5	54-56	5	189-213	0.709	MW086754
Panel 8	TB2C396	CTCCTCTCAGTGCTAATCCA GTATTTCAACAGGCCAAAAG	HEX	(TG)22	54-56	7	188-216	0.658	MW086755
	TB2C311	TATGCCACATTATCACATGC CACACACAACCTCAGAAAGA	TET	(CA)16	54-56	6	188-218	0.575	MW086756
Panel 9	TB3C-10	ATTGCACTTGAGGTGAAAGA ATCCTGGGTTGTGTCTGAG	FAM	(GGA)6	54-56	2	162, 171	0.310	MW086757
	TB2C446	CCCTGAGGAAAAAGAAAAAG ACCCATGCACACATACTGA	HEX	(TG)18	54-56	8	134-162	0.737	MW086758

Multiplex Panel No	Loci name	Primer sequence 5′-3′	Fluorescent label	Repeat motif	Tm (°C)	N_A	Allele size (bp)	PIC	Accession no.
Panel 10	TB2C479	AAAAAGTGAAGAAGGGTGTG AATGGAGGTGAGGAGTGTG	FAM	(CA)18	54-56	3	196,200,202	0.503	MW086759
	TB2C110	GGACTAATGAGGAGCTGATG AGAATAAATGCGATGGACTG	HEX	(CA)18	54-56	9	160-198	0.782	MW086760
Panel 11	TB3C-20	CTATTGATGCTGCTGCTCT ACACCCTGTCTGTCCTGTC	FAM	(ACC)5	54-56	5	114-144	0.573	MW086761
	TB3C-22	CGTTCTGAGGAAACGAAT GGCGCTAGTGATCTACACA	HEX	(GAT)5	54-56	6	114-144	0.517	MW086762
Panel 12	TB3C-95	ATGAGAACTGTTTCCACCAC ACTTGACTGAACCTGAACCT	TET	(ATG)7	54-56	5	123-144	0.385	MW086763
	TB5C-3	TACACTGTTTTGCCAGTTCC GAGTCCCTGCTTTTACTCCT	FAM	(TAATA)7	54-56	8	145-180	0.775	MW086764
	TB3C-53	ACACAAAGGAGCAAAAGAAG ACCTGAAAGTGGCTGAGAG	HEX	(CAG)7	54-56	16	123-180	0.896	MW086765
Panel 13	TB3C-66	GTCGGGTTAAAGTTTCAGG GTGGAGACACCAGGAATAAA	HEX	(TTG)8	54-56	8	162-183	0.793	MW086766
	TB3C-78	TTTACAGCTCTGGAAAGGAA AACTGCTACATGGTGAGAAA	TET	(GAT)11	54-56	9	159-183	0.798	MW086767
Panel 14	TB2C360	CTCTTATATCCTCGGTTACTCG CTTTTTCTCCCTTCCTCTCT	FAM	(TG)22	54-56	13	110-172	0.896	MW086768
	TB3C-8	ACTGCAGTTGACTGAGTGC CATGAGGAATGCCTTTTATC	HEX	(GCC)5	54-56	7	147-171	0.800	MW086769
	TB3C-32	TAAAGACCGTGTGTTTGGAT ACTGCTACTCTGTGAGAGACG	TET	(AAT)8	54-56	8	117-171	0.820	MW086770

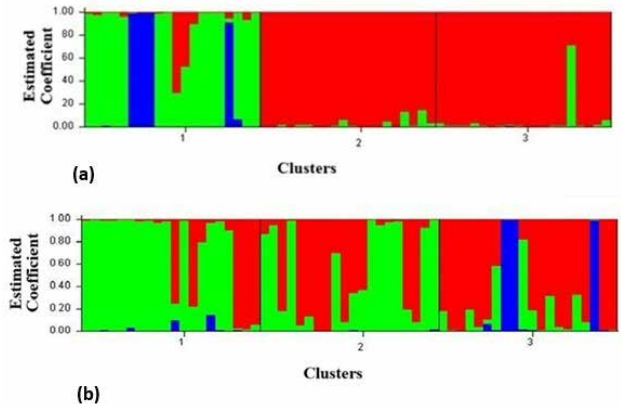

Fig. 1. The plot of K results from the STRUCTURE analysis of a)*Thunnus albacares* into two populations and b) *Trachinotus blochi* into three populations (K values ranged from 1 to 7). The panel displays the results of bar plots of the population; each colour represents a cluster

Yellowfin tuna is considered to be a species that shows transboundary migrations. The developed primers were validated using samples from Kochi, Andamans and Visakhapatnam. Though YFT contributes to a significant fishery in the Indian Ocean, attempts to identify the genetic stocks in this region are limited, except for a few (Nishida *et al*., 2001; Dammannagoda *et al*., 2008; Kunal *et al*., 2013; Barth *et al*., 2017). Sampling locations *viz.* Kochi, Visakhapatnam, and Andamans were similar in both studies, and the studies of Kunal *et al.* (2013) could not differentiate the stock of Andamans using mt DNA marker. The nuclear markers developed in the study are polymorphic, necessary enough to reveal the stock structuring within YFT populations. A holistic approach to the genetic structuring of *T. albacares* in the Indian Ocean region remains unexplored. The marker panel developed for both species in this study will help form a rapid and economical method for detecting the genetic stock of a highly valued shared species. This study does not aim to examine the genetic stock structure of YFT in Indian waters. Rather, the focus is on creating low-cost and polymorphic marker panels that can be utilized by researchers and policymakers worldwide. These panels can aid in the conservation and management of *T. albacares*, spanning across its natural distribution. Cross-species transferability of the identified polymorphic microsatellite markers was also tested in *E. affinis*. Analysis of the cross-transferability of 26 loci showed amplification at 12 genomic sites in *E. affinis.* A total of 4 loci exhibited polymorphism with 2–6 alleles, eight loci showed monomorphism, and most loci (14) failed to amplify in *E. affinis*. The loci amplified in *E. affinis* were TA2C16, TA2C38, TA2C39, and TA3C15, and

these markers can be used for population genetic works in *E. affinis*.

The current study also aimed to develop polymorphic microsatellite markers for genetic stock identification of Silver pompano, to form baseline information to initiate hatchery programs for this culture-important species. In the present study, a large panel of microsatellite markers was developed and confirmed for use *T. blochii* diversity studies. The panel comprises 36 polymorphic loci with a cost-effective multiplex PCR with a universal fluorescent primer (M13) approach. Statistical analysis indicated a high degree of polymorphism with a fine-scale genetic structuring revealed that the characterised microsatellites in this work will be useful to explore the population genetics and stock structure of these highly valued aquaculture species.

Conclusion

Microsatellite markers are used to assess genetic diversity and structure in populations. Genotyping individuals from *T. blochii* and *T. albacares* can reveal genetic variation, gene flow, and connectivity and these factors which are crucial for defining genetic stocks for conservation. Microsatellite markers developed in this study can identify unique populations, assess inbreeding, species' genetics, behaviour, and ecological roles and guide management strategies. To summarise, this information can support conservation efforts and aid in these valuable species' sustainable use and protection.

Acknowledgements

The authors acknowledge the Director, ICAR-NBFGR, for providing support and facilities for the study.

Author contributions

Conceptualization: KKL; Methodology: DPR, RKS, VM; Writing Original Draft: DPR, LJ; Data Analysis: LJ, SP, DMJ; Data Collection: BVS, RK; Supervision: DPR

Data availability

The data are available and can be requested from the corresponding author

Conflicts of interest

The authors declare that they have no conflict of financial or non-financial interests that could have influenced the outcome or interpretation of the results.

Ethical statement

No ethical approval is required as the study does not include activities that require ethical approval or involve protected organisms/ human subjects/ collection of sensitive samples/ protected environments.

Funding

This research was carried out as part of an outreach activity on genetic stock identification of fishes funded by the Indian Council of Agricultural Research.

Publisher's note

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