Vol. 22, No. 2, Summer, 2026, 68-79 *Original Article*
DOI: 10.22055/ivj.2025.536829.2823
Journal Homepage: http://www.ivj.ir/

Characterization and phylogenetic analysis of a cDNA encoding an EF-hand domain-containing calumenin-like protein in *Strongyloides ratti*

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Received: 24.07.2025 Accepted: 16.10.2025

Abstract

Strongyloides ratti serves as an experimental model for diagnosing strongyloidiasis in humans. The secretory EF-hand-containing calumenin-like protein functions as calcium signaling molecule from rat was characterized. An 849-nucleotide cDNA fragment was amplified using RT-PCR, and a GenBank search revealed that the amplified product shared 98.54% identity with an incomplete mRNA sequence for *S. ratti*. Using this fragment as a probe, a search was conducted in the Expressed Sequence Tags (EST) database, identifying a 998 bp cDNA fragment through overlapping regions designated as *Sr*CAL. The encoded protein consists of 314 amino acids and has a molecular weight of 36.74 kDa, and an isoelectric point (pI) of 4.61. The sequence includes a signal peptide, which has a cleavage site located between amino acids 21 and 22. A protein domain database search identified five highly conserved EF-hand calcium-binding motifs (cd16226), classifying *Sr*CAL within the Efh_CREC_Calumenin-like protein superfamily, spanning amino acids 29 to 296. Structural prediction revealed that *Sr*CAL is composed of 39.18% alpha-helices, 18.21% beta-strands, and 42.61% loops. Three dimensional (3D) structural modeling focused on 232 amino acids, covering 74% of the total coding sequence, with 99.8% confidence, showing strong structural resemblance to the crystal structure of an EF-hand protein from *Danio rerio*. Genetic distance and phylogenetic analysis revealed that *Sr*CAL shares the lowest genetic distance (0.19%) with the only known protein sequence isolated from *S. ratti* (XP_024499492.1). *Sr*CLA plays a crucial role in calcium signaling and may serve as a housekeeping gene with strong potential as a nematode-specific drug target.

Key words: Nematodes, EF-hand domain, *Strongyloides ratti*, RT-PCR, Calumenin-like proteins

Introduction

Strongyloides ratti is a soil-transmitted gastrointestinal nematode that primarily infects rodents. This parasite exhibits a unique life cycle, alternating between free-living non-parasitic and obligatory parasitic generations (Buonfrate, et al, 2020). During infection, infective L3 larvae (iL3) penetrate the host's skin, migrate to the lungs, and eventually reach the small intestine, where they mature into parthenogenetic adult female worms that produce eggs. Due to its biological similarities, S. ratti is maintained in laboratories as a model organism for *S. stercoralis*, a human-infecting nematode responsible for strongyloidiasis, a neglected tropical disease ranked among the most widespread human infections (Viney and Kikuchi, 2017; Hotez et al, 2008). Despite belonging to different phylogenetic clades, nematodes such as *Ascaris*, *Toxocara*, *Necator*, and *Strongyloides* follow similar

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migration patterns and elicit comparable immune responses in their hosts (Maizels et al, 2009; Finkelman et al, 2004).

The EF-hand calcium-binding loop motif is a structural domain found in various calcium-binding proteins, including calumenin. Also known as crocalbin or CBP-50, calumenin plays a critical role in protein folding and trafficking within the endoplasmic reticulum (ER). Similarly, RCN-1 and RCN-3 are involved in calcium-dependent processes, influencing cell signaling and protein interactions (Grabarek, 2006). The calumenin-like protein family contains five EF-hand motifs in *Caenorhabditis elegans* and six in Homo sapiens, exhibiting a relatively low affinity for Ca^{2+} (Cho et al, 2009; Yabe et al, 1997). These proteins play essential roles in intracellular calcium regulation, contributing to various cellular functions.

The EF-hand protein family has been identified in a diverse range of organisms, including bacteria, protozoa, helminths, arthropods, and mammals (Nagamune et al, 2008; Orans et al, 2010; Chazin, 2011). Genomic analysis estimates that 250 EF-hand genes exist in plant species such as Arabidopsis (Day et al, 2002). Several secretory acidic and rich in cysteine (SPARC) Ca^{2+}-binding proteins have been cloned from cDNA libraries of parasitic nematodes *Strongyloides ratti* (Anandarajah et al, 2016) and *Necator americanus* (Mason et al, 2014), as well as trematodes (Ruiz de Eguino et al, 1999) and *Clonorchis sinensis* (Zhou et al, 2013). These proteins are involved in host-parasite interactions. In Caenorhabditis elegans, calumenin plays a role in Ca^{2+}-regulated behaviors, including pharyngeal pumping and defecation (Cho et al, 2009). Experimental studies have demonstrated that CaM-null individuals of *Drosophila melanogaster* survive embryogenesis but die within two days of hatching as first-instar larvae (Heiman et al, 1996). Furthermore, the previous research using RNA interference (RNAi) to deplete the single nematode CaM gene (cmd-1) resulted in lethal embryos, exhibiting abnormalities in morphogenesis, cell migration, apoptosis, cell proliferation, and cytokinesis (Karabinos et al, 2015).

In this study, we successfully identified a full-length cDNA sequence from *S. ratti*, encoding an EF-hand calcium-binding protein. This highlights the functional significance of EF-hand calcium-binding proteins, particularly calumenin-like proteins, in mediating host-parasite interactions and regulating calcium-dependent cellular processes

Materials and Methods

Total RNA isolation and cDNA Synthesis

Total RNA was extracted from approximately 100,000 infective larvae (iL3) of *S. ratti* (Anandarajah et al, 2016) using RNX Plus solution (Cinnagen, Iran), following the manufacturer's protocol. A 12 µL aliquot, containing 2 µg of total RNA, was incubated with 0.5 µg of oligo(dT) primer at 70°C for 10 minutes, followed by brief centrifugation. The reaction was then placed on ice before adding 1 µL of RNasin (Cinnagen, Iran), 1 µL of a 120 mM dNTP mixture, 2.5 µL of 5X enzyme buffer, and 1 µL (200 units) of Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase. The mixture was subsequently incubated at 42°C for 1 hour, followed by heat inactivation at 70°C.

RT-PCR amplification

The selected coding region was amplified using specific primers: CAL-F (5´-GAACATGGACATGGAAGTGAACA) and CAL-R (5´-ATCATGTTTTTGAAGTTGCTCACC), designed based on a partial mRNA sequence encoding the EF-hand domain (XM_024643085) using the Primer3Plus program. The PCR reaction contained 5 µL of the reverse transcription product, 0.2 µM of each primer, 250 µM of each dNTP, and

1 U of *Taq* DNA polymerase in a standard PCR buffer. Amplification conditions included an initial denaturation at 94°C for 3 minutes, followed by 30 cycles of 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 7 minutes. The resulting PCR products were analyzed using electrophoresis on a 1% (w/v) agarose gel to assess amplification efficiency.

DNA sequence analysis

The amplified cDNA fragments were sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Homology searches were conducted using the NCBI BLAST program available at NCBI. The complete cDNA sequences were assembled from overlapping fragments. Primers were designed using the Primer3Plus program (www.primer3plus.com). For sequence analysis, multiple alignments were performed using CLUSTAL_W (Thompson et al, 1994) and refined using BOXSHADE software (www.ch.embnet.org/software/BOX_form.html). The theoretical molecular weight (MW) and isoelectric point (pI) was calculated using ExPASy-Compute pI/Mw tool (web.expasy.org/compute_pi/). Protein domain predictions were performed using ExPASy Prosite (prosite.expasy.org/scanprosite), and DeepLoc 2.0 (services.healthtech.dtu.dk/services/DeepLoc-2.0/) was used to determine subcellular localization of eukaryotic proteins. Phylogenetic analysis and genetic distance estimations were carried out using the neighbor-joining method (Saitou, 1987) with 1,000 bootstrap replicates in MEGA11 software (Tamura et al, 2021). Three-dimensional structure predictions were generated using the Phyre2 program (Kelley and Sternberg, 2009).

Results

RT-PCR amplification and sequence analysis

RT-PCR amplification was performed using aliquots of *S. ratti* cDNA as the template, yielding an 849 bp amplified fragment (Figure 1). The resulting product was consistent with the expected sequence, as predicted through manual calculation, confirming the accuracy of the amplification process.

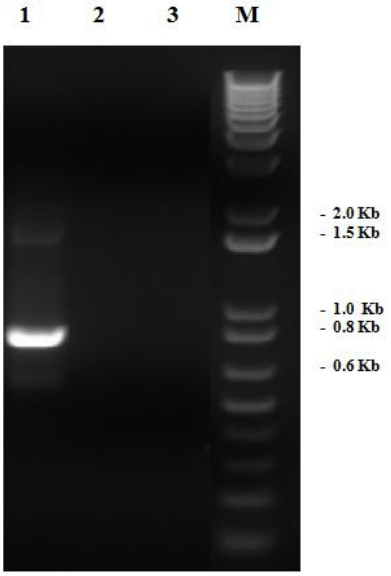


Figure 1: Agarose gel electrophoresis of RT-PCR products for the SrCLA transcript from infective *S. ratti* larvae. Lanes: M, 1 Kb plus DNA ladder; 1, RT-PCR amplification products; 2. No-template negative control; 3-empty.

To compare the amplified cDNA fragment with existing sequences, the BLASTn program available on the NCBI website was used with the Highly Similar Sequences (Megablast) algorithm. Sequence analysis revealed that the amplified fragment exhibited 99.06% identity to the only available *S. ratti* sequence, consisting of a 945 bp partial mRNA encoding an EF-hand domain (XM_024643085). Additionally, the amplified sequence showed identity to the *S. papillosus* genome assembly (LM525584) and a partial mRNA sequence from *Brugia malayi* (XM_043073392) encoding an EF hand family protein (Bma-

calu-1) at 90.26% and 76.03 %, respectively. Furthermore, a search of the Expressed Sequence Tags (EST) database using the amplified sequence as a probe identified four overlapping sequences: FC821077 (919 bp), FC821696 (832 bp), BI450405.1 (515 bp), and BI142485.1 (521 bp). The complete nucleotide sequence of *Sr*CAL was assembled from overlapping cDNA fragments. Sequence analysis revealed that the full-length *Sr*CAL sequence consists of 998 bp, encompassing the complete coding region along with 9 bp and 44 bp non-coding regions at the 5´ and 3´ ends, respectively (Figure 2). The start codon (nucleotides 10-12) and stop codon (952-954) were identified within the sequence. The open reading frame (ORF) spans 942 nucleotides, encoding a 314-amino acid protein with a molecular mass of 36.74 kDa and an isoelectric point (pI) of 4.61. The protein sequence contains a complete ORF of 291 amino acids, categorized into aliphatic (84, 26.75%), aromatic (28, 8.92%), basic (54, 17.2%), and acidic (98, 31.21%) side groups. Among these, glutamate is the most abundant amino acid (40, 12.74%), whereas cysteine is the least represented (2, 0.64%). The total number of negatively charged residues (Asp + Glu) is 78, while positively charged residues (Arg + Lys) amount to 40.

```
ccagttgagA TGCGTTTTTT AATTTTAATA TCTCTTTTAC TTATTGGCAT TACATTAGCT      60
GAACATCATA CTGATCCATT ATCTGATAAA GAACATGGAC ATGGAAGTGA ACATAATAAA     120
CAATATGATC ATGAAGCTTT CTTAGGTAAG GATACTGCTG CTGAATTTGA TGAATTAACA     180
CCTGAAAAAT CTAAAGAAAA ACTTGCTTCA CTTATTCCAA AAATGGATAG TAATGGTGAT     240
GGATTTGTTG AAGAGGATGA ACTTAGAAAT CATATTAATT TTATGCAACA AAGATATGTT     300
AATAATGATG TTGAACGTAC ATGGAAAAAT TATAAAGCTG AAGAGATAGC TGATGGTAAA     360
TTGTCATGGG CTGATTATAG AGAAATTGTT TATAGACATC CAACAGGTGA AGGACAAGAT     420
TTATCTGATG AGTATAAAAA AATGATTACT AGAGATGAAC GTAGATGGAA AGTTGCTGAT     480
TATGATTCAG ATGAAAAATT AGATAGAACT GAGTATGGAT GTTTTATGCA CCCTGAAGAT     540
TGTGAATCAA TGAGAGATGT TGTTGCTACA GAAACTATTG AAGATATTGA TAAAAATAAG     600
GATGGATTTG TTGATATTAA TGAATATATT AGTGATATGT ATAGACCAGA AGATTATCCT     660
GAATTAAATG GTAAAGAACC AGAATGGGTT GAATCTGAAA GAGAACTTTT CAAAGAACAT     720
CGTGATAAGA ATAATGATGG TAAATTAGAT CGTGATGAGA TGCGTGATTG GATTATGCCA     780
ATTGGTTTTG ATCATGCTGA TGCTGAGGCA AAGCATTTAA TTGGAATTGC TGATGAAAAT     840
AAGGATGGAA AACTTTCACC AGAAGAGATT ATCCAACATT ATGATACATT TGTTGGATCA     900
CAAGCAACTG ATTATGGTGA GCAACTTTCA AAACATGATC CATCTGAATT ATAAaaaaaa     960
aaaaaaaaaa ctcgagacta gttctctctt tctctctc                            998
```

Figure 2: Nucleotide sequence of *Sr*CLA. Non-coding sequences are in lowercase, start and stop codons are bolded, and primer binding sites are underlined.

A protein domain database search identified a highly conserved domain containing the EF-hand calcium-binding motif (cd16226), with an e-value of 4.23e-124, classifying it within the Efh_CREC_Calumenin-like protein superfamily spanning amino acids 29 to 296. Further domain analysis predicted the presence of five EF-hand Ca^{2+}-binding domains at residues 6-95, 144-179, 181-216, 239-261, and 262-297 in *Sr*CAL (Figure 3). This domain superfamily consists of five EF-hand structural units, each composed of two helices connected by a twelve-residue calcium-binding loop, essential for calcium coordination and protein function.

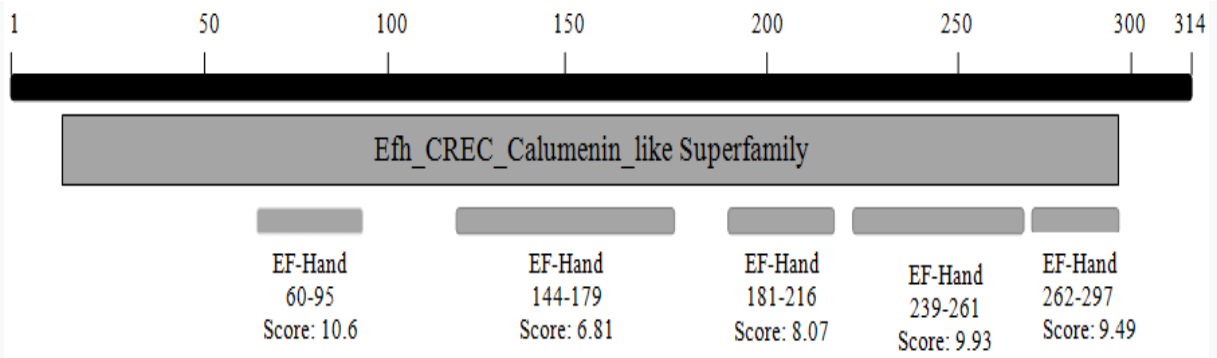


Figure 3: Schematic representation of the *Sr*CLA domain, highlighting its structural organization, key functional elements, and defining features essential for its biological role.

Taxonomic analysis of *Sr*CLA at the nucleotide level revealed similarities with three sequences within the *Rhabditida* order, two of which specifically belong to the genus *Strongyloides*. Among these, *S. ratti* exhibited the highest similarity, while the third sequence corresponded to *S. papillosus*. At the protein level, *Sr*CAL displayed homology with 126 sequences within the Nematode phylum, of which 105 were classified under the *Rhabditida* order. Within this subset, only three sequences were assigned to the *Strongyloidoidea* family, with two sequences specifically matching *S. ratti*.

Multiple alignments

The amino acid sequence identity of *Sr*CLA in aligned regions with *Mesorhabditis belari* (CAJ0935108.1), *Steinernema hermaphroditum* (KAK0423442.1), *Ostertagia ostertagi* (KAK6033036.1), *Ancylostoma caninum* (RCN51010.1), *Nippostrongylus brasiliensis* (WKY16943.1), and *Necator americanus* (XP_064070443.1) was 75.32%, 74.80%, 75.39%, 75.15%, 75.07%, and 75.20%, respectively (Figure 4). Sequence analysis using SignalP predicted that SrCAL contains a signal peptide, which is cleaved at Ala17-Glu18. Additionally, DeepLoc-2.0, a program for eukaryotic protein subcellular localization, determined that *Sr*CLA is primarily localized in the endoplasmic reticulum (ER) and is considered non-cytoplasmic with a probability of 0.8562. Further analysis revealed the presence of a C-terminal ER retention signal sequence (PSEL) at positions 311-314, consistent with the previous findings (Honore and Vorum 2000). The critical C-terminal residues (EL) are conserved, aligning with observations in other homologs (Yabe et al. 1997).

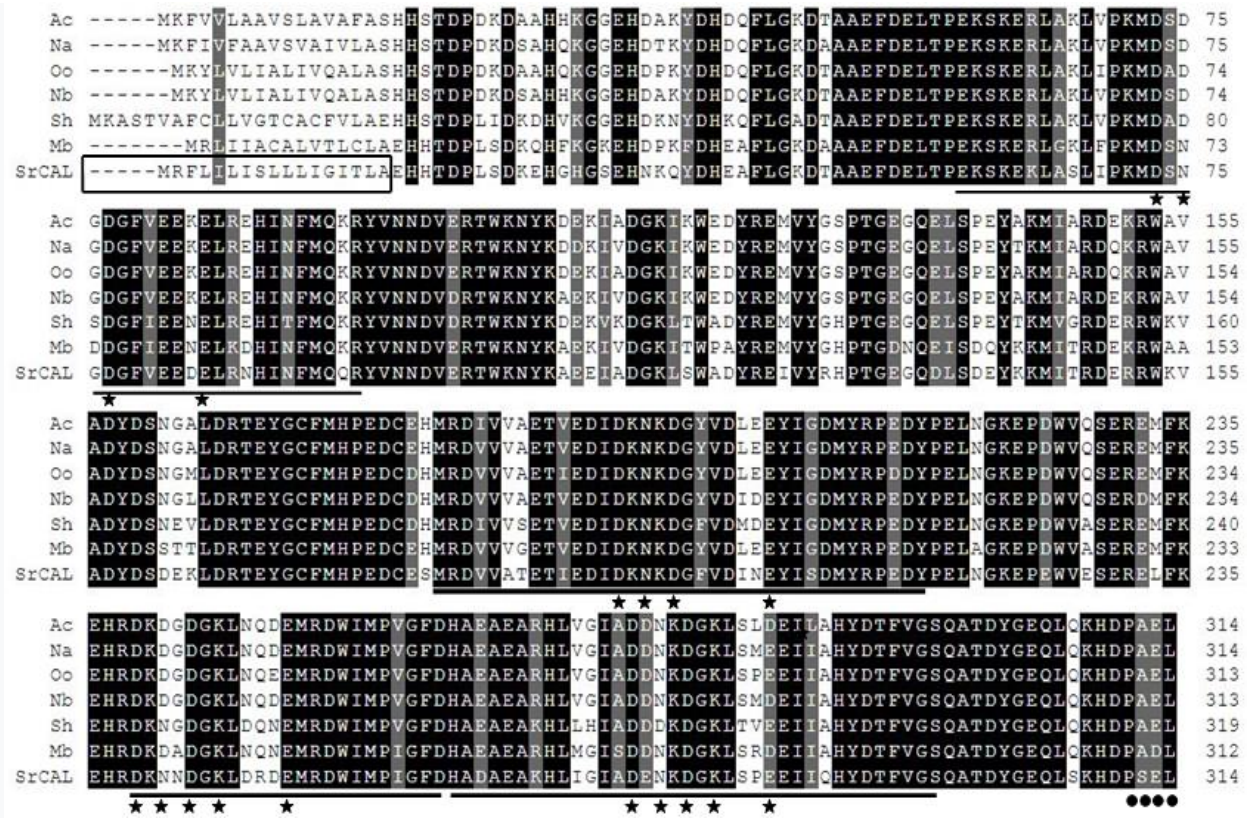
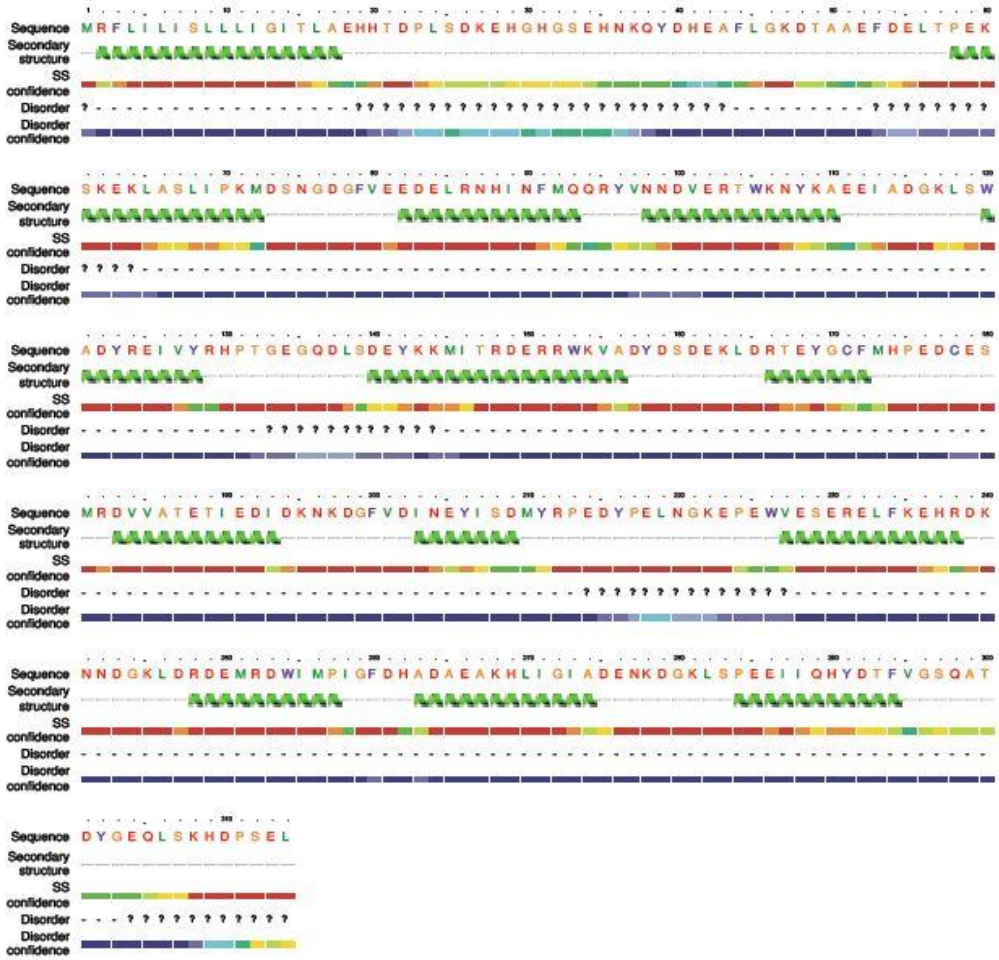


Figure 4: Multiple sequence alignment of the *Sr*CAL sequence with its homologous counterparts. The aligned sequences include *Ancylostoma caninum* (Ac), *Necator americanus* (Na), *Ostertagia ostertagi* (Oo), *Nippostrongylus brasiliensis* (Nb), *Steinernema hermaphroditum* (Sh), and *Mesorhabditis belari* (Mb). Identical residues are shaded black, while conservative substitutions are highlighted gray. The EF-hand domain is underlined, active sites are marked with stars, and the signal peptide is enclosed within a box. C-terminal tetra-peptides associated with endoplasmic reticulum (ER) retention are indicated by black circles.

Molecular characterization and secondary structure

The secondary structure of *Sr*CAL protein was predicted using the PSIPRED protein sequence analysis method, where each amino acid was assigned values for alpha-helix, beta-sheet, and loops within a 7-amino acid window (Figure 4). By applying these parameters and calculating the probability for each residue, the protein structure was predicted with high confidence. Further PSIPRED analysis determined that the *Sr*CAL sequence consists of 39.18% alpha-helices, 18.21% beta-sheets, and 42.61% loops, highlighting its structural composition (Figure 5A). Structurally, the EF-hand domain is arranged in a characteristic helix-loop-helix conformation, facilitating calcium-binding interactions. For 3D structural modeling, the analysis focused on 232 amino acids, covering 74% of the total coding sequence, with 99.8% confidence, revealing a strong resemblance to the crystal structure of an EF-hand protein from *Danio rerio* dr.36843 (Figure 5B).

A)



B)

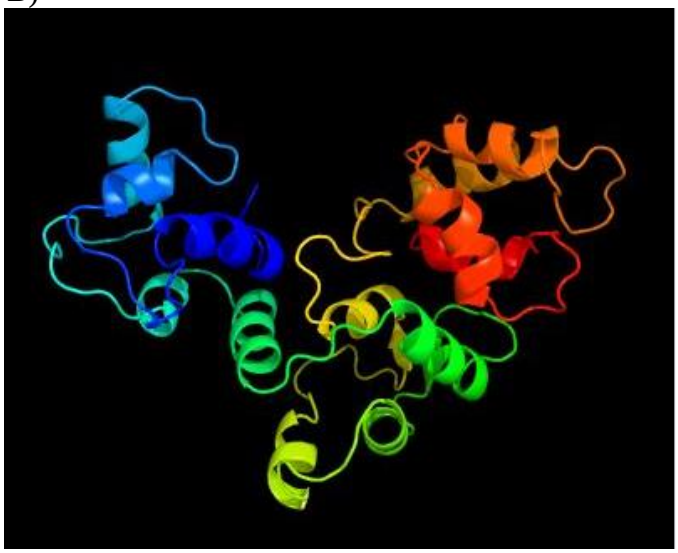


Figure 5: Structural visualization of *Sr*CAL, showcasing its secondary structure (A) alongside its three-dimensional (3D) molecular model (B).

Phylogenetic analysis and genetic distances

To examine *Sr*CLA within the broader context of EF-hand domain-containing proteins, a phylogenetic tree of the *Sr*CAL protein sequence was generated using the Neighbor-Joining method in MEGA11 software, incorporating 16 homologous sequences from the parasitic nematode family. As illustrated in Figure 6, the constructed phylogram reveals two distinct clusters. In the major cluster, *Sr*CAL is positioned alongside other nematode parasite sequences, forming a sub-cluster that groups exclusively with the only available *S. ratti* sequence (XP_024499492.1), supported by a moderate bootstrap value of 49%. Meanwhile, all filarial nematode parasites are categorized into a separate cluster, which is strongly supported by a bootstrap value of 96%, indicating a high degree of confidence in their phylogenetic grouping. To ensure accurate phylogenetic placement, *Trichinella papuae* (KRZ78707.1) was included as an out-group sequence, providing a reference point for evolutionary divergence.

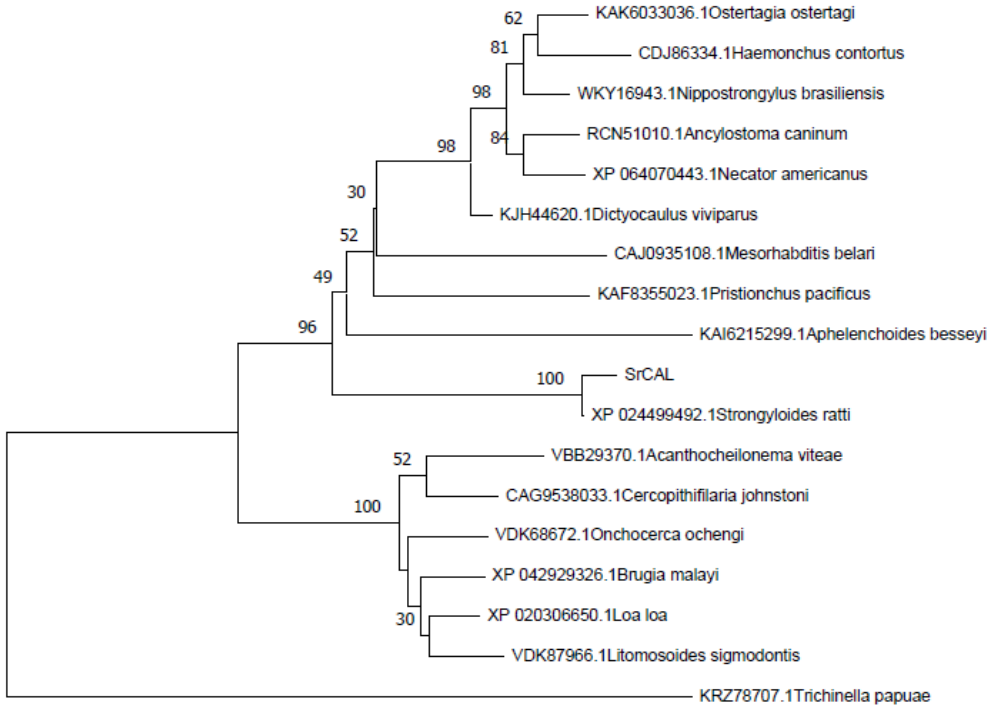


Figure 6: Phylogenetic analysis of *Sr*CLA amino acid sequences and their parasite homologs, constructed using the Neighbor-Joining method. Bootstrap values, derived from 1,000 replicates, indicate the reliability of branching patterns. Numbers preceding species names represent GenBank accession numbers corresponding to related gene variants, while the numbers above the branches depict phylogenetic relationships between groups.

As presented in Table 1, the genetic distance of *Sr*CLA from related EF-hand-containing calumenin-like protein sequences was calculated using MEGA11 software. Among the 14 homologous sequences available in GenBank, *Sr*CAL exhibited the lowest genetic distance (0.19%) with the only known protein sequence isolated from Strongyloides ratti (XP_024499492.1). In contrast, the genetic distance between *Sr*CAL and other related nematode species ranged from 23.5% to 33.6%, highlighting significant sequence divergence within this family.

Table 1: Genetic pairwise distances of *Sr*CAL, comparing its sequence similarity to related S. ratti protein sequences

	1	2	3	4	5	6	7	8	9	10	11	12	13
1-SrCAL													
2-XP 024499492.1 S. ratti	0.019												
3-CAJ0935108.1 M. belari	0.275	0.262											
4-KAK0423442.1 S. hermaphroditum	0.274	0.257	0.271										
5-RCN51010.1 A. caninum	0.282	0.261	0.222	0.208									
6-KAK6033036.1 O. ostertagi	0.258	0.238	0.226	0.209	0.073								
7-WKY16943.1N. brasiliensis	0.262	0.242	0.218	0.201	0.066	0.059							
8-XP_064070443.1 N. americanus	0.282	0.261	0.206	0.208	0.059	0.076	0.076						
9-KAI1725696.1 D. destructor	0.267	0.255	0.251	0.239	0.205	0.222	0.226	0.217					
10-KAF8355023.1 P. pacificus	0.274	0.252	0.228	0.252	0.215	0.220	0.216	0.219	0.228				
11-CDJ86334.1 H. contortus	0.301	0.279	0.254	0.213	0.108	0.073	0.073	0.108	0.251	0.241			
12-KAI6215299.1 A. besseyi	0.336	0.318	0.306	0.331	0.301	0.297	0.302	0.292	0.284	0.292	0.333		
13-KJH44620.1 D. viviparus	0.235	0.210	0.186	0.186	0.071	0.067	0.058	0.071	0.193	0.172	0.093	0.230	
14-KRZ78707.1 T. papuae	0.645	0.627	0.693	0.636	0.649	0.645	0.652	0.649	0.664	0.663	0.671	0.693	0.529

Discussion

Parasitic worms have evolved elaborate strategies to thrive within diverse host environments. To improve their chances of survival, they secrete excretory/secretory proteins that effectively suppress the host's immune response. Recent advancements in *Strongyloides ratti* genome analysis have provided researchers with a vast dataset of Expressed Sequence Tags (ESTs), enabling deeper molecular investigations. A targeted approach to anti-parasitic drug development focuses on exploiting metabolic differences between parasites and hosts to design enzyme inhibitors, paving the way for effective therapeutic solutions.

Calumenin-like proteins appear to be conserved across a diverse range of nematode species. Homologs have been identified in both animal parasitic nematodes, including *Ascaris sum, Brugia malayi*, and *Trichinella spiralis*, as well as plant parasitic nematodes such as *Meloidogyne hapla* and *Bursaphelenchus xylophilus*. Additionally, calumenin homologs are present in free-living nematodes like *Caenorhabditis elegans* and *C. briggsae*. The broad distribution of these orthologs, including *C. elegans* protein (CAA96655.1), which has been linked to embryo development (Jantsch-Plunger and Glotzer, 1999), suggests that *Sr*CLA homologs may serve a fundamental housekeeping function in nematodes. However, the exact biological roles and mechanisms of these proteins remain to be fully elucidated.

Calumenin is an endoplasmic reticulum (ER)-resident protein that contains five EF-hand motifs in *Caenorhabditis elegans* and six in humans, exhibiting a relatively low affinity for Ca^{2+}. Calcium-binding proteins generally contain two to eight EF-hand motifs, each consisting of a paired EF-hand structure that facilitates Ca^{2+} ion coordination, emphasizing their functional importance (Nakayama *et al.*, 2000). Multiple sequence alignment and phylogenetic analysis of *Sr*Cal confirm that it encodes an EF-hand domain-containing protein. The findings indicate that *Sr*Cal belongs to the Calumenin-like protein subfamily, with a high degree of conservation in both soil-transmitted gastrointestinal nematodes and parasitic filarial nematodes. Given its strong evolutionary preservation, developing calumenin-targeting drugs could represent a promising strategy for treating lymphatic filariasis and gastrointestinal nematode infections, including *Strongyloides*.

The presence of the signal peptide in the N-terminal region of *Sr*CLA indicates that this protein is secreted from the cell as an extracellular protein. The results indicate that *Sr*CLA contains a signal peptide, a short amino acid sequence located at the N-

terminus of the protein, which directs it to the endoplasmic reticulum (ER) during translation. Also, the retention signal (PSEL) at the C-terminus part of *Sr*CLA sequence ensures its ER localization, preventing its unrestricted distribution in the cytoplasm (Yabe et al, 1997).

Procollagen biosynthesis involves chaperones and folding-modifying enzymes, which continue to function after secretion from the endoplasmic reticulum (ER) (Lamande and Bateman, 1999). ER-resident enzymes such as protein disulfide isomerase (PDI) and peptidyl prolyl cis-trans isomerase (PPIase) play essential roles in procollagen modification, and their activity has been reported to be regulated by Ca^{2+} (Primm et al, 1996). Given this, it is plausible that *Sr*CLA may contribute to maintaining ER Ca^{2+} levels, thereby influencing the enzymatic processes required for proper procollagen modification. Additionally, studies have shown that calumenin mutants in *Caenorhabditis elegans* exhibit reduced fertility and severe cuticle defects (Cho et al, 2009). Due to its involvement in cuticle formation, *C. elegans* calumenin has been proposed as a potential drug target for human-infective nematodes, particularly filarial worms (Choi et al, 2018). It is also possible that *Sr*CLA plays a possible role in various cuticle development processes.

In conclusion, Calumenin-like proteins are gaining increasing research interest due to their involvement in various cellular functions as calcium signaling molecules. However, their physiological and pathological mechanisms are not yet fully understood. Due to their essential role as housekeeping genes in related nematodes and their high conservation across soil-transmitted gastrointestinal, the proteins in this parasite are considered as potential candidates for nematode-specific drug targets.

Acknowledgments

This study was financially supported by a research grant from the Vice President of Research Affairs Office at Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Conflict of interest

The authors declare no conflicts of interest.

Funding

The authors declare that no funding was received from any private or commercial organization.

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Received: 24.07.2025
Accepted: 16.10.2025

DOI: 10.22055/ivj.2025.536829.2823

شناسایی و بررسی فیلوژنتیک یک cDNA کدکننده پروتئین کالومنین حاوی دامنه EF-hand در *Strongyloides ratti*

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تاریخ دریافت: ۱۴۰۴/۵/۲ تاریخ پذیرش: ۱۴۰۴/۷/۳۰

چکیده

Strongyloides ratti به عنوان یک مدل تجربی برای تشخیص استرونژیلوئیداز در انسان عمل می‌کند. در این مطالعه پروتئین ترشحی کالومنین حاوی دامنه EF-hand از رت که به عنوان یک مولکول سیگنالینگ کلسیم عمل می‌کند، توالی یابی و شناسایی گردید. یک قطعه ژن به طول ۸۴۸ نوکلئوتید با RT-PCR افزوده سازی شد. در جستجوی بانک ژن نشان داده شد که این محصول ۹۸/۵۴ درصد با یک توالی mRNA ناکامل از *Strongyloides ratti* مشابهت داشت. با استفاده از این قطعه ژن به عنوان جستجوگر، پایگاه داده ژنی Expressed Sequence Tags (EST) مورد جستجو قرار گرفت که متعاقب آن، یک قطعه ژن ۹۹۸ نوکلئوتیدی از طریق مناطق همپوشان شناسایی شد که *Sr*CAL نامگذاری گردید. این ژن یک پروتئین شامل ۳۱۴ اسید آمینه را کد می‌کند که وزن مولکولی آن ۳۶/۷۴ کیلو دالتون و نقطه ایزوالکتریک (pI) آن ۴/۶۱ است. این پروتئین حاوی یک پپتید سیگنال با محل برش بین اسیدهای آمینه ۲۱-۲۲ می‌باشد. جستجوی در پایگاه داده‌های ژنی نشان داد که این پروتئین دارای پنج دامنه EF-hand (cd16226) به منظور اتصال به کلسیم است که بسیار حفاظت شده می‌باشند. نتایج نشان داد که *Sr*CAL بر اساس تشابه اسید آمینه‌های بین ۲۹ تا ۲۹۶ در ابرخانواده پروتئین‌های Efh_CREC_Calumenin طبقه‌بندی می‌شود. پیش‌بینی ساختار ثانویه پروتئینی نشان داد که *Sr*CAL از ۳۹/۱۸ درصد مارپیچ آلفا، ۲۱/۱۸ درصد رشته‌های بتا و ۴۲/۶۱ درصد لوپ تشکیل شده است. مدل‌سازی ساختار سه‌بعدی که با استفاده از ۲۳۲ اسید آمینه که ۷۴ درصد از کل توالی ناحیه کدگذاری شده را شامل می‌شود، نشان داد که این پروتئین با ۹۹/۸ درصد اطمینان مشابهت با ساختار کریستالی یک پروتئین حاوی دامنه EF-hand از *Danio rerio* می‌باشد. بررسی فیلوژنتیک *Sr*CAL کم‌ترین فاصله ژنتیکی (۰/۱۹ درصد) را با تنها توالی پروتئینی موجود جدا شده از *S. ratti* (XP_024499492.1) دارد. *Sr*CAL می‌تواند نقش مهمی در سیگنالینگ کلسیم ایفا کند که ممکن است به عنوان یک ژن خانه‌دار با پتانسیل قوی به عنوان یک هدف دارویی اختصاصی نماتودی عمل کند.

کلمات کلیدی: نماتود، دامنه EF-hand، *Strongyloides ratti*، RT-PCR، کالومنین

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