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Positive selection in the mitochondrial protein coding genes of teleost regional endotherms: Evidence for adaptive evolution

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Original Article

Abstract

Mitochondrial oxidative phosphorylation genes play critical role in energy metabolism, aerobic potential and thermogenesis. These genes were thought to evolve neutrally, however increasing evidence suggests that mitogenome is susceptible to selection and adaptive variation. Organisms that have encountered selective forces to improve their metabolic potential or adapt to cooler environment can be suitable candidates to study the pattern and impact of selection on mitochondrial genome. Tunas, billfishes, butterfly mackerel and opah are the only teleost fishes to exhibit regional endothermy. They might have experienced strong selective forces to enhance their metabolic potential making them a suitable candidate group to search for positive selection. Mitochondrial protein coding genes of 16 regionally endothermic teleosts retrieved from NCBI GenBank were used to examine the pattern of selection using different ω-based approaches implemented in DATAMONKEY and TreeSAAP to analyze the changes in physicochemical properties of the amino acids. We found evidence for positive selection in different mitochondrial protein subunits across several branches of the phylogeny. Changes found in the subunits ND5 and ND6 might have modified the proton pumping efficiency and assembly of complex I respectively and the substitutions found in the subunit ATP6 might have an impact on the rotor performance of the complex V. Further studies on assessment of metabolic consequences of OXPHOS substitutions are essential to understand the importance of these substitutions on the performance of the fishes.

Keywords: *Positive selection, regional endothermy, mitochondrial genome, oxidative phosphorylation (OXPHOS), adaptive variation*

Introduction

Regional endothermy, the capacity of an organism to produce body heat endogenously, retain and/or warm selected tissues higher than the ambient temperatures, have been well documented in mammals and birds. The occurrence of regional endothermy in other clades remained overlooked until Davy (1837) recorded elevated body temperature than the ambient waters in skipjack tunas, which were conventionally regarded as cold blooded or ectotherms. It is now known that at least four lineages of teleost fishes (Legendre and Davesne, 2020) have evolved distinct specializations to increase their body temperature, *viz*., opah (*Lampris guttatus,* Lampridiformes), butterfly mackerel (*Gasterochisma melampus,* Scombridae), billfish (Istiophoridae and Xiphiidae) and tunas (Scombridae). Larger body size, pelagic lifestyle, open-sea predation and fast swimming capacity are other similar features common to these groups. They have the ability to migrate longer distances and dive vertically below the thermocline thereby coming across a wide temperature range. In spite of several shared morphological and physiological adaptations, these lineages do not share a recent common ancestor and are consistently regarded to be phylogenetically distinct (Orrell *et al.,* 2006; Santini and Sorenson, 2013; Legendre and Davesne, 2020). The authors also recently confirmed the phylogenetic separation of regional endothermic teleosts using mitochondrial data. Hence, regional endothermy cannot be considered as a plesiomorphic character or a primitive trait, which remained unchanged during the evolutionary process (Wagele, 2005). Various advantages of regional endothermy in teleosts have been proposed including enhanced vision and neural activity as in cranial endothermy (Block and Carey, 1985), deep cold water foraging (Fujioka *et al.,* 2018; Stoehr *et al.,* 2020), higher digestion and absorption rate (Stevens and

McLeese, 1984; Goldman, 1997), higher rate of gonadal and somatic growth and rapid recuperation from anaerobic burst (Brill, 1996). However, increased swimming performance and ecological niche expansion, which enabled the endothermic teleosts for large scale migration, is the most accepted adaptive benefit of regional endothermy (Dickson and Graham, 2004; Watanabe *et al.,* 2015). This might be the major driving force for the convergent evolution of regional endothermy (Fujioka *et al.,* 2018; Stoehr *et al.,* 2020; Legendre and Davesne, 2020) in distinct teleost fishes as well as in extinct Mesozoic marine organisms like mosasaurs, plesiosaurs and ichthyosaurs (Bernard *et al.,* 2010) at least at the phenotypic level.

Fishes exhibiting regional endothermy are highly migratory and therefore should experience high energy expenditure when compared to the non-migratory species (Gross *et al.,* 1987; Sun *et al.,* 2011). This group of teleosts with high aerobic capacity possesses high mitochondrial content, indicating the association of mitochondrial genes to thermoregulation and energy metabolism (Dalziel *et al.,* 2006). The mitochondria under aerobic conditions generates up to 95% of a cell's energy in the form of ATP in eukaryotes through Oxidative phosphorylation (OXPHOS). Vertebrate mitochondrial genome comprises 13 protein coding genes and the peptides encoded together with the peptides of nuclear origin assemble into five multimeric-protein complexes (Barshad *et al.,* 2017). The peptides of mitochondrial origin includes, seven subunits (ND1-6, ND4L) of complex I or NADH dehydrogenase, one cytochrome b subunit (cytb) of complex III or bc1 complex, three cytochrome oxidase subunits (COI-III) of complex IV or cytochrome c oxidase and two ATP subunits (ATP6 and 8) of complex V or ATP synthase (Wallace, 2007). These large protein complexes are involved in the electron transport chain and the phosphorylation of ADP to ATP there by playing a major role in tissue bioenergetics (Little, 2009). Besides this OXPHOS plays an important role in energy consuming processes like ion pumping and muscle contraction, thereby assisting overall metabolic and swimming performance of the fishes (Sun *et al.,* 2011).

The evolution of mitochondrial genome under the assumption of neutrality is now being increasingly questioned suggesting that the genetic variation of the mitochondrial DNA is not solely shaped by random genetic drift. In spite of the functional constraints, the mitochondrial protein coding genes are susceptible to selection and adaptation in accordance to the environmental conditions like high temperature (Morales *et al.,* 2017), low temperature (Cheviron *et al.,* 2014; Stier *et al.,* 2014), reduced oxygen level or hypoxia (Scott *et al.,* 2010), availability of nutrients (da Fonseca *et al.,* 2008), anoxia (Tomasco and Lessa, 2011), high energy demands for flight (Shen *et al.,* 2010), variation in gene expression (Mishmar *et al.,* 2003; Morales *et al.,* 2015) and high altitude (Hochachka *et al.,* 1983; Yu *et al.,* 2011) in diverse organisms including humans. Even though marine organisms are less studied unlike other terrestrial organisms, patterns of selection in mitochondrial DNA have been reported in several marine species in response to various factors like sea surface temperature as in Japanese sand lance (Deng *et al.,* 2019), temperature clines as in North Pacific walleye Pollock (Grant *et al.,* 2006; Atlantic herring, Teacher *et al.,* 2012; European anchovy, Silva *et al.,* 2014; Marbled rockfish, Xu *et al.,* 2017), latitudinal clines as in killer whale (Foote *et al.,* 2011; Pacific salmon, Garvin *et al.,* 2011), osmotic environment as in Killifish (Brennan *et al.,* 2016), hydrological and physiological factors as in *Prochilodus* sp. (Moyer *et al.,* 2005).

Given the significant roles of OXPHOS genes in energy production and thermoregulation, it is possible that any amino acid change may influence the protein function causing significant fitness and metabolic consequences in the species (Ballard and Pichaud, 2014). Hence, we hypothesize that variations in energy requirements might have induced selection pressures on the mitochondrial OXPHOS genes so that they can meet the metabolic demand and adapt to the new environment. Despite being phylogentically distinct, such mitochondrial energy adjustments have also been reported in birds and mammals wherein, the convergent evolution of the trait like thermogenesis regulation occurred. In this study, we take the benefit of complete mitogenome sequences of teleost regional endotherms that are deposited in the GenBank and make an attempt to analyze the pattern of selection experienced by the OXPHOS genes during the course of evolution.

Material and methods

The complete mitogenome sequences of 16 endothermic teleosts representing four families (Xiphiidae, Istiophoridae, Scombridae and Lampridae) (Johnson, 1986; Nelson, 2006) were retrieved from NCBI GenBank (Table 1). Initially datasets for thirteen protein coding genes (COI-III, ND1-6, ND4L, ATP6, ATP8 and Cytb) were made, aligned separately, edited manually and concatenated excluding the stop codons with the Clustal X v1.81 algorithm. The pattern by which protein coding genes undergoes selection is usually estimated by the ratio of non-synonymous to synonymous substitutions (dN/dS or ω). The genes in question are thought to be under (i) positive selection if the ratio is >1, (ii) negative or purifying selection if the ratio is <1 and (iii) neutral if the value equals 1 (Zhang *et al.,* 2006). We calculated the dN/dS ratio using DnaSP software 6.12.01 (Rozas *et al.,* 2017). The final concatenated matrix of 11,379bp was used to create an unrooted phylogenetic tree using the maximum likelihood method in RAxML v.8.2.10 (Stamatakis, 2014) with the GTRCAT model for each partition. A 10000 nonparametric bootstrapping replicates were used to analyze the support for nodes. The phylogenetic tree reconstructed

Table 1. The GenBank accession numbers of the endothermic teleost species used in this study

No	Accession Number	Species	Family	Suborder
1	KY400011.1	*Thunnus obesus*	Scombridae	Scombrioidei
2	KF906721.1	*Thunnus orientalis*		
3	KF906720.1	*Thunnus thynnus*		
4	AP006033.1	*Gasterochisma melampus*		
5	AB470301.1	*Xiphias gladius*	Xiphiidae	
6	KJ510416.1	*Istiompax indica*	Istiophoridae	Xiphoidei
7	AB470306.1	*Istiophorus platypterus*		
8	NC030010.1	*Kajikia albida*		
9	AB470304.1	*Makaira mazara*		
10	AB470303.1	*Tetrapturus angustirostris*		
11	AP006035.1	*Istiophorus albicans*		
12	KU315126.1	*Kajikia audax*		
13	KU315120.1	*Makaira nigricans*		
14	NC030009.1	*Tetrapturus georgii*		
15	NC030007.1	*Tetrapturus pfluegeri*		
16	AP002924.1	*Lampris guttatus*	Lampridae	Lampriformes (order)

using the maximum likelihood method was used along with the sequence alignment in further analysis to infer the sites undergoing positive selection using various methods.

Codon based methods, which could estimate site specific dN/dS in the concatenated mitochondrial protein coding genes were used to detect the signs of selection. Mixed effects model of evolution (MEME) is a mixed effect model which uses maximum likelihood approach to identify both pervasive and episodic positive selection at each site by analyzing the distribution of dN/dS ratio to differ from sites to sites (the fixed effect) and also from branch to branch (the random effect) at a site (Murrell *et al.,* 2012). Fixed Effects Likelihood (FEL) uses maximum likelihood method to deduce the dN/dS ratio of each site for a given sequence alignment and corresponding phylogenetic tree by assuming that the selection pressure acting on each site is constant across the whole phylogeny (Pond and Frost, 2005). A Fast, Unconstrained Bayesian Approximation for Inferring Selection (FUBAR) is faster and robust method to detect positive selection even for larger datasets since it uses flexible parameters and thus are less susceptible to model requirements (Murrell *et al.,* 2013). All the analyses discussed above were performed using DATAMONKEY (Adaptive Evolution server) (Weaver *et al.,* 2018). The threshold *P*-value were set to $p<0.05$, <0.1 and posterior probability value >0.9 for MEME, FEL and FUBAR respectively.

TreeSAAP uses a sliding window approach to compare the expected amino acid replacement patterns to the observed replacements at the positively selected sites assumed from a phylogenetic tree under the assumption that every amino acid replacement has equal probability to occur (Woolley *et al.,* 2003). These are then used to analyze the significant changes caused by the replacements in the physicochemical properties of the amino acids using different models under neutral conditions. Replacement of each amino acid and subsequent change in physicochemical property is classified into eight categories based on the magnitude. Categories 1-3 represent the most conservative (minimal effect on the amino acid property or stabilizing selection) and categories 6-8 represent the radical changes (destabilizing selection) which can bring about changes in the protein biochemistry and are exposed to the selection pressures (Teacher *et al.,* 2012). A z-test was utilized to estimate the kind of selection at each sliding window and the significance; negative values indicating purifying (or negative) selection and positive values indicating positive selection or the occurrence of higher amount of non-synonymous mutations under neutrality (Morales *et al.,* 2015). To minimize the detection of false positive, only the amino acid replacements with magnitude category ≥6, z-scores less than or greater than 3.09 with significance value, $p<0.001$ and the amino acid positions supported by at least two separate methods were considered to be under positive selection (McClellan *et al.,* 2005; Sun *et al.,* 2018). Finally, the construction of 3D homology model of protein subunits was done using the SWISS-MODEL server (Schwede *et al.,* 2003) using the best hit templates. The sites undergoing positive selection were located in the three-dimensional protein subunit structure.

Results and discussion

The average dN/dS ratio for the concatenated protein coding genes of 16 teleost species was found to be 0.0447, a value much less than 1, a signature for negative purifying selection in which the evolutionary pressures conserves the protein in its ancestral state (Yang and Bielawski, 2000). However, this statistical method to identify molecular adaptation is found to be highly biased and hence not satisfactory (Ngatia *et al.,* 2019). Due to their functional constraints and active involvement in the cell metabolism, mitochondrial protein coding genes are highly conservative. Therefore, few amino acid changes which can even lead to positive selection and further adaptation are masked by the sites undergoing purifying selection (Meiklejohn *et al.,* 2007; da Fonseca *et al.,* 2008).

The unrooted phylogenetic tree constructed via Maximum

likelihood method confirmed the systematic placement of the species as per previous molecular methods (Fig. 1). Monophyly of the endothermic tunas is well supported with strong bootstrap values (Jondeung and Karinthanyakit, 2010) and the butterfly mackerel (*Gasterochisma melampus*) formed the basal scombrid (Jondeung and Karinthanyakit, 2010; Van Sebroeck, 2015). Within Xiphoidei, monotypic Xiphiidae formed a separate lineage from the Istiophorids (Santini and Sorenson, 2013). Opah (*Lampris guttatus*) formed a distinct lineage from all the other endothermic teleosts (Chen *et al.,* 2003). The phylogeny built using the maximum likelihood method was used along with the nucleotide data to test the signals for positive selection.

Even though strong purifying or negative selection to conserve the function of the mitochondrial proteins was expected, several candidate sites which evolved under positive selection were identified in the present study (Table 2). Among the 3793 sites tested, MEME analysis found evidence for episodic positive or diversifying selection at 30 sites. FEL found evidence of pervasive positive or diversifying selection at 4 sites and pervasive purifying selection at 2808 sites. FUBAR found evidence of episodic positive selection at 2 sites and episodic purifying selection at 3461 sites. Among the 36 codons identified by all the three methods 1, 1, 9, 2, 2, 4, 1, 3, 6 and 7 codons were found in the genes ND1, ND2, COI, ATP8, ATP6, COIII, ND4L, ND4, ND4 and ND6 respectively. TreeSAAP detected 12 significant radical changes in physicochemical properties of amino acids in the genes COI (3), ATP8 (3), ATP6 (1), COIII (1) and ND5 (4). Cytb which forms the only membrane bound unit of the respiratory complex III (cytochrome bc1 complex) showed no evidence for positive selection. It catalyses the reversible transfer of electrons to cytochrome c from the ubiquinol (Q-cycle) along with the translocation of protons against the electrochemical gradient (Trumpower, 1990; Saraste, 1999; Ngatia *et al.,* 2019). The extreme functional constraints due to its essential role in the respiratory chain (Iwata *et al.,* 1998; Da Fonseca *et al.,* 2008) can explain the absence of positively selected sites in the present study.

Among the 13 protein coding genes, ND genes encoding the subunits of the mitochondrial complex I, (NADH dehydrogenase complex or Respiratory complex I or NADH: ubiquinone oxidoreductase) harbored highest number of candidate sites under positive section. Mitochondrial complex I, is the first and largest membrane bound multimeric enzyme of the respiratory complex with total mass of about 1000Da (Walker, 1992; Nakamaru *et al.,* 2003). It comprises 46 subunits (bovine enzyme) of which seven are coded by mitochondrial DNA and includes the hydrophobic components designated as ND1-ND6 and ND4L (Carroll *et al.,* 2003; Lenaz *et al.,* 2006). The enzyme comprises three different regions: a dehydrogenase and hydrogenase like components constituting the hydrophilic peripheral arm which protrudes into the matrix/cytoplasm and a hydrophobic transporter component which is embedded within the inner mitochondrial membrane, together making an L-shaped structure (Mathiesen and Hagerhall, 2002). The subunits encoded by the mitochondrial genome are embedded within the membrane arm and possess a minimum of one bound ubiquinone (Shinzawa *et al.,* 2010). The core subunits that abode the catalytic function are highly conserved from prokaryotes to humans indicating similar energy production mechanism of complex I for all species (Efremov and Sazanov, 2011). The

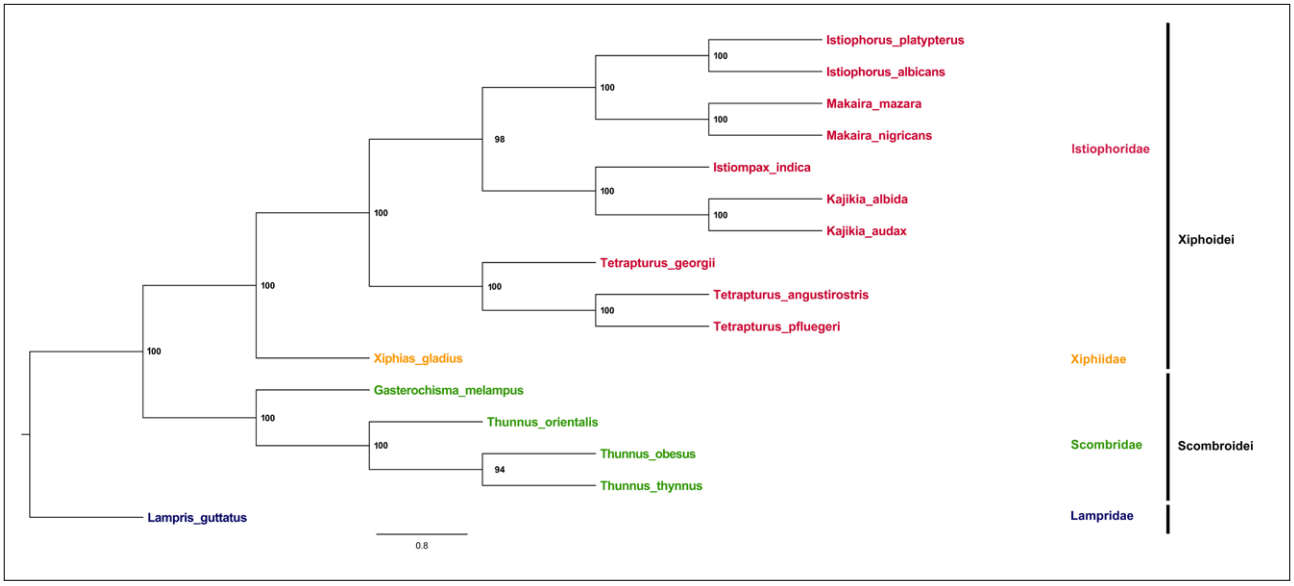

Fig. 1. Phylogenetic tree obtained from the concatenated sequences of 13 coding regions 16 endothermic teleost species. Numerals at the nodes indicate the bootstrap value

enzyme provides proton flux (about 40%) across the inner membrane to power the ATP synthesis by using the reducing potential of the NADH to convert quinine to quinol. Transfer to two electrons from the NADH to ubiquinone (Coenzyme Q, a fat soluble electron carrier) is coupled with the translocation of four protons across the mitochondrial membrane creating a proton motive force (Sazanov, 2007; Kampjut and Sazanov, 2020). Increased susceptibility of the ND subunits or the genes to mutations and adaptive selection may be related to the location of the ND genes in the mitochondrial genome. They are placed immediately upstream to the origin of light strand replication (O_L) and/or downstream to the origin of heavy strand replication (O_H). During the process of replication these genes remain single stranded for much more time when compared to the other mitochondrial genes and hence they are vulnerable to high mutation rate (Marshall *et al.,* 2008). Evidence for positive

Table 2. Outline of the results obtained from various tests conducted on the OXPHOS genes of sixteen regionally endothermic teleosts to detect signals of positive selection

				MEME	FEL	FUBAR	TreeSAAP	Distribution of amino acid replacements across lineages
Gene	Amino acid position	From codon to codon	From amino acid to amino acid	P value	P value	Posterior probability	Physicochemical properties (Category)	
ND1	245	GCC-ACC	Ala-Thr	0.1				*Makaira nigricans*
ND2	220	CTC-ATT	Leu-Ile	0				*Tetrapturus angustirostris*
COI	21	GTA-CTG	Val-Leu	0				*Tetrapturus pfluegeri*
COI	117	GGG-GCC	Gly-Ala	0.06				*Tetrapturus pfluegeri*
COI	262	TCA-GCC	Ser-Ala	0.09			Coil tendencies (6)	*Thunnus thynnus*
COI	331	GCT-TCT	Ala-Ser	0.01				Istiophoridae
COI	338	CTT-ATA	Leu-Met	0.08				*Tetrapturus pfluegeri*
COI	390	GTT-ATG	Val-Met	0.09				*Thunnus thynnus*
COI	416	GTA-CTC	Val-Leu	0.06				*Tetrapturus pfluegeri*
COI	449	CTA-GTC	Leu-Val	0.1				*Tetrapturus pfluegeri*
COI	481	GAA-TTA	Glu-Leu	0.05			Chromatographic index (8), Hydropathy (8)	*Tetrapturus pfluegeri*
COI	507	GAG-CAG	Glu-Gln	0.1				Xiphoidei
ATP8	47	CCA-ACA	Pro-Thr	0.05				Xiphoidei
ATP8	51	AAC-CCC	Asn-Pro	0.03			Compressibility (6), Power to be at the C-terminal (6), Thermodynamic transfer hydrohphobicity (6)	*Gasterochisma melampus*
	2	ACA-GTA	Thr-Val	0.08			Solvent accessible reduction ratio (7)	Istiophoridae
ATP6	112	GCA-GTA	Ala-Val	0.07				*Kajikia albida*
COIII	50	CTT-CCT	Leu-Pro	0.09			Compressibility (6)	*Lampris guttatus*
COIII	98	TTC-TTA	Phe-Leu	0.04				*Istiophorus* albicans
COIII	121	ATT-CTA	Ile-Leu	0.09				Tunas
COIII	175	TTC-CTC	Phe-Leu	0.07				*Kajikia albida*
ND4L	24	ACC-TAC	Thr-Tyr	0.01				Xiphoidei
ND4	422	GCA-CAC	Ala-His	0.08				*Lampris guttatus*
ND4	446	ATC-GCC	Ile-Ala	0.04	0.094			*Gasterochisma melampus*
ND5	17	ACA-TCA	Thr-Ser	0.08	0.084			Xiphoidei
ND5	337	ACT-CCT	Thr-Pro	0.07			Compressibility (6)	*Lampris guttatus*
ND5	404	GCC-ACC	Ala-Thr	0.08				Xiphoidei + Scombroidei
ND5	538	ACA-GCA	Thr-Ala	0.01				Xiphoidei + Scombroidei
ND5	604	ACC-GCC	Thr-Ala	0.05			Hydropathy (6), Solvent accessible reduction ratio (8), Surrounding hydrophobicity (8)	Tunas
ND6	11	GGT-TCT	Gly-Ser	0.03				Xiphoidei
ND6	95	ATG-CTG	Met-Leu	0.01	0.024	0.901		*Gasterochisma melampus*
ND6	103	GTG-GCG	Val-Ala	0.03	0.021	0.96		Xiphoidei + Scombroidei

selection in the ND genes of the complex I has been reported in several fishes (Teacher *et al.,* 2012; Caballero *et al.,* 2015; Consuegra *et al.,* 2015; Jacobsen *et al.,* 2016).

Eleven sites; COI (#262, #481), ATP8 (#51), ATP6 (#2), COIII (#50), ND4 (#446), ND5 (#17, #337, #604), ND6 (#95, #103), which showed evidence for positive selection by more than any one of the four approaches used (MEME, FEL, FUBAR, TreeSAAP) were selected for further analysis to avoid false positives. However, seven sites among them were private or were found in the nodes leading to a single individual but not in other test individuals. Hence, the significance of these terminal mutations is unable to verify. Four positively selected sites were found to occur at the basal region of a particular lineage leading to multiple individuals and therefore are likely to play an important role in the evolution and adaptation of the species. Among them, three were found in the complex I (NADH dehydrogenase): (i) two sites in the gene ND5 (#17 and #604) at the base of the branch leading to the suborder Xiphoidei and endothermic tunas respectively and (ii) one site in the gene ND6 (#103) at the base of the branch leading to the entire suborder Scombroidei and Xiphoidei. A significant mutation in the gene ATP6 (#2) was found at the base of the branch leading to the family Istiophoridae.

These sites were then located in the three-dimensional structure of the protein subunit predicted using appropriate templates. The best hit homology protein subunits or templates for each gene were: ND5 (SMTL ID: 6zkb.1.B), ND6 (SMTL ID: 6qc5.1.U) and ATP6 (SMTL ID: 6zpo.1.U). The sites that exhibited signatures for positive selection in the mitochondrial complex I (ND5 and ND6) (Fig. 2 and 3) are located in the trans membrane helix. The mitochondrial subunits ND2, ND4 and ND5 are supposed to be associated with proton pumping due to their sequence homology to bacterial Na+/H+ antiporters (Brandt, 2006) and possess H^+ translocation sites favoring indirect proton pumping mechanism (Nakamaru *et al.,* 2010). Also, the subunits ND2 and ND4 are joined together by the ND5 arm facilitating a coordinated switch in proton pumping (Hunte *et al.,* 2010). ND6 plays an important role in the assembly and arrangement of the complex I by forming salt bridges and hydrogen bonds to their neighboring subunits (Efremov *et al.,* 2011). The subunit is also suspected to be involved in the formation of quinine-binding site within the membrane arm of the complex (Efremov *et al.,* 2010).

The site which showed evidence for positive selection in the ATP6 (Fig. 4) is located in the speculated internal helix loop region. ATP6 is a subunit of the membrane inserted F_0 proton channel of the complex V (ATP synthase) which forms the final and fifth complex of the oxidative phosphorylation pathway (Saraste, 1999). Complex V catalyzes the conversion of ADP to ATP by using the proton electrochemical gradient generated during the transport of protons from the intermembrane space to the matrix through the pore formed by the F_0 region (Jonckheere *et al.,* 2012). A portion of the F_0 region rotates during this process (rotary catalysis; Devenish *et al.,* 2008) and the subunit ATP6 plays an important role in the rotation of the protein (Spikes *et al.,* 2020). Thus, changes occurring in the gene ATP6 will be associated with variations in the energy production (Sun *et al.,* 2018) and such selective changes associated with metabolism and energy kinetics have been reported in other taxa also (Fontanillas *et al.,* 2005; da Fonseca *et al.,* 2008; Kucharczyk *et al.,* 2010; Finch *et al.,* 2014; Jacobsen *et al.,* 2015).

Altogether, changes in the subunits ND5, ND6 and ATP6 may affect the proton pumping, protein-protein interactions and functioning of the metabolic chain which can enhance the proton-pumping efficiency (da Fonseca *et al.,* 2008; Ngatia *et al.,* 2019). Even though we could not identify the direct relationship

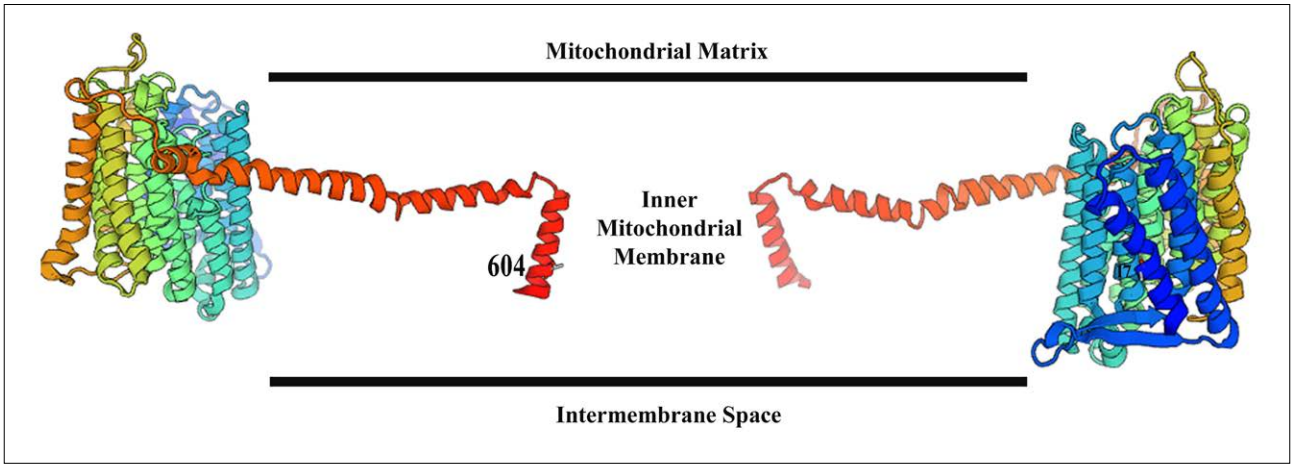

Fig. 2. Three dimensional representation of the mitochondrial subunit ND5 of respiratory complex I created using SWISS-MODEL. Site showing positive selection is marked in bold

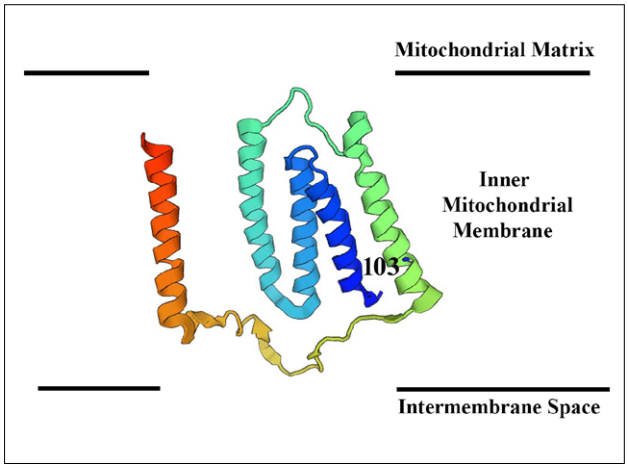

Fig. 3. Three dimensional representation of the mitochondrial subunit ND6 of respiratory complex I created using SWISS-MODEL. Site showing positive selection is marked in bold

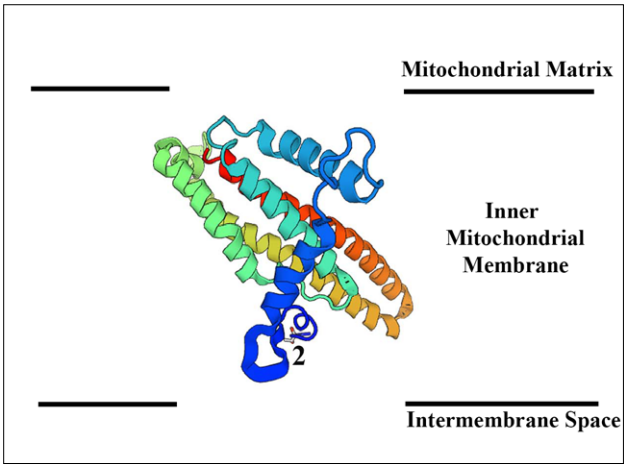

Fig. 4. Three dimensional representation of the mitochondrial subunit ATP6 of complex V (ATP synthase) created using SWISS-MODEL. Site showing positive selection is marked in bold

of the positive selections detected to the functional implications and fitness consequences on the species, we could conclude that these variations can improve the overall ATP production, which could theoretically augment the heat production and metabolic potential of the individual. Temperature is one of the chief selective force acting on mitochondrial DNA (Ballard and Whitlock, 2004) and positive selection on mitogenome have been known to increase the aerobic capacity (Grossman *et al.,* 2004) and thus adaptation to low temperatures (Blier *et al.,* 2001; Mishmar *et al.,* 2003; Ballard and Whitlock, 2004; Grossman *et al.,* 2004; Jobson *et al.,* 2004; Dalziel *et al.,* 2006).

However, molecular evidence of positive selection does not always imply adaptation (Morales *et al.,* 2015). It is a challenge to distinguish between random genetic drift and natural selection, because both the forces interplay mutually to shape genetic variation (Sun *et al.,* 2018). Due to their co-evolution, the selective forces acting upon the nuclear genome may indirectly affect the mitogenome also (Blier *et al.,* 2001; Levin *et al.,* 2014). Such patterns of co-evolution have been reported among cytochrome c oxidase (a mitochondrial DNA product) and cytochrome c protein (a nuclear DNA product) of primates (Osheroff *et al.,* 1983), and also among the NDUFA1 (nuclear encoded) and ND1/ND4 (mitogenome encoded) subunits of humans (Gershoni *et al.,* 2010). Moreover, OXPHOS coupling is not the only way to produce ATP and heat; protons can bypass the complex V or the ATP synthase by proton leak (uncoupling mechanism) to produce heat (Brand, 2000), a key factor, which distinguishes the ectothermic and endothermic mitogenomes (Brand *et al.,* 1991). Reduction of OXPHOS efficiency to enhance thermo genesis has benefited human populations during their thermal niche expansion and their migration towards cooler habitats (Mishmar *et al.,* 2003; Ruiz-Pesini *et al.,* 2004). Increased aerobic potential can also be achieved by elevated enzyme quantity such as in tunas (Korsmeyer and Dewar, 2001; Dalziel *et al.,* 2005). Hence, further studies including enzyme activity assays, protein structure mapping, nutritional and metabolic assessments and experimental evidences are essential in order to elucidate the substantial selective force and their functional consequences in endothermic teleosts.

Our study provided evidence for positive selection in teleost regional endotherms against a background of strong negative selection in the mitochondrial OXPHOS genes. These mutations might have facilitated improvements in thermo genesis, metabolic potential, successful thermal niche expansion and further adaptation to the new environment for this group of species. Moreover, the results obtained provide exciting opportunities for further research on co-evolution of nuclear genes, physiology and various other aspects which will eventually help us to understand how a species can adapt to climate change in future.

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