



Mitochondrial DNA marker reveals shallow genetic structuring in *Priacanthus hamrur* (Forsskål, 1775) along the Indian coast

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Original Article

Abstract

Priacanthus hamrur (Forsskål, 1775), a marine perch belonging to the family Priacanthidae commonly known as “bull’s eye” has started emerging as an important fishery resource in the trawl landings along both the west and east coasts of India. In the present study, genetic stock structure of *P. hamrur* inhabiting Indian coastal waters was ascertained with mitochondrial DNA sequences from the cytochrome b (*cyt-b*) gene using samples of the species collected in two samplings from five different geographical locations, viz. Chennai and Visakhapatnam (east coast) and Cochin, Mumbai and Veraval (West coast). Partial sequence of *cyt-b* gene of *P. hamrur* from 5 representative regions along the coastal zones was amplified by PCR. The *cyt-b* marker revealed high haplotype diversity coupled with very low nucleotide diversity within each population, as well as low genetic distance, high gene flow, and high mitochondrial DNA similarity among all five populations. Phylogenetic trees and pairwise analyses demonstrated a very small divergence (0.43-0.64%) between the populations, suggesting the lack of population subdivisions. The overall lack of genetic subdivision among samples was also detected by the analysis of molecular variance, and pairwise F_{ST} values. Furthermore, the results of this study revealed a pattern of high nucleotide homology among the adjacent populations, and a small number of nucleotide changes among disjunct populations, leading us to conclude that there is a genetic connectivity

among the populations of *P. hamrur* inhabiting the coastal waters of India.

Key words: Bulls eye, *Priacanthus hamrur*, mitochondrial DNA, *cyt-b*, genetic diversity

Introduction

The awareness for diversification of fishing activity has been given top priority in recent years in order to augment marine fish production in our country. Further increase in the marine fish landings can be achieved only through the extension of fishing activities to deeper water for exploiting the non-conventional demersal resources. Priacanthids has been identified as one of the major demersal resources suitable for such exploitation. Among the unconventional finfish resources of the deep sea, they have attained greater importance along the coasts of India as an abundant group (Premalatha, 1997). Priacanthids comprise a relatively small circumtropical family (18 species

currently known) of marine percid fishes. They reach maximum diversity in the Indo-Pacific region with one species confined to the Eastern Pacific and two to the Atlantic Ocean (Starnes, 1988). Priacanthids are a potential deepwater resource located all along the west and east coasts in the 50-400m depth zone with a peak concentration in the 100-200 m depth zone (Premalatha, 1997). Priacanthids made their appearance in the fish landing centres in nineties of last century after the commercial trawlers ventured upto 90-100 m depth. *P. hamrur* (Forsskal, 1775), a marine perch belonging to the family Priacanthidae commonly known as "bull's eye" has started emerging as an important fishery resource in the trawl landings along both the west and east coasts (Sivakami *et al.*, 2001) of India. Bull's eye resource, of late is increasing in the commercial landing all along the Indian coast. According to John and Sudarsan (1988) the biomass estimate for bullseye in the Indian EEZ is 1.17 lakh tonnes. It is generally marketed fresh, may be salted or dried. Besides, they have good export potential as they are considered excellent food fishes in Japan and other South east Asian countries. Bullseye are among those fishes used for the preparation of fish balls, noodles, sauces and seasonal minces in the South East Asian countries. They are found to be a suitable raw material for the preparation of surumi.

Management of fish stocks should be based on reliable biological data for sustainable exploitation of biological marine resources (Johnson and Jonsson, 1995). For a given resource, genetic population analysis can provide basic information on the geographic limits of stocks and gene flow among subpopulations, allowing the identification of self-recruiting units (Ryman and Utter, 1987; Carvalho and Hauser, 1998; Hauser and Ward, 1998). For the management of fish populations, studying genetic diversity is critical. Nevertheless, studies on the geographic distribution and genetic population structure of many commercial fish species stocks have not been completed (Atarhouch *et al.*, 2005). To the best of our knowledge the genetic diversity of *P. hamrur* populations has yet to be investigated using modern DNA technology. Because lack of genetic variability causes a decline in the survival fitness of local populations (Hutchings, 2000; Jackson *et al.*, 2011; Knutsen *et al.*, 2003), lack of data on genetic diversity raises concerns, especially in those cases in which there is overexploitation of local stocks, which in turn may cause a genetic bottleneck and consequent depletions.

Analysis of mitochondrial DNA (mtDNA) has proved to be a powerful tool for addressing issues of genetic diversity among a great number of organisms (Hewitt, 1996; Avise, 2000; Saccone *et al.*, 2000). Because of mtDNA'S very important molecular features, such as compact organization, primarily maternal inheritance, and absence of recombination, it has provided a unique tool for such studies (Stabile *et al.*, 1996; Saccone *et al.*, 2000). Furthermore, a large number of studies have shown

it to be, in general, a good phylogenetic marker for vertebrate phylogenetic analysis (Samonte *et al.*, 2000; Sebastio *et al.*, 2001). The cytochrome b (*cyt-b*) gene has been specifically chosen by many investigators because of its sufficient point mutation rate, its usefulness in discriminating closely related fish species, and in determining the degree of intraspecific variability in pelagic fish species for population identification (Reilly and Ward, 1999; Jerome *et al.*, 2003; Lecomte *et al.*, 2004). Analysis of differences in mtDNA sequences has led to the identification of Mediterranean and Eastern Atlantic populations of the sardinella, *Sardinella aurita* (Chikhi *et al.*, 1997).

The objective of the present study was to reveal the genetic stock structure of *P. hamrur* along the Indian coast using mitochondrial *cyt-b* gene.

Material and Methods

The bullseye samples were collected from five different geographical locations along the Indian coast, three from the west coast *viz.*, Cochin, Kerala (lat 9° 58' N, long 76° 17' E); Mumbai, Maharashtra (lat 18° 55' N, long 72° 54' E); Veraval, Gujarat (lat 20° 53' N, long 73° 26' E); and two from the east coast *viz.*, Chennai, Tamilnadu (lat 13° 04' N, long 80° 17' E) and Visakhapatnam, Andhra Pradesh (lat 17° 42' N, long 83° 15' E) (Fig. 1) throughout the range of species distribution along the Indian coast during 2008 and 2009. Samples were obtained from commercial vessels immediately after the catch. Upon arrival at the laboratory, individuals were morphologically recognized as belonging to the species *P. hamrur* and then the total length,

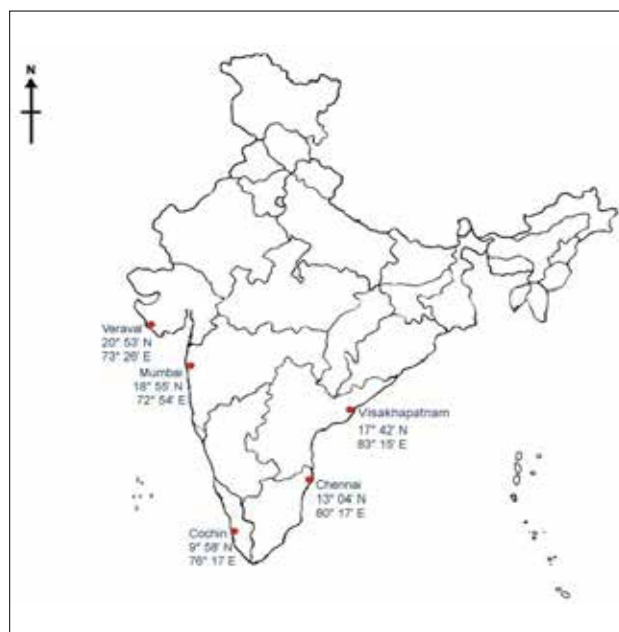


Fig. 1. Locations selected for sampling of *Priacanthus hamrur* along the Indian coast (map not to scale)

standard length and body weight for each fish were measured and recorded separately for each region.

Total genomic DNA was isolated from muscle tissue/fins using the phenol chloroform method (Sambrook and Russell, 2001) with slight modification. DNA concentration was estimated colorimetrically, measuring the optical density (OD) set at 260 nm using a UV-visible spectrophotometer (SPECORD 205, Qiagen, Limburg, Netherlands).

PCR amplification and Sequencing

The partial sequence of cytochrome *b* genes was PCR amplified using universal primers *cyt-bA* (5' – CCA TGA GGA CAA ATA TCA TTY TGR GG–3') and *cyt-bC* (5' – CTA CTG GTT GTC CTC CGA TTC ATG T–3') (Bossuyt and Milinkovitch, 2000). In this study, 25 sequences (5 individuals per each sampling site) were used to analyse the population structure of the species.

The PCR amplifications were performed in 25 μ l assay volume containing 2.5 μ l 10x assay buffer (100mM Tris, 500mM KCl, 0.1% gelatin, pH 9.0) with 1.5mM MgCl₂ (Genei, Bangalore, India), 5 pmoles of each primer, 200 μ M of each dNTP (Genei, India), and 1.5 U Taq DNA polymerase, 18 ml de ionized water and 20 ng of genomic DNA. The thermal conditions used to amplify *cyt-b* gene consisted of an initial denaturation of 95°C for 5 min; followed by 30 cycles of 95°C for 30 s, annealing at 500 C for 30 s, extension at 72°C for 1 min and a final extension at 72°C for 10 min. To check DNA contamination, a negative control was set up omitting template DNA from the reaction mixture. PCR product along with a marker (100 bp DNA ladder) was loaded in 1.5% agarose gel, visualized and documented using Image Master VDS. The PCR product was purified using GeNei™ Quick PCR purification kit (Genei, India) followed by sequencing PCR with same primer pairs by cycle sequencing using ABI PRISM Big Dye® Terminator v3.1 Cycle Sequencing kit, (Applied Biosystems, USA).

The raw DNA sequences were aligned and edited using BIOEDIT sequence alignment editor version 7.0.5.2 (Hall, 1999). Multiple alignment of sequences was performed using CLUSTAL X version 2 (Larkin *et al.*, 2007) alignment editor. Alignment was then manually checked and corrected. Haplotype number, haplotype frequency, nucleotide sequence characteristics as well as standard genetic diversity indices like haplotype diversity (*h*) and nucleotide diversity (π) within populations were analysed using the program DnaSP v 5.10.01 (Librado and Rozas, 2009). All haplotype sequences were submitted in NCBI GenBank (Accession Numbers HM037255 – HM037266). Phylogenetic trees were then constructed based on neighbor-joining (Saitou and Nei, 1987) and maximum likelihood (Eck and DayHoff, 1966) analyses, using MEGA Version 4 (Tamura *et al.*, 2007). *Lutjanus sebae cyt-b* sequences (GeneBank accession number:

AY651959) were used an outgroup in the construction of the phylogenetic tree. Pairwise sequence divergence among populations, the number of transitions and transversions and the rate of transitions / transversions were also calculated using the MEGA program, according to Kimura's 2-parameter model (Kimura, 1980). Pairwise nucleotide divergence was analyzed using both the rate of transition/ transversion, and percent divergence. Estimated pair wise genetic distances (based on Kimuras'2- parameter model) were used to construct both Neighbour joining tree and maximum likelihood tree.

The analysis of molecular variance (AMOVA) framework (Excoffier *et al.*, 1992) implemented in the program ARLEQUIN Version 3.5 (Excoffier and Lischer, 2010) was used to test the overall genetic heterogeneity of the samples. In this statistical method, a nested analysis of variance was carried out based on the partitioning of molecular variability at different arbitrarily defined hierarchical levels (from the individual level to population level). In our analysis a group genetic structure of samples was defined by pooling them according to the different collecting areas (East coast and West coast). F_{ST} was calculated to estimate the genetic divergence between the east and west coast samples and among the populations within the coasts and within populations. Estimates of genetic differentiation between all the five populations was done using F-statistics (Wright, 1951). Significance threshold of pairwise comparisons ($P < 0.005$) was always adjusted by applying sequential Bonferroni correction (Rice, 1989).

Results

Cytochrome *b* Sequence Variation

A total of 422 bp sequence of *cyt-b* gene was obtained from samples of five geographic locations along the Indian coast. A total of 7 variable positions with 12 haplotypes including 6 parsimony informative sites and 1 singleton variable sites were obtained (Table 1). The average frequencies of four nucleotides for all samples of *P. hamrur* were A: 22.8%, T: 32.1%, C: 33.6%, G: 11.5%. Most nucleotide variation resulted from transitions (86.67%) followed by transversions (13.33%) with a ratio (Ts/Tv) of 4.7. Unique haplotypes were observed within all populations but

Table 1. Nucleotide sequence characteristics of *cyt-b* gene across five populations of *P. hamrur*

<i>cyt-b</i> sequence characteristics	Total
Characters / sites	422
Invariable (monomorphic) sites	415 (98.34%)
Variable (polymorphic) sites	7 (1.66%)
Singleton variable sites	1 (0.24%)
Parsimony informative sites	6 (1.42%)

there was no characteristic geographic clustering of haplotypes. 7 haplotypes were identified from west coast and 5 haplotypes were identified from east coast. Veraval and Chennai populations possessed the maximum number of haplotypes, three haplotypes each out of five samples. Remaining 3 populations possessed 2 haplotypes each. Out of the 7 haplotypes identified from the west coast, 5 haplotypes were found to be sharing between Veraval and Mumbai populations. All the 5 haplotypes identified from the east coast were found to be sharing between Chennai and Visakhapatnam populations which indicated significant gene flow between these populations. There was no characteristic geographic distribution pattern for the haplotypes. Tests of neutrality were not carried out, as the number of haplotypes observed was few. Distribution of haplotypes among the populations is given in Table 2.

Table 2. Distribution of haplotypes of 422 bp fragment of the *cyt-b* gene among the populations of *Priacanthus hamrur*

Site	Haplotype (Representative Sample Name)											nt	nh		
	A	B	C	D	E	F	G	H	I	J	K			L	
Cochin	3	2	5	2											
Mumbai	1	2	2	5	2										
Veraval	1	2	2	5	3*										
Chennai	2	2	1	5	3**										
Visakhapatnam	1	1	3	5	2										
Total	2	2	2	2	2	3	2	2	2	2	1	3	25	12	

nt - number of individuals analyzed per site; nh - number of haplotypes per site

* haplotype shared between Mumbai and Veraval

** haplotype shared between Chennai and Visakhapatnam

Mean pair-wise distances between populations were very low ranged from 0.00432-0.006472 indicating homogeneity in population (Table 3). The mean nucleotide diversity for all the samples from five locations of *P. hamrur* was found to be 0.00412, whereas haplotype diversity was recorded as 0.74. The haplotype diversity of samples were high and nucleotide diversity of samples were generally very low (Table 4). All the haplotypes were submitted to the NCBI GenBank (HM037255-HM037266)

Table 3. Mean pairwise distances between populations of *P. hamrur* based on *cyt-b* gene sequences

Population names	Veraval	Mumbai	Cochin	Chennai	Vizag
Veraval					
Mumbai	0.004992				
Cochin	0.00432	0.00432			
Chennai	0.005072	0.005856	0.005432		
Vizag	0.005376	0.005568	0.006472	0.004788	

Table 4. Intrapopulation nucleotide (π) and haplotype (h) diversities of *Priacanthus hamrur* based on *cyt-b* gene sequences

Population Names	Nucleotide diversity (π)	Haplotype diversity (h)
Cochin	0.0028	0.6000
Mumbai	0.0056	0.8000
Veraval	0.0028	0.8000
Chennai	0.0042	0.8000
Visakhapatnam	0.0052	0.7000

The AMOVA carried out by considering the variability based on haplotype frequency differences, corrected for the interhaplotype sequence divergence, revealed the lack of genetic structuring between the east and west coast samples and among the populations within the coasts. 18.74% and 7.93% of variation was observed between east and west coast samples and among the populations of *P. hamrur* within the coasts respectively which were not significant. F_{ST} values were found to be significant within populations. AMOVA analysis showed that 73.34% of variation is accounted within populations (Table 5). The overall *cyt-b* homogeneity of *P. hamrur* samples was further supported by pairwise F_{ST} (Fixation index) values (Table 6) most of which were low and statistically not significant which did not show any differentiation between five populations of *P. hamrur*.

Table 5. Results of the hierarchical analysis of molecular variance (AMOVA) of populations of *Priacanthus hamrur* based on mitochondrial *cyt-b* gene sequences

Source of variation	df	% total variance	Φ	P-value
Among groups	1	18.74 NS	$\Phi_{CT} = 0.18736$	0.1002±0.006
Among populations within groups	3	7.93 NS	$\Phi_{SC} = 0.09754$	0.1385±0.006
Within populations	20	73.34**	$\Phi_{ST} = 0.26662$	0.0053±0.001

Groups- east and west coast, (** $P \leq 0.01$) NS- Non Significant

Table 6. Pairwise F_{ST} values for between populations of *Priacanthus hamrur* based on *cyt-b* gene sequences

<i>Priacanthus hamrur</i>	Veraval	Mumbai	Cochin	Chennai
Veraval	-	-	-	-
Mumbai	0.1346	-	-	-
Cochin	0.2895	0	-	+
Chennai	0.2925	0.1393	0.2967	-
Visakhapatnam	0.2411	0.1541	0.3357	0

+, significant pairwise F_{ST} at $P < 0.005$ after sequential Bonferroni adjustment
-, not significant at this level

Sequence-Based Phylogeny

The phylogenetic trees were constructed using neighbour - joining and maximum likelihood methods (Fig. 2). Both trees showed almost same topology, which revealed very little difference in the *cyt-b* region investigated in this study. When *Lutjanus sebae* was included as an out group in the

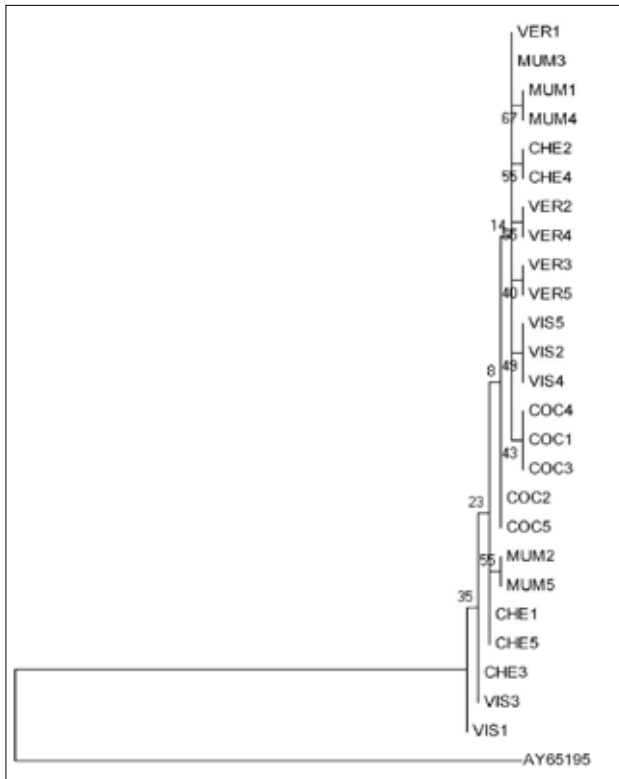


Fig. 2. Maximum likelihood tree of the populations of *Priacanthus hamrur* inferred from haplotype sequence variation of the mitochondrial DNA *cyt-b* gene. Numbers at nodes indicate the bootstrap values. AY65195 (*Lutjanus sebae*) is from the genbank and included as outgroup species

construction of the phylogenetic tree, the outgroup did reveal a great difference from the samples included in this study. The data obtained from pairwise nucleotide divergence analysis, varying between 0.43% to 0.64%, also further confirmed the result obtained from phylogenetic tree analysis. The transition-to-transversion rate (Ts/Tv) estimate for the ingroup in the present study was 4.7, another important feature affecting the divergence rate among populations. It has been reported that, in general, transitions cause less divergence than transversions (Hewitt, 1996; Saccone *et al.*, 2000).

Discussion

Priacanthids are widely distributed in the Indian Seas. Bulls eye (*P. hamrur*) is one of the important constituents of the fishes available in the outer shelf in the EEZ of India. No attempt was hitherto made to study the genetic diversity of *P. hamrur* populations inhabiting along the Indian coast. Knowledge of genetic diversity and population distribution is vital for the effective management and sustainability of any regional fishery (Reilly and Ward, 1999). Mitochondrial DNA is only a part of the whole genome but showing hyper variability and can be used for population studies (Fuchs *et al.*, 2008). The presence of 2 anchovy populations in the Mediterranean Sea was successfully confirmed by molecular phylogenetic DNA

analysis (Borsa, 2002; Borsa *et al.*, 2004). Furthermore, the signature of the early genetic bottleneck in a population of Moroccan Sardines (*Sardina pilchardus*) was also determined using mtDNA analysis (Atarhouch *et al.*, 2005).

The phylogenetic trees constructed using neighbor joining and maximum likelihood methods (Fig. 2) determined that the *P. hamrur* stocks investigated in the present study were not from different populations. In both trees, all populations were clustered in close proximity. Moreover, divergence between 0.43% to 0.64% further proves how closely they are related. Based on the results obtained from mtDNA analyses, there is a lack of isolated populations and heterogeneity among the *P. hamrur* populations inhabiting the coasts of India. Despite the high disproportion in size between sample collection sites observed in the present study, phylogenetic analysis did not reveal any genetic diversity among the populations. This phenomenon was also observed by Sarmasik *et al.* (2008) among the *Sardina pilchardus* populations inhabiting the coast of Turkey. The same phenomenon was also observed for Aegean Sea (Spanakis *et al.*, 1989), Spanish Mediterranean coast (Ramon and Castro, 1997), and Adriatic and Ionian stocks of sardine (Tinti *et al.*, 2002). The overall lack of genetic subdivision of Adriatic and Ionian stocks of *Sardina pilchardus* was observed by Tinti *et al.* (2002) through sequence variation analysis of a 307-bp cytochrome b gene fragment. Genetic analysis did not detect significant differences either within the Adriatic stock of sardines or between samples collected from the Adriatic Sea and surrounding areas. Tinti *et al.* (2002) reported that there is gene flow between Adriatic-Ionian and Spanish sardines. The lack of genetic heterogeneity in the Adriatic and Ionian sardine stocks suggests that they belong to a large self-recruiting population whose boundaries are greater than the Adriatic Sea and adjacent areas of the Ionian Sea. Furthermore, they concluded that these sardine stocks might be part of a large population in this region. Low-level nucleotide diversity of mtDNA among the Sardinops inhabiting zones of the Indian-Pacific Ocean was reported by Grant and Bowen (1998).

Species with high rates of dispersal, exhibit a low level of genetic diversification in population structure, whereas species with little capability for dispersal have a significant degree of interpopulation genetic diversity (Stabile *et al.*, 1996). The degree of gene flow can act as a strong force in the maintenance or homogenization of genetic differences among adjacent and disjunct populations (Sarmasik *et al.*, 2008). The low and significant F_{ST} values among populations of *P. hamrur* in the present study suggests the existence of high gene flow among the populations. Migratory behaviour of Priacanthids were reported by many earlier workers. (Premalatha, 1997; Vijaykumar and Naik, 1988a; Pillai *et al.*, 1999; James and

Pillai, 1990; Sivakami *et al.*, 2001). *P. hamrur*, though demersal, is a highly mobile species which reportedly migrates both shoreward as well as along shore (southerly and northerly), across the shelf and parallel to the shelf along the Indian coast, which probably facilitates gene exchange among different geographical locations which results in mixing of stocks. Similar results were obtained by Kinsey *et al.* (1994) for Spanish sardine (*Sardinella aurita*), which is a highly mobile pelagic species migrates north and south along the east Florida continental shelf (Hildebrand, 1963). Kinsey *et al.* (1994) observed the gene flow was high and effectively homogenized genetic variation among sample locations of Spanish sardine indicating that a single panmictic population of Spanish sardines exists at least from South Carolina to Florida panhandle. The homogeneity of the goosefish populations off the eastern coastline of the United States suggests that there is unrestricted gene flow across the region (Chikarmane *et al.*, 2000).

According to Grant and Bowen (1998), high h and low π are interpreted as population bottle neck followed by rapid population growth and accumulation of mutations. The present finding suggests the scenario with high haplotype with low nucleotide diversity. Similar results were obtained in *Pampus argenteus* (Divya *et al.*, 2015) and *Coilia dussumieri* (Kathirvelpandian *et al.*, 2014). The observed high haplotype diversity and low nucleotide diversity are interpreted as occurrence of population bottleneck followed by rapid population expansion due to accumulation of new mutations. Management measures are to be devised for conservation and sustainable utilization of this species.

In conclusion, the analysis of *P. hamrur* using mitochondrial *cyt-b* gene revealed low level of genetic differentiation between samples collected from east and west coast of India. The findings of the present study can be used in the management of this species as a unit stock in Indian waters.

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