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SHORT COMMUNICATION

Genetic Divergence and Molecular Phylogenetic Analysis of Rainbow Trout, *Oncorhynchus mykiss* (Walbaum, 1792), From the Indian Upland Coldwater Regions

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Abstract

The popular coldwater fish, the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), was first brought to the Indian subcontinent in the 20th century and was established in the coldwater upland regions. This study examines the molecular phylogenetic linkages and genetic divergence of the *O. mykiss* that was introduced in India's North-Western Himalayas and Southern Western Ghats. The genetics of Indian *O. mykiss* are compared with Pacific trout in the genus *Oncorhynchus*. Twenty-two accessions were gathered from four distinct geographic regions, and the nBLAST homology method was used to analyse the 650 bp mtDNA COX1 segment. The results showed an intra-species divergence of 0.0 %–0.4 %, which confirms the study species to be *O. mykiss*. However, including nine other *Oncorhynchus* populations from the Pacific lineage resulted in an inter-species divergence of 0.2 % to 1.7 %. Phylogenetic analysis by the Bayesian and BEAST methods showed a distinct lineage of Pacific trout and salmon groups in separate clades. The phylogenetic trees obtained confirmed that *O. clarkii* was a sister clade to *O. mykiss*, *O. gilae, and O. g. apache*. All of the Pacific salmonid species were clearly distinguished from the clade that contained the Indian *O. mykiss* stocks. The study's findings fill the current knowledge gap about the genetic composition of Indian *O. mykiss* stocks in comparison to other stocks throughout the world and provide insight into how to manage the genetic variety of Indian *O. mykiss* stocks.

Keywords: Pacific trout, cytochrome c oxidase subunit I, genetic diversity, maximum likelihood

Introduction

The fish genus *Oncorhynchus*, which belongs to the Salmonidae family, is indigenous to Mexico and the Western United States. Every *Oncorhynchus* species and subspecies is endangered and threatened, and some are regarded as "species of conservation concern" both in their natural habitats and in the few nations where they were introduced (Wilson and Turner, 2009). One of the many salmonid species that was introduced globally for food, sport, and recreational fishing is the rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792), which is also widely cultivated. From the Pacific Ocean to the coastal portions of North America (from Alaska south to Mexico), the endemic range of *O. mykiss* has been expanded to include the eastern parts of North America as well as the continents of Africa, Asia, Australasia, Europe, and South America (MacCrimmon, 1971).

Since its introduction to India in the late nineteenth century, rainbow trout have become a popular sport and leisure fishery as well as a significant aquaculture species (Crawford and Muir, 2008). Reared in the Indian uplands, they make a substantial economic and revenue contribution to states in North India, including Jammu and Kashmir, Himachal Pradesh, Sikkim, and Arunachal Pradesh. They are also regarded as an essential component of healthy diet and a rich source of polyunsaturated fatty acids, accounting for 25 % of the total fatty acids (Sheeshka and Murkin, 2002). In 1863, trout fisheries were first established in Southern

India in Ooty, which is situated in the Western Ghats. These days, rainbow trout can be found in a few mountainous areas of the Southern Indian Western Ghats, including Ooty, Kodaikanal, and Munnar (Sehgal, 1999). Despite being effectively introduced and established in Southern India, *O. mykiss* in India is today endangered (Devaa et al., 2021; Devaa and Ramesh, 2022; Devaa and Ramesh 2023). While research on wild trout stocks indicated little genetic diversity (Devaa et al., 2024), investigations on farmed trout stocks in India demonstrated a considerable level of genetic variety (Barat et al., 2015).

Because the phylogenetic structure of the Indian trout stocks has not been widely studied since their introduction, this study aimed to understand the trout phylogenetic structure with DNA barcodes obtained using mtDNA cytochrome c oxidase subunit 1 marker. Most animal species can be distinguished using just one gene sequence from the mtDNA cytochrome c oxidase subunit 1 (Hebert et al., 2003), which is also used as a global genetic marker. In general, this gene area shows significant divergence between species, allowing for species differentiation, but limited variation within species (Hebert et al., 2003; Pentinsaari et al., 2016). This method compares an unknown sample's DNA barcode to a reference sequence and assigns a species when the query sequence only matches one of the reference library's species. The global Fish Barcode of Life Initiative (FISH-BOL) is currently assembling a DNA barcode reference library for every species of fish (Ward et al., 2009). With more than 6500 species records, it has demonstrated unambiguous discrimination of about 93 % of freshwater species and 98 % of marine species (Pappalardo et al., 2011).

DNA barcoding shows great promise for species identification, monitoring, and biodiversity assessment (Nithaniyal et al., 2014; Devaa et al., 2023). However, because they might not have the diagnostic sites needed for distinction, some salmonid species that comprise a closely related group of anadromous and non-anadromous species with obvious intra-species diversity might not be evaluated by DNA barcoding (Waples et al., 2001), suggesting a possible overlap between intra and inter-species divergence.

Furthermore, the rate of nucleotide substitution in mitochondrial DNA (mtDNA) increases by 2 % per million years (Smith, 1992). The COX1 gene, a reliable and accessible marker, can be extensively employed for monitoring the biodiversity of fish species (Shen et al., 2013). Because of its quick rate of evolution, maternal inheritance, and lack of recombination, mtDNA is a good tool for analysing genetic diversity (Avise, 2000). Numerous fish species have been phylogenetically studied based on the mtDNA COX1: snow trout, *Schizothorax richardsonii* (Gray, 1832) (Ali et al., 2014); barred knifejaw, *Oplegnathus fasciatus* (Temminck & Schlegel, 1844) (Park et al., 2018); Asian swamp eel, *Monopterus albus* (Zuiew, 1793) (Zhou et al., 2020); and silver croakers, *Pennahia argentata* (Houttuyn, 1782) (Supmee and Suppapan, 2022). Using the mtDNA COX1 marker, this study aimed to understand the genetic diversity and phylogenetic linkages of the *O. mykiss* stocks in the Indian subcontinent.

Materials and Methods

Ethical approval

All necessary permissions were obtained from the relevant authorities including the Director and Additional Director, Tamil Nadu Fisheries Department, Ooty, Tamil Nadu, India (Permit No. 3790/F1/2019, dated 25 March 2019), the Chief Projects Officer, Fisheries Department of Kokernag, Kashmir, India (Permit No. CPO/KOK/2016/260, dated 12 May 2016), the Chairman, High Range Angling Association, KDHP Company, Munnar, Kerala, India (Ref. 44 of KDHP records, dated 13 November 2019) and Management Officials, Palani Hills Conservation Council, Kodaikanal, Tamil Nadu, India (verbal communication/approval).

Sample collection

Using an angler's rod, rainbow trout (*O. mykiss*) were captured from Munnar, Kerala (n = 5), Ooty (n = 7) and Kodaikanal in Tamil Nadu (n = 5), and Kokernag, Kashmir (n = 5) (Table 1, Fig. 1). Caudal fin clippings were taken from each fish, fixed in 90 % ethanol (Hayman Specialty Products, Essex, UK), and transported to the fisheries laboratory for further DNA analyses.

DNA extraction, amplification and sequencing

The proteinase-K digestion method and the phenol-chloroform-isoamyl alcohol method, as described by Russell and Sambrook (2001), were used to extract genomic DNA. A UV spectrophotometer (Shimadzu, Japan) and 1 % agarose gel electrophoresis with 1× Tris-boric acid-EDTA (TBE) buffer stained by ethidium bromide were used to quantify the concentration of the isolated DNA. A UV transilluminator (Bio-Rad, USA) was then used to qualitatively visualise the resulting concentrate for the presence of DNA. The 650 bp sequence of the COX1 gene was amplified by employing the following nucleotide primers: forward (5′-TCAACCAACCACAAAGACATTGCCAC-3′) and reverse (5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′) primers (Ward et al., 2005). COX1 gene was amplified 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. The reaction was carried out in a thermal cycler (SureCycler 8800, Agilent Technologies, USA). The PCR

Table 1. Coordinates, sampling locations, sources, sampling type, species and size in the study of rainbow trout *Oncorhynchus mykiss* genetics and phylogeny in Indian upland coldwater regions.

Location	Sampling source	Type of sampling	Species	Sample size	Coordinates
Kokernag, Kashmir	Kokernag Stream	Random	Rainbow trout (*O. mykiss*)	5	33°59′04.99″N, 75°29′35.25″E
Munnar, Kerala	Rajamallay Stream	Random	Rainbow trout (*O. mykiss*)	5	10°15′37.2″N, 77°03′64.68″E
Ooty, Tamil Nadu	Lakkidi Stream	Random	Rainbow trout (*O. mykiss*)	7	11°2′97.28″N, 76°58′75.61″E
Kodaikanal, Tamil Nadu	Gundar Stream	Random	Rainbow trout (*O. mykiss*)	5	10°2′15.99″N, 77°42′87.60″E

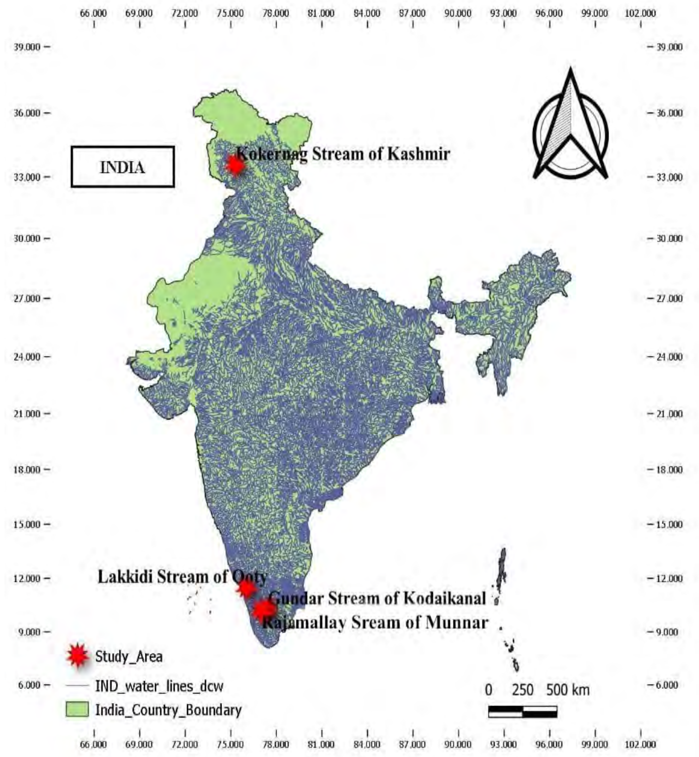

Fig. 1. Map showing the sampling locations of trout stocks from Ooty, Kodaikanal and Munnar in the Western Ghats and Kokernag, Kashmir in the North-Western Himalayas in India.

conditions for the COX1 gene were as follows: initial denaturation at 95 ºC for 2 min followed by 35 cycles of strand denaturation at 94 °C for 30 sec; annealing at 54 °C for 30 sec; and extension at 72 °C for 1 min with a final extension at 72 °C for 10 min. The amplified PCR products were then electrophoresed on a 1 % TBE agarose gel stained with ethidium bromide (20 ng.µL^{-1}), observed with 1 kb DNA ladder (Thermo Scientific, USA) using UV transilluminator. A molecular imager ChemiDoc XRS+ with Image Lab Software (Bio-Rad, USA) was then used for documentation. The amplified bands were gel-eluted with gel extraction kit (FavorPrep FAGPK 001, Favorgen, Taiwan). The positive purified amplicon was sequenced by Sanger's dideoxy chain termination method with the same PCR primer (F/R) by using Applied Biosystems Automated DNA Sequencing System (3500 series Genetic Analyser, Applied Biosystems, USA).

DNA sequence assembly and annotation

The base calling quality of each *O. mykiss* DNA barcode sequence was examined, and Sequence Scanner Software v.1.0 (Applied Biosystems, USA) was used for analysis. Full-length consensus sequences were annotated and assembled using Codon Code Aligner version 4.2.4 (CodonCode Corporation, USA). The NCBI-BLAST homology search program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to validate primary COX1 sequence data. The validated sequences were then added to the NCBI database for public access with accession numbers listed in Table 2. Using Taxon DNA v.1.6.2, the genetic distances between and within species were determined by matching a query sequence to its closest match based on genetic divergence (Meier et al., 2006). According to

Table 2. Locality and COX1 depositions for Indian rainbow trout *Oncorhynchus mykiss* samples.

Location	COX1 depositions	GenBank deposition No.
Kokernag Stream, Kokernag, Kashmir, North-Western Himalayas, India	Kashmir 1	OR573660.1
	Kashmir 2	OR573676.1
	Kashmir 3	OR573690.1
	Kashmir 4	OR573699.1
	Kashmir 5	OR574990.1
Rajamallay Stream, Munnar, Kerala, Southern India	Munnar 1	OR569672.1
	Munnar 2	OR569683.1
	Munnar 3	OR573689.1
	Munnar 4	OR573477.1
	Munnar 5	OR572103.1
Lakkidi Stream, Ooty, Tamil Nadu, Southern India	Ooty1	MW172282.1
	Ooty2	MW168255.1
	Ooty3	MW168249.1
	Ooty4	MW168795.1
	Ooty5	MW168256.1
	Ooty6	MW168250.1
	Ooty7	MW168253.1
Gundar Stream, Kodaikanal, Tamil Nadu, Southern India	Kodaikanal 1	OR178022.1
	Kodaikanal 2	OR569066.1
	Kodaikanal 3	OR569133.1
	Kodaikanal 4	OR569167.1
	Kodaikanal 5	OR569170.1

Nithaniyal et al. (2021), the percentage divergence was computed as follows:

(Number of mismatched nucleotides / Total number of aligned nucleotides) × 100

MEGA v.11 was used to compute the sequence statistics, including conserved sites, variable sites, and parsimony informative sites (Tamura et al., 2011).

Phylogenetic analysis

Using strict criteria, the datasets of Indian *O. mykiss* stocks and other closely similar taxonomic sequences were obtained from the NCBI's GenBank database (https://www.ncbi.nlm.nih.gov/) for our work (Sivaraj et al., 2018). A total of 56 COX1 sequences from Pacific trout and Pacific salmon groups made up the concatenated dataset, which was then employed for the phylogenetic analysis. To determine the evolutionary relationships between the introduced Indian populations and other related species, a gene tree was built using the maximum likelihood method and the Bayesian methodology in MrBayes 3.2.5 (Ronquist et al., 2012).

Bayesian analysis

The GTR substitution model and the likelihood parameters found in MrBayes 3.2.5 were used to perform Bayesian studies. Using the default parameters, the Monte Carlo Markov Chain (MCMC) was run for 10 M generations. When the average standard deviation (SD) of split frequencies reached 0.003, the cold and heat chains were terminated. Following the removal of the original trees, the resulting trees were condensed into a 50 % majority rule consensus tree. Using FigTree 1.4.2, the phylogenetic tree was labelled with branch support of posterior probability (pp) (http://tree.bio.ed.ac.uk/software/figtree).

Results

By computing the pairwise combinations of 22 COX1 accessions of the *O. mykiss* stocks gathered from the North-Western Himalayan region of Kashmir and the Western Ghats regions of Ooty, Munnar, and Kodaikanal, genetic divergence was assessed. Intra-species divergence ranged from 0.0 to 0.4 %, and the nucleotide composition study of the accessions revealed that all of them included 54.3 % AT and 45.7 % GC. Additionally, 541 bp of conserved sites, 3 bp of variable sites, and 3 bp of parsimony informative sites were identified. Fewer variable sites and parsimony informative sites denoted higher genetic stability of the Indian rainbow trout stocks. However, no further variation was observed within the population.

A total of 56 COX1 sequences from nine *Oncorhynchus* species were examined for genetic divergence. The intra- and inter-species divergence was evaluated using 1,540 pairwise combinations representing all Indian trout stock accessions and other salmonids from Pacific Rim countries. The inter-species divergence varied from 0.2 % to 1.7 %, whereas intra-species divergence was between 0.0 % and 0.2 %. Additionally, in Indian trout stocks, a barcoding gap was found with lower intra-species divergence compared to minimum inter-species divergence (in other species). The AT and GC contents were 54.2 % and 45.8 %, respectively, and the number of conserved sites, variable sites and parsimony informative sites were 403 bp, 141 bp and 136 bp, respectively. For more details on the variable sites of the Indian trout COX1 and other salmonid COX1 trout sequences see supplementary Table 1.

A total of 56 COX1 sequences of *O. clarkii* (Richardson, 1836), *O. gilae* (Miller, 1950), *O. keta* (Walbaum, 1792), *O. kisutch* (Walbaum, 1792), *O. mykiss* (Walbaum, 1792), *O. nerka* (Walbaum, 1792), *O. gorbuscha* (Walbaum, 1792), *O. masou* (Brevoort, 1856) and *O. tshawytscha* (Walbaum, 1792) were used to construct the phylogenetic tree using the maximum likelihood method. In order to comprehend the genetic link, the brown trout, *Salmo trutta* Linnaeus, 1758, was employed as an outgroup in the phylogenetic tree. Five clades that constitute the Pacific trout and salmon group were identified using the Bayesian tree and are as follows: (i) Clade one comprised 22 Indian *O. mykiss* stock accessions that were sampled from Ooty, Kodaikanal, Munnar, and Kashmir. Furthermore, it included 16 stocks from the following countries: Davis, USA; Quebec, Canada; Tasmania, Australia; Bang Kapi and Phayao, Thailand; Chonging, China; and Azad Jammu and Kashmir, Pakistan. Although the Indian *O. mykiss* stocks displayed less genetic divergence, they were found to belong to single clade. The findings indicated that the *O. mykiss* stocks from Ooty were nested between Davis and Tasmania, USA, and Australia, respectively, while the stocks from Kodaikanal were comparable to those from Bang Kapi and Phayao, Thailand. The Munnar *O. mykiss* stocks, on the other hand, formed a distinct cluster and exhibited modest genetic divergence with those from Pakistan and Kashmir. Additionally, the monophyly origin of *O. mykiss* was formed by the nesting of the Canadian sequences of *O. gilae* within a basal clade formed by *O. mykiss*. (ii) *O. clarkii* species, which formed a sister clade to *O. mykiss* stocks, made up Clade 2. In the phylogenetic tree, the Pacific trout group—*O. mykiss*, *O. gilae*, and *O. clarkii*—formed a distinct lineage with 100 % posterior probability. (iii) Clade 3 included *O. kisutch* and *O. tshawytscha*, which demonstrated a strong genetic bond. (iv) *O. gorbuscha*, *O. keta*, and *O. nerka* made up Clade 4. Stocks of *O. keta* from Japan and Thailand and *O. gorbuscha* from Mexico and Russia were grouped together. The *O. nerka* sequences from Thailand and Japan were categorised as a subclade of the highly genetically related *O. gorbuscha* and *O. keta* sequences. The Pacific salmon group displayed two lineages: *O. gorbuscha*, *O. keta*, and *O. nerka* as a distinct lineage with 100 % posterior probability, and *O. kisutch* and *O. tshawytscha* as an individual lineage. (v) Clade 5 included the Atlantic outgroup *S. trutta fario* and *O. masou* (Fig. 2).

Discussion

According to recent research by Devaa et al. (2025), the percentage identification of Indian *Oncorhynchus mykiss* stocks ranges from 99.24 % to 100 %. This makes it easier to accurately authenticate the commercial salmonid species in India's highland areas. Due mostly to significant inbreeding depression, no genetic changes were found in the mtDNA COX1 gene within the sampled Indian *O. mykiss* populations used in the current investigation. Reduced genetic diversity results from inbreeding depression; when closely related individuals reproduce constantly, the population's heterozygosity decreases and the allele frequencies of inbred individuals change. A higher risk of extinction may result from this inbreeding depression, which can have a number of detrimental effects, including heightened disease susceptibility, decreased reproductive success, and heightened susceptibility to environmental changes (Pavlova et al., 2017; Frankham et al., 2019; Monson and Sadler, 2010). Microsatellite markers have been used to identify the issue of inbreeding depression in Indian *O. mykiss* populations (Devaa et al., 2024), and the mtDNA COX1 gene has been used to demonstrate this. Additionally, the meagre supply of wild fish in the research areas is a contributing factor to the inbreeding depression.

The availability of the wild fish stocks may have been significantly impacted by ongoing human interactions (high fishing pressure) and competition with other species (Devaa et al., 2021; Devaa and Ramesh, 2022; Devaa and Ramesh, 2023; Devaa et al., 2023). In Kashmir, the invasive brown trout dominates the wild streams and causes a negative impact on the native species and also on *O. mykiss*. That said, the number and size of the population may suffer as a result of the loss of the wild fish that are currently present. Previous investigations using different molecular markers such as OmyY1 Y-chromosome marker and microsatellites, have also demonstrated that Indian *O. mykiss* stocks have little genetic variation (Devaa et al., 2023; Devaa et al., 2024). The current study highlights the need for conservation management of the Indian salmonid stocks, as the *O. mykiss* stocks in Munnar, Ooty, and Kodaikanal regions of Southern India are currently in an endangered state (Devaa et al., 2021; Devaa and Ramesh, 2022; Devaa and Ramesh, 2023). Continuous stocking can help in both stock recovery and in the enhancement of genetic diversity. In 2021, new *O. mykiss* stocks were brought to Munnar from Uttarakhand (ICAR-DCFR Annual Report, 2021; Devaa et al., 2024). Similarly in 2022, *O. mykiss* eyed ova were stocked for Ooty from Kashmir to genetically improve the Southern Indian wild trout populations (Devaa et al., 2024; Muthuswamy et al., 2024).

Indian populations of *O. mykiss* had minimal genetic variety, as evidenced by the intraspecies divergence of 0.2 % and the average genetic diversity of 0.4 % within these populations (compared to other *O. mykiss* populations). Some salmonid species had low between-species divergence values of less than 1.0 %, according to earlier research by Schlei et al. (2008) and Hubert et al. (2008). Similarly, our study discovered that the stocks of Indian *O. mykiss* and other Pacific salmonids had a modest interspecies divergence of 1.7 %. According to the Bayesian tree, the Indian *O. mykiss* populations were grouped with other salmonid species

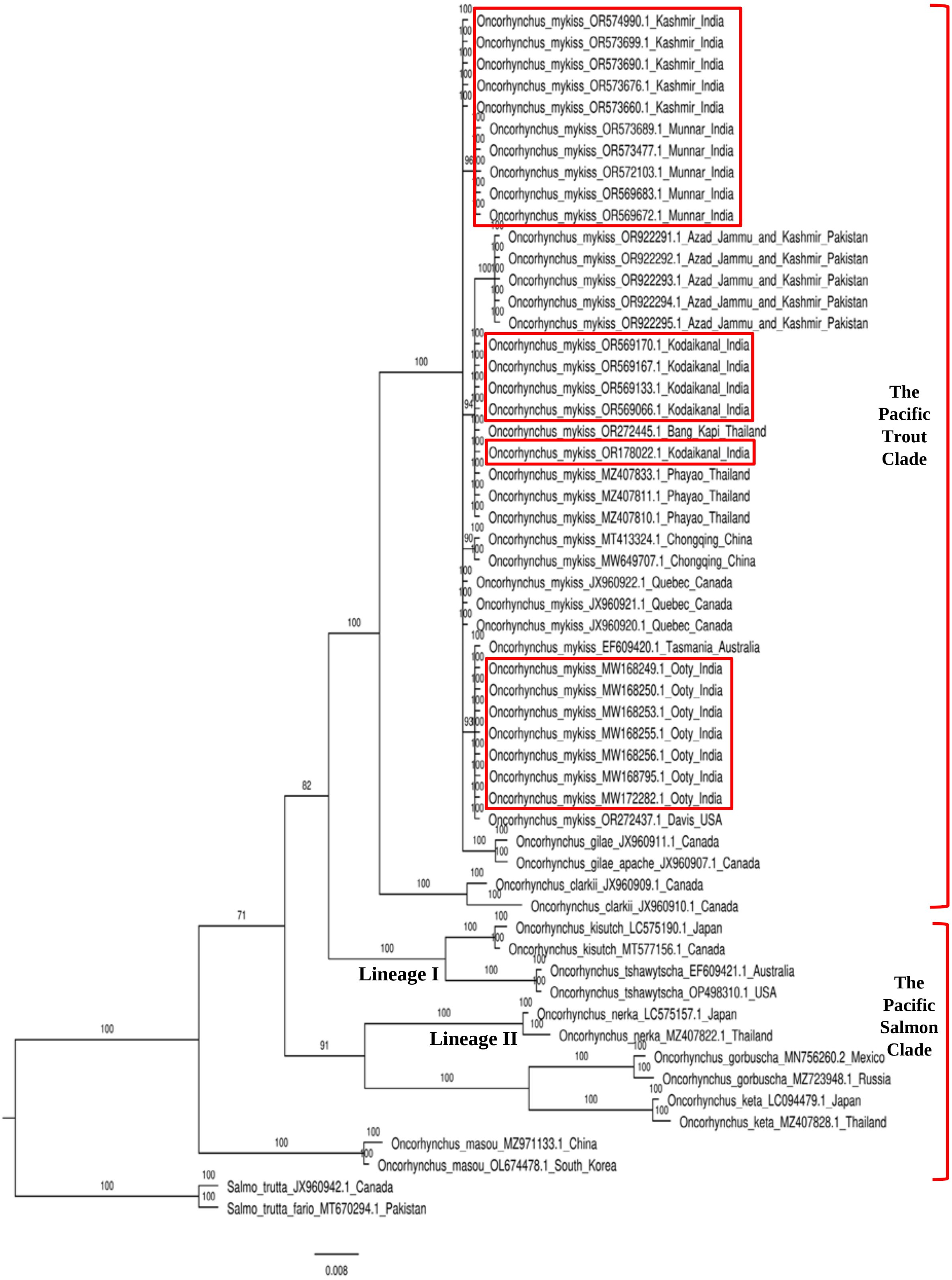

Fig. 2. Bayesian phylogeny based on COX1 sequences for the twenty-two Indian rainbow trout *Oncorhynchus mykiss* specimens. Numbers above branches are posterior probability values. Highlighted in red outline are COX1 sequences used in this study; red brackets denote the Pacific trout and Pacific salmon group.

in their genera. This could be due of their close evolutionary relationship within the genus *Oncorhynchus*. Their notable genetic resemblance is not the only reason for this; other factors include their extreme geographic isolation and barrier, environmental adaptations like habitat diversity and differences, and natural selection pressures in the particular environments they were introduced into, which have resulted in adaptations in their external traits like size, colour, and behaviour. Life history variations may also be a contributing factor. Early introduction efforts are another factor that may have contributed to the differences between and within the Indian *O. mykiss* populations under investigation. In order to increase the populations, *O. mykiss* was reportedly brought from Kashmir to Ooty in 1920. Later, it was brought from Ooty to Munnar in 1938 and from Munnar to Ooty in 1989 (Mackay 1945; Gopalakrishnan et al. 1999). This led to phenotypic plasticity (Devaa et al., 2025). Due to early introductions from Munnar to Kodaikanal, we discovered that the *O. mykiss* population from Kashmir shared genetic similarities with the populations from Munnar and Kodaikanal. After *O. mykiss* was introduced from Kashmir to the Ooty region, multiple further introductions were made to improve the stock (Gopalakrishnan et al., 1999). These recurrent introductions have resulted in hybrid formation (Gopalakrishnan et al., 1999) and which is the possible reason for them to be sub-cladded separately from other Indian *O. mykiss* populations with minor degrees of variation.

According to genetic research, the Ooty trout stock is typical of *O. mykiss* (Devaa et al., 2025). Therefore, the early introduction efforts, habitat diversity and geographical locations contribute to variations between the Indian *O. mykiss* populations. However, genome-wide association studies (GWAS) can detect genetic variants, including SNPs linked to fish temperature tolerance, and further enable genetic improvement targeted by marker assisted selection, allowing for the genetic improvement of *O. mykiss* stocks. By analysing a large population of fish, GWAS can identify potential genes and genomic areas linked to characteristics like survival time in higher temperatures. This can technically be utilised to choose breeding individuals with desirable alleles at these loci, which will accelerate the genetic improvement to temperature tolerance. Thus far, GWAS has been used to predict *O. mykiss* tolerance to acute hypoxia (Prchal et al., 2023) and to investigate growth and survival under chronic heat stress (Gallardo-Hidalgo et al., 2021). However, research is still needed to increase *O. mykiss*'s genetic tolerance to temperature. This method of genetic improvement to higher temperatures has been successfully tested in fish species like large yellow croaker, *Larimichthys crocea* (Richardson, 1846) (Wu et al., 2021); Olive flounder, *Paralichthys olivaceus* (Temminck & Schlegel, 1846) (Udayantha et al., 2023; San et al., 2024); and largemouth bass, *Micropterus salmoides* (Lacépède, 1802) (Li et al., 2024). *Oncorhynchus mykiss* from Ooty formed the basal clade to all other Indian *O. mykiss* populations in this study, according to an analysis of COX1 data. The fact that *O. mykiss* from other nations (Pakistan, Thailand, Australia, Canada, and China) claded in between the Indian stocks indicates that they may have shared a common location. Additionally, we found that the Indian *O. mykiss* stocks and all salmonid species were clearly separated (100 percent posterior probability).

Furthermore, as previously noted by Rasmussen et al. (2009), the results revealed that all of the individual species clustered together and that there were no shared or overlapping barcodes. COX1 DNA barcode analysis significantly differentiated *O. mykiss* stocks from other *Oncorhynchus* species, which had delineated from *O. clarkii*, *O. gilae*, *O. keta*, *O. kisutch*, *O. nerka*, *O. gorbuscha*, *O. masou* and *O. tshawytscha*. Our study clearly demonstrated that the low divergence within *O. mykiss*, *O. clarki* and *O. gila* was due to a close genetic association, and high divergence between *O. kisutch* and *O. tshawytscha* was due to distant genetic relationship. Two clades were identified by phylogenetic analysis of COX1: the Pacific trout group and the Pacific salmon group. According to Osinov and Lebedev (2000), Crespi and Fulton (2004), and Phillips et al. (2004), the Pacific salmon formed a monophyletic clade. In line with Wilson and Turner (2009) and Crespi and Fulton (2004), the COX1 gene analysis of the current study revealed a sister relationship between *O. gorbuscha* and *O. nerka*, *O. tshawytscha* and *O. kisutch*. Additionally, their findings demonstrated that, with a posterior probability of >94 %, the Pacific salmon formed a monophyletic clade with two sub-clades: *O. gorbuscha* + *O. nerka* and *O. tshawytscha* + *O. kisutch*.

The Pacific salmon group formed two lineages – the chinook salmon (*O. tshawytscha*) and coho salmon (*O. kisutch*) comprised Lineage I, whereas the pink salmon (*O. gorbuscha*), chum salmon (*O. keta*), and sockeye salmon (*O. nerka*) comprised Lineage II. This Pacific salmon group was basal to the pacific trout group (Fig. 2). According to Kitano et al. (1997), the Pacific trout group only inhabits Western America, whereas the Pacific salmon group inhabits the entire Northern Pacific Ocean region. This supports the idea that the Pacific trout and Pacific salmon groups cladded separately in the tree.

In line with research by Oohara et al. (1997), *O. masou* formed a basal clade with the remainder of the tree that comprised the Pacific trout groups (cladded above Pacific salmon clades) and the Pacific salmon (separate clades) as analysed by the Bayesian results of mtDNA COX1 (Fig. 2). Using GH1C (growth hormone intron), Phillips et al. (2004) found that *O. masou* was associated with Pacific salmon but not with Pacific trout. The Pacific trout's inclusion in the *Oncorhynchus* genus is supported by the COX1 results of the current study. The Pacific trout clade consisted of two subclades: *O. mykiss* + *O. gilae* and *O. clarki* (Fig. 2).

Smith and Stearley (1989) examined the inclusion of Pacific trout in the *Oncorhynchus* versus *Salmo* genera based on osteological and molecular characteristics. They showed that in the Pacific trout group, the clade supporting *O. clarkii* was basal to all the clades supporting *O. mykiss*. On the basis of a phenogram made using similarity coefficients of meristic and non-meristic characters, Gold (1977) demonstrated that the Western trouts (*O. gilae*, *O. chrysogaster*, *O. apache*, *O. m. aguabonita*, *O. m. newberrii* ("redband trout"), and *Oncorhynchus m. gairdneri* (*O. gairdneri*)) formed basal clades to *O. clarkii* sp. *Oncorhynchus clarkii* clades were shown to be basal to *O. mykiss* by Rasmussen et al. (2009). *Oncorhynchus gilae* and *O. apache* were distinct from *O. mykiss* and did not form a cluster.

However, the tree built using the mtDNA COX1 data from our work demonstrated that *O. gilae* formed a sister clade to *O. mykiss* and cladded with *O. mykiss* and *O. clarkii* (Fig. 2), which was also demonstrated by Nielsen et al. (1998) utilizing the 3′ end of the mtDNA regulatory region. *Oncorhynchus mykiss* clades from India showed an association with *O. mykiss* from other countries, which indicates genetic similarity. According to results from allozyme data, Loudenslager et al. (1986) found at least three subgroups in *O. mykiss*: *O. g. apache* + *O. g. gilae*; *O. mykiss*; and *O.* sp. (Rio Mayo, Mexico). This finding is consistent with Nielsen et al. (1998) and the findings of the current study (Fig. 2). Similar findings were reported by Colín et al. (2023), who found a tight link between *O. mykiss* and *O. gilae*; *O. gilae* were divergent yet formed a sister clade to the *O.* mykiss clade. According to this study, *O. mykiss* populations in India are one of the main lineages of Pacific trout and a varied group that descended from *O. clarkii*, which is present in North American Pacific coastal regions.

However, a study by Devaa et al. (2023) revealed that the *O. mykiss* stocks of Southern India were of the steelhead trout ecotype (*O. m. irideus*) and originated from the British Columbia, Washington, and Oregon regions of north-western America. This could be because the *O. mykiss* in this study and the *O. mykiss* from these regions had the same haplotype number. Rasmussen et al. (2009) also noted this feature, demonstrating that *O. mykiss* individuals gathered from various places shared a haplotype, with some haplotypes being specific to individuals found in Oregon and Washington. As demonstrated by earlier research using different mitochondrial markers (Nielsen et al., 1998; Phillips et al., 2004; Wilson and Turner, 2009), this work collectively demonstrated that the Pacific trout lineage has split from the Pacific salmon, and a similar event was noted using the COX1 marker. In summary, our study demonstrates the effectiveness of the COX1 marker as a molecular marker for comprehending the genetic diversity and phylogenetic links in *O. mykiss*.

Conclusion

The results of this study demonstrated that the populations of *O. mykiss* in India and other parts of the world were genetically related. Additionally, there was concern about the genetic diversity of the Indian *O. mykiss* stocks, which led to the necessity for population maintenance and genetic diversity management. Appropriate conservation measures have been applied to improve the genetic diversity of Indian *O. mykiss*. Natural selection forces, environmental adaptations, and a wide geographic barrier are the primary causes of the differences seen in Indian *O. mykiss*. Most significantly, it was discovered that the Pacific trout formed a monophyletic clade comprising subclades, *O. mykiss*, *O. gilae* and *O. clarkii*. This indicates that the populations of Indian *O. mykiss* worldwide have diverged from those of the Pacific coastline regions of North America. By employing the mtDNA COX1 marker, our work thereby fills the knowledge gap on the genetic diversity and phylogenetic linkages of Indian *O. mykiss* stocks.

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Author contributions: J.C. Walter Devaa: Sample collection, investigation, formal analysis, writing – original draft. Stalin Nithaniyal: Formal analysis, writing – original draft. Vimal Panneerselvam: Investigation. Kasi Marimuthu: Formal analysis, writing – original draft. Ramesh Uthandakalaipandian: Supervision, funding acquisition.

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Supplementary Table 1. Details of variable sites in Indian rainbow trout COX1 and other salmonid COX1 trout sequences. Indian rainbow trout COX1 sequences are highlighted in green colour.

Species Name	Nucleotide Sequences (bp)																																																																																																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99
Oncorhynchus clarkii JX960909.1 Canada	A	A	C	G	T	G	A	T	C	G	T	C	A	C	A	G	C	C	C	A	T	G	C	C	T	T	C	G	T	T	A	T	G	A	T	T	T	T	C	T	T	T	A	T	A	G	T	T	A	T	G	C	C	A	A	T	T	A	T	G	A	T	C	G	G	A	G	G	C	T	T	T	G	G	A	A	A	C	T	G	A	T	T	A	A	T	T	C	C	C	T	T	A	A	T	A	A	T	C
Oncorhynchus clarkii JX960910.1 Canada	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus gilae apache JX960907.1 Canada	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Oncorhynchus gilae JX960911.1 Canada	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Oncorhynchus gorbuscha MN756260.2 Mexico	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	G	.	.	.
Oncorhynchus gorbuscha MZ723948.1 Russia	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	G	.	.	.
Oncorhynchus keta LC094479.1 Japan	.	.	T	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	G	.	.	.
Oncorhynchus keta MZ407828.1 Thailand	.	.	T	.	.	A	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	A	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	G	.	.	.
Oncorhynchus kisutch LC575190.1 Japan	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	G	.	.	.
Oncorhynchus kisutch MT577156.1 Canada	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	.	.	.
Oncorhynchus masou MZ971133.1 China	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
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Oncorhynchus mykiss MW168250.1 Ooty India	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MW168253.1 Ooty India	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MW168255.1 Ooty India	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MW168256.1 Ooty India	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MW168795.1 Ooty India	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MW172282.1 Ooty India	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
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Oncorhynchus mykiss MZ407810.1 Phayao Thailand	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
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Oncorhynchus mykiss OR272445.1 Bang Kapi Thailand	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR569066.1 Kodaikanal India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
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Oncorhynchus mykiss OR569672.1 Munnar India	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
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Oncorhynchus mykiss OR573689.1 Munnar India	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
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Oncorhynchus clarkii JX960909.1 Canada	G	G	G	G	C	C	C	C	T	G	A	T	A	T	G	G	C	A	T	T	C	C	C	T	C	G	A	A	T	A	A	A	T	A	A	C	A	T	A	A	G	C	T	T	C	T	G	A	C	T	C	C	T	T	C	C	T	C	C	A	T	C	C	T	T	T	C	T	C	C	T	C	C	T	C	C	T	A	T	C	T	T	C	A	T	C	T	G	G	A	G	T	T	G	A	A	G	C	C
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Oncorhynchus mykiss MW168795.1 Ooty India	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MW172282.1 Ooty India	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
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Oncorhynchus mykiss OR569133.1 Kodaikanal India	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
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Oncorhynchus mykiss OR569170.1 Kodaikanal India	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
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Nucleotide Sequences (bp)

Species Name	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297
Oncorhynchus clarkii JX960909.1 Canada	G	G	C	G	C	T	G	G	T	A	C	T	G	G	A	T	G	A	A	C	A	G	T	T	T	A	C	C	C	C	C	C	T	C	T	G	G	C	C	G	G	T	A	A	C	C	T	C	G	C	C	C	A	T	G	C	A	G	G	A	G	C	C	T	C	T	G	T	T	G	A	T	T	T	A	A	C	T	A	T	C	T	T	C	T	C	C	C	T	C	C	A	T	T	T	A	G	C	T
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Oncorhynchus gilae apache JX960907.1 Canada	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.
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Oncorhynchus gorbuscha MZ723948.1 Russia	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.	A	.	.	C	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	…	…	…	…	…	…	…	…	…C
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Oncorhynchus keta MZ407828.1 Thailand	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	.	.	G	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	A	.	.	.	.	.	A	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	G	.	.	A	.	.	.	.	.	C	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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Oncorhynchus clarkii JX960909.1 Canada	G	G	A	A	T	C	T	C	C	T	C	A	A	T	T	T	T	G	G	G	A	G	C	C	A	T	T	A	A	T	T	T	T	A	T	T	A	C	G	A	C	C	A	T	T	A	T	T	A	A	C	A	T	A	A	A	A	C	C	T	C	C	G	G	C	T	A	T	C	T	C	T	C	A	A	T	A	C	C	A	A	A	C	C	C	C	C	C	T	T	T	T	T	G	T	T	T	G	A
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Species Name	Nucleotide Sequences (bp)																																																																																																					
	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495
Oncorhynchus clarkii JX960909.1 Canada	G	C	T	G	T	G	C	T	A	G	T	C	A	C	T	G	C	T	G	T	C	C	T	T	C	T	A	T	T	A	C	T	T	T	C	C	C	T	T	C	C	C	G	T	C	C	T	G	G	C	A	G	C	A	G	G	C	A	T	T	A	C	T	A	T	G	T	T	A	C	T	T	A	C	A	G	A	C	C	G	A	A	A	T	C	T	A	A	A	C	A	C	C	A	C	T	T	T	C
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Oncorhynchus mykiss OR922295.1 Azad Jammu and Kashmir	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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Species Name	Nucleotide Sequences (bp)																																															
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Oncorhynchus clarkii JX960909.1 Canada	T	T	T	G	A	C	C	C	G	G	C	A	G	G	C	G	G	A	G	G	A	G	A	C	C	C	A	A	T	T	C	T	A	T	A	C	C	A	A	C	A	C	C	T	C	T	T	T	T
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Oncorhynchus gilae apache JX960907.1 Canada	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
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Oncorhynchus gorbuscha MN756260.2 Mexico	.	.	.	.	.	.	.	.	A	.	.	G	.	.	G	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-
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Oncorhynchus keta LC094479.1 Japan	.	.	.	.	.	.	.	.	A	.	.	G	.	.	T	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus keta MZ407828.1 Thailand	.	.	.	.	.	.	.	.	A	.	.	G	.	.	T	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
Oncorhynchus kisutch LC575190.1 Japan	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
Oncorhynchus kisutch MT577156.1 Canada	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oncorhynchus masou MZ971133.1 China	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus masou OL674478.1 South Korea	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss EF609420.1 Tasmania Australia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
Oncorhynchus mykiss JX960920.1 Quebec Canada	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss JX960921.1 Quebec Canada	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss JX960922.1 Quebec Canada	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MT413324.1 Chongqing China	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
Oncorhynchus mykiss MW168249.1 Ooty India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MW168250.1 Ooty India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MW168253.1 Ooty India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MW168255.1 Ooty India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MW168256.1 Ooty India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MW168795.1 Ooty India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MW172282.1 Ooty India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MW649707.1 Chongqing China	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.
Oncorhynchus mykiss MZ407810.1 Phayao Thailand	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MZ407811.1 Phayao Thailand	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss MZ407833.1 Phayao Thailand	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR178022.1 Kodaikanal India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR272437.1 Davis USA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR272445.1 Bang Kapi Thailand	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR569066.1 Kodaikanal India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR569133.1 Kodaikanal India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR569167.1 Kodaikanal India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR569170.1 Kodaikanal India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR569672.1 Munnar India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oncorhynchus mykiss OR569683.1 Munnar India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oncorhynchus mykiss OR572103.1 Munnar India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oncorhynchus mykiss OR573477.1 Munnar India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oncorhynchus mykiss OR573660.1 Kashmir India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR573676.1 Kashmir India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR573689.1 Munnar India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oncorhynchus mykiss OR573690.1 Kashmir India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR573699.1 Kashmir India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR574990.1 Kashmir India	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus mykiss OR922291.1 Azad Jammu and Kashmir							A	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-
Oncorhynchus mykiss OR922292.1 Azad Jammu and Kashmir							A	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-
Oncorhynchus mykiss OR922293.1 Azad Jammu and Kashmir							A	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-
Oncorhynchus mykiss OR922294.1 Azad Jammu and Kashmir							A	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-
Oncorhynchus mykiss OR922295.1 Azad Jammu and Kashmir							A	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-
Oncorhynchus nerka LC575157.1 Japan	.	.	.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oncorhynchus nerka MZ407822.1 Thailand	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	G	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Oncorhynchus tshawytscha EF609421.1 Australia	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	-
Oncorhynchus tshawytscha OP498310.1 USA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	T	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	-
Salmo trutta fario MT670294.1 Pakistan	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	T	.	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Salmo trutta JX960942.1 Canada	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.