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GENETIC AND PHYLOGENETIC VARIATIONS OF YELLOWFIN TUNA (THUNNUS ALBACARES) AS A BASIS FOR SUSTAINABLE FISHERY RESOURCES MANAGEMENT IN NORTH MOLUCCAS

MUHAMMAD ARIS*[1], NEBUCHADNEZZAR AKBAR[1], RUSMAWATI LABENUA[1]

[1]Faculty of Fisheries and Marine Science, Khairun University, Ternate Indonesia

ABATRACT

Yellowfin tuna, a big pelagic fish, is highly abundant in the waters of North Molucca. Increases fishing activities result in a decrease in population stock. The sustainability and resources status can be understood through genetically approaches. The objective of this research is to understand phylogenetic and genetic variations of yellowfin tuna in the North Molucca waters. Sample collection was done in Mei-July 2016 in the islands of Morotai, Obi, and Sanana, while the secondary data were from the islands of Ternate, Bacan, and Ambon. In total, there were 72 samples collected and analyzed. The results showed that the genetic variations of yellowfin tuna were 0.97 in Morotai, 1.00 in Sanana, 0.97 in Obi, 0.98 in Bacan, and 1.00 in Ternate and Ambon. Nucleotide variations analysis resulted 0.022 in Morotai, 0.026 in Sanana, 0.018 in Obi, 0.029 in Bacan, 0.027 in Ternate, and 0.025 in Ambon. The genetic variations value found suggests that yellowfin tuna is still in good condition based on criteria. The high variety of genetic diversity and specific haplotypes provides an illustration that there has been no genetic change in yellowfin tuna populations. Haplotype distribution analysis found 44 haplotype-specific, 11 haplotype mixed among locations, and 4 similar haplotype. Phylogenetic reconstruction showed that yellow fin tuna is panmixia population indicating all samples do not have significant genetic differences. The strategy for sustainable fishery management has to be done based on several proposed recommendations; first knowledge of genetic information on an ongoing basis covering the deployment area. Second is a population measurement based on morphological size information temporally. Third is the increase of tuna fish stock through restoking activities. The recommendations are important to be implemented in order to keep the stability and sustainability of yellow fin tuna population in the area.

KEYWORDS: ***genetic variations, phylogenetic, yellowfin tuna, North Moluccas.***

MUHAMMAD ARIS*

Faculty of Fisheries and Marine Science, Khairun University, Ternate Indonesia

Corresponding Author

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INTRODUCTION

[1]Yellowfin tuna is a big pelagic fish and has high economic values. [2]This species is found in almost entire world's sea waters as it has high migratory ability. [3]Tuna fishing has been done for more than five decades and the fishing rates are steadily increased by 5.45% per year. Reported that the fishing production of yellowfin tuna in the Pacific Ocean in 2010 was 2,296,000 tonnes which decreased by 3% from the previous year, in the Indian Ocean the production in 2010 was 843,000 tonnes which decreased by 4% from 2009, and in the Atlantic Ocean the production was 419,600 tonnes which increased by 2% from the previous year. The potency of large pelagic fishes, especially tuna, is found in the waters of North Molucca. In 2014, fishing production in the area was 216,000 tonnes. The export of tuna in 2010-2015 was 142.00 tonnes, increased by 15. This total potency indicates that the North Molucca waters are part of the migration routes of large pelagic fishes. The abundance of tuna resources is affected by the geographical location that directly connected to the Pacific Ocean, Seram Sea, Molucca Sea, Halmahera Sea and Banda Sea which are the path of the Indonesian throughflow.[4] The North Molucca waters are also the spot of biodiversity as it is in the world coral triangle area. [5]High fishing rate leads to a decrease of yellowfin stock size in terms of weight-length, individual size and population size.[2] Stated that the status of yellowfin tuna in the coral triangle area is fully exploited and that of big eye tuna is overfishing.[6] If fishing of these species is done continuously, it could decrease the population as their life cycle may be. [2]Therefore, the program of tuna management and conservation through genetic conservation is needed that can be used to establish the policy of management and genetic conservation of yellow fin tuna. Research reported[7] on yellow fin tuna variations in the Pacific Ocean, [8, 1] in the Pacific and Indian Oceans, [9]in the Indian Ocean, and at along the coast of India. [10]Population structure can be determined through a morphology study and population sustainability can be determined through a genetic analysis.[11] Genetic variations relate to sustainable resources utilization and conservation aspects, and also affect the development of paired organs in animals. [12, 13, 14, 15, 16] The information of tuna genetic variations is useful for population structure identification optimization, breeding programs, stock development and management for sustainable resources.[1] The high commercial and economic values of yellowfin tuna become the reason of the importance of genetic variation understanding for an effective sustainable fishery resources management.[17] The knowledge about the structure can be used in fishery management and conservation efforts for commercial valuable pelagic fishes in Indonesia. The objective of this research is to understand the genetic and phylogenetic variations of yellowfin tuna in North Molucca waters. [18]PCR-sequencing method was used to find the information of genetic variations of a population. This method can be used to obtain the alkali sequence of nucleotides in DNA moleculer. [19] Had been research on the genetic variations and structures of yellowfin tuna in North Molucca, Indonesia. These researches, however, do not provide yet complete information about tuna genetic variations, therefore additional information from some other areas in the North Molucca waters is needed. The information of genetic variations of yellowfin tuna in the waters of North Molucca is scarce, but the information is needed to know the population status and to formulate a management policy. This study correlates the results of field data and secondary data from.

Research methodology

Samples and Methods

Samples of tuna were collected in May-June 2016 in three locations in the North Molucca Province, i.e. Morotai Island (10 samples), Obi Island (10 samples), dan Sanana Island (10 samples). The tuna fishing area is located in the waters of Morotai Island in the north and west, on the island of Obi the fishing operation is in the south and east while for Sanana Island itself the fishing area is in the south, east and west. The location of the fishing is based on the migration of the tuna waters of each island. Samples were fish caught in the waters around the research locations that landed at Fish Landing Centers (Pangkalan Pendaratan Ikan, PPI) and Archipelagic Fishing Port (Pelabuhan Perikanan Nusantara, PPN) (Figure 1). The Sec ondary data in the form of tuna sequences are taken from[19] research results located in Ternate, Bacan and Ambon. All secondary data were from the previous research. Each sampled fish was pictured and measured its length. A piece of about 3 cm in length was taken from pectoral fins and placed inside a tube contained 96% ethanol for preservation. Samples analysis were carried out in the Indonesian Biodiversity Research Center (IBRC) Bali.

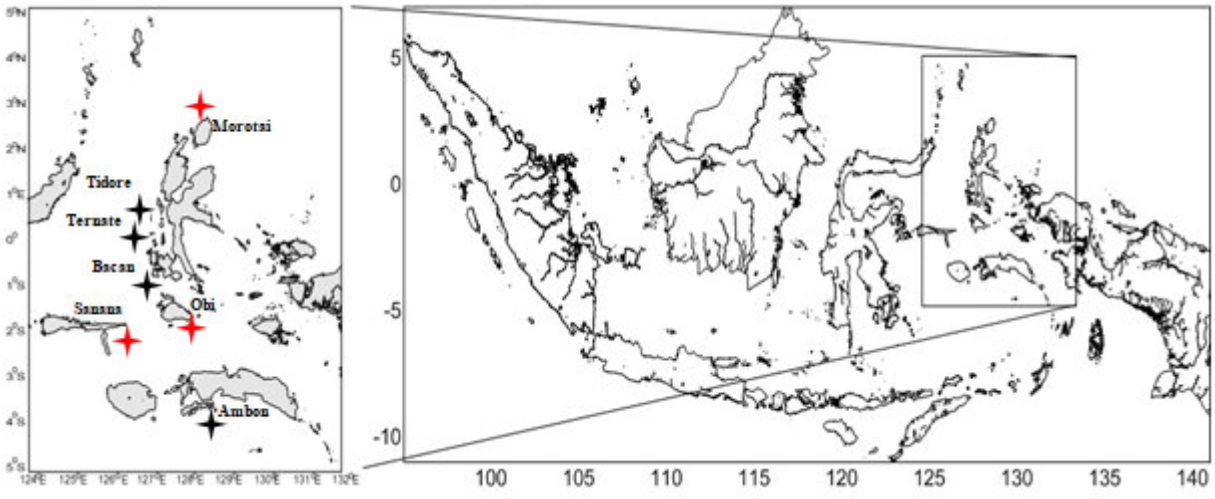


Figure 1
Sampling locations of yellow fin tuna (Thunnus albacores) in the waters of North Molucca (red stars = primary data, black stars = secondary data)

[20]The DNA samples were isolated using Chelex 10% solution. Extraction was started with putting a sample in a tube and then vortexed and centrifuged for ± 20 seconds. Next, it was heated using a heat block at 95°C for ± 45 minutes. After that, the tube was re-vortexed dan centrifuged for 20 seconds. [21]The Polymerase Chain Reaction amplification process was focused on the locus of mtDNA control region with forward primer CRK 5'-AGCTC AGCGC CAGAG CGCCG GTCTT GTAAA-3' and reverse primer CRE 5'-CCTGA AGTAG GAACC AGATG-3'. The procedure in PCR was started with a predenaturation at 94°C for 15 seconds, 38 cycles including denaturation at 94°C for 30 seconds, annealiing at 50°C for 30 seconds, and extension at 72°C for 45 seconds and then further extension at 72°C for 5 minutes. The quality of DNA produced by PCR was examined using an electrophoresis. In this stage a 1 gr agarose gel was made and put in an erlenmeyer, added TAE 1x 75mL and heated in a microwave and then added EtBr 4 uL. After that, the agarosa gel was poured into a tray that had a wells maker comb and left for 30 minutes.[18] The PCT products were sent to Berkeley Sequencing Facility using Sanger method.

Data Analysis

The analysis of mtDNA control region sequence used MEGA5 software consisting of genetic distances, phylogenetic using neighbour joining method, Kimura's 2-parameter evolution model and 1000 bootstraps replications. Species identification used Blast (Basic Local Aligment Tools) application. Variation analysis of haplotype (H_d) and nucleotide (π) in the mtDNA sequences was based on[26] using DnaSP 4.0 application[23].

RESULTS and DISCUSSION

Molecular Characteristics

The fragment of mtDNA control region was obtained 512 bp (base pairs) in length of 72 yellow fin tuna (*T. albacares*) samples including 42 secondary data from Ternate (12 samples), Bacan (23 samples), and Ambon (8 samples). The obtained DNA fragment was similar to the findings of[7, 19] i.e. 517 bp and of i.e. 500 bp. Other tuna research found short DNA fragments, for example[21] found 400 bp, [1]366 bp, and [7]304 bp. These fragment length variations are due to the quality of DNA samples and the number of samples collected. [24]These variations, however, do not indicate any effects on the results of sequence analysis. In fact, some research done, for example, by[25,26] found 600–700 bp in shark samples, while[23] found 860 bp in big eye tuna, and[24] found 526 bp in grouper *Epinephelus* spp.

Genetic variations

Genetic variations were found to be very high with varied of haplotype total and nucleotide variations (Tabel 1). Haplotype (*Hd*) variations in each location samples showed that Sanana (*nucleotide* 0.026), Ternate (*nucleotide* 0.027), and Ambon (*nucleotide* 0.025) had the highest genetic variations of 1.00. In Bacan (*nucleotide* 0.029), the variation value was 0.98, while in Morotai (*nucleotide* 0.022) and Obi (*nucleotide* 0.018) had 0.97 variation value. High genetic variation value of yellow fin tuna had been reported, for examples [7]0.840 in the Pacific Ocean, [8]0.878, 0.992 in the western Pacific Ocean and [1]0.999 in the western Indian Ocean, and [9]0.998. [27, 28, 29, 30, 31, 32, 15, 16, 17]High genetic variations also had been reported for other migratory species like big eye tuna (*Thunnus obesus*), albacore (*Thunnus alalunga*), skipjack (*Katsuwonus pelamis),* frigate mackerel (*Auxis thazard*), bonito (*Euthynnus affinis*), Spanish mackerel (*Scomberomorus commerson*), stripped mackerel (*Rastrelliger kanagurta*). [33]Migration ability allowing inter-population encounters, and high genetic variations in tunas are characteristics of *Scrombridae* family.

Table 1
The number of haplotype (Hn), haplotype variation (Hd), nucleotide variation (μ) and the number of samples (n) of yellow fin tuna (Thunnus albacares).

Location	*n*	H_n	H_d	π	*Base Pairs*
Morotai	10	9	0,97	0,022	512
Sanana	10	10	1,00	0,026	
Obi	10	9	0,97	0,018	
Bacan	23	20	0,98	0,029	
Ternate	11	11	1,00	0,027	
Ambon	8	8	1,00	0.025	

[22]Based on the criteria of genetic variations, the findings indicated that the yellow fin tuna is still in good condition. Even though this species is the target catch in all areas, but with their migration ability and fast reproduction, this tuna population could continue to exist. [34]Stated that tuna migration rate is higher than other saltwater fishes, so the probability to meet and inter-breed with other populations is even greater. [35, 36]Genetic variations provide the ability to adapt to climate and environmental change and diseases, and indicate that the species has large population size. The results of genetic variation analysis revealed 58 haplotype from 72 samples (Table 2). Overall, the haplotype variations indicated that there were 44 haplotype-specific, 11 haplotype mixed among locations, and 4 similar haplotype which occurred in two species from the same location. The tuna fish are migration and have migration routes at each research site The overall research sites are feeding areas and tuna migration paths. This provides evidence that the waters around North Maluku are where live habitats tuna fish.

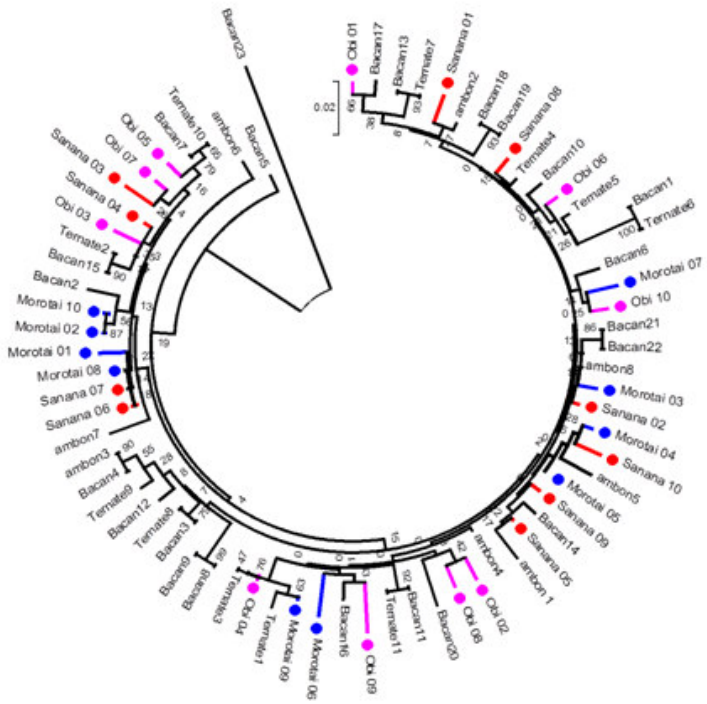
Table 2
The number of haplotype (Hd) variation in yellow fin tuna (Thunnus albacares)

No	*Haplotipe* (*Hd*)	No	*Haplotipe* (*Hd*)
1	Hap_1: 1 [Morotai]	30	Hap_30: 2 [Bacan3 Ternate8]
2	Hap_2: 2 [Morotai Morotai]	31	Hap_31: 2 [Bacan4 ambon3]
3	Hap_3: 1 [Morotai]	32	Hap_32: 1 [Bacan5]
4	Hap_4: 1 [Morotai]	33	Hap_33: 1 [Bacan6]
5	Hap_5: 1 [Morotai]	34	Hap_34: 2 [Bacan7 Ternate10]
6	Hap_6: 1 [Morotai]	35	Hap_35: 2 [Bacan8 Bacan9]
7	Hap_7: 1 [Morotai]	36	Hap_36: 1 [Bacan10]
8	Hap_8: 3 [Morotai Sanana Sanana]	37	Hap_37: 2 [Bacan11 Ternate11]
9	Hap_9: 1 [Morotai]	38	Hap_38: 1 [Bacan12]
10	Hap_10: 1 [Sanana]	39	Hap_39: 2 [Bacan13 Ternate7]
11	Hap_11: 1 [Sanana]	40	Hap_40: 1 [Bacan14]
12	Hap_12: 1 [Sanana]	41	Hap_41: 2 [Bacan15 Ternate2]
13	Hap_13: 1 [Sanana]	42	Hap_42: 1 [Bacan16]
14	Hap_14: 1 [Sanana]	43	Hap_43: 1 [Bacan17]
15	Hap_15: 1 [Sanana]	44	Hap_44: 2 [Bacan18 Bacan19]
16	Hap_16: 1 [Sanana]	45	Hap_45: 1 [ambon]
17	Hap_17: 1 [Sanana]	46	Hap_46: 1 [ambon2]
18	Hap_18: 1 [Obi]	47	Hap_47: 1 [ambon4]
19	Hap_19: 1 [Obi]	48	Hap_48: 1 [ambon5]
20	Hap_20: 1 [Obi]	49	Hap_49: 1 [ambon6]
21	Hap_21: 2 [Obi Ternate3]	50	Hap_50: 1 [ambon7]
22	Hap_22: 1 [Obi]	51	Hap_51: 1 [ambon8]
23	Hap_23: 1 [Obi]	52	Hap_52: 1 [Bacan20]
24	Hap_24: 1 [Obi]	53	Hap_53: 2 [Bacan21 Bacan22]
25	Hap_25: 1 [Obi]	54	Hap_54: 1 [Bacan23]
26	Hap_26: 1 [Obi]	55	Hap_55: 1 [Ternate1]
27	Hap_27: 1 [Obi]	56	Hap_56: 1 [Ternate4]
28	Hap_28: 2 [Bacan1 Ternate6]	57	Hap_57: 1 [Ternate5]
29	Hap_29: 1 [Bacan2]	58	Hap_58: 1 [Ternate9]

High genetic variations and varied haplotype-specific indicate that there are no genetic structure changes yet in yellow fin tuna population because it still has various gene variations. Haplotype variations had been reported by[1], where there were 111 different haplotype of 124 total samples. Moreover, [37]from 28 samples found 18 haplotype -specific in each individual fish, 8 haplotype distributed in two individuals and 1 haplotype distributed in 4 individuals. [17]Reported 172 haplotype from 177 analyzed samples. [15]found 98 haplotype from 118 total samples observed in big eye tuna. Yellow fin tuna that widely distributed in the world oceans has big population with high haplotype variations. This indicates that the population has high survival against environmental disturbances. [38]Various haplotype composite types are also contributed highly to increase the genetic variations of a population. [22]Several reports on haplotype variations in populations including in a big population, the environmental variations and the history of threatened population life were steadily going up.

Phylogenetic Thunnus albacares

The reconstruction of family relationship among yellow fin tuna population in each location found that there occur an individual mixture (Figure 1). Reported similar finding that an individual mixture occurred among populations. Moreover, [9]also found no genetically difference in the Indian Ocean based on phylogenetic reconstruction. [39]Reported that a mixture had been occurred between yellow fin tuna from the Philippines and from Spain. [40]Various researches had also reported the close family relationship based on phylogenetic tree in the Pacific Ocean, [31, 32]in the Atlantic Ocean, [15]China Sea, the Philippines and the western Pacific Ocean, and [16]in the Indian Ocean for big eye tuna (*T. obesus*). This indicates that the population has a genetic closeness and shows a strong relationship among populations. [41]Explained that in order to reveal low population difference, migration involving several individuals per generation could be a key to produce far genetic homogeneity. Geographically, the North Molucca has many islands. The distance between research locations, i.e. Morotai, Obi and Sanana are quite far, this however, did not limit the movement and mobility of tuna. [39]Stated that tuna generally can migrate for long distance as they are able to adapt to ocean environment changes, and then in winter they migrate to tropical waters. Besides, the North Molucca waters are the route of the Indonesian throughflow that helps to distribute fish partially in each location. [42]Stated that ocean flow could affect population distribution and fish genetic structures. [43]Revealed that gene exchange processes occurred among populations in the tropical Indo-Pacific resulted in a genetic closeness among populations, and therefore giving a chance for tuna to meet in the area with a big population and from different locations.



Gambar 1

Phylogenetic tree of yellow fin tuna (Thunnus albacares) analyzed using a neighbor-joining method with Kimura 2-parameter, (Primary Data: Pink Dot = Obi, Blue Dot = Morotai, and Red Dot = Sanana).

The constructed phylogenetic tree supported by quite high bootstrap values at each branches. This indicates that reconstructed family tree has a good accuracy. The bootstrap values ranged from 55 to 99%. The analysis result concludes that the yellow fin tuna is panmixia population, which explains that there are no genetic differences among populations. Other tuna species had been reported by [15]that big eye population is panmixia mixed among populations of the South China Sea, Philippines waters, western Pacific Ocean. In addition, [31]reported that there is genetic closeness in big eye tuna of the Indo-Pacific and the Atlantic Ocean. Furthermore, [16]provided evidence of individual mixing of big eye tuna in the Indian Ocean. [44]This big eye tuna similarity occur as there are gene flows from the western Pacific Ocean to the Indian Ocean and then to the Atlantic Ocean. [45, 46,

[47] Pelagic fishes are found to have small genetic differences even though they are far separated geographically as they have big populations and high dispersal distances.

Tuna fishery management concept

The potency of fish resources in Indonesian waters is high. Globally, the annual catch tends to increase. [48]The catch data indicated that tuna fishing production in Indonesian waters reached 1.2 million tonnes in 2013. Economic incentives generated by fishing activities were 3.3 million work forces and US$ 68 millions due to export of skipjack, tuna and others. Reported that there was an increase of 10.96% in 2015 comparing in 2013 with the production value of 236 billion, while the total of fish capture production reached 6.4 million tonnes. Showed that the global fishing of skipjack and yellow fin tuna tended to increase reached 2,2 and 1,4 million tones, respectively, in 2003, and the catch of big eye tuna was 493,000 tonnes in 2000 which was lower than the previous year. Surely, the high potency of big eye tuna resource needs a special attention. This is useful in conserving and guaranties the sustainability of tuna resources in Indonesia. [49,50,51]Fishing activities have potential impacts on maturity processes and the reduction of population density and body size. Therefore, in the future the knowledge of catch and stock status becomes crucial managerial information for fishery resources management. [52]Besides, the use of technology in fishing raises a big problem, i.e. predominantly young fish in pole and line fleets catches. [53, 54]Consequently, a nonstop fishing can threaten the sustainability of the existed fish resources. The high genetic variations found in the North Molucca waters indicate that the tuna populations are still able to adapt to the occurred environmental changes. However, it is needed an action to avoid biological lost including genetic aspect. Activities to be carried out to safeguard tuna resource populations; First knowledge of genetic information on an ongoing basis covering the extent of the dispersal area, it is important to serve as the basis for determining population status.[55, 56]This is important primary data in determining population status. Genetic observations are unlimited efforts in potency management of commercial species. [57]Besides, genetic information can become a reason to conserve a species. The second is population measurement based on temporal information of morphological measurements. [58]An approach to know the population status can be done through measurement based on morphology. [59]This analysis is as the first step to observe the existence of a big size population based on phenotypic features. [52]Biological data set like this can be made as scientific evidences in developing a sustainable fishery management. The third is to increase tuna stock through restocking action. This process is started with collecting tuna juveniles through fishing and then cultured. This action aims to create stock development and stability in a sea. The restocking can, however, be done regularly using juveniles from hatcheries. We suggest this action to be made as a basis in tuna fishery management in the future.

CONCLUSION

All the results show that genetic variations and haplotype composites are still high and inter-population relation are very strong. This information indicates that yellow fin tuna population in the waters of North Molucca is not vulnerable to the prevailing environmental change. A strong fishery resources management has to be implemented as a form of protection and conservation of tuna for the future.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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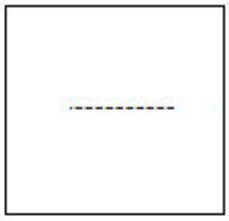

Ince Ayu Khairana Kadriah

Research Institute for Coastal Aquaculture, South Sulawesi, Indonesia

Asso.Prof.Dr. R. Usha, MSc, M.Phil, Ph.D.

Associate Professor, Department of Microbiology, Karpagam University, Eachanari (PO), Coimbatore - 641 021, Tamil Nadu, India.

Prof.Dr.K.Suriaprabha

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