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Integrated Bioinformatics Characterization of the Hypothetical Protein RvY_05612 from Ramazzottius varieornatus

Arifa Jahan Bithi
East West University https://orcid.org/0009-0001-4105-9696
Mahatab Hasan Rahat
rahathasanmahatab@gmail.com

East West University

Research Article

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Abstract

Background: The genome of the extremotolerant tardigrade *Ramazzottius varieornatus* contains numerous hypothetical proteins with unknown biological functions. Computational annotation of these proteins is essential for understanding their structural and functional roles. This study aimed to characterize the hypothetical protein RvY_05612 using an integrated bioinformatics approach.

Methods: The amino acid sequence of RvY_05612 was retrieved from the NCBI database and analyzed using multiple bioinformatics tools. Physicochemical properties were evaluated with ExPASy ProtParam, conserved domains were identified using the Conserved Domain Database (CDD), sequence homology was assessed through BLASTp and HHpred, secondary structure was predicted using SOPMA and PSIPRED, three-dimensional structure was modeled with SWISS-MODEL, and functional annotation and subcellular localization were predicted using UniProt, InterPro, and CELLO.

Results: RvY_05612 was identified as a 98-amino-acid basic protein with a predicted molecular weight of 9.86 kDa and a theoretical isoelectric point of 11.65. Conserved domain analysis identified a highly significant Sec61β domain (PF03911), while homology analyses demonstrated strong similarity to Sec61β proteins from other eukaryotes. Structural analyses predicted a predominantly α-helical protein containing a transmembrane helix. Functional annotation and localization analyses consistently indicated that RvY_05612 is associated with the endoplasmic reticulum membrane and likely participates in intracellular protein transport.

Conclusion: Integrated computational analyses support the annotation of RvY_05612 as a putative Sec61β family protein involved in the endoplasmic reticulum translocon complex. These findings provide a comprehensive computational characterization of this previously uncharacterized protein and establish a foundation for future experimental validation.

1. Introduction

Tardigrades, commonly known as water bears, are microscopic ecdysozoan invertebrates renowned for their extraordinary ability to survive a wide range of environmental extremes, including desiccation, ionizing radiation, ultraviolet exposure, extreme temperatures, high hydrostatic pressure, and even the vacuum of outer space (Hashimoto et al., 2016). Their remarkable resilience is largely attributed to cryptobiosis, a reversible ametabolic state in which cellular metabolism is drastically reduced, allowing survival under otherwise lethal conditions. Because of these exceptional physiological adaptations, tardigrades have become valuable model organisms for investigating molecular mechanisms of stress tolerance, genome stability, and cellular protection, with potential applications in biotechnology, medicine, agriculture, and astrobiology (Hashimoto et al., 2016).

The publication of the *Ramazzottius varieornatus* genome has significantly advanced the understanding of tardigrade biology by revealing numerous genes associated with stress tolerance and identifying several proteins that appear to be unique to tardigrades (Hashimoto et al., 2016). These include proteins

involved in DNA protection, antioxidant defense, and cellular homeostasis, many of which have been implicated in the organism's exceptional resistance to environmental stress. Despite these advances, a substantial proportion of predicted proteins encoded within the genome remain annotated as hypothetical proteins. These proteins are identified through computational gene prediction but lack experimental evidence regarding their biological function, molecular interactions, or cellular localization. Consequently, functional annotation of hypothetical proteins remains an important challenge in modern genomics and proteomics.

The rapid accumulation of genome sequencing data has substantially increased the number of hypothetical proteins deposited in public biological databases. Although these proteins represent a significant fraction of predicted proteomes across diverse organisms, many remain functionally uncharacterized due to the absence of experimental validation. Computational approaches have therefore become indispensable for predicting the structural and functional properties of hypothetical proteins. Integrated bioinformatics analyses combining sequence similarity searches, conserved domain identification, structural modeling, evolutionary analysis, and functional annotation provide an efficient strategy for inferring protein function and prioritizing candidates for future experimental investigation (Lu et al., 2020; The UniProt Consortium, 2023; Varadi et al., 2022).

Among the computational methods available, physicochemical characterization provides preliminary insights into protein stability, charge distribution, hydrophobicity, and potential biological behavior. Conserved domain analysis enables identification of evolutionarily conserved functional regions that frequently correspond to catalytic or structural elements, while homology-based approaches such as BLASTp and HHpred facilitate the detection of homologous proteins with known biological functions (Söding, 2005). Furthermore, advances in protein structure prediction and homology modeling have greatly enhanced the accuracy of functional inference by enabling visualization of three-dimensional protein architectures, particularly when combined with resources such as SWISS-MODEL and the AlphaFold Protein Structure Database (Varadi et al., 2022; Waterhouse et al., 2018). Functional annotation using integrated databases such as UniProt and InterPro, together with Gene Ontology (GO) classification and subcellular localization prediction, further strengthens computational predictions by providing biological context for newly characterized proteins (The UniProt Consortium, 2023).

The hypothetical protein RvY_05612 from *Ramazzottius varieornatus* is currently annotated with limited functional information despite sequence evidence suggesting similarity to members of the Sec61β protein family. The Sec61 complex is an evolutionarily conserved heterotrimeric protein translocation channel located within the endoplasmic reticulum membrane of eukaryotic cells and is responsible for mediating the translocation and integration of newly synthesized proteins into the secretory pathway (Lewis et al., 2024). The β-subunit, although smaller than the α-subunit, contributes to stabilization of the translocon complex and participates in efficient protein translocation and membrane organization. Identifying whether RvY_05612 belongs to this conserved protein family may improve our understanding of protein transport systems in tardigrades and provide additional insights into the molecular components that support cellular function in extremotolerant organisms.

Therefore, the present study employed an integrated bioinformatics workflow to comprehensively characterize the hypothetical protein RvY_05612 from *Ramazzottius varieornatus*. Multiple computational approaches were applied to evaluate its physicochemical properties, conserved domains, evolutionary relationships, secondary and tertiary structural features, predicted cellular localization, and putative biological function. The findings provide a comprehensive computational annotation of RvY_05612 and establish a foundation for future experimental studies aimed at validating its biological role.

2. Materials and Methods

2.1 Protein Sequence Retrieval

The amino acid sequence of the hypothetical protein RvY_05612 from *Ramazzottius varieornatus* was retrieved from the National Center for Biotechnology Information (NCBI) protein database using the GenBank accession number GAU93716.1. Protein identity, sequence length, taxonomic information, and associated annotations were further verified using the UniProt Knowledgebase (UniProtKB). The retrieved FASTA sequence served as the input for all subsequent computational analyses.

2.2 Physicochemical Characterization

The physicochemical properties of RvY_05612 were analyzed using the ExPASy ProtParam server. Parameters including amino acid composition, molecular weight, theoretical isoelectric point (pI), instability index, aliphatic index, grand average of hydropathicity (GRAVY), estimated half-life, and the number of positively and negatively charged residues were calculated directly from the amino acid sequence. These parameters were used to evaluate the basic biochemical characteristics, stability, and hydrophobic nature of the protein.

2.3 Conserved Domain Analysis

To identify conserved functional domains, the protein sequence was analyzed using the Conserved Domain Database (CDD) available through the National Center for Biotechnology Information. Conserved domains were identified based on sequence similarity to curated protein families, and the corresponding domain architecture, domain location, and statistical significance (E-value) were recorded. The identified domains were used to infer the putative biological function of the protein.

2.4 Homology Analysis

Sequence homology was investigated using the Basic Local Alignment Search Tool for proteins (BLASTp) against the NCBI non-redundant protein database. Homologous proteins were identified based on sequence similarity, allowing evaluation of the evolutionary conservation of RvY_05612 across different organisms.

To improve the sensitivity of homology detection, the amino acid sequence was further analyzed using HHpred, which employs hidden Markov model (HMM)-based profile comparisons to identify remote

homologous proteins and structural templates. The highest-scoring homologous proteins, probability scores, E-values, and predicted structural features were recorded for functional inference.

2.5 Secondary Structure Prediction

The secondary structural elements of RvY_05612 were predicted using SOPMA and PSIPRED. These tools estimate the distribution of α-helices, β-strands, β-turns, and random coils based on amino acid sequence composition and evolutionary information. The predicted secondary structure was used to provide insight into the structural organization of the protein.

2.6 Three-Dimensional Structure Modeling

Homology-based three-dimensional structure prediction was performed using the SWISS-MODEL server. Appropriate structural templates were automatically selected based on sequence similarity to experimentally resolved or computationally predicted protein structures. The quality of the generated model was evaluated using the Global Model Quality Estimation (GMQE) score, template sequence identity, and structural validation parameters. The predicted model was subsequently examined to identify structural features, including the presence of transmembrane helices.

2.7 Functional Annotation

Functional annotation of RvY_05612 was performed by integrating information obtained from UniProt, InterPro, HHpred, and conserved domain analyses. Predicted Gene Ontology (GO) terms describing cellular component and biological process were used to infer the probable biological role of the protein. Structural information generated from homology modeling was incorporated to support functional predictions.

2.8 Subcellular Localization Prediction

The subcellular localization of RvY_05612 was predicted using the CELLO v2.5 server. Localization prediction was performed based on sequence-derived physicochemical characteristics and machine learning algorithms. The predicted localization was compared with the functional annotation results to evaluate the consistency of the computational predictions.

3. Results

3.1 Sequence Characteristics

The hypothetical protein RvY_05612 from *Ramazzottius varieornatus* was retrieved from the NCBI protein database (GenBank accession: GAU93716.1). The protein consists of 98 amino acids and is annotated in UniProt under the accession A0A1D1UVK7. Taxonomic annotation confirmed that the protein belongs to the tardigrade *R. varieornatus* (strain YOKOZUNA-1). The retrieved amino acid sequence served as the basis for all subsequent computational analyses.

Table 1. General characteristics of hypothetical protein RvY_05612.

Parameter	Result
Organism	*Ramazzottius varieornatus*
Protein ID	RvY_05612
GenBank Accession	GAU93716.1
UniProt Accession	A0A1D1UVK7
Protein Length	98 amino acids

3.2 Physicochemical Properties

Physicochemical characterization using ExPASy ProtParam revealed that RvY_05612 is a relatively small protein with a predicted molecular weight of 9,857.37 Da and a theoretical isoelectric point (pI) of 11.65, indicating a highly basic protein. The instability index was calculated as 40.75, classifying the protein as marginally unstable under in vitro conditions. The aliphatic index of 66.84 suggested moderate thermostability, whereas the GRAVY value (0.086) indicated a slightly hydrophobic nature.

Analysis of amino acid composition demonstrated enrichment of alanine (15.3%), glycine (15.3%), threonine (11.2%), and serine (10.2%). In addition, the protein contained substantially more positively charged residues (12) than negatively charged residues (2), consistent with its high theoretical pI.

Table 2. Physicochemical properties of hypothetical protein RvY_05612.

Property	Value
Protein length	98 amino acids
Molecular weight	9,857.37 Da
Theoretical pI	11.65
Instability index	40.75
Aliphatic index	66.84
GRAVY	0.086
Negatively charged residues (Asp + Glu)	2
Positively charged residues (Arg + Lys)	12

3.3 Conserved Domain Analysis

Conserved domain analysis identified a highly significant Sec61β domain belonging to the Pfam family PF03911. The domain spanned amino acid residues 56–93 and was detected with an E-value of 5.11×10^{-15}, indicating a highly confident match. This finding classified RvY_05612 as a putative member of the Sec61β protein family, which is associated with the Sec61 protein translocation complex.

Table 3. Conserved domain identified in RvY_05612.

Parameter	Result
Conserved domain	Sec61β
Pfam accession	PF03911
Domain position	Residues 56–93
E-value	5.11×10^{-15}

Predicted function Component of the Sec61 protein translocation complex

3.4 Homology Analysis

BLASTp analysis demonstrated significant sequence similarity between RvY_05612 and previously annotated Sec61β proteins from multiple eukaryotic organisms, particularly other tardigrade species. The highest similarity was observed with proteins annotated as Sec61β or Sec61β-like proteins, indicating strong evolutionary conservation.

Remote homology detection using HHpred further supported these findings by identifying Sec61β family proteins with approximately 100% probability and an E-value of 1.30×10^{-20}. HHpred also predicted the presence of a transmembrane segment, consistent with the conserved architecture of Sec61β proteins.

3.5 Secondary Structure Prediction

Secondary structure prediction using SOPMA and PSIPRED indicated that α-helices constitute the predominant structural element of RvY_05612. Specifically, α-helices accounted for 47.96% of the protein sequence, followed by random coils (36.73%), extended β-strands (13.27%), and β-turns (2.04%).

The predominance of α-helical structures together with the predicted transmembrane region suggested a structural organization typical of membrane-associated proteins.

Table 4. Predicted secondary structure composition of RvY_05612.

Secondary Structure	Percentage (%)
Alpha helix	47.96
Extended strand	13.27
Beta turn	2.04
Random coil	36.73

3.6 Three-Dimensional Structural Modeling

Homology modeling using SWISS-MODEL generated a three-dimensional structural model based on the AlphaFold prediction of the corresponding protein. The selected template exhibited 100% sequence identity, while the predicted model achieved a GMQE score of 0.57, indicating moderate confidence.

Structural analysis identified a characteristic transmembrane helix, consistent with the conserved structural organization of Sec61β proteins. Evaluation of the predicted model using the Ramachandran plot demonstrated acceptable stereochemical quality, supporting the reliability of the generated structural model.

Table 5. Structural modeling summary.

Parameter	Result
Modeling server	SWISS-MODEL
Selected template	AlphaFold model (A0A1D1UVK7)
Sequence identity	100%
GMQE score	0.57
Predicted structural feature	Transmembrane helix

3.7 Functional Annotation

Functional annotation integrating UniProt, InterPro, conserved domain analysis, and HHpred consistently indicated that RvY_05612 is a putative component of the Sec61 translocon complex. Gene Ontology annotation associated the protein with the endoplasmic reticulum membrane (GO:0005784) and the biological process of intracellular protein transport (GO:0006886). These predictions were consistent across multiple annotation platforms, providing complementary evidence supporting the inferred biological function.

Table 6. Functional annotation of RvY_05612.

Category	Annotation
Protein family	Sec61β family
Cellular component	Endoplasmic reticulum membrane (GO:0005784)
Biological process	Intracellular protein transport (GO:0006886)
Predicted role	Component of the Sec61 translocon complex

3.8 Subcellular Localization

Subcellular localization analysis performed using CELLO v2.5 predicted that RvY_05612 is localized to the endoplasmic reticulum. This prediction was fully consistent with the conserved domain analysis,

structural modeling, and Gene Ontology annotation, all of which support

localization within the endoplasmic reticulum membrane as a component of the Sec61 protein translocation machinery.

4. Discussion

The present study employed an integrated bioinformatics approach to characterize the hypothetical protein RvY_05612 from *Ramazzottius varieornatus*. By combining sequence analysis, physicochemical characterization, conserved domain identification, homology detection, structural modeling, functional annotation, and subcellular localization prediction, a comprehensive computational profile of the protein was established. The results consistently suggest that RvY_05612 is a putative member of the Sec61β protein family and is likely involved in intracellular protein translocation across the endoplasmic reticulum (ER) membrane.

Physicochemical characterization indicated that RvY_05612 is a relatively small protein consisting of 98 amino acids with a molecular weight of approximately 9.86 kDa. The predicted theoretical isoelectric point (pI) of 11.65 suggests that the protein is highly basic, reflecting an abundance of positively charged amino acids. The instability index classified the protein as marginally unstable under in vitro conditions; however, such predictions are based solely on amino acid composition and do not necessarily reflect the stability of proteins within their native cellular environment. The moderate aliphatic index and slightly positive GRAVY value further suggest that RvY_05612 possesses physicochemical characteristics compatible with membrane-associated proteins, which is consistent with its predicted biological function.

One of the most significant findings of this study was the identification of the highly conserved Sec61β domain (PF03911) with a very low E-value, indicating a high level of confidence in the domain assignment. Members of the Sec61β family constitute an integral component of the Sec61 translocon complex, which serves as the primary channel for the translocation and insertion of newly synthesized proteins into the endoplasmic reticulum membrane. Although the β-subunit is not directly responsible for forming the protein-conducting channel, it contributes to the structural organization and efficient functioning of the translocon complex (Lewis et al., 2024). The identification of this conserved domain strongly supports the functional annotation of RvY_05612 as a Sec61β family protein.

Homology analyses performed using both BLASTp and HHpred further reinforced this prediction. Significant sequence similarity was observed with annotated Sec61β proteins from multiple eukaryotic organisms, including other tardigrade species. HHpred identified the Sec61β family with near-complete probability, demonstrating that the protein shares substantial structural and evolutionary similarity with previously characterized homologs. The conservation of homologous sequences across diverse taxa suggests that this protein family has maintained its biological function throughout evolution, highlighting the essential role of Sec61-mediated protein translocation in eukaryotic cells.

Secondary structure prediction revealed that nearly half of the amino acid residues are organized as α-helices, whereas structural modeling identified a characteristic transmembrane helix within the protein. This structural organization is consistent with the known architecture of Sec61β proteins, which typically contain a single membrane-spanning α-helix that anchors the protein within the ER membrane. The SWISS-MODEL-generated three-dimensional structure, supported by a template with 100% sequence identity, further strengthens the structural prediction. Although the GMQE score indicated moderate confidence, the agreement among multiple computational analyses provides additional support for the reliability of the predicted structure.

Functional annotation using UniProt, InterPro, and Gene Ontology databases consistently associated RvY_05612 with the endoplasmic reticulum membrane and intracellular protein transport. These findings were further corroborated by CELLO, which predicted localization of the protein to the endoplasmic reticulum. The concordance among independent computational approaches considerably increases confidence in the proposed functional annotation. Rather than relying on a single prediction method, the integration of multiple complementary bioinformatics tools provides stronger evidence supporting the assignment of RvY_05612 to the Sec61β protein family.

The characterization of proteins from *Ramazzottius varieornatus* is of particular interest because this extremotolerant tardigrade has evolved remarkable adaptations that enable survival under severe environmental stress. Although the present study does not directly investigate mechanisms of cryptobiosis or stress tolerance, accurate functional annotation of previously uncharacterized proteins contributes to improving genome annotation and expanding our understanding of tardigrade molecular biology. Computational studies such as this provide valuable baseline information that can guide future functional genomics and experimental investigations.

Despite the consistency of the computational predictions, several limitations should be acknowledged. All functional assignments reported in this study are based exclusively on bioinformatics analyses and therefore remain predictive in nature. Protein expression, biochemical activity, intracellular localization, and physiological function require experimental validation through molecular and cellular approaches. Consequently, the findings presented here should be considered computational evidence supporting the proposed annotation rather than definitive experimental confirmation.

Overall, the integration of multiple computational analyses produced highly consistent results that collectively support the classification of RvY_05612 as a putative Sec61β family protein associated with the endoplasmic reticulum translocon complex. This study demonstrates the effectiveness of combining complementary bioinformatics approaches for functional annotation of hypothetical proteins and provides a useful computational framework for future studies involving uncharacterized proteins from extremotolerant organisms.

5. Conclusion

This study presents a comprehensive in silico characterization of the hypothetical protein RvY_05612 from *Ramazzottius varieornatus* using an integrated bioinformatics workflow. Sequence analysis, physicochemical characterization, conserved domain identification, homology detection, secondary structure prediction, three-dimensional modeling, functional annotation, and subcellular localization prediction consistently indicated that RvY_05612 is a putative member of the Sec61β protein family. The identification of a conserved Sec61β domain, together with strong sequence homology, the presence of a predicted transmembrane helix, and localization to the endoplasmic reticulum, collectively supports its proposed role as a component of the Sec61 translocon complex involved in intracellular protein transport.

Although the present investigation is entirely computational, the integration of multiple independent bioinformatics tools provided complementary evidence that strengthened the confidence of the functional predictions. These findings contribute to the ongoing annotation of hypothetical proteins within the *R. varieornatus* genome and demonstrate the value of bioinformatics approaches for generating functional hypotheses in the absence of experimental data.

In conclusion, this work provides a robust computational characterization of RvY_05612 and illustrates how integrated sequence- and structure-based analyses can facilitate the functional annotation of previously uncharacterized proteins. The results establish a useful foundation for future experimental studies aimed at validating the predicted biological function and further exploring the molecular biology of this extremotolerant tardigrade.

Declarations

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Figures

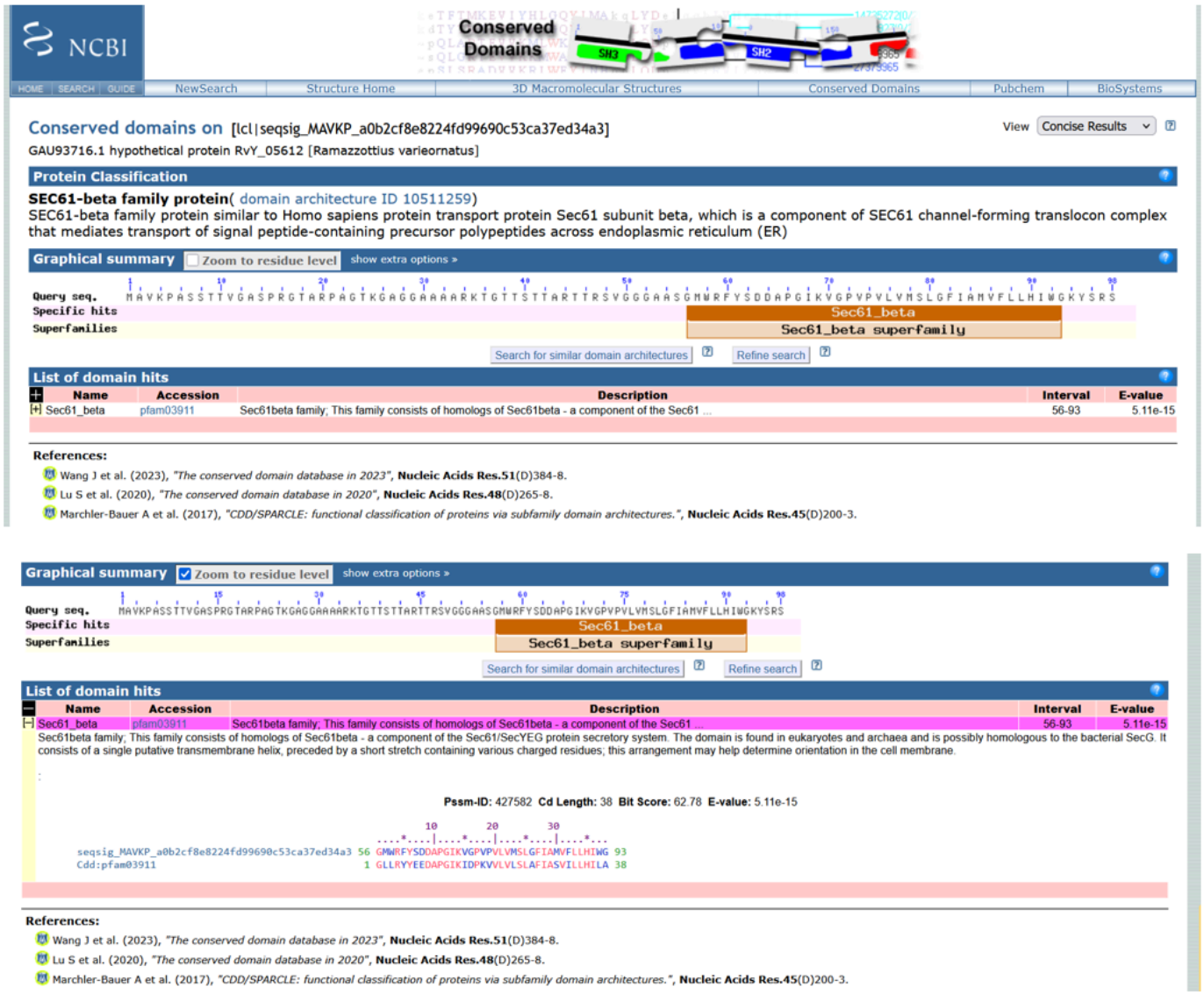


Figure 1

Conserved Domain Analysis (CDD Search)

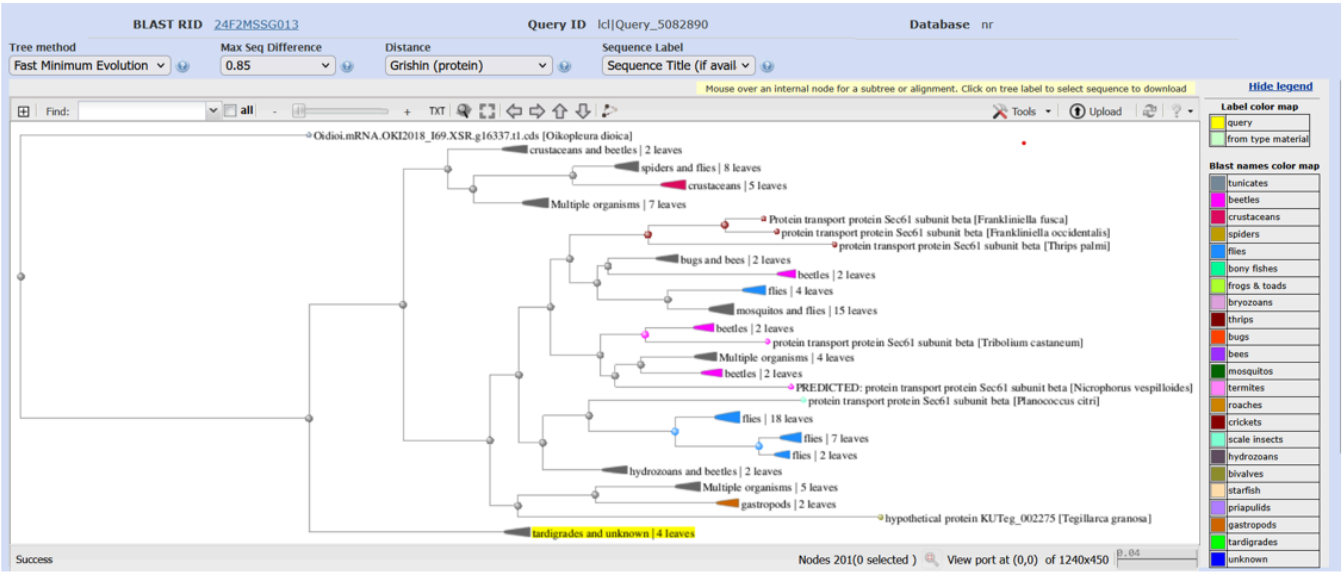


Figure 2

Phylogenetic Tree from the BLASTp



Figure 3

Homologous Sequences from NCBI-BLASTp and HHpred

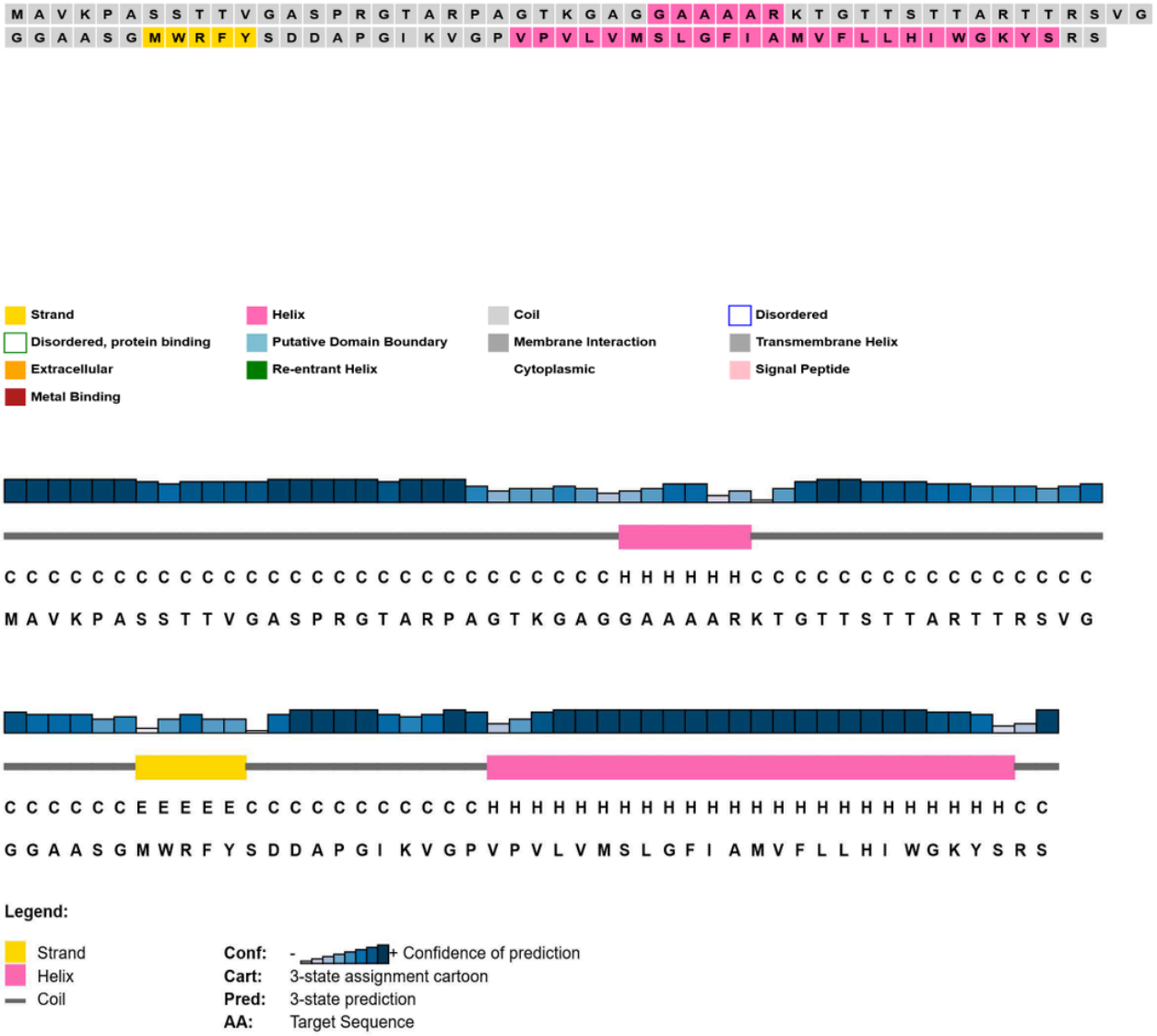


Figure 4

Secondary Structure Prediction (SOPMA, PSIPRED)

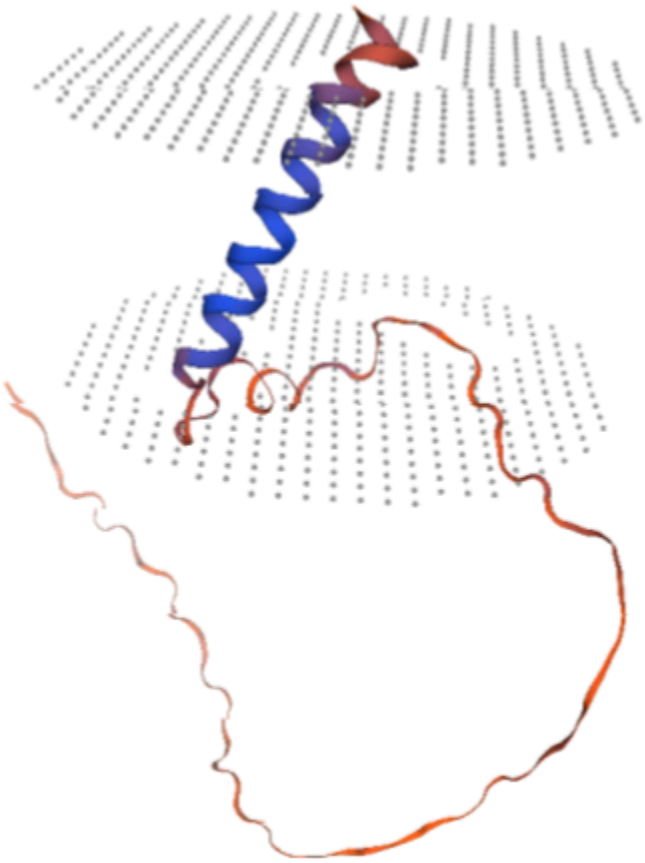


Figure 5

The SWISS 3D Model.

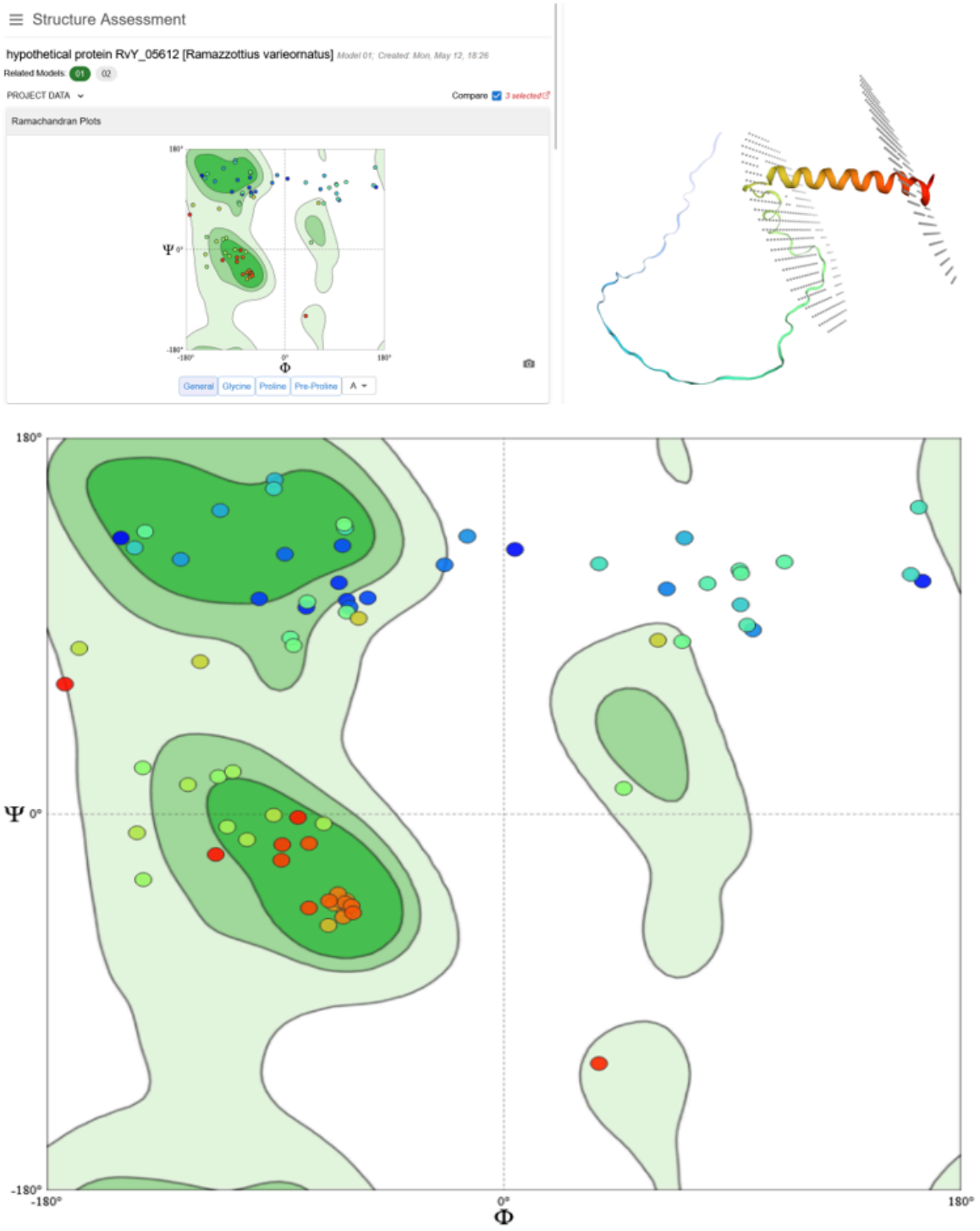


Figure 6

The Ramachandra Plot prediction from the SWISS Model

CELLO RESULTS

SeqID: GAU93716.1 hypothetical protein RvY_05612 [Ramazzottius varieornatus]

Analysis Report:

SVM	LOCALIZATION	RELIABILITY
Amino Acid Comp.	Nuclear	0.261
N-peptide Comp.	Mitochondrial	0.666
Partitioned seq. Comp.	Chloroplast	0.327
Physico-chemical Comp.	Mitochondrial	0.425
Neighboring seq. Comp.	Mitochondrial	0.247

CELLO Prediction:

	Mitochondrial	1.562 *
	Nuclear	0.861
	Chloroplast	0.823
	PlasmaMembrane	0.771
	Extracellular	0.400
	Cytoplasmic	0.388
	Lysosomal	0.041
	Peroxisomal	0.041
	Vacuole	0.040
	ER	0.032
	Cytoskeletal	0.021
	Golgi	0.018

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Figure 7

Protein Localized by CELLO

Supplementary Files

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- AppendixA.docx