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Identification, Genomic Characterization, and Phylogenetic Relationship of the Heat Shock Protein Beta-1 (HSPB1) in Placental Mammals

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Identification, Genomic Characterization, and Phylogenetic Relationship of the Heat Shock Protein Beta-1 (HSPB1) in Placental Mammals

Running title: Genomic characterization of HSPB1 in Mammals

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Abstract: Heat Shock Protein Beta-1 (HSPB1), a molecular chaperone crucial for celluar response and proteostasis, exhibits evolutionary conservation woth potential lineage-specific adaptations in placental mammals, warranting detailed comparative genomic investigation. The study investigated the characteristics, evolutionary links, motifs, secondary structure, and genetic organization of Heat Shock Protein Beta-1 **(**HSPB1) protein across twelve distinct mammals. Significant sequence conservation was identified using multiple sequence alignments (MSA), with over 70% identity in specific areas among the chosen organisms. Physiochemical analysis revealed that all species' protein sequences exhibited an acidic nature, while instability indices indicated inherent protein instability. The GRAVY analysis referred to hydrophilic properties, while the aliphatic index showed heat stability. Phylogenetic analysis revealed five distinct clades, corresponding to major placental mammals' groups (e.g. *Homo sapiens*, *Bos Taurus*), which underscores deep evolutionary divergences and conserved stress-response adaptations across lineages. Motif analysis revealed distinctive patterns in several species, and InterProScan results revealed membership in the "Homologous superfamily HSP20_like_Chapserson" family. An examination of the genetic organization indicated differences among organisms in the upstream, downstream, intron, and CDS regions, and the presence of conserved regions suggested their identity and similarity matrices. The current study conducted a computational approach and supporting evidence that HSPB1 is a novel heat shock responsive protein identified in placental mammals The current study findings provide a foundational framework delving into HSPB1 evolutionary and lineage-specific diversification, offering valuable insights into stress adaptation mechanisms and their implications for biomedical or evolutionary studies in mammals.

Keywords: domain; gene structure; genomics; HSPB1; motif; phylogeny

Introduction

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In response to environmental stress, all organisms— ranging from archaea and bacteria to plants and animals—produce heat shock proteins. A subgroup of these heat shock proteins is known as small heat shock proteins (sHSPs). HSPB1 also referred toknown as Heat Shock Protein 27, is a member of of this family of sHSPs. The production of these Heat Shock Protein (HSPs) is triggered when cells are exposed to elevated temperatures [1].The sHSP family is the most prevalent class of molecular chaperones, which are proteins responsible for assisting in the proper folding or unfolding of other proteins and the assembly and disassembly of macromolecular structures,. The functions of molecular chaperones, particularly sHSPs, is critically dependent on their structural properties. sHSPs typically from large oligomeric complexes that resemble spheres or barrels. These Oligomers are macromolecular complexes composed of multiple monomers, usually ranging from 12 to 24 monomers, formed through non-covalent interactions between proteins [2]. Structural studies, including crystallography, reveal that dimers are the fundamental building blocks that aggregate to form these larger oligomers [3]. This oligomerization process is essential for the chaperones function of sHSPs, which involves preventing the aggregation of misfolded proteins and assisting in their proper refolding under stress condition [4]. sHSP are present in virtually all organisms, and their role in cellular protection under stress has led to increasing interest from researchers in recent years To facilitate the study of sHSPs[5], a dedicated database, known as sHSPdb has been developed. This database serve as a resource to analyze the structure, function and evolving research trends related to sHSPs [6].Heat Shock Protein 27, also known as HSPB1, is a component of the family of proteins known as small heat shock proteins, or sHSPs. However, stress situations such as heat shock can cause the cell to become exposed, which in turn leads to the folding of proteins and stimulates the production of HSPB1.The production of HSPB1 in the cell results in increased resistance of the cell to the damaging effects of heat shock and oxidative stress. HSPB1 are molecular chaperones, which are proteins that aid in the conformational folding or unfolding as well as the construction or disassembly of other macromolecular structures, and they share an ATP independent holdase activity [7]. HSPB1 are ATP-independent chaperon; they engage with proteins that have not fully folded, and it is this association that prevents the proteins from aggregating under stressful conditions [8] and promotes the storage of the proteins in a refolding competent state. In the event that these partially folded or misfolded proteins do not interact with HSPB1, then this leads to irreversible protein aggregation, which can be harmful to cells.

In addition to this, HSPB1 is particularly significant since it is associated with diabetic kidney disease as well as viral infection [9,10]. Osteoblasts are very critical cells for the development of bones, and HSPB1 plays a role in the functionality of osteoblasts. Both TNF- and IL-6 are considered to be inflammatory cytokines, and both become active during the process of inflammation. Interleukin (IL)-6 plays an important role in the development of an immune response, the formation of B cells, and the production of neutrophils in the bone marrow. It is also produced when tissue is damaged or infected. The production of tumor necrosis factor alpha (TNF-a), which has a role in the development of resistance to infection and cancer, takes place during acute inflammation. In osteoblast cells such as MC3T3-E1, HSPB1 acts as a regulator for the TNF--stimulated production of IL-6 [11]. The current study will provide vital information pertaining to the structure, functions, and evolutionary links of the heat shock protein (HSPB1) among mammals.

This work aims to systematically identify and characterize HSPB1 orthologs across placental mammals by analyzing their genomic features, evolutionary conservation and lineage-specific adaptations. Additionally, it seeks to elucidate the phylogenetic relationships of HSPB1, tracing its functional divergence and role in stress adaptation mechanisms within eutherian lineages.

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Materials and Methods

Data Collection

The CDS, protein, and DNA sequences were retrieved from the National Centre for Biotechnology Information (NCBI) https://www.ncbi.nlm.nih.gov/ [12]. The data for all organisms are shown in Table 1, together with their respective accession ids, protein ids, and the databases from which they were downloaded.

Table 1. Information about data source, organism nucleotide and amino acid sequence length, and accession ID.

Species	Protein-ID	Length	Database	Accession-ID	Database
Ochotona princeps	XP_004587250.1	233	NCBI	XM_004587193.1	NCBI
Cavia porcellus	XP_003470158.1	200	NCBI	XM_003470110.4	NCBI
Lynx Canadensis	XP_030157330.1	205	NCBI	XM_030301470.1	NCBI
Bos Taurus	NP_001020740.1	204	NCBI	NM_001025569.1	NCBI
Ovis aries	XP_027817273.1	201	NCBI	XM_027961472.2	NCBI
Camelus ferus	XP_032315487.1	201	NCBI	XM_032459596.1	NCBI
Capra hircus	XP_017896392.1	201	NCBI	XM_018040903.1	NCBI
Pteropus Alecto	XP_006918629.1	207	NCBI	XM_006918567.3	NCBI
Homo sapiens	NP_001531.1	205	NCBI	NM_001540.5	NCBI
Gorilla gorilla	XP_004045665.1	205	NCBI	XM_004045617.3	NCBI
Erinaceus europaeus	XP_007518007.1	199	NCBI	XM_007517945.2	NCBI
Suncus etruscus	XP_049643837.1	209	NCBI	XM_049787880.1	NCBI

Multiple Sequence Alignment

All of the HSPB1- protein sequences were aligned using Multiple Align Show (https://www.bioinformatics.org/sms/ multi_align.html) [13], which allowed for the detection and visualization of sequence variants as well as insertions and deletions.

Phylogenetic tree

The neighbor joining (NJ) approach was used to generate a phylogenetic tree of closely related species using MEGA 11 [14]. The bootstrap was set to 1000 repeats, and the tree was constructed using this method. ITOL version 4.2.4 was utilized in order to visualize and investigate the findings of phylogeny and multiple sequence alignment.

Motif and Domain Analyses

The MEME (Multiple Expectation Maximization) tool [15] was utilized in order to locate motifs within the protein sequences of each and every organism. The generation of motifs permitted any number of repetitions, and the maximum number of motifs that could be used was 10. In order to locate domains in each and every organism, the InterProScan [16] was utilized. It was possible to determine which protein families an organism belonged to and, as a result, whether or not it included any functional domains.

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Gene structure Analysis

The application of Gene Structure Display Server (GSDS) version 2.0 [17] was utilized in order to determine the configuration of conserved elements, exons, and introns, as well as their respective localities.

Physiochemical Properties

ProtParam an online tool [18] that is available on the Expert Protein Analysis System (Expasy) Server which was used to compute physiochemical properties, including the molecular weight (MW), amino acids (AA), theoretical pI, instability index (II), aliphatic index (AI) and grand average of hydropathy (GRAVY). The WOLFPSORT software is used to determine where in the subcellular environment each and every organism's protein resides. The Hydropathy Plot was created with the support of the ProtScale software and then analyzed further.

Structural Characterizations

Using a method for the prediction of secondary structures called GORIV [19], which is used to calculate the secondary structure parameters (Alpha helix, Beta Bridge, Beta Turn, Extended strand, and Random Coil). The properties of all of an organism's secondary structures are listed in Table 5. The 3D model of protein was generated using online tool Phyre2, a completely web-based homology modelling tool for protein structures.

Results

Multiple Sequence Alignment

The Multiple Sequence Alignment (MSA) of each organism's protein was analyzed and displayed using the Multiple Align show. In Figure 1, the light red color represents the identical amino acid, while the blue color represents amino acids that are related. Although the color light yellow indicates that there are no similarities between the organisms. More than 70 percent of the sequences are identical along these locations that have been highlighted.

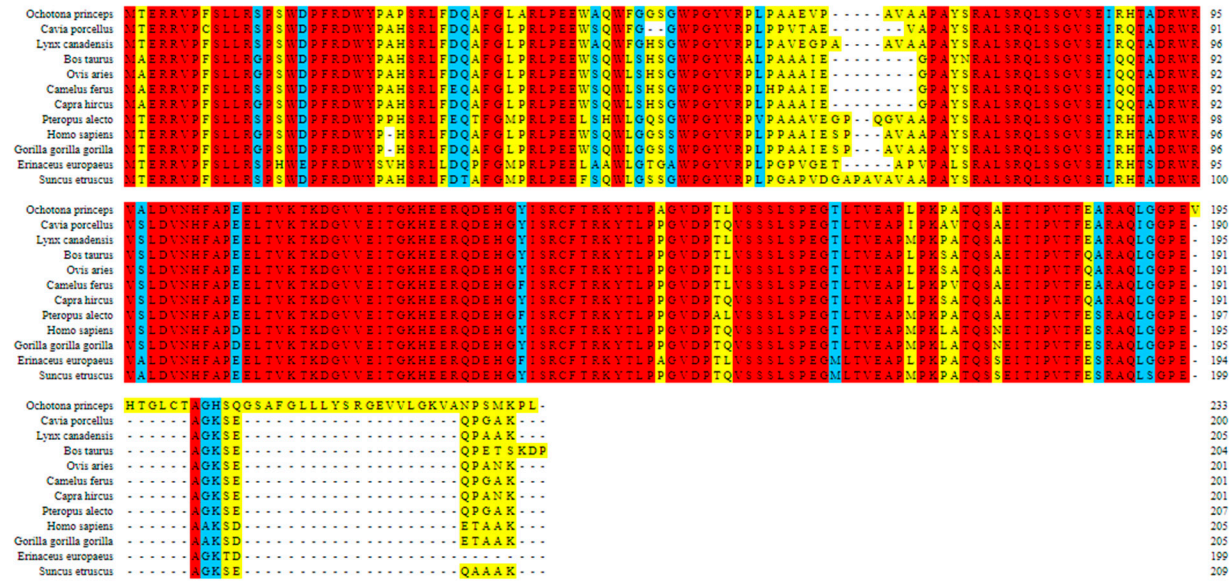

Figure 1. The multiple sequence alignment of the twelve proteins that was obtained by using Multiple align is displayed here. The protein sequences in the red dark regions share an identity of greater than 70 percent.

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Physiochemical Analysis

The results showed that there were changes in the physiochemical parameters of the organisms (Table 2). The species *E. europaeus* has the lowest number of amino acids (199), while the species *O. princeps* had the highest number of amino acids (233). Because all of the organisms have theoretical PI values that are lower than 7, it may be deduced that these are acidic in their natural state. All of the organisms' instability indices were higher than 40, indicating that the proteins of all of the selected organisms were unstable. The aliphatic index is a measure that may be used to evaluate the thermal stability of proteins; in this case, its value was greater than 50% for every protein. This suggests that aliphatic side amino acids covered over half of the volume of these proteins. The fact that the GRAVY values were less than 0 demonstrates that the hydrophilic quality of proteins. It was noted that the nucleus and the mitochondria both contain proteins from the organisms that were studied. Additionally, It was observed that the proteins from certain organisms are water-soluble. The hydropathy plot, generated for all of the species and shown in Figure 2, demonstrated these organisms tend to exhibi a greater affinity for hydrophobicity. The physiochemical parameters of each organism are provided in Table 2, including the amino acid composition, extinction coefficient, theoretical pI, instability index, aliphatic index, molecular weight, grand average of hydropathicity (GRAVY) and localization of each protein.

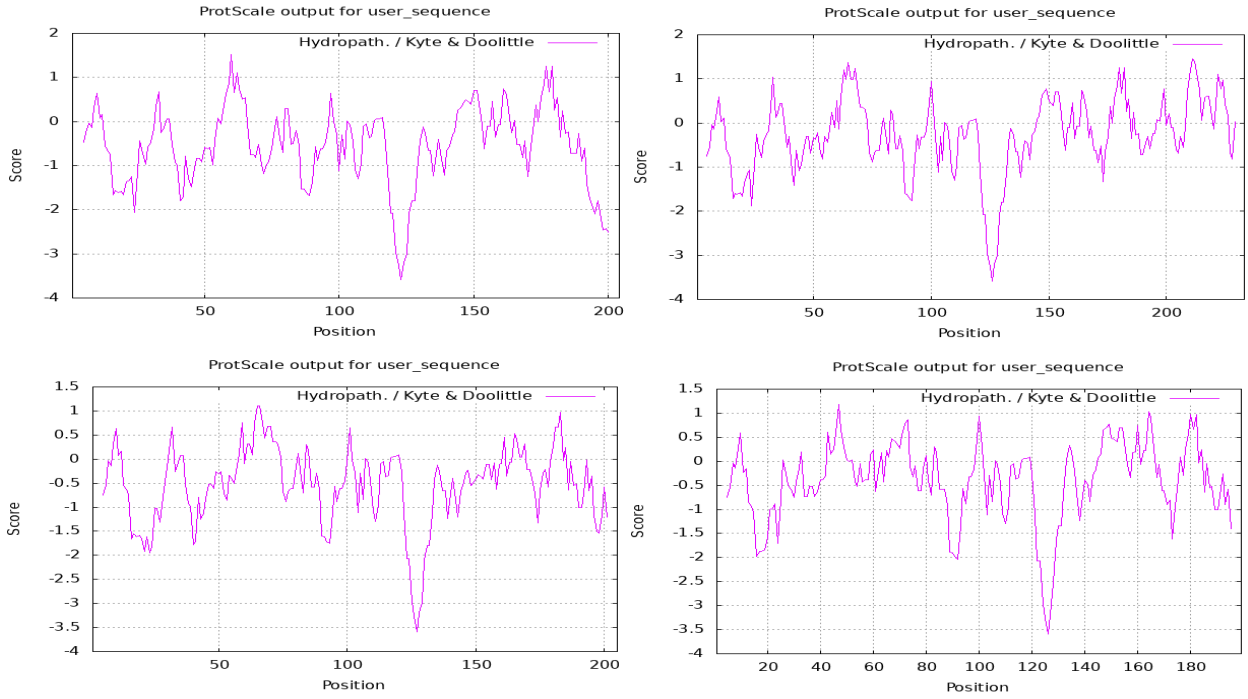

Figure 2. The hydrophobicity plot for these four creatures illustrates that, in their natural states, they are all more inclined to be hydrophilic.

Table 2. Physiochemical characteristics of different organisms.

Species	AA length	Molecular Weight	Theoretical PI	Instability index	Aliphatic index	Gravy	Localization
Ochotona princeps	233	25476.85	6.60	58.61	77.47	-0.289	Mitochondria
Cavia porcellus	200	22284.03	6.12	61.85	68.25	-0.562	Nuclear

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Lynx Canadensis	205	22720.55	6.23	65.03	67.61	-0.524	Mitochondria
Bos Taurus	204	22679.34	5.77	59.53	69.36	-0.597	Mitochondria
Ovis aries	201	22334.03	6.22	63.46	70.40	-0.568	Nuclear
Camelus ferus	201	22410.17	6.09	65.52	70.85	-0.551	Nuclear
Capra hircus	201	22349.00	6.22	62.71	68.46	-0.604	Nuclear
Pteropus alecto	207	22853.71	6.32	73.49	66.43	-0.549	Nuclear
Homo sapiens	205	22782.52	5.98	62.82	68.54	-0.567	Nuclear
Gorilla gorilla	205	22782.52	5.98	62.82	68.54	-0.567	Nuclear
Erinaceus europaeus	199	22032.94	6.08	68.37	75.43	-0.438	Nuclear & Mitochondria
Suncus etruscus	209	22988.85	6.22	69.20	65.84	-0.492	Nuclear & Mitochondria

Phylogenetic Analysis

Within the evolutionary tree that was constructed with MEGA 11, five distinct groups, or clades, emerged. Figure 3 illustrates quite clearly that members of clade 1, represented by the blue color, *S. etruscus* and *E. europaeus*, have descended from the same ancestor and are closely related to one another. In the second group, represented by the color red, *H. sapiens* and *G. gorillas* are closely related to one another since they share a common ancestor. *C. porcellus*, *L. canadensis*, and *O. princeps* are the three species that originated from the same ancestor and make up the third clade, which is colored peach. All four of these species, *B. taurus*, *O. aries, C. ferus, and C. hircus*, belong to the same clade and are closely related to one another. *P. alecto* is not closely related to any other organism and is depicted as a single entity in the phylogenetic tree even though it is located in clade 5, which is denoted by the branch color purple.

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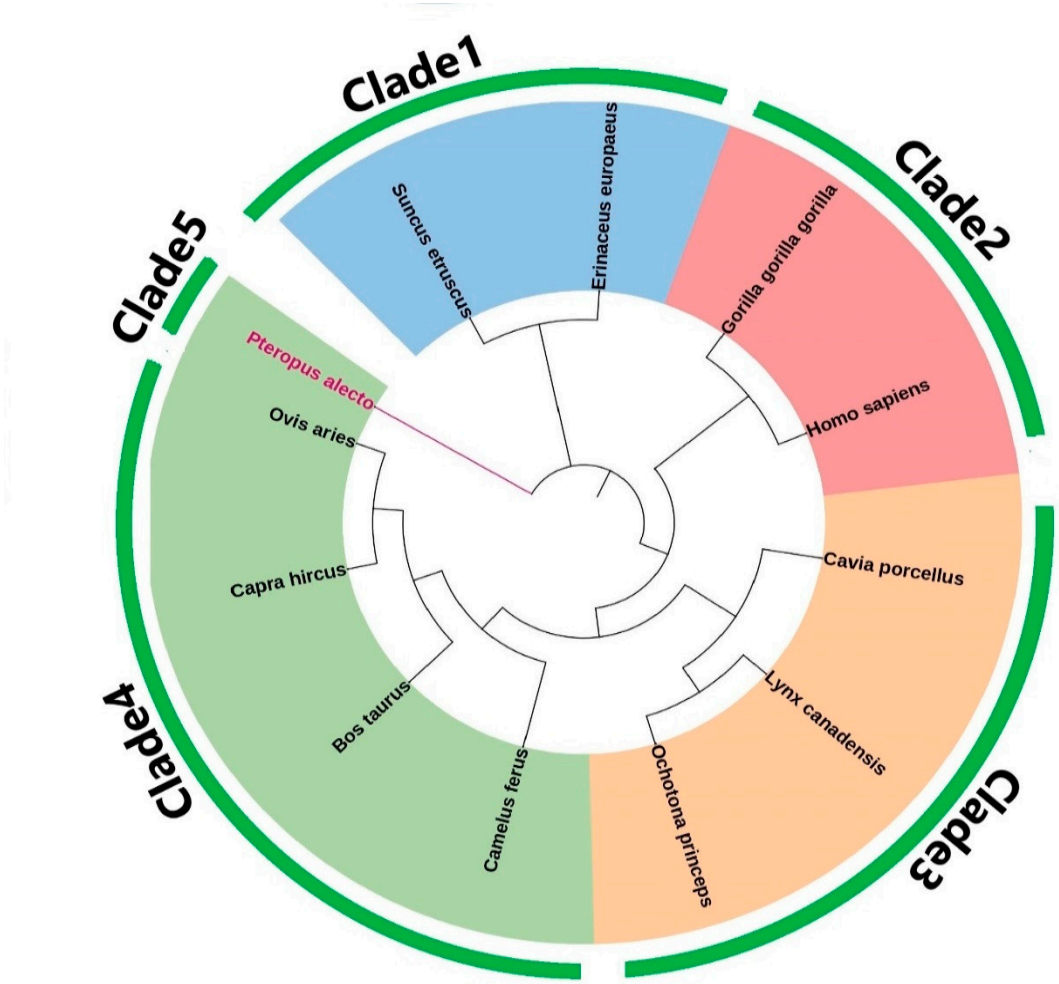

Figure 3. The phylogenetic tree was constructed by using the maximum likelihood approach in conjunction with the bootstrap test (1000 replications) in MEGA 11 and ITOL.

Motif Analysis

The investigation that was carried out with the assistance of the MEME tool revealed that motifs 1 through 7 were existing in the protein sequences of all species with the exception of *E. europaeus*. Only the protein sequences of two different species, namely *S. etruscus* and *O. princeps*, contained the Motif 8 pattern. Two more species, namely *P. alecto* and *O. princeps*, were identified to have a motif 9 in their DNA. Motif 10 was unique to *O. princeps*, as illustrated in Figure 4, and was only identified in that species.

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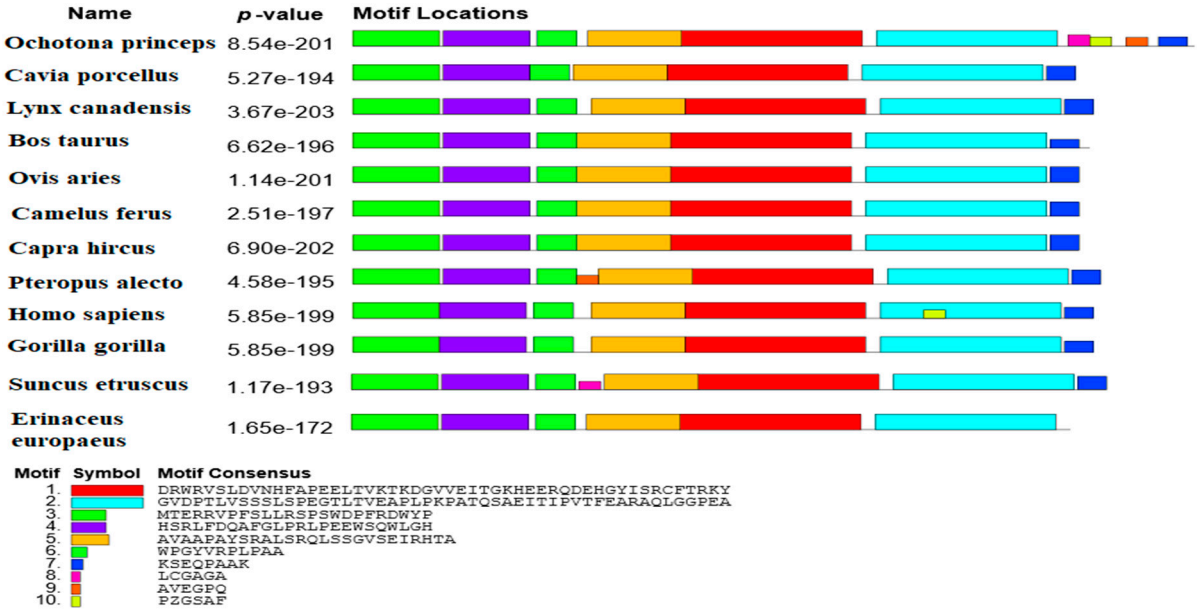

Figure 4. The motifs found in the protein sequences of 12 distinct organisms, with each motif represented by a unique color.

Domain Analysis

According to the results of the InterProScan study, all of the proteins from the species that were selected are members of the same homologous family called "Homologous super family HSP20_like_Chaperson," having the accession number IPR008978. The accession numbers (IPR002068 and IPR037876) for both of the DNA building domains known as "ACD_HSPB1 and A Crystalline" for each species as shown in Table 3.

Table 3. Positions of homologous super families and domains present in the selected organisms.

Organism Name	Homologous Super family HSP20_like_Chaperone IPR008978	Domain 1 ACD_HSPB1 IPR037876	Domain 2 A Crystalline IPR002068
Ochotona princeps	69-189	83-168	75-183
Cavia porcellus	62-187	79-164	71-179
Lynx canadensis	69-191	76-184	84-169
Bos Taurus	67-187	72-180	80-165
Ovis aries	67-187	72-180	80-165
Camelus ferus	65-187	80-165	72-180
Capra hircus	67-187	72-180	80-165
Pteropus alecto	72-193	78-186	86-171
Homo sapiens	69-197	84-169	76-184
Gorilla gorilla	69-197	84-169	76-184
Erinaceus europaeus	69-190	83-168	75-183
Suncus etruscus	69-190	83-168	75-183

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Gene Structure Analysis

The analysis of upstream, downstream, intron, and CDS regions was conducted using GSDS for each species. The term upstream and downstream refer to the relative positions of the genetic information within RNA or DNA, respectively. Specifically, downstream refers to the 3' end of the coding strand, while upstream refers to the 5' end of the coding strand. Both *B. taurus* and *O. princeps* possess all four components, upstream, downstream intron and CDS regions. Other species, including *C. porcellus, L. canadensis, O. aries, C. ferus, C. hircus, P. alecto, H. sapiens, G. gorilla, E. europaeus, and S. etruscus* only possess intron and CDS regions of the gene.

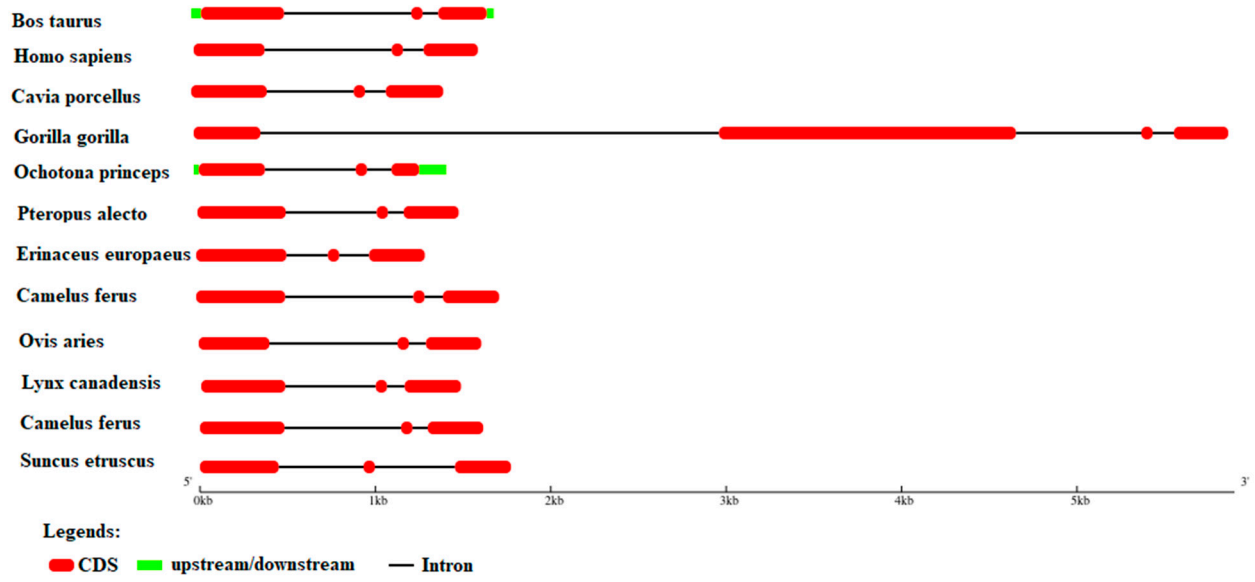

Figure 5. A Graphical representation of GSDS indicating proteins that are upstream, downstream, in the intron, and in the CDS portion.

Percentage Identity and Similarity of HSPB1 Gene

The percentage of amino acids that are identical to one another and the identity matrix of amino acids are crucial factors in predicting evolutionary patterns and distinguishing different species. Tables 4 and 5 present the the identity percentage and the similarity percentage of various species, respectively. A higher identity and similarity percentage between species typically indicates a closer relationship, suggesting that their structure and functions are are more likely to be comparable. In particular, sequences with a similarity percentage of approximately 70% are indicative of shared homology, functional similarity, and highly conserved regions HSPB1 gene. This gene plays a crucial role in cellular stress response and protein maintenance. The pair of species exhibiting a similarity of around 70% in their HSPB1 gene likely share similar functions and evolutionary histories.

Table 4. The percentage of amino acids identical across different organisms.

	Ochotona princeps	*Cavia porcellus*	*Lynx Canadensis*	*Bos taurus*	*Ovis aries*	*Camelus ferus*	*Capra hircus*	*Pteropus alecto*	*Homo sapiens*	*Gorilla gorilla*	*Erinaceus europaeus*	*Suncus etruscus*
Ochotona princeps	100%	76.56%	81.58%	75.31%	76.98%	76.15%	76.56%	75.73%	76.15%	76.15%	71.54%	75.31%
Cavia porcellus	76.56%	100%	92.46%	88.28%	90.79%	92.05%	91.21%	87.44%	88.28%	88.28%	81.58%	86.19%
Lynx canadensis	81.58%	92.46%	100%	89.12%	92.05%	91.21%	91.63%	90.37%	90.37%	90.37%	83.68%	89.53%
Bos taurus	75.31%	88.28%	89.12%	100%	96.65%	93.3%	96.23%	85.35%	86.19%	86.19%	79.91%	82.84%
Ovis aries	76.98%	90.79%	92.05%	96.65%	100%	95.81%	99.58%	87.86%	89.12%	89.12%	82%	85.77%
Camelus ferus	76.15%	92.05%	91.21%	93.3%	95.81%	100%	95.39%	89.53%	87.86%	87.86%	82.42%	85.35%
Capra hircus	76.56%	91.21%	91.63%	96.23%	99.58%	95.39%	100%	87.44%	89.53%	89.53%	81.58%	86.19%
Pteropus alecto	75.73%	87.44%	90.37%	85.35%	87.86%	89.53%	87.44%	100%	89.12%	89.12%	83.68%	89.12%
Homo sapiens	76.15%	88.28%	90.37%	86.19%	89.12%	87.86%	89.53%	89.12%	100%	100%	82.42%	88.7%
Gorilla gorilla	76.15%	88.28%	90.37%	86.19%	89.12%	87.86%	89.53%	89.12%	100%	100%	82.42%	88.7%
Erinaceus europaeus	71.54%	81.58%	83.68%	79.91%	82%	82.42%	81.58%	83.68%	82.42%	82.42%	100%	82.84%
Suncus etruscus	75.31%	86.19%	89.53%	82.84%	85.77%	85.35%	86.19%	89.12%	88.7%	88.7%	82.84%	100%

Table 5. The Percentage of amino acids similar across different organisms.

	Ochotona princeps	*Cavia porcellus*	*Lynx canadensis*	*Bos taurus*	*Ovis aries*	*Camelus ferus*	*Capra hircus*	*Pteropus alecto*	*Homo sapiens*	*Gorilla gorilla*	*Erinaceus europaeus*	*Suncus etruscus*
Ochotona princeps	100%	75.73%	80.33%	74.47%	75.73%	75.73%	75.31%	76.56%	75.73%	75.73%	71.12%	76.56%
Cavia porcellus	75.73%	100%	78.66%	74.89%	76.15%	78.24%	76.56%	75.73%	75.73%	75.73%	69.45%	75.31%
Lynx canadensis	80.33%	78.66%	100%	76.15%	77.82%	77.82%	77.4%	78.24%	77.4%	77.4%	71.12%	77.82%
Bos taurus	74.47%	74.89%	76.15%	100%	82%	79.49%	81.58%	74.89%	74.47%	74.47%	68.2%	73.22%

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Ovis aries	75.73%	76.15%	77.82%	82%	100%	80.75%	83.68%	76.15%	76.15%	76.15%	69.03%	74.89%
Camelus ferus	75.73%	78.24%	77.82%	79.49%	80.75%	100%	80.33%	76.98%	75.73%	75.73%	69.45%	75.31%
Capra hircus	75.31%	76.56%	77.4%	81.58%	83.68%	80.33%	100%	75.73%	76.56%	76.56%	68.61%	75.31%
Pteropus alecto	76.56%	75.73%	78.24%	74.89%	76.15%	76.98%	75.73%	100%	78.24%	78.24%	72.38%	78.66%
Homo sapiens	75.73%	75.73%	77.4%	74.47%	76.15%	75.73%	76.56%	78.24%	100%	85.77%	70.71%	78.66%
Gorilla gorilla	75.73%	75.73%	77.4%	74.47%	76.15%	75.73%	76.56%	78.24%	85.77%	100%	70.71%	78.66%
Erinaceus europaeus	71.12%	69.45%	71.12%	68.2%	69.03%	69.45%	68.61%	72.38%	70.71%	70.71%	100%	73.22%
Suncus etruscus	76.56%	75.31%	77.82%	73.22%	74.89%	75.31%	75.31%	78.66%	78.66%	78.66%	73.22%	100%

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Structural Analysis

GORIV tool was used to make the prediction about the secondary structure of the HSPB1 gene for the 12 organisms that were chosen. According to table 6, the HSPB1 gene has an alpha helix, a random coil, and extended strands as part of its secondary structure. The HSPB1 gene has an alpha helix, a random coil, and extended strands as part of its secondary structure. The *L. canadensis* alpha helix takes up a total of 26.83 percent of the structure. The portion of the structure that is occupied by the alpha helix, the least by *H. sapiens* and *G. gorilla* is 13.66 percent. In addition, the extended strand for the *B. taurus* was the longest at 17.75%, while the extended strand for the *C. porcellus* was the shortest at 11.00%. On the other hand, random coils make up 73.17% of the structure of *H. sapiens* and *G. gorilla*, which is the largest percentage of random coils among other creatures. For HSPB1 protein sequence, a 12, 3D structure templates were created, and all of the models were successfully constructed from Phyre2 (Figure 6). The 3D structure of HSPB1 protein revealed details about their folding, stability, and domain interactions.

Table 6. All the criteria and percentages for the secondary structure of the organisms.

Organism Name	Alpha helix	Beta Bridge	Beta Turn	Extended strand	Random Coil
Ochotona princeps	26.61%	0.00%	0.00%	14.16%	59.23%
Cavia porcellus	21.00%	0.00%	0.00%	11.00%	68.00%
Lynx Canadensis	26.83%	0.00%	0.00%	12.20%	60.98%
Bos Taurus	21.08%	0.00%	0.00%	17.75%	61.27%
Ovis aries	20.90%	0.00%	0.00%	14.93%	64.18%
Camelus ferus	19.90%	0.00%	0.00%	16.40%	63.68%
Capra hircus	20.90%	0.00%	0.00%	12.94%	66.17%
Pteropus Alecto	18.36%	0.00%	0.00%	13.53%	68.12%
Homo sapiens	13.66%	0.00%	0.00%	13.17%	73.17%
Gorilla gorilla	13.66%	0.00%	0.00%	13.17	73.17%
Erinaceus europaeus	16.58%	0.00%	0.00%	17.59%	65.83%
Suncus etruscus	18.66%	0.00%	0.00%	12.44%	68.90%

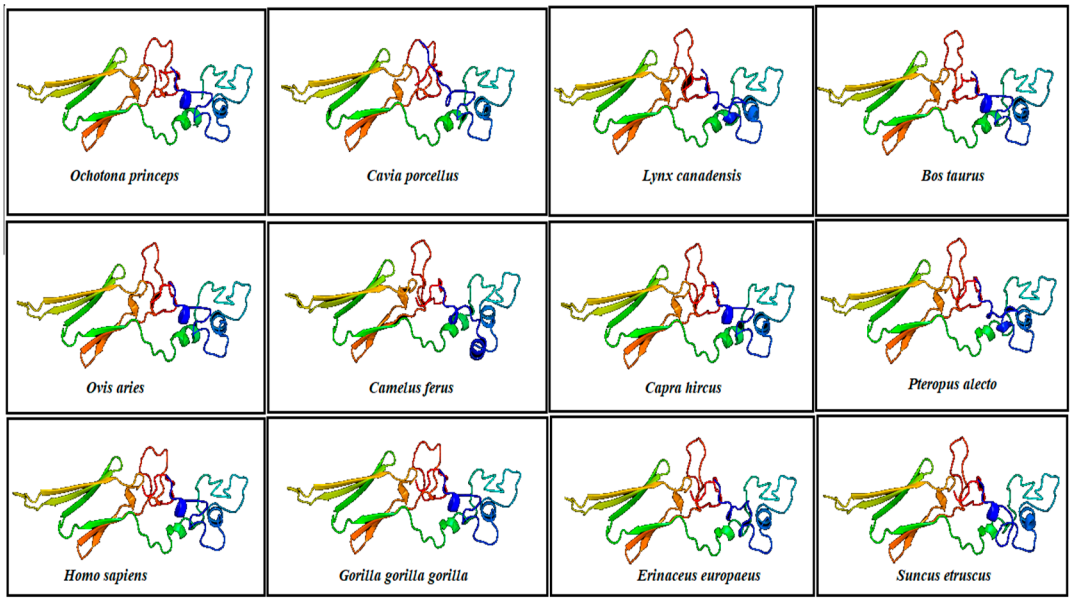

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Figure 6. Protein tertiary structures predicted by Phyre2.

Discussion

Heat shock protein 27, also known as HSPB1, is a member of the family of proteins known as small heat shock proteins, or sHSPs. This protein contributes to the development of a stable state during times of high stress, such as when the body is subjected to heat shock or oxidative damage [20]. The findings of this study were derived from a computational analysis of the HSPB1 protein found in a variety of species. During this computational analysis, a number of analyses were carried out in order to identify Domains, multiple sequence alignment, physiochemical properties, motifs, quaternary structure, secondary structure parameters, protein interaction with its functional proteins in a variety of organisms, similarity and identity matrix, and the Position and composition of conserved elements, introns, exons in protein.

A gene family's genetic characterization can reveal details about the genes' origins, duplication events, and diversification throughout their evolutionary history. The conserved regions, which are crucial for protein structure and functional studies, can be found using multiple sequence alignment (MSA) [21]. In nucleus majority of the proteins were found, while the mitochondria only contained a small percentage. Except for *B. taurus* and *O. princeps*, all creatures had just CDS parts, downstream and upstream in their protein sequences. These two species have CDS parts, Introns, downstream and upstream in their protein sequences. When referring to the relative placement of the genetic code in DNA or RNA, the terms "downstream" and "upstream" are both used. Upstream is towards the 5' end of the coding strand of the protein that is now being analyzed, while downstream is towards the 3' end of the coding strand. Both ACD_HSPB1 IPR037876 and A Crystalline IPR002068 were identified as the domains, present in each and every organism. The homologous superfamily found in all organisms was designated as IPR008978 Hsp20. The motif from 1 to 7 was found in the protein sequences of all organisms, with the exception of *E. europaeus*. Only the protein sequences of two different organisms, namely *S. etruscus* and *O. princeps*, contained the Motif 8 pattern. Two more organisms, namely *P. alecto* and *O. princeps*, were found to have a motif 9 in their DNA. While Motif 10 was unique to *O. princeps*, it was detected in both species. Furthermore, the varied patterns of the introns and downstream/upstream untranslated regions (UTRs) in the gene structure suggest that it may be due to the inclusion and removal of retroposons [22]. A prior study [23] was also responsible for collecting amino acids, determining % similarity and identity, phylogenetic relationship, determining physiochemical qualities, and identifying motifs.

The phylogenetic tree revealed the presence of five distinct clades. In clade 1(represented by blue color), the species *S. etruscus* and *E. europaeus* share common ancestor, indicating their close evolutionary relationship. In the second clade (marked in red), *H. sapiens* and *G. gorillas* are closely related, as evidenced by to their shared common ancestor. The third clade, colored peach, consists of *C. porcellus*, *L. canadensis*, and *O. princeps*, which all originated from a common ancestor, highlighting their evolutionary proximity. The fourth clade includes *B. taurus*, *O. aries*, *C. ferus*, and *C. hircus*, which are all closely related, stemming from a shared lineage. Lastly, *P. alecto* is depicted as a distinct entity in clade 5 (marked in purple), isolated from other species, despite being located within this clade. The phylogenetic analysis also revealed a high degree of sequence homology among the species, reinforcing their evolutionary relationships as observed in other studies [24].

Furthermore, assessing the physiochemical characteristics of the gene encoding the gene is necessary to comprehend the protein structure and function of the gene family [25]. According to the findings, every organism has a greater tendency towards hydrophilicity. The aliphatic index is a measure that may be used to evaluate the thermal stability of proteins; in this case, its value was greater than 50% for every protein. This suggests that aliphatic side amino acids covered over half of the volume of these proteins. In addition, GRAVY values that are lower than zero indicate that the hydrophilicity of these proteins is greater than that of their surroundings. The fact that all of the organisms have theoretical PI values that are lower than 7 demonstrates that they are all acidic in

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their natural state. Because the instability index for every creature was greater than 40, it may be deduced that every protein in every organism, with one exception, is unstable. Similarly, since GRAVY indicates a protein's hydrophilicity (GRAVY <0) or hydrophobicity (GRAVY >0), assessing it would aid in a better understanding of its tertiary structure or shape [26]. Similar genetic characterization was done in previous studies including the assembly of amino acids and nucleotides, the computation of similarity and identity matrices, the assessment of physiochemical properties, the analysis of evolutionary relationships, the characterization of structural features, and the generation of a hydrophobicity plot [27]. Hence in recent years, determining and quantifying the evolutionary processes that contributed to genetic variety has emerged as an exciting area of research.

The secondry structure of the HSPB1 gene in 12 species was predicted using GORIV tool, indicating the presence of alpha helices, random coils and extended strands. *L. canadensis* showed the highest alpha helix content, while *H. sapiens* and *G. gorillas* had the largest proportion of random coils. The three-dimensional models generated for the HSPB1 protein provided insights into its folding. Stability and domain interactions. These findings align with the previous studies on protein structure-function relationship and emphasize the evolutionary conservation of the HSPB1 gene across the species [28,29].

Conclusion

This study provides comprehensive insights into the evolutionary history, functional motifs, physiochemical characteristics and structural traits of the HSPB1 gene across various species. The results revealed the vital role of HSPB1 proteins in cellular processes, emphasizinf their acidic nature and high sequence conservation. Phgylogenetic analysis highlighted the evolutionary relationship among species, depicting their shared ancestry, The GRAVY values pointed to the hydrophilic nature of these proteins while the aliphatic index indicated their heat stability, further emphasizing their functional significance. The study also unveiled conserved motifs and homologous superfamily memberships which underscore the functional conservation of HSPB1 gene across the species. Variations in genetic organization, like the presence of distinct upstream and downstream regions in certain species reflect the complexity of gene regulation. Furthermore, the presence of conserved regions within the supports the notion of functional similarity across the species. The identity and similarity matrices confirmed that the species with higher similarity percentages in the HSPB1 gene likely shre common functions and evolutionary backgrounds. The analysis of secondary structure, including the distribution of alpha helices, random coils and extended strands, provided valuable insights on the protein folding, stability and domain interactions, the three-dimensional structure of HSPB1 protein offered further insights into its functional architecture. This work identifies HSPB1 as functionally significant and underscores the need for additional research to fully comprehend the function and mechanism of HSPs.

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