Origin and History of Introduction of Rainbow Trout, *Oncorhynchus mykiss* (Walbaum, 1792) Stocks in Southern India As Inferred From Y-linked Marker

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Abstract

Rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), is a popular cold-water fish widely distributed and farmed globally. In the nineteenth century, rainbow trout were introduced into India, and since then, it has gradually spread and established itself as one of the most prevalent non-native fish. In Southern India, rainbow trout were introduced from New Zealand in 1909 in the Ooty region of the Western Ghats, followed by introductions in other coldwater regions in the Peninsular uplands such as Munnar and Kodaikanal. Continuous introductions were done from various geographical locations at different periods to increase *O. mykiss* stocks in the Peninsular upland regions. Despite being regularly introduced in the streams of Munnar, Ooty, and Kodaikanal of Southern India, they still have not become self-sustaining, and genetic diversity has been suggested as a potential underlying factor. Therefore, this study aimed to resolve these questions and explore the structure and origin of Southern Indian stocks by conducting a population genetic study. The Y-linked marker of the trout stocks sampled from Munnar, Ooty, and Kodaikanal was compared with that of the native populations from North America. The results showed less proportion of inter-population genetic variation, suggesting that Southern Indian stocks were derived from multiple origins of population, with a great majority of parental populations belonging to the coastal rainbow trout from North America. This study revealed no considerable genetic differences among the Southern Indian stocks and reported the major ecotype as the Steelhead trout, *Oncorhynchus mykiss irideus* (Gibbons, 1955).

Keywords: *Oncorhynchus mykiss*, *Salmo trutta fario*, OmyY1, trout introduction, Western Ghats, maximum likelihood

Introduction

Salmonids like rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), are highly preferred by many anglers as a sport and recreational fish. Rainbow trout are aquaculture species (Crawford and Muir, 2008) that have been widely introduced across the globe since the nineteenth century, and currently, they are found in 75 countries (Singh et al., 2016). Since their global introduction, they have successfully established themselves as self-sustaining populations (Stanković et al., 2016). The rainbow trout is also an economically important food fish, native to the Pacific Basin of the North American region, the northern region of Mexico, and the western Pacific regions of Kamchatka. Behnke (2002) has thoroughly reviewed the subspecies and classification of rainbow trout. Within the United States, stocking practices have distributed the coastal subspecies of rainbow trout widely throughout the range of the inland subspecies. These lineages are recognised by differences in colour and numbers of pyloric caeca, scales along the lateral line, vertebrae, and gill rakers (Behnke, 1992).

Microsatellite differences and allozyme frequencies have often been used to study hybridisation events between these subspecies (Utter, 2001; Knudsen et al., 2002; Small et al., 2007). The discovery of polymorphic Y-linked marker in rainbow trout by Brunelli et al. in 2008 has helped in the study of geographic distribution among rainbow trout populations. This marker was initially identified through a homology sequence,

isolated in the closely related Chinook salmon, *Oncorhynchus tshwytscha* (Walbaum, 1792), from an amplified fragment length polymorphism (AFLP) band present in males but not in females (Brunelli and Thorgaard, 2004), as only the male-specific region of the sex chromosome provides an accurate legacy of paternal lineages (Brunelli et al., 2010).

This study aimed to consolidate the history of the introduction and geographical distribution of rainbow trout stocks in Southern India. In Southern India, trout fisheries were first established by Francis Day in 1863 in Ooty (11°,22',30"N 76°,45',30"E), located in the Western Ghats. Brown trout, *Salmo trutta fario*, Linnaeus 1758, and Loch Leven trout, *Salmo levensis* Linnaeus, 1758, were the first species to be introduced, but the attempts to successfully establish fisheries in this region were not successful (Jhingran and Sehgal, 1978). In 1909, Henry. C. Wilson successfully introduced rainbow trout from New Zealand. However, the number and size of the stocks in this region diminished over time. Therefore, to overcome this issue, rainbow trout stocks from Kashmir, North India (Mackay, 1945), were introduced in 1920. The existing stock was further increased by introducing salmonids [golden trout, *Oncorhynchus aquabonita* (Jordan, 1892), tiger trout (*Salmo trutta* Linnaeus, 1758 × *Salvelinus fontinalis* (Mitchill, 1814), sockeye salmon, *Oncorhynchus nerka* (Walbaum, 1792), and brown trout, *Salmo trutta fario* Linnaeus, 1758, of which golden trout alone survived] from Japan in 1968 (Anon, 1987), followed by other stocking efforts in 1989 by introducing rainbow trout from Munnar, which resulted in the formation of hybrids.

Further efforts were again initiated in 1997 by the National Bureau of Fish Genetic Resources through a crossbreeding program, which failed (Thakur et al., 1997) with a hatching percentage of 0.5 %. Several attempts at introductions and translocations of trout resulted in unclear taxonomic identity. Therefore, the genetic profile of this trout stock needs to be verified (Gopalakrishnan et al., 1999), which is currently challenging due to the endangered state of the stocks (Devaa and Ramesh, 2022).

Brown trout, *Salmo trutta fario*, fisheries were first established in 1909 in the Munnar High Range (10°05'21"N 77°03'35"E) located in the Western Ghats region and were successfully established until 1914. This was initially successful, but further maintenance and stocking operations were not possible due to the First World War. Therefore, no further documentation on trout fishing was done until 1932 (Mackay, 1945). Subsequently, rainbow trout were introduced in 1932 from Ooty and in 1941 from Sri Lanka (Mackay, 1945) and successfully established. Similarly, the Palani Hills Game Association introduced rainbow trout in the Kodaikanal Hills (10°21'60.3"N 77°42'87.4"E) of the Western Ghats in 1943. However, no evidence on trout's existence in this region is available since their introduction (Kuruppan, 1989). Currently, rainbow trout stocks in Munnar (Devaa et al., 2021) and Kodaikanal are endangered.

In contrast, rainbow trout and brown trout have been successfully established in the North Indian states such as Jammu and Kashmir, Himachal Pradesh, Sikkim, and Arunachal Pradesh, which has boosted the states' economy. Although trout have been successfully introduced in Southern India, they are currently endangered (Devaa et al., 2021; Devaa and Ramesh, 2022), and no sufficient studies on the Southern Indian stocks are available. Genetic studies on the North Indian stocks have revealed significant genetic variability among the stocks (Barat et al., 2015). However, no genetic studies have been done on the trout stocks in extreme Southern Indian regions. Tracing the history of the introduction of trout in the Southern Indian regions is complex, as trout were introduced at various intervals and from various geographical locations. For example, in Ooty, trout were introduced from various locations such as New Zealand; Kashmir, North India; Japan; and Munnar, Southern India. Similarly, in Munnar, trout were introduced from two geographical locations: Ooty (Southern India) and Sri Lanka. However, the source of the introduction of trout stock in Kodaikanal is unknown.

The rainbow trout is well known for its high nutritional profile (Devaa et al., 2021); therefore, the demand is ever-increasing, ultimately declining the population status. The population status of rainbow trout is a prerequisite for conservationists, anglers and aquaculturists. Therefore, genetic studies have been done by using independent molecular systems (mitochondrial DNA sequences, allozymes, microsatellite loci, MHC, SNPs, and even genome-wide studies (Bagley and Gall, 1998; Nielsen, 1999; Johnson et al., 2007; Heath et al., 2008; Stephens et al., 2009; Brunelli et al., 2010; Simmons et al., 2010; Hecht et al., 2012, 2013). However, these studies show little intraspecific variation due to complex evolutionary history. Very few studies have analysed the origins of introduced trout stocks, and those studies are from Argentina (Riva Rossi et al., 2004), Chile (Colihueque et al., 2019) and Europe (Stanković et al., 2016) and all three studies have proposed the representation of multiple lineage sources. Such studies are not available in a biodiversity-rich country like India.

Therefore, this study aimed to investigate the genetic relationships to find the multiple lineage sources of rainbow trout stocks introduced from the North American regions to the three locations of Southern India. Studies have shown that Y-chromosome markers (OmyY1 Locus) are ideal genetic markers with a fixed locus that can be exploited for identifying trout species and revealing phylogenies (Brunelli et al., 2013). Therefore, this study employed the OmyY1 marker to gain insight into the origins of the Southern Indian naturalised rainbow trout stocks. In addition, other reference OmyY1 sequences were assembled

using the haplotypic data available for native populations from North America.

Materials and Methods

Ethical approval

All suitable approvals and permissions were received from the Director and Additional Director of Tamil Nadu Fisheries Department for the collection of caudal fin clippings of the suspected hybrid trout stock from the Ooty region of the Southern Western Ghats of India (No. 3790/F1/2019, dated 25/03/2019).

Study area and sampling

Twelve tissue samples (fin clips) of male rainbow trout were collected from three localities of the Western Ghats in Southern India – (1) Gundar Stream (n = 5; 10°21'60.3"N 77°42'87.4"E), Kodaikanal, Tamil Nadu (TKT: Trout from Kodaikanal, Tamil Nadu), situated at an elevation of 2073 metres (MSL); (2) Lakkidi Stream (n = 4; 11°15'59.9"N 76°33'51.3"E), Upper Bhavani Reservoir, Ooty, Tamil Nadu (TOT: Trout from Ooty, Tamil Nadu), situated at an elevation of 2263 metres (MSL); and (3) Rajamallay Stream (n = 3; 10°,15',41"N 77°00'50"E), Munnar, Kerala (TMK: Trout from Munnar, Kerala), situated at an elevation of 1973.58 metres (MSL) (Fig. 1). Due to unavailability of male rainbow trout samples, a considerable portion of samples were taken. The size varied from 1 to 5 depending on the locality. Fin clips were taken and stored in 95 % ethanol (Hayman, United Kingdom).

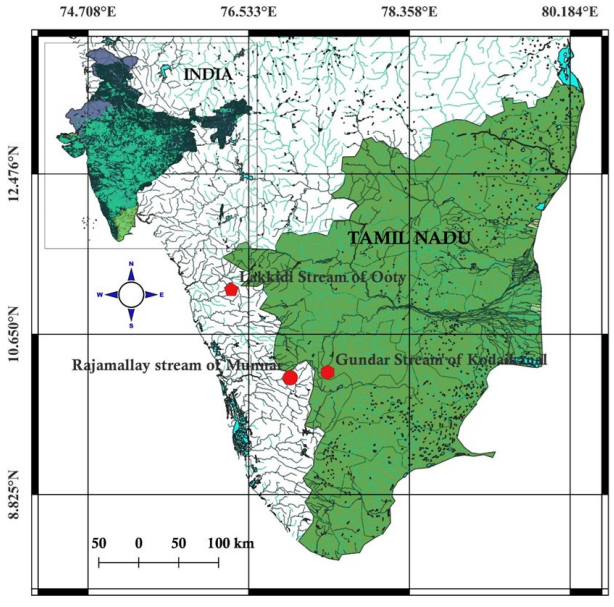

Fig. 1. Locations from which fin tissue samples of *Oncorhynchus mykiss* were collected for Y-linked marker analysis: Gundar Stream, Kodaikanal, Tamil Nadu, Southern India; Lakkidi Stream, Ooty, Tamil Nadu, Southern India; Rajamallay Stream, Munnar, Kerala, Southern India. Inset map: India. Blue line in the figure indicates non-perennial/intermittent/fluctuating water areas and black line indicates perennial/permanent water areas.

DNA extraction, amplification, and sequencing of Y-chromosome (OmyY1)

Genomic DNA was extracted by proteinase-K digestion method followed by phenol-chloroform-isoamyl alcohol protocol as described by Russell and Sambrook (2001). The concentration of the extracted DNA was estimated using a UV spectrophotometer (Shimadzu, Japan) and 1 % agarose gel electrophoresis with 1× Tris-boric acid-EDTA (TBE) buffer stained by ethidium bromide. The concentrate obtained was qualitatively visualised for the presence of DNA using a UV transilluminator (BIORAD, USA). The 1058 bp sequence of the OmyY1 locus was amplified by employing OmyY1 SNP evaluation primers – forward primer (5′-GACAGTTGTGGCAATAGATA-3′) and reverse primer (5′-CGATTAGAAAGGCCTGCTTG-3′) (Brunelli et al., 2008). Amplification of OmyY1 locus was carried out by adding a final concentration of 50 ng.μL^{-1} of sequenced DNA to a 40 µL reaction mixture containing 20 µL of Ampliqon Taq DNA Polymerase 2× Master Mix RED with 1.5 mM $MgCl_2$ (Ampliqon, Denmark), 5 pmol (0.8 µL) of forward primer and 5 pmol (0.8 µL) of reverse primer, 4 µL of 50 ng template DNA, and 15.2 µL of sterile Milli Q, and the reaction was carried out in SureCycler 8800 thermal cycler (Agilent Technologies, USA). The PCR conditions for the OmyY1 Locus were as follows: initial denaturation at 95 °C for 3 min followed by 30 cycles of strand denaturation at 94 °C for 50 s; annealing at 60 °C for 30 s; and extension at 72 °C for 50 s with a final extension at 72 °C for 4 min. The amplified PCR products were then electrophoresed on a 1 % TBE agarose gel stained with ethidium bromide (20 ng/µL), observed with 1 kb DNA ladder (Thermo Scientific, USA) using UV transilluminator, and documented using molecular imager, ChemiDoc XRS+ with Image Lab Software (BIORAD, USA) gel documentation system. The amplified bands were gel-eluted using gel extraction kit (FavorPrep FAGPK 001, Favorgen, Taiwan). The positive purified amplicon was sequenced by Sanger's dideoxy chain termination method using the same PCR primer (F/R) by Applied Biosystems Automated DNA Sequencing System (3500 series Genetic Analyser, Applied Biosystems, USA).

DNA sequence assembly and annotation of OmyY1

The quality of each OmyY1 sequence obtained from *O. mykiss* was analysed using Sequence Scanner Software v.1.0 (Applied Biosystems, CA, USA). Full-length sequences were assembled, and consensus sequences were annotated using Codon Code Aligner version 4.2.4 (CodonCode Corporation, MA, USA). Sequence data were primarily validated by homology search using NCBI-BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Validated OmyY1 sequences were submitted to NCBI database for open access under accession numbers MT721865.1–MT721859.1 (Table 1). Intra-specific

Table 1. Locality, sample size, haplotypes and depositions of OmyY1 sequences for samples of Southern Indian rainbow trout, *Oncorhynchus mykiss*.

Location	Latitude and longitude	Individuals	Haplotypes observed	Y-Haplotypes depositions	GenBank deposition No.
Rajamallay Stream, Munnar, Kerala, Southern India	10°15'41"N 77°00'50"E	3	1	TMK 1	MT721857
				TMK 2	MT721858
				TMK 3	MT721859
Upper Bhavani Reservoir, Ooty, Tamil Nadu, Southern India	11°15'59.9"N 76°33'51.3"E	4	1	TOT 1	MT721865
				TOT 2	MT721866
				TOT 3	MT721867
				TOT 4	MT721868
Gundar Stream, Kodaikanal, Tamil Nadu, Southern India	10°21'60.3"N 77°42'87.4"E	5	1	TKT 1	MT721860
				TKT 2	MT721861
				TKT 3	MT721862
				TKT 4	MT721863
				TKT 5	MT721864

TOT, trout from Ooty, Tamil Nadu; TMK, trout from Munnar, Kerala; TKT, trout from Kodaikanal, Tamil Nadu.

genetic distances were calculated by assigning a query sequence to its closest match based on the genetic divergence using Taxon DNA v.1.6.2 (Meier et al., 2006). Divergence was calculated as follows: per cent divergence = no. of mismatched nucleotides / total no. of aligned nucleotides × 100. The sequence statistics were calculated using MEGA v.5.1. (Tamura et al., 2011).

Phylogenetic analysis

The phylogenetic tree was constructed using the combined dataset that comprised our DNA sequence data and sequences retrieved from the GenBank database of NCBI (https://www.ncbi.nlm.nih.gov/). Species relationship related to parental inheritance was analysed by retrieving OmyY1 sequences representing 31 *Oncorhynchus mykiss* and 16 other *Oncorhynchus* species from the NCBI database. All the sequences were retrieved from the GenBank database according to Sivaraj et al. (2018) using stringent criteria. Phylogenetic analysis was performed by the maximum likelihood method using Clustal W alignment tool in MEGA v. 5.1 (Tamura et al., 2011). The total number of nucleotides in the aligned data set was 924 bp. All positions containing gaps and missing data were eliminated from the analysis. The K2P distance was set as an evolutionary model, and the bootstrap support was analysed with 1000 replications. The tree with a 70 % bootstrap value was generated and viewed in FigTree version 1.3.1 (http://tree.bio.ed.ac.uk/software/figtree).

Results

Divergence analysis of OmyY1 in Southern Indian trout stocks

Genetic divergence was estimated by calculating pairwise combinations of 12 OmyY1 sequences of the *O. mykiss* collected from Ooty, Munnar, and Kodaikanal. The results showed a minimum of 0.0 % and a maximum of 1.3 % divergence among the Southern Indian stocks. The AT and GC contents were 64.6 % and 35.4 %, respectively. The number of conserved sites, variable sites, and parsimony informative sites were 791 bp, 189 bp, and 71 bp, respectively. In summary, divergence analysis of OmyY1 locus showed that the Southern Indian stocks belonged to C haplotype (coastal haplotype) and contained a single haplotype. No further variation was observed.

Divergence analysis of OmyY1 in Southern Indian trout and other salmonid OmyY1 sequences

Genetic divergence was estimated by calculating pairwise combinations of 45 OmyY1 sequences belonging to seven species of the *Oncorhynchus* genus. The results showed a minimum intra-specific divergence of 0.0 % and a maximum divergence of 0.5 %. Inter-species divergence ranged from 0.2 % to 12.4 %. Moreover, the sequencing gap was observed with lower intra-specific divergence (in Southern Indian stocks) than minimum inter-specific divergence (in other trout species). The AT and GC contents were 64 % and 36 %, respectively, and the number of conserved sites, variable sites, and parsimony informative sites were 726 bp, 242 bp, and 92 bp, respectively. Details of variable sites in Southern Indian Trout OmyY1 and other salmonid OmyY1 Trout sequences are provided in Supplementary Table.

Maximum likelihood tree analysis

The maximum likelihood tree constructed using OmyY1 markers contained 45 sequences representing seven species – *Oncorhynchus clarkii* (Richardson, 1836), *Oncorhynchus gilae* (Miller, 1950), *Oncorhynchus keta* (Walbaum, 1792), *Oncorhynchus kisutch* (Walbaum, 1792), *O. mykiss*, *O. nerka*, and *Oncorhynchus tshawytscha* (Walbaum, 1792). All 10 sequences of *O. clarkii* formed a separate clade, which shows clear

segregation from other species. The lone sequences representing *O. keta, O. nerka, O. kisutch,* and *O. tshawytscha* formed basal clades with *O. clarkii*. Thirty sequences of *O. mykiss* (including Southern Indian stocks) showed distinct sub-clades indicating the species' genetic diversity. The sequences of *O. gilae* were nested within the clades formed by the *O. mykiss* populations (Fig. 2). The OmyY1 sequences of *O. mykiss* analysed by maximum likelihood tree showed a mix of introduction events among the Southern Indian stocks and other *O. mykiss* sequences. The results showed that TOT 1, TOT 3, TKT 3, and TMK 2 formed a distinct clade with TKT 1 and TOT 2. Similar observations were seen when TMK 1, TMK 3, TKT 2, TKT 4, TKT 5, and TOT 4 formed a separate clade. This revealed the events of introduction and hybridisation within the stocks. All the OmyY1 sequences of the Southern Indian trout stocks (TOT, TMK, and TKT) belonged to the coastal haplogroup (C1, C2, C3, C4, C5, C6, and C7) and were clustered and differentiated within the OmyY1 sequences of *Oncorhynchus mykiss irideus*, with low divergence. The results revealed several introduction and hybridisation events within the Southern Indian *O. mykiss* stocks and among the coastal rainbow trout types (Fig. 2).

Discussion

Lineage and origin of the Southern Indian trout stocks

The OmyY1 is an ideal genetic marker with a fixed locus for species-specific identification and phylogeny development (Brunelli et al., 2013). In this study, we used the OmyY1 marker to trace the origin of the naturalised Southern Indian trout by comparing the data available for Southern Indian trout with the haplotypic data available for native populations from North America. Y-marker pattern obtained showed consistency between samples and haplogroups. Single-copy Y-linked OmyY1 marker was evaluated for variation over 800–900 bases from 12 males sampled at 3 localities of Southern India**.** Phylogenetic analysis revealed the classification of OmyY1 haplotypes (Fig. 2) to be consistent with that of the salmonid species. This study showed that the phylogenetic tree at the bottom supports the clades of the cutthroat trout (*O. clarkii*) followed by the salmon species such as chinook, coho, sockeye, and chum, which is in accordance with the findings of Brunelli et al. (2013).

Moreover, the clades supporting the rainbow trout sequences (*O. mykiss*) consisted of gila trout (*O. gilae*) that were nested within the *O. mykiss* populations, which is due to past events of hybridisation within the *Oncorhynchus* species. The Steelhead trout, *O. mykiss irideus* and the Southern Indian trout sequences sampled from Ooty, Munnar, and Kodaikanal cladded together to form a coastal haplogroup lineage. Furthermore, inland rainbow trout, *O. mykiss gairdnerii* and golden trout, *O. aquabonita*, belonged to haplotypes I1, I3, I4, I5 (inland). They were grouped separately in the *Oncorhynchus* genus, which is in good concordance with other reports. For example, Brunelli et al. (2010) found that haplotype networks revealed two distinct groups: the coastal and inland haplogroups. This study clarified an interesting issue; the lineage composition of naturalised Southern Indian stocks based on comparison with haplotypic data of native source populations. As revealed by the maximum likelihood phylogenetic tree, the haplotype frequency of all the Southern Indian trout sequences ranged from C1 to C7 (Fig. 2). But the OmyY1 locus in Southern Indian translocated stocks revealed a single haplotype (Table 2), which shows that they belong to a specific native ecotype and are derived from steelhead trout, *O. mykiss irideus*. They might have possibly arrived from the Moosevale and Canyon Creeks and the river tributaries of Yakoun, Zymoetz, Morice, and Cowichan, British Columbia; Touchet River and Abernathy Creek, Washington; and the river tributaries of Alsea, Hood, and Warm Springs and the Bake Oven, Buck, and Witham Creeks in Oregon of the North Western American regions (Table 2; Brunelli et al., 2010). Genetic distance analysis showed corroboration between the Southern Indian stocks and native populations classified according to the ecotype, as Southern Indian stocks are related to steelhead trout.

The results of this study infer that the stocks introduced in Southern India are derived from multiple sources, and the history of introductions suggests that the genetic pool of this salmonid comprises many strains. Multiple origin hypothesis was also examined for naturalised trout stocks from Chile (Colihueque et al., 2019). Previous studies also have reported on other naturalised trout populations from Europe (Stanković et al., 2016), Argentina (Riva Rossi et al., 2004), and Missouri (Dillman and Koppelman, 2006). Riva Rossi et al. (2004) found similar genetic patterns between naturalised anadromous and resident rainbow trout inhabiting the Patagonia River in Argentina, which showed that most populations could have originated from North America. Stanković et al. (2016), according to CR sequence studies, found that translocated trout populations from Europe had a higher level of allelic richness and genetic diversity than native trout populations and were clustered in four well-defined haplogroups, which proved that the genetic pool of these populations should reflect multiple origins, but OmyY1 locus showed no variation in the European translocated populations.

Similar results were also observed for the OmyY1 locus of Southern Indian trout stocks. It can be inferred that the Southern Indian stocks belonging to the coastal haplogroup must have arrived from various regions of the world. For instance, according to the available historical records, trout were first introduced in Ooty in 1909 from New Zealand, during which period

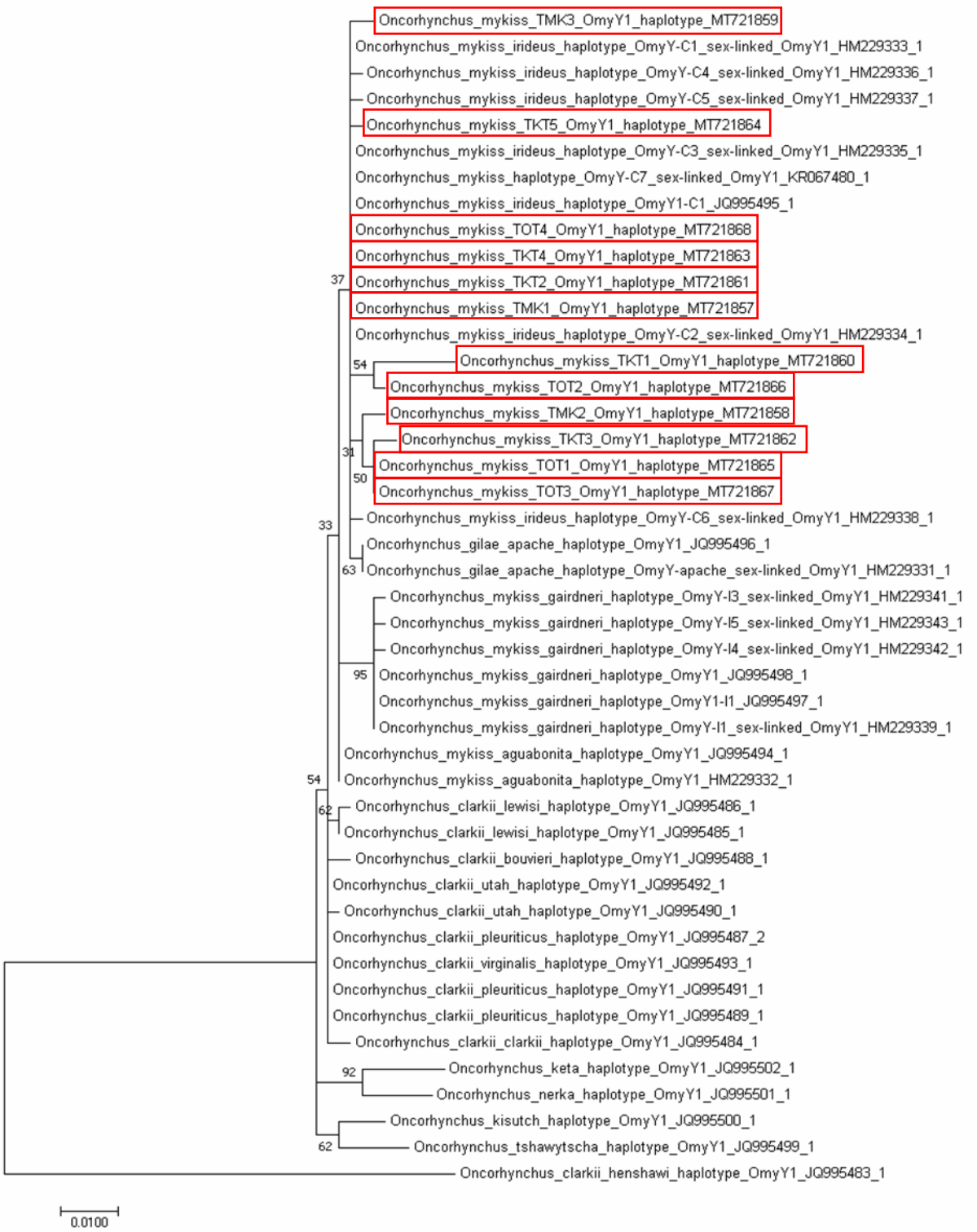

Fig. 2. Maximum likelihood (ML) phylogenetic tree based on OmyY1 sequences of the Southern Indian rainbow trout (*Oncorhynchus mykiss*) stocks (TOT1, TOT2, TOT3, TOT4, TMK1, TMK2, TMK3, TKT1, TKT2, TKT3, TKT4 and TKT5). All OmyY1 Southern Indian trout cladded within the rainbow trout, *Oncorhynchus mykiss* that belonged to the coastal haplotype. TOT, trout from Ooty, Tamil Nadu; TMK, trout from Munnar, Kerala; TKT, trout from Kodaikanal, Tamil Nadu. Highlighted in red outline are sequences used in this study.

steelhead stock could have possibly been introduced, and the same was introduced to New Zealand in 1883 (MacCrimmon, 1971). Subsequently, in 1920, another stock of steelhead trout was introduced in Ooty from Kashmir. Furthermore, other salmonid species such as golden trout, *O. aquabonita*, tiger trout (*Salmo trutta fario* × *Salvelinus fontinalis*), sockeye Salmon (*O. nerka*), and brown trout (*Salmo trutta fario*) were introduced at different time points to upgrade the Ooty stock (Anon, 1987), but none survived. However, previous studies

Table 2. Sampling and geographic origins of rainbow trout, *Oncorhynchus mykiss* with various OmyY1 haplotypes (Brunelli et al., 2010).

No.	Location	Y-marker Haplotype (Freq.)	No.	Location	Y-marker Haplotype (Freq.)
1.	Voyampolka R., Russia	C2(2)	30.	Hood R., OR	C1(5); C4(1); I1(1); I2(1); I3(1)
2.	Sedanka R., Russia	C2(2)	31.	Little Sheep Crk., OR	I3(16)
3.	Zhupanova R., Russia	C1(2); C2(1)	32.	Clearwater R., ID	I2(9)
4.	Swanson R., AK	C1(9)	33.	Rapid R., ID	I2(1); I3(6)
5.	Sashin Crk., AK	C1(4)	34.	Pahsimeroi Hatchery, ID	I2(1); I3(7)
6.	Ealue Lake, BC	C2(4)	35.	Alsea R., OR	C1(1)
7.	Moosevale Crk, BC	C1(1)	36.	Warm Springs R., OR	C1(1); C2(2); I1(5); I2(4); I3(5)
8.	Turnagain R., BC	C1(9)	37.	Bake Oven Crk.,	C1(1); I1(5); I2(4); I3(3)
9.	Yakoun R., BC	C1(7); C2(1)	38.	Rogue R., OR	C1(2); C2(4)
10.	Copper R., BC	C1(3); C2(4)	39.	Buck Crk., OR	C1(1)
11.	Canyon Crk, BC	C2(1)	40.	Bridge Crk., OR	I1(2)
12.	Zymoetz R., BC	C2(1)	41.	Upper Williamson R., OR	C1(4)
13.	Morice R., BC	C2(1)	42.	Witham Crk., OR	C1(4); C5(1)
14.	Blackwater R., BC	C3(8)	43.	Threemile Crk., OR	I1(4)
15.	Tzenzaicut Lake, BC	I2(9)	44.	Bridge Crk., OR	C2(3)
16.	Pennask Lake, BC	I2(1); I3(8)	45.	Mud Crk., OR	I1(3)
17.	Cowichan R., BC	C1(3); C3(1)	46.	Thomas Crk., OR	C1(3)
18.	W. Fork Trout Crk, WA	C1(3); I2(5)	47.	Honey Crk., OR	C1(2)
19.	Kootenay Lake, BC	I2(2)	48.	N. Fork Little Deep Crk, OR	I2(1)
20.	Basin Crk, MT	I2(7); I4(2)	49.	W. Little Owyhee R., OR	I1(2)
21.	Fisher River, MT	I2(4); I5(2)	50.	Sheepheaven Crk., CA	C1(2)
22.	Hoh R., WA	C1(5)	51.	Hayspur Hatchery, ID	C1(16)
23.	White R., WA	C1(2)	52.	South Tacoma Hatchery, WA	C1(7)
24.	Wells Hatchery, WA	C1(2); I1(1)	53.	Spokane Hatchery, WA	C1(9)
25.	N. Fork Little Deep Crk., WA	C1(2); I3(6)	54.	Scott Crk., CA	C1(5)
26.	Touchet R., WA	C1(1); I2(3); I3(1)	55.	Whale Rock Res., CA	C1(6)
27.	Abernathy Crk., WA	C1(10); C4(1); C6(1)	56.	Volcano Creek, CA (Golden trout)	V(5)
28.	Washougal R., WA	C1(4)	57.	Black R., AZ (Apache trout)	A(7)
29.	Kalama R., WA	C1(12)			

BC – British Columbia; WA – Washington; OR – Oregon; MT– Montana; ID – Idaho; CA – California; AZ – Arizona.
C - Coastal; I - Inland; V - Golden trout in California; A - Apache trout in Arizona.
Highlighted Y-marker Haplotype (Freq.) in green are indicative of the native ecotype/origin to which the Southern Indian trout stocks have originated, based on their haplotype frequency.

have reported on the survival of golden trout (Sehgal, 1999; Kuruppan, 1989), but this study revealed that the OmyY1 sequences of Ooty stocks (TOT 1, TOT2, TOT 3, TOT 4) cladded with the coastal haplotype. Golden trout (*O. aquabonita*) could have possibly once dominated the cold streams of Ooty, but over the years, this strain might have disappeared due to continuous poaching and animal intrusion.

Moreover, according to the records, the steelhead trout strain of Ooty was introduced in Munnar in 1932, during which a small consignment of 5000-eyed ova of steelhead trout was introduced. However, of the 5000-eyed ova, only 2000 fingerlings survived. However, the phylogenetic analysis of this study showed the coastal steelhead type to be well established in the Munnar, Kodaikanal and Ooty regions. Furthermore, information obtained from an official of the KDHP Company shows that in Kodaikanal, trout were introduced from Munnar, and in 1943, a one-time introduction of the stock should have happened. The maximum likelihood analysis of this study revealed that the OmyY1 sequences of trout stocks sampled from Ooty, Munnar, and Kodaikanal had cladded together, which indicates the several mixes of introduction events within the Southern Indian stocks. These Southern Indian stocks belong to the coastal haplogroup and have survived for many decades in the southern cold-water regions.

Limitations

The stocks sampled from Ooty, Munnar, and Kodaikanal showed no variation in the OmyY1 locus, mainly because of the less availability of male fish. Male *O. mykiss* were naturally unavailable due to environmental conditions such as cold temperatures and other climatic conditions. Wild fish populations can be masculinised in warmer water temperatures and with temperature-dependent sex determination;

however, warm and cold-water temperatures masculinise fish populations with mid-range conditions producing at most 50% females (Honeycutt et al., 2019). This phenomenon was also observed in rainbow trout, where high temperatures increased the masculinisation rate of all female (XX) rainbow trout populations (Valdivia et al., 2014). Other reasons for the unavailability of male fish are continuous human interactions (poaching and overfishing) and animal intrusion, for example, by otters, *Lutra nair* (Linnaeus, 1758) (Devaa et al., 2021; Devaa and Ramesh, 2022), which can have a considerable impact on the availability of fish stocks. This way, the available male fish can be lost along with the female fish. This study showed no genetic variation in the OmyY1 locus of the sampled stocks, which might be due to overfishing (Vitorino et al., 2017; Yan et al., 2021). The presence of low genetic variation has been proved by microsatellite markers (Devaa et al., pers. comm). However, continuous stocking can help in stock recovery and the upgradation of genetic diversity.

Conclusion

Rainbow trout, *Oncorhynchus mykiss*, is a popular cold-water fish widely distributed and farmed across the globe. This study, aimed to gain insight into the origins of Southern Indian trout stocks by using OmyY1 marker and comparing the obtained data with the haplotypic information available for native populations from North America. The results revealed that the Southern Indian rainbow stocks exhibited no genetic structure. This might be because trout introduced to Southern India belonged to multiple origins populations likely represented by lineages originating from different regions of western North America, such as river tributaries and creeks of British Columbia, Washington, and Oregon. The presence of coastal lineages C1–C7 suggests that Southern Indian stocks comprise only one major ecotype: the Steelhead trout (*Oncorhynchus mykiss irideus*).

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Author contributions: J.C. Walter Devaa: Field visit, sample collection, molecular genetics work, statistical assessment and wrote the manuscript. Stalin Nithaniyal: Assisted in the molecular genetics work and statistical assessment. Vimal Panneerselvam: Assisted in the molecular genetics work and statistical assessment. Ramesh Uthandakalaipandian: Supervision.

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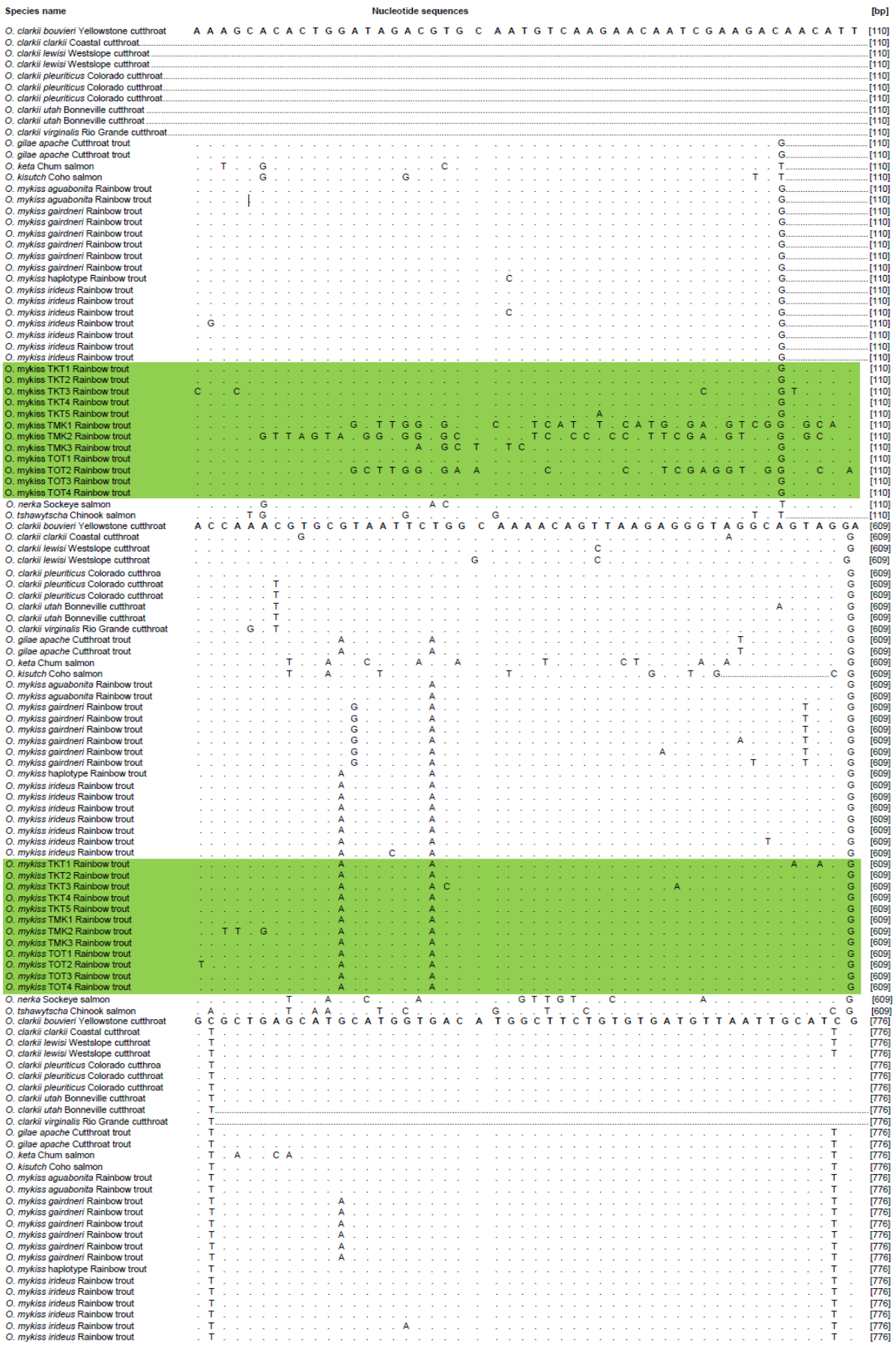
Supplementary Table. Details of variable sites in Southern Indian trout OmyY1 and other salmonid OmyY1 trout sequences. Southern Indian rainbow trout OmyY1 sequences are highlighted in green colour.



Supplementary Table. Continued.