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Characterization and gene mining of BAC resource of *Clarias magur* for their potential applications in genomic research

Rashmi Verma[1,2], Basdeo Kushwaha[1*], Uzma Afaq[2*], Vishwamitra Singh Baisvar[1], Satyamvada Maurya[1], Murali S. Kumar[1], Akhilesh Kumar Mishra[1], Uttam Kumar Sarkar[1], Ravindra Kumar[1]

[1]ICAR-National Bureau of Fish Genetic Resources, Lucknow, India.
[2]Department of Biosciences, Integral University, Lucknow, India.

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ABSTRACT

Bacterial artificial chromosomes (BAC) libraries are valued genomic resources for preserving the genetic material of an organism. The end sequences of BAC clones can help in determining overlapping regions of clones to improve genome assembly and to select clones having genes of interest. The mapping of the ends' sequences of BAC clones using bioinformatic tools resulted in 18 BAC clones mapping on 17 scaffolds of *Clarias magur* genome on which 38 genes were present. Gene enrichment analysis indicated the involvement of several genes in vital functions. Protein– Protein Interaction Network analysis revealed two types of interactions among 14 nodes (genes) and 17 edges (*adamts5, cth1, mta2, nos2a, odc1, slc7a5, taf6l, TIMM21, herc1, kif18a, rb1cc1, scly, uba2, adamts1, crocc2, smyhc3,* and *tpma*). The *ADAMTS1, ADAMTS5,* and *uba2* genes were involved directly and indirectly in growth. Several genes playing key roles in the functions of gonad, brain, and nervous systems were discovered. Physical mapping of selected clones using FISH revealed the presence of DNA probe signals on the 11th and 12th chromosome pairs of *C. magur*. The study indicated the presence of important genes on the 18 undertaken clones and has helped in understanding the genome structure of the species and their evolution with respect to related species by comparative genomics.

1. INTRODUCTION

Fish and other aquatic animals are susceptible to changing environments and suitable efforts are needed to replenish the species. A lack of appropriate management strategies may lead to further critical decline for the species in the years to come [1,2]. *Clarias magur* (Hamilton, 1822), commonly called walking catfish (magur), is an important freshwater clariid species native to India and neighboring nations with promising aquaculture prospects. A variety of known and unknown factors have contributed to a severe drop in the availability of this species, hence, it is categorized as "*Endangered (A3cde + 4acde)*" in IUCN Red List Status [3]. This species has gained attention and considerable investigations were carried out in the field of aquaculture, conservations, genomics, and so on, including the development of bacterial artificial chromosomes (BAC) genomic resources, to replenish this species in a sustainable aquaculture system.

BACs are DNA constructs used to clone large DNA fragments in bacteria. They are designed to carry and replicate large inserts of DNA, typically ranging from 100,000 to 300,000 base pairs in size. BACs are widely used in molecular biology and genomics research to study and manipulate genes and genomes. They are particularly useful for projects involving sequencing large stretches of DNA. BAC insert DNA can be used for high-throughput BAC end (BE) sequencing, chromosomal mapping, genome sequencing, and other related studies [4,5]. Numerous investigations, such as chromosome mapping using BAC-FISH [6,7] chromosomal investigations using Giemsa, C-banding, Ag-NOR, CMA_3 staining, and 18S as well as 5S rDNA-FISH [7], molecular studies, like genome sequencing [8], transcriptome analysis [9], thermal stress studies [10], and so on, were undertaken to better characterize and improve this species. A BAC library of 55,141 clones constructed from *C. magur* genome is maintained at ICAR-National Bureau of Fish Genetic Resources, Lucknow, India. Moreover, the whole genome assembly of *C. magur* (GenBank ID: GCA_013621035.1) is also available [8]. The present study was focused on analyzing the BAC clones using their end sequences and whole genome information for gene and simple sequence repeats (SSR) mining and pathway analysis which could aid in genomic selection programs or genetic diversity studies and complement aquaculture production. BAC clone DNA probe that hybridizes on an individual chromosome could be used to identify that

*Corresponding Author
Basdeo Kushwaha, ICAR-National Bureau of Fish Genetic Resources, Lucknow, India. E-mail: basdeo.kushwaha @ icar.gov.in and Uzma Afaq, Department of Biosciences, Integral University, Lucknow, India. Email: mailto:uzmaafaq89 @ gmail.com

particular chromosome. This approach can also be utilized to resolve chromosomal identity in ambiguous morphological conditions. The study will be helpful to identify overlapping clones, select clones for restriction endonuclease fingerprints, identify appropriate clones for FISH mapping, select clone(s) containing genes of interest, and so on. It will also support United Nations' sustainable development goals (SDGs), particularly SDG 14 of promoting research on life below water, and SDG2 on achieving food security and promoting sustainable agriculture by species conservation and complementing aquaculture production.

2. MATERIALS AND METHODS

2.1. BAC Clone Revival and Culture

The BAC library of *C. magur* genome consisting 55,141 clones stored in 144 plates of 384-well was utilized in this study. One 384-well plate (ID: 012) was randomly selected and revived. The revived clones were further sub-divided into 96-well plate format for ease of maintenance as well as handling and stored at −80°C for (BE) sequencing and other use. One 96-well plate was randomly selected and thawed after which, 15 µl of each clone was transferred in 15.0 ml centrifuge tubes for revival, culture, and isolation of insert DNA, as per protocol [6,7].

2.2. BAC DNA Isolation and BE Sequencing

Selected clones were subjected to BAC insert DNA isolation, utilizing an alkaline lysis protocol [6]. Briefly, the *E. coli* culture was incubated overnight and centrifuged at 3,300 rcf for 10 minutes at room temperature. The supernatant was decanted and tubes containing pallets were inverted to remove traces of media. The remaining pellets were pooled in an 8 ml culture tube and used for insert DNA isolation. The isolated BAC inserts DNA were purified with 25 µl magnetic beads using 96-well format recommended protocol of polymerase chain reaction purification procedure of Agencourt AMPure XP, Beckmann Coulter (https://www.beckmancoulter.com/wsrportal/techdocs?docname=B37419). The quality and quantity of isolated insert DNA were determined in a transilluminator with 0.4% agarose gels and in NanoDrop2000 spectrometer (Thermo Fisher Scientific, USA). Both ends of the clones were sequenced in Genetic Analyzer ABI 3500 (Thermo Fisher Scientific, USA) using T7 forward (5′-TAATACGACTCACTATAGGG-3′) and pbRP1 reverse (5′-CTCGTATGTGTGGAATTGTGAGC-3′) primers (http://www.epibio.com). The sequence quality was checked and good-quality sequences of BEs were used in analysis.

2.3. BE Mapping, Gene Mining, and Functional Annotation of Mapped Genes

The qualified BE sequences were mapped on the genome assembly of *C. magur*) using *BLASTN* tool with 10^{-5} *e*-value. Custom Perl scripts were used for identifying the end-to-end sequence length of BAC insert DNA fragments. The clone-wise mapped end-to-end sequences on the scaffolds were then retrieved and a fasta file of the same was created. Gene prediction, gene mining, and annotation of complete sequences of clones were carried out using *OmicsBox* platform, wherein InterPro mapping, and annotation using UniProt and NR databases were used. SSRs present in the clone sequences were mined from using MISA tool [11].

2.4. Probe Labelling and BAC-FISH

One µg of isolated BAC inserts DNA from K10 and A23 well clones of 012 plate, containing a comparatively higher number of genes as determined bioinformatically from whole genome data, were taken for physical chromosome mapping. The DNA of K10 and A23 clones were labeled with green fluorescein-12-dUTP (Fermentas, Vilnius, Lithuania) and red tetramethyl-rhodamine-5-dUTP (Roche, Basel, Switzerland) fluorophores using nick translation technique. FISH was carried out to hybridize probes on metaphase chromosomes for 2 to 3 days, following the protocol of Kumar [6]. After hybridization, the chromosomes were counterstained for an hour using VectraShield mounting media (Vector Labs, Burlingame, CA) containing DAPI and antifade. Metaphase spreads hybridized with DNA probes were then examined under a fluorescence microscope (Leica, Wetzlar, Germany) under 3 band filters, *viz*. DAPI filter (340–380 nm excitation, 461 nm emission) for chromosome visualization in blue, N2.1 filter (515–560 nm excitation, 595–605 nm emission) for rhodamine-labeled probe visualization in red, and I3 filter (450–490 nm excitation) for fluorescein-labeled probe visualization in green color. Chromosomes (visualized in DAPI) and probes (visualized in N2.1 or I3 filters) images were superimposed using Karyo4000 software (Leica, Germany) to determine and visualize the location of the probes' signals on specific chromosomes. Individual hybridized metaphase spreads were then manually karyotyped and a consensus karyotype was generated.

2.5. Comparative Genomics and Functional Enrichment Analysis

The synteny analysis of annotated genes, present on clones, was performed by searching them against NCBI and Ensembl databases and generating detailed information of the genes using genomes of *Danio rerio* and *Ictalurus punctatus* as a query. The complete scaffolds with ID numbers, containing annotated genes of *C. magur* genome and genes discovered in *D. rerio* and *I. punctatus* but present on different chromosomes, were considered for synteny. To make clear image visualization, only one gene of the scaffold was considered for comparisons, though the rest genes were also located on the same scaffold. *Circos* program [12] was used in synteny visualization.

Functional enrichment analysis of the nodes within the cluster network was carried out using PANTHER gene ontology (GO) tool [13]. GO framework was employed to annotate gene sets, offering a comprehensive structure for functional categorization. Additionally, the KEGG pathway database [14] was applied to provide information on molecular interactions and network reactions. The PANTHER gene list analysis involved a statistical over-representation test, which was employed to identify functionally enriched gene networks within the GO Biological Process category. This statistical test relied on binomial statistical comparison to determine over-represented biological functions associated with the gene set.

2.6. Phylogenetic Analysis

Phylogenetic analysis was carried out for the annotated genes present on undertaken clones of *C. magur* and genes commonly found in *I. punctatus* and *D. rerio* genomes. Neighbour-joining (NJ) methods based on *p*-distances approach were carried out using MEGA 7 software [15]. The phylogenetic topology of DNA sequences for predicting evolutionary lineages was presented using the interactive tree of life v5.5 tool [16].

2.7. Protein–Protein Interaction (PPI) Network Analysis

The PPI network analysis of annotated genes was carried out using STRING 12.0 [17] and visualized using Cytoscape v3.6.0 tool [18]. The network was built based on a stringent confidence score at a

threshold value of 0.04, signifying that only interactions with a high level of confidence were considered for inclusion in the network.

2.8. Functional Annotation

GO-based functional annotation of identified genes was performed through BLAST using UniProtKB/Swiss-Prot database (http://www.uniprot.org/). The most significant BLAST hits were selected which met the following criteria: *E*-value <1e–5 and similarity >80%.

3. RESULTS AND DISCUSSION

3.1. BEs Mapping, Gene Mining, and Functional Annotation

A few BAC libraries have been established as genomic resources in fishes [19–24]. BAC libraries and their characterization using end sequencing will provide insights into the genome. The isolated plasmid DNA quality was good which was used for forward and reverse-end sequencing. Out of 96 clones revived, good-quality DNA was obtained in 36 clones which were further used for end sequencing. A sufficient amount of BAC insert DNA is required for the BE sequencing of clones. The sequence quality check resulted in 32 clones, out of which 18 clones were sequenced with both ends, and the rest had either forward or reverse end sequence only. Both end sequences of 18 clones were used for further bioinformatic analysis.

The lengths of forward and reverse end sequences of 18 clones ranged from 154 bp (forward end sequence of clone ID: 012B11) to 914 bp (reverse end sequence of clone ID: 012H6) and most of the end sequences were over 500 bp in size (Table 1), indicating good size. Bioinformatic mapping of BE sequences on magur genome revealed their distribution on 17 scaffolds. The mapped 17 scaffolds covered 30,945,289 bp of magur genome and their size ranged from 195,099 bp (Scaffold882) to 6,784,174 bp (Scaffold3) (Table 1). The sizes of the clones were bioinformatically predicted by aligning both the end sequences of the clones on the scaffolds of the magur genome, which ranged from 90.615 kb (012A5 present on Scaffold118) to 150.071 (012H19 present on Scaffold170). A total of 38 genes were present on these clones and identified, annotated, and characterized (Table 1, Supplementary Table 1). A total of 1974 SSRs were identified in 18 clones' sequences using MISA tool (Table 1). The clone ID: 012A6 contained maximum SSRs (310), while ID: 012H12 contained minimum SSRs (47).

Making use of the genome sequence's advantages and BAC library of magur, a BAC-based partial physical map of *C. magur* genome was constructed [25]. There are several databases of BE sequences developed for numerous model species, including human, rice, mouse, and sea urchin [26]. In several studies, BE sequences were generated and utilized for genomic studies [25,27,28] indicating BACs as an important source of live genetic material in different biological contexts. BE sequences are also used for comparative analysis with other model genomes. This will help in determining the sequence orientation of a large genome region on the chromosome for gene discovery.

Metaphase chromosomal complements of fair quality were generated manually with a diploid chromosome (2n) count of 50. Based on morphology and chromosome number, the karyotype is 14m+20sm+8st+8t, with a fundamental arm number of 90 (Fig. 1a and b). A similar karyotype has been obtained by Indian workers in *C. magur* [25,29].

BAC-FISH is an important tool in physical chromosome mapping, which includes many steps, namely selecting an appropriate clone and obtaining high-quality chromosomal spread for probe signal detection and distinguishing chromosomes based on their morphology [25] employed BAC-FISH to establish its utility as a

Table 1. Details of BE mapping on the scaffolds of *C. magur* genome.

S.No.	Clone ID	BE sequence length		Scaffold ID of *C. magur* genome where clone mapped	Scaffold length (bp)	BE position mapped on *C. magur* scaffold		Estimated clone size (kb)	No of SSRs present on the clone	No of genes identified in clones
		Forward end (T7)	Reverse end (pbRP1)			Start	End			
1.	012A23	626	547	Scaffold390	664,982	358,207	490,361	132.155	90	4
2.	012K10	426	807	Scaffold199	1,234,627	576,729	712,507	135.779	76	4
3.	012A10	851	465	Scaffold25	3,982,592	2,796,995	2,901,886	104.892	80	3
4.	012A6	697	427	Scaffold191	1,256,143	459,700	606,285	146.586	310	4
5.	012B6	693	535	Scaffold37	3,370,182	2,714,669	2,841,806	127.138	87	2
6.	012B8	537	308	Scaffold882	195,099	55,411	154,774	99.364	102	3
7.	012H2	890	893	Scaffold88	2,101,350	1,613,820	1,784,901	171.082	96	3
8.	012H4	865	875	Scaffold87	2,117,253	978,919	1,104,567	125.649	113	2
9.	012H5	881	897	Scaffold438	578,457	378,898	499,847	120.95	175	2
10.	012H6	879	914	Scaffold87	2,117,253	978,919	1,104,567	125.649	113	2
11.	012H19	662	541	Scaffold170	1,405,293	119,619	269,689	150.071	87	2
12.	012H10	615	735	Scaffold346	763,522	64,112	209,355	145.244	105	1
13.	012H12	722	820	Scaffold3	6,784,174	248,831	347,673	98.843	47	1
14.	012H18	308	190	Scaffold464	546,807	369,594	483,743	114.15	62	1
15.	012C5	342	600	Scaffold159	1,493,528	1,115,554	1,232,702	117.149	67	1
16.	012B3	906	951	Scaffold169	1,415,417	1,206,038	1,325,900	119.863	91	1
17.	012B11	154	617	Scaffold65	2,520,177	511,088	618,116	107.029	209	1
18.	012A5	160	740	Scaffold118	1,826,015	140,655	231,269	90.615	64	1

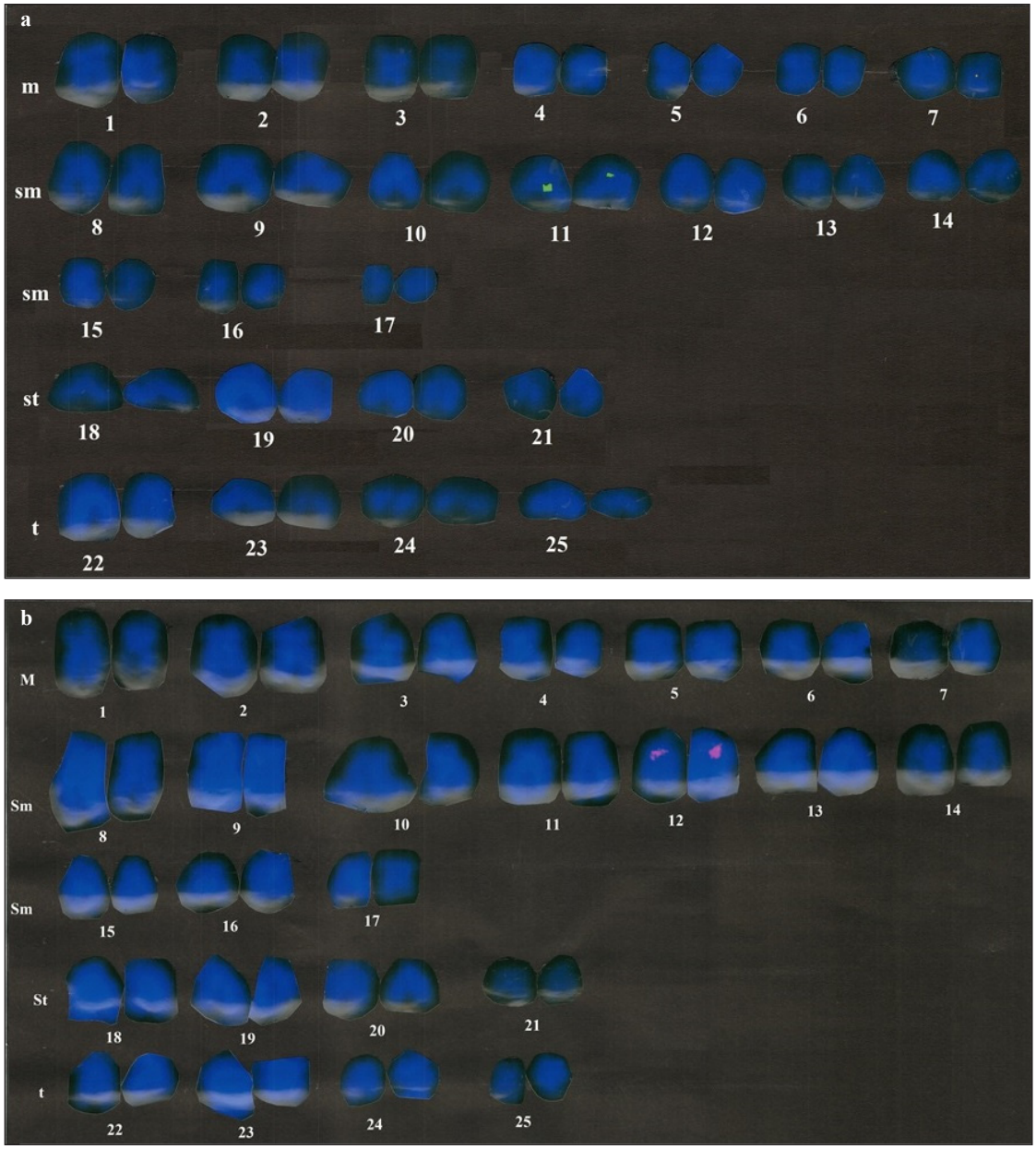

Figure 1. (a) 012K10 clone signal on 11th submetacentric chromosome. (b) 012A23 clone signal on 12th submetacentric chromosome.

cytological marker for identifying individual chromosomes. In the present study, clone 012K10, labeled with green color fluorophore, fluorescein 12-dUTP, contained 4 genes (Table 1) and was mapped on 11th pair of sub-metacentric chromosomes (Fig. 1a), while clone 012A23, labeled with a red color fluorophore, rhodamine-5-dUTP, contained 4 genes (Table 1) and were mapped on the 12th pair of sub-metacentric chromosomes (Fig. 1b), with gene descriptions presented in Supplementary Table 1. The genes were well-defined and localized on the chromosomes, and compared with channel catfish and zebrafish in terms of gene localization and their pathways (Fig. 2).

3.2. Functional Gene Enrichment

Gene enrichment analysis illuminates a diverse array of biological processes and molecular functions linked to the studied gene set. The cellular anatomical entity (GO: 0110165) has the maximum Go term, involving 13 genes, under the cellular component. Enrichment, such as cellular anatomical entity and protein-containing complex, under cellular components, points towards a focus on cellular structures and protein interactions. Higher-order processes, like "multicellular organismal process" and "response to stimulus", hint at responses to environmental cues and organismal functions. Notably, the enrichment in "binding" and "catalytic activity" suggests significant involvement in these functions within the studied context. Additionally, "transcription regulator activity" and "ATP-dependent activity" underscore their regulatory and energy-related roles. In biological processes, the enrichment in "cellular process", "biological regulation", and "metabolic process" emphasizes their importance in various cellular activities and regulatory pathways (Fig. 3). However, the presence of unclassified entries suggests potential novel functions requiring further investigation.

Four pathways, providing insights into potential cellular mechanisms, were predicted using PANTHER tool. Nicotinic acetylcholine receptor signaling pathway (P00044), PI3 kinase pathway (P00048),

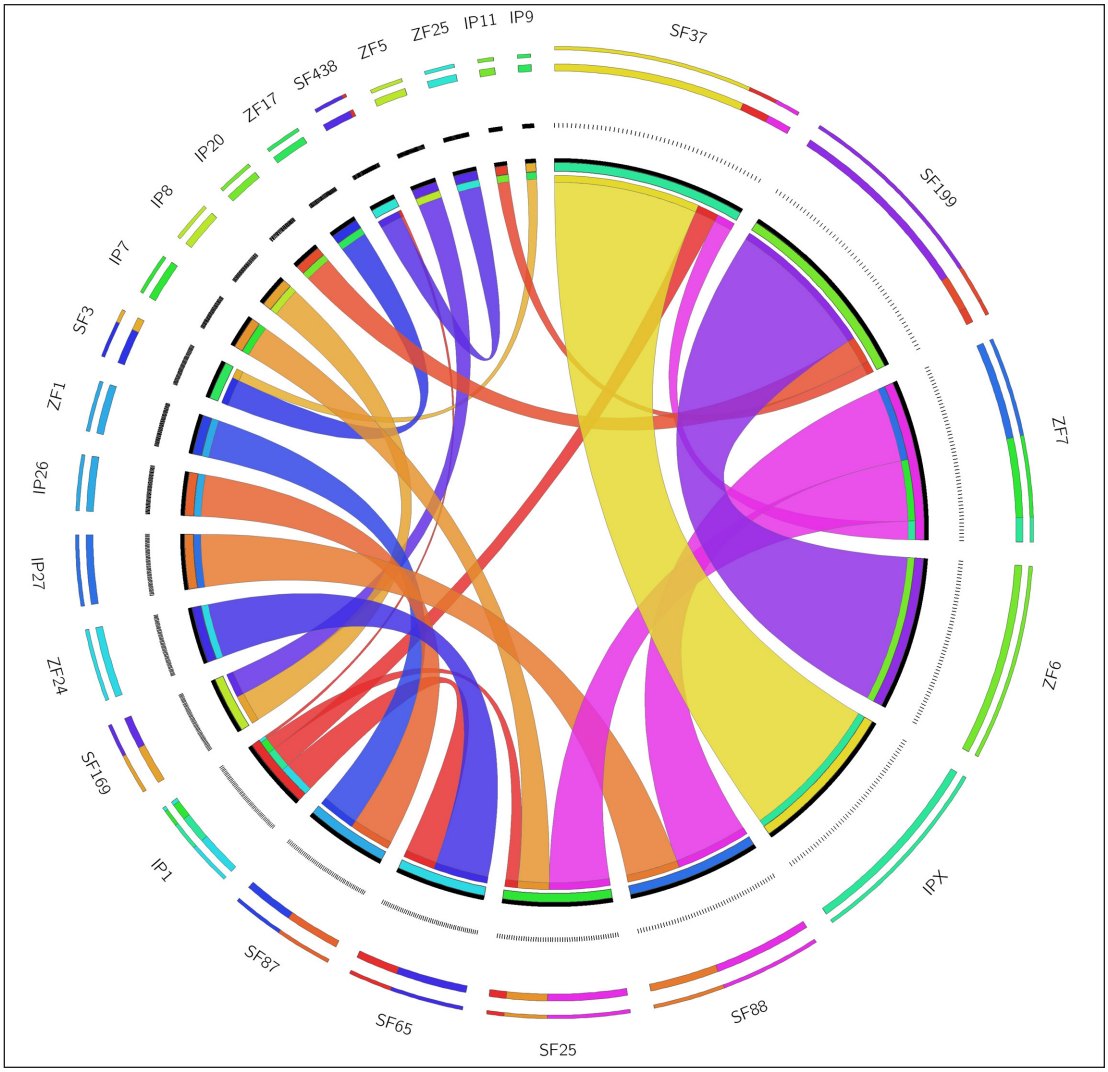

Figure 2. Circos plot showing synteny of 38 genes present on 17 genome scaffolds of *C. magur* with *I. punctatus* and *D. rerio*. SF represents scaffold of *C. magur*, IP represents chromosome of *I. punctatus* and ZF represents chromosome of *D. rerio* on which gene were present.

p53 pathway (P00059), and Ubiquitin proteasome pathway (P00060) offered glimpses into synaptic transmission, cellular signaling, stress response, and protein turnover, respectively (Fig. 4). However, a significant portion of unclassified entries indicates either a lack of classification or the presence of potential novel pathways, necessitating further exploration. Overall, these analyses offer comprehensive insights into the biological processes, molecular functions, and potential pathways associated with the studied gene set, paving the way for a deeper understanding of their roles in cellular activities and interactions.

3.3. Comparative Genomics and Genetic Diversity

By aligning end sequences of 18 clones representing 38 genes (Supplementary Table 1) mapped on the *C. magur* genome, 9 genes were synchronized with *D. rerio* and *I. punctatus* chromosomes. Circos graphic displays gene information and their locations on scaffolds and chromosomes in three species (Fig. 2). Remaining 29 genes present on clones were either present in chromosomes of either of the two species (*I. punctatus* or *D. rerio)*, hence, were not considered for synteny visualization. The 38 genes located on 18 clones of magur genome were annotated with gene id, protein id, amino acid (AA), and gene (exon) size in bp. *Herc1* gene, present on clone 012A6, showed a maximum 3919 AAs, while DAT39_0118 gene, present on clone 012K10, was the smallest with 61 AAs (Supplementary Table 1).

Four NJ-phylogenetic circular unrooted trees were constructed based on the p-distances for clear visualization of 20 common genes found on the chromosomes of *I. punctatus, D. rerio,* and on *C. magur* genome scaffolds (Fig. 5 a–d). The *Scly* gene of *I. punctatus* did not cluster with *D. rerio* and *C. magur*. The NJ-phylogeny of genes present on scaffolds SF_390, SF_390, SF_199, SF_199, SF_25, and SF_191 of *C. magur* with zebrafish and *I. punctatus* generated 3 clusters (Fig. 5a), while SF_191, SF_37, SF_882, SF_88, and SF_87 generated 4 clusters (Fig. 5b). Likewise, SF_88, SF_87, and SF_438 grouped into 3 clusters (Fig. 5c) and SF_170, SF_3, SF_159, SF_169, SF_65, and SF_118 generated into 4 clusters with *I. punctatus* and *D. rerio* (Fig. 5d).

3.4. Protein-Protein Interaction

A network-based approach was undertaken to identify and prioritize candidate genes in clone-mapped *C. magur* scaffolds with emphasis on PPI network and its role in unraveling the functional relationships

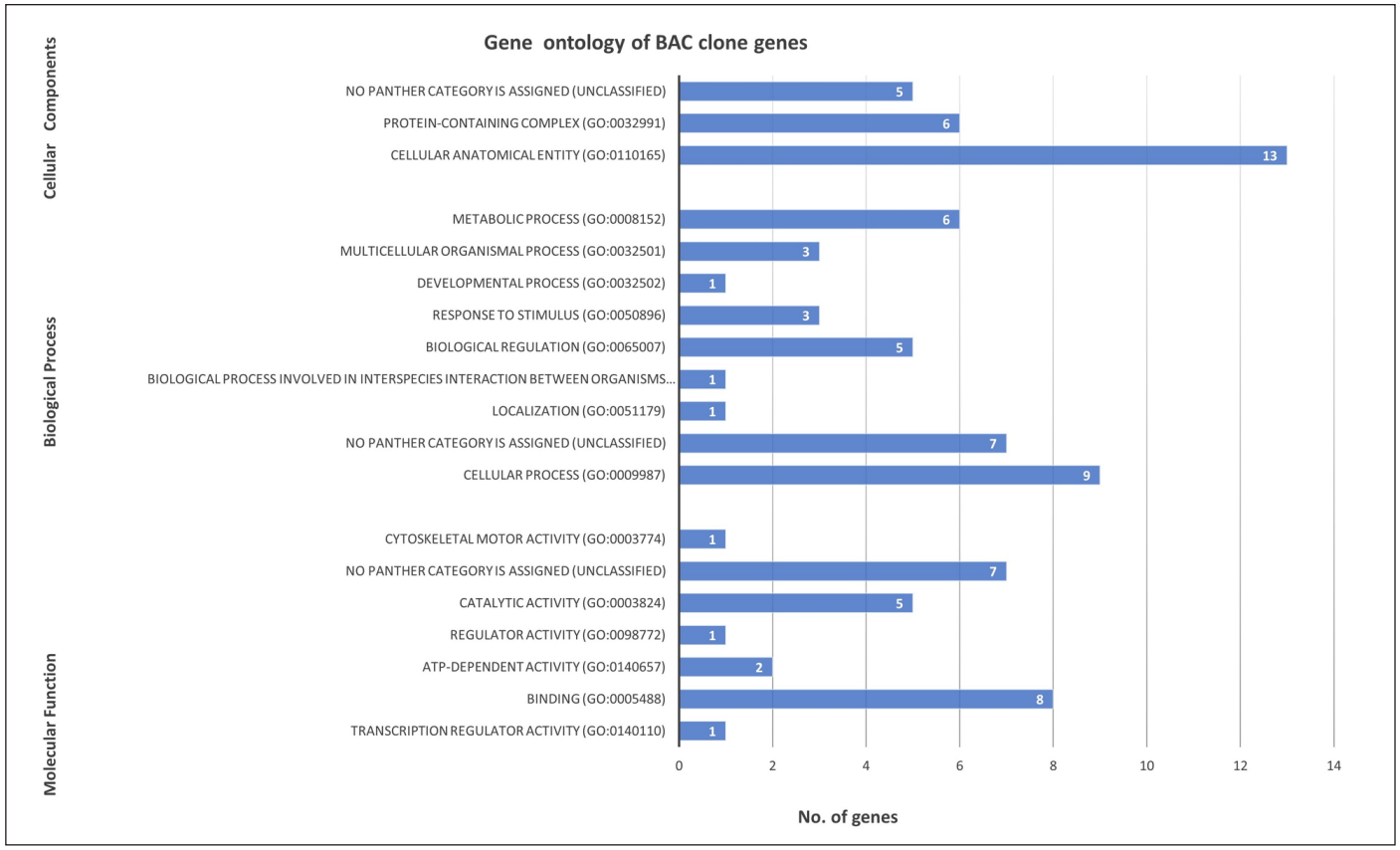

Figure 3. Panther database analysis showing GO terms associated with molecular function, biological process and cellular components.

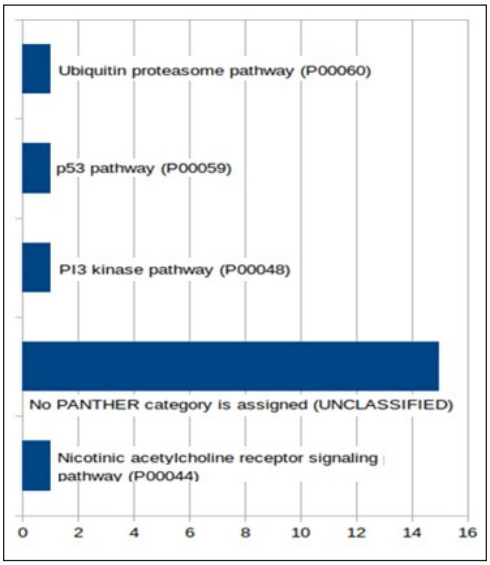

Figure 4. Bar chart showing results of protein class of genes present on 18 clones in Panther analysis.

between the genes or proteins. The gene co-occurrence can computationally be predicted by examining genomic datasets from various samples or environments including gene expression profiles and genomic sequencing data. The constructed PPI network revealed two types of interactions among 14 nodes (genes) and between 17 edges (*adamts5, cth1, mta2, nos2a, odc1, slc7a5, taf6l, TIMM21, herc1, kif18a, rb1cc1, scly, uba2, adamts1, crocc2, smyhc3,* and *tpma*) (Fig. 6a). These interactions were assessed based on various parameters, including their source in the database, experimental evidence, co-expression patterns and text mining, with 0.004 confidence score and 0.00415 PPI enrichment value (*p*-value). The clustering analysis of 17 genes in *C. magur* resulted in 3 distinct clusters represented by red, green, and blue colors (Fig. 6b). The red color cluster contained largest number of 7 genes (*adamts5, cth1, mta2, nos2a, odc1, slc7a5,* and *taf6l*), green color cluster had 6 genes (*TIMM21, herc1, kif18a, rb1cc1, scly,* and *uba2*) and blue color cluster consisted of 4 genes (*adamts1, crocc2, smyhc3,* and *tpma*). The genes exhibiting interaction include *uba2-scly* and *mta2-cth1*. Computational inferences of gene neighborhood, fusions, and co-occurrence can reveal some hypothesized interactions and provide insights into putative gene interactions.

3.5. Gene Ontology

The genes discussed exhibit diverse functions and locations within cells. The listed genes encompass a diverse array of biological functions and cellular localizations (Table 2). The gene *MFSD7* encodes a protein with RNA binding activity primarily localized in the membrane. *NCBP1* is involved in various mRNA processing and capping processes, localized in the nucleus, specifically in the nuclear cap-binding complex and nucleus. Nuclear cap-binding proteins *NCBP1* and *NCBP3* have been reported in some eukaryotes to form a complex that binds to elements of the mRNA processing machinery, enhancing poly(A) RNA production in higher eukaryotes [30]. *RPLX* gene is primarily localized in the large ribosomal subunit and participates in translation processes with RNA binding activity. *SCLY*, involved in lyase activity, is primarily found in the cytosol. *SLC7A5* has been reported to be essential for mTORC1 protein complex activation during fin regeneration in zebrafish, with leucine and glutamine signaling enhancing cell proliferation and regeneration

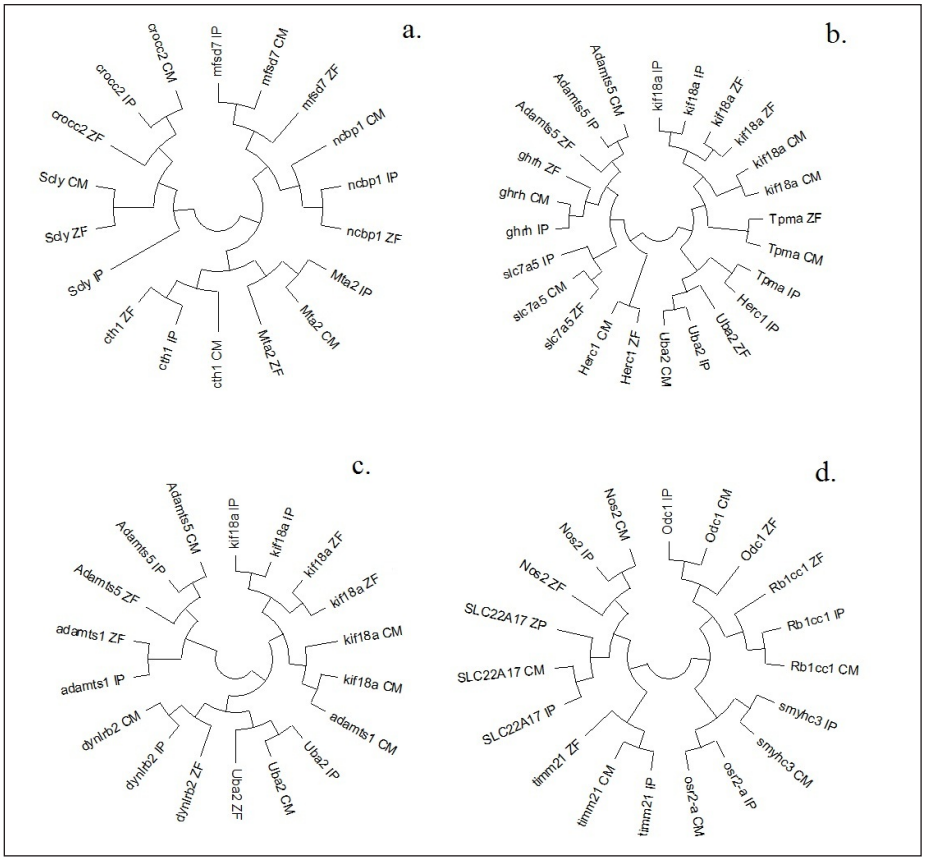

Figure 5. (a–d) Phylogenetic trees constructed by NJ method based on p-distance of common genes present on chromosomes of *I. punctatus* and *D. rerio* with *C. magur.*

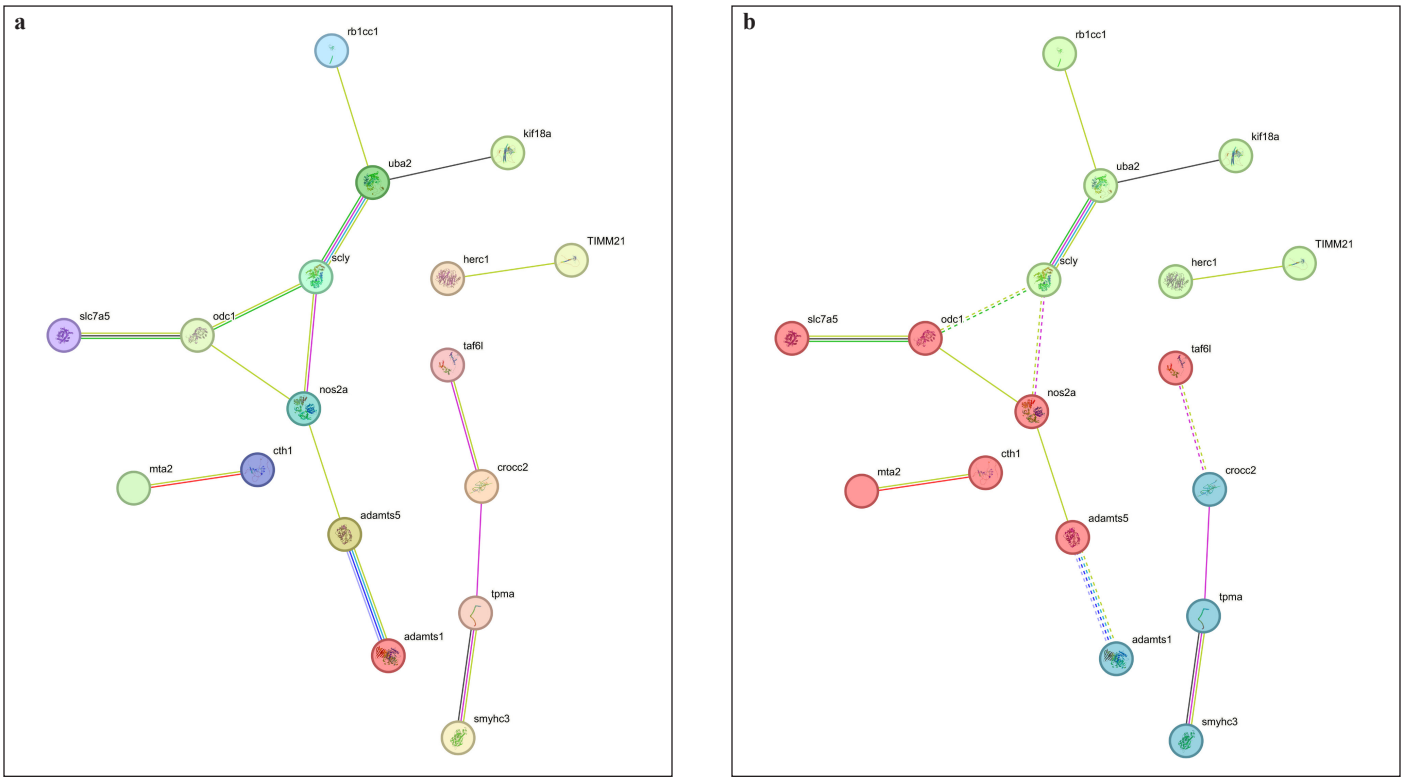

Figure 6. (a) PPI network and cluster analysis. (b) PPI network of 17 genes present on clones in *C. magur* resulting in three distinct clusters, denoted by red, green and blue.

Table 2. Annotation of genes located on mapped scaffold of *C. magur* genome.

S.No.	Gene name	Protein names	GO		
			Biological process	Molecular function	Cellular component
1.	mfsd7	Major facilitator superfamily domain-containing protein 7		RNA binding [GO:0003723]	membrane [GO:0016020]
2.	ncbp1	Nuclear cap-binding protein subunit 1	7-methylguanosine mRNA capping [GO:0006370]; mRNA export from nucleus [GO:0006406]; nuclear-transcribed mRNA catabolic process, nonsense-mediated decay [GO:0000184]; RNA splicing [GO:0008380]	RNA cap binding [GO:0000339]	nuclear cap binding complex [GO:0005846]; nucleus [GO:0005634]
3.	rplX	Large ribosomal subunit protein uL24 (60S ribosomal protein L26)	translation [GO:0006412]	RNA binding [GO:0003723]; structural constituent of ribosome [GO:0003735]	large ribosomal subunit [GO:0015934]
4.	il17b	Interleukin-17B-like	NA	NA	NA
5.	crocc2	Rootletin-like isoform X2	NA	NA	NA
6.	DAT39_011845	Espin-like protein	NA	NA	NA
7.	DAT39_011846	Espin-like protein	sensory perception of sound [GO:0007605]	NA	stereocilium [GO:0032420]
8.	scly	Selenocysteine lyase (EC 4.4.1.16)		lyase activity [GO:0016829]	cytosol [GO:0005829]
9.	mta2	Metastasis-associated protein MTA2 isoform X1	regulation of DNA-templated transcription [GO:0006355]	chromatin binding [GO:0003682]; metal ion binding [GO:0046872]; sequence-specific DNA binding [GO:0043565]	nucleus [GO:0005634]
10.	cth1	mRNA decay activator protein ZFP36 (Zinc finger protein 36)	3'-UTR-mediated mRNA destabilization [GO:0061158]; positive regulation of nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay [GO:1900153]	metal ion binding [GO:0046872]; mRNA 3'-UTR AU-rich region binding [GO:0035925]	cytoplasm [GO:0005737]; nucleus [GO:0005634]; ribonucleoprotein complex [GO:1990904]
11.	taf6l	TAF6-like RNA polymerase II CBP-associated factor-associated factor	transcription initiation at RNA polymerase II promoter [GO:0006367]	potassium channel activity [GO:0005267]; protein heterodimerization activity [GO:0046982]; RNA polymerase II general transcription initiation factor activity [GO:0016251]	membrane [GO:0016020]; SAGA complex [GO:0000124]; SLIK (SAGA-like) complex [GO:0046695]; transcription factor TFIID complex [GO:0005669]
12.	gnao1	Guanine nucleotide-binding protein G(O) subunit alpha	G protein-coupled receptor signaling pathway [GO:0007186]	G-protein beta/gamma-subunit complex binding [GO:0031683]; GTP binding [GO:0005525]; GTPase activity [GO:0003924]	NA
13.	gnao1	Guanine nucleotide-binding protein G(O) subunit alpha	G protein-coupled receptor signaling pathway [GO:0007186]	G-protein beta/gamma-subunit complex binding [GO:0031683]; GTP binding [GO:0005525]; GTPase activity [GO:0003924]	NA
14.	tpma	Tropomyosin alpha-1 chain	NA	actin binding [GO:0003779]	cytoskeleton [GO:0005856]
15.	herc1	Putative E3 ubiquitin-protein ligase HERC1 isoform X1	NA	ubiquitin-protein transferase activity [GO:0004842]	
16.	DAT39_003239	EH domain-binding protein 1-like protein 1	NA	NA	NA
17.	ghrh	Somatoliberin (Growth hormone-releasing factor) (Growth hormone-releasing hormone)	neuropeptide signaling pathway [GO:0007218]	neuropeptide hormone activity [GO:0005184]	extracellular region [GO:0005576]

Continued

S.No.	Gene name	Protein names	GO		
			Biological process	Molecular function	Cellular component
18.	DAT39_004143	Phosphatase and actin regulator	NA	actin binding [GO:0003779]; protein phosphatase inhibitor activity [GO:0004864]	NA
19.	slc7a5	Large neutral amino acids transporter small subunit 1-like	NA	transmembrane transporter activity [GO:0022857]	membrane [GO:0016020]
20.	slc7a5	Large neutral amino acids transporter small subunit 1-like	NA	transmembrane transporter activity [GO:0022857]	membrane [GO:0016020]
21.	slc7a5	Large neutral amino acids transporter small subunit 1-like	NA	transmembrane transporter activity [GO:0022857]	membrane [GO:0016020]
22.	DAT39_021308	Carbonic anhydrase (EC 4.2.1.1)	NA	carbonate dehydratase activity [GO:0004089]; zinc ion binding [GO:0008270]	NA
23.	uba2	SUMO-activating enzyme subunit 2 (EC 2.3.2.-)	protein sumoylation [GO:0016925]	ATP binding [GO:0005524]; metal ion binding [GO:0046872]; SUMO activating enzyme activity [GO:0019948]	SUMO activating enzyme complex [GO:0031510]
24.	DAT39_007712	Bestrophin-1-like	NA	NA	membrane [GO:0016020]
25.	kif18a	Kinesin-like protein KIF18A	NA	NA	NA
26.	kif18a	Kinesin-like protein	microtubule-based movement [GO:0007018]; system development [GO:0048731]	ATP binding [GO:0005524]; microtubule binding [GO:0008017]; microtubule motor activity [GO:0003777]	microtubule [GO:0005874]
27.	kif18a	Kinesin-like protein KIF18A	NA	NA	NA
28.	kif18a	Kinesin-like protein KIF18A	microtubule-based movement [GO:0007018]; system development [GO:0048731]	ATP binding [GO:0005524]; microtubule binding [GO:0008017]; microtubule motor activity [GO:0003777]	NA
29.	kif18a	Kinesin-like protein	microtubule-based movement [GO:0007018]; organelle organization [GO:0006996]; system development [GO:0048731]	ATP binding [GO:0005524]; microtubule binding [GO:0008017]; microtubule motor activity [GO:0003777]	microtubule [GO:0005874]; spindle [GO:0005819]
30.	adamts5	A disintegrin and metalloproteinase with thrombospondin motifs 5	proteolysis [GO:0006508]	metalloendopeptidase activity [GO:0004222]; zinc ion binding [GO:0008270]	collagen-containing extracellular matrix [GO:0062023]; extracellular region [GO:0005576]
31.	adamts1	A disintegrin and metalloproteinase with thrombospondin motifs 1	extracellular matrix organization [GO:0030198]; proteolysis [GO:0006508]	metal ion binding [GO:0046872]; metalloendopeptidase activity [GO:0004222]	extracellular region [GO:0005576]; membrane [GO:0016020]
32.	dynlrb2	Dynein light chain roadblock-type 2	microtubule-based movement [GO:0007018]	NA	cytoplasm [GO:0005737]; cytoplasmic dynein complex [GO:0005868]; microtubule [GO:0005874]
33.	DAT39_016899	Genetic suppressor element 1-like isoform X3	NA	NA	
34.	SLC22A17	Solute carrier family 22 member 17	NA	transmembrane transporter activity [GO:0022857]	membrane [GO:0016020]
35.	smyhc3	Myosin-7-like	system development [GO:0048731]	actin filament binding [GO:0051015]; ATP binding [GO:0005524]; cytoskeletal motor activity [GO:0003774]	myosin complex [GO:0016459]
36.	DAT39_015308	Roundabout 2-like isoform X2	NA		membrane [GO:0016020]
37.	Odc1	ornithine decarboxylase (EC 4.1.1.17)	methylation [GO:0032259]; polyamine biosynthetic process [GO:0006596]	carboxy-lyase activity [GO:0016831]; histone methyltransferase activity [GO:0042054]	nucleus [GO:0005634]

Continued

S.No.	Gene name	Protein names	GO		
			Biological process	Molecular function	Cellular component
38.	al5ap	Arachidonate 5-lipoxygenase-activating protein	leukotriene biosynthetic process [GO:0019370]	enzyme activator activity [GO:0008047]	endoplasmic reticulum membrane [GO:0005789]; nuclear membrane [GO:0031965]
39.	timm21	Mitochondrial import inner membrane translocase subunit Tim21	protein import into mitochondrial matrix [GO:0030150]	NA	TIM23 mitochondrial import inner membrane translocase complex [GO:0005744]
40.	NOS2	nitric-oxide synthase (NADPH) (EC 1.14.13.39)		NA	NA
41.	NOS2	nitric-oxide synthase (NADPH) (EC 1.14.13.39)	nitric oxide biosynthetic process [GO:0006809]	metal ion binding [GO:0046872]; nitric-oxide synthase activity [GO:0004517]	NA
42.	NOS2	Nitric oxide synthase (EC 1.14.13.39)	nitric oxide biosynthetic process [GO:0006809]; translation [GO:0006412]	calmodulin binding [GO:0005516]; flavin adenine dinucleotide binding [GO:0050660]; FMN binding [GO:0010181]; heme binding [GO:0020037]; metal ion binding [GO:0046872]; NADP binding [GO:0050661]; nitric-oxide synthase activity [GO:0004517]; structural constituent of ribosome [GO:0003735]	ribosome [GO:0005840]
43.	rb1cc1	RB1-inducible coiled-coil protein 1	autophagosome assembly [GO:0000045]		NA
44.	osr2-a	Protein odd-skipped-related 2 isoform X1		metal ion binding [GO:0046872]	nucleus [GO:0005634]

[31]. *MTA2* regulates DNA-templated transcription and is located in the nucleus. *CTH1* participates in mRNA destabilization and is found in the cytoplasm and nucleus. *TAF6L*, involved in transcription initiation, is localized in the membrane and transcription factor complexes. *TAF6L* has been implicated in the regulation of transcription, cell cycle, cell division, and stem cell maintenance, potentially interacting with c-MYC to activate target gene expression in humans [32]. *GNAO1* is part of the G protein-coupled receptor signaling pathway and is localized in the cytoplasm. It has been reported to be part of signal transduction pathways and has been linked to tumorigenesis in humans [33].

The growth hormone receptor (GHR) is found in various organs of fish, including the liver, brain, and gonads, and plays a role in growth and development [34–37]. *GHRH* gene, participating in neuropeptide signaling, is primarily found in the extracellular region. *SLC7A5* encodes a transmembrane transporter found in the membrane. KIF18A is involved in microtubule-based movement and system development and is primarily localized in the microtubule. *AL5AP* participates in leukotriene biosynthesis and is located in the endoplasmic reticulum and nuclear membrane. NOS2 participates in nitric oxide biosynthesis and is localized in the ribosome. *RB1CC1* is involved in autophagosome assembly. OSR2-A exhibits metal ion binding activity and is localized in the nucleus. *OSR2*-A exhibits metal ion binding activity and has been implicated in chondrocyte development and fin morphogenesis in *D. rerio* [38]. Selenocysteine lyase (*Scly*) is an enzyme that decomposes selenocysteine (Sec) into selenide, providing selenium for the synthesis of new selenoproteins, crucial for various biological functions in mice [39]. The metastasis-associated (*MTA*) gene family, which includes *Mta2* and *Mta3*, are reported to be involved in *D. rerio* embryogenesis [40]. *Zfcth1* gene in *D. rerio*, akin to the mammalian TIS11 family, encodes a protein with CCCH zinc fingers and is involved in RNA expression during oogenesis and early embryogenesis, playing a role in cell fate determination and oocyte maturation [41]. *ADAMTS5* [42] and *ADAMTS1* [43] participate in proteolysis and are localized in the extracellular matrix and membrane, respectively. *DYNLRB2* is involved in microtubule-based movement and localized in the cytoplasm and cytoplasmic dynein complex [44] *ODC1* is involved in methylation and polyamine biosynthesis and primarily found in the nucleus [45]. *TIMM21* is involved in protein import into the mitochondrial matrix, primarily found in the *TIM23* mitochondrial import inner membrane translocase complex [46]. Through GO study, genes targeting a diverse array of biological functions could be traced, which may be employed for understanding biological processes to particular stimuli or stress or phenotypes.

4. CONCLUSION

The BAC clone mapping provides a valuable source of identification of genes of interest and is also useful to detect the genes' order and genetic markers present in the genome. Here, the study provides important information on 38 genes and 1974 SSRs present in the selected 18 clones of *C. magur* genome. The genes identified in 18 clones using mapping of BE sequences on scaffolds are well-described in terms of gene description, complete gene annotation, protein-protein network analysis, and synteny studies, which are useful resources for undertaking the population dynamics and structural genomics and have application in genomic selection as well as cytogenetic studies. Our study can also lead to the development of the BE sequence and BAC-wise genes database of *C. magur,* which will be a valuable resource for the formulation of gene-specific constructs for genetic manipulation studies.

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6. LIST OF ABBREVIATIONS

AA, Amino acid; AL5AP, Arachidonate 5-lipoxygenase-activating protein; BAC, Bacterial artificial chromosome; BE mapping, Bac end mapping; *C. magur*, Clarias magur; CTH1, mRNA decay activator protein ZFP36; *D. rerio*, *Danio rerio*; DAPI, 4′,6-diamidino-2-phenylindole; DYNLRB2, Dynein light chain roadblock-type 2; *E. coli*, *Escherichia coli*; FISH, Fluorescence in situ hybridization; GO, Gene ontology; GHRH, Growth hormone-releasing hormone; GNAO1, Guanine nucleotide-binding protein G(O) subunit alpha; GSLC7A5, Large neutral amino acids transporter small subunit 1-like; HR, Growth hormone receptor; *I. punctatus*, *Ictalurus punctatus*; Kb, Kilobase; KEGG, Kyoto encyclopedia of genes and genomes; KIF18A, Kinesin-like protein KIF18A; MFSD7, Major facilitator superfamily domain-containing protein 7; MTA, Metastasis-associated protein MTA2 isoform X1; MTA2, Metastasis-associated protein MTA2 isoform X1; NCBP1, Nuclear cap binding protein subunit 1; NJ, Neighbour joining; NOS2, Nitric oxide synthase; ODC1, ornithine decarboxylase; OSR2-A, Protein odd-skipped-related 2 isoform X1; PPI, Protein-protein interaction; RB1CC1, RB1-inducible coiled-coil protein 1; Rcf, Relative centrifugal force; RPLX, Large ribosomal subunit protein; SCLY, Selenocysteine lyase; SF, Scaffolds; SLC7A5, Large neutral amino acids transporter small subunit 1-like; SSR, Simple sequence repeats; TAF6L, TAF6-like RNA polymerase II CBP-associated factor-associated factor; TIMM21, Mitochondrial import inner membrane translocase subunit Tim21.

7. AUTHOR'S CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

8. CONFLICT OF INTEREST

The authors have declared that none of their known financial conflicts or interpersonal connections could have influenced the work presented in this paper.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. SUPPLEMENTARY MATERIAL

The supplementary material can be accessed at the journal's website: Link here [https://jabonline.in/admin/php/uploadss/1297_pdf.pdf].

12. PUBLISHER'S NOTE

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13. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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