

Phylogenetic analysis of cubilin (CUBN) gene

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Abstract:

Cubilin, (CUBN; also known as intrinsic factor-cobalamin receptor [Homo sapiens Entrez Pubmed ref NM_001081.3; NG_008967.1; GI: 119606627]), located in the epithelium of intestine and kidney acts as a receptor for intrinsic factor – vitamin B12 complexes. Mutations in CUBN may play a role in autosomal recessive megaloblastic anemia. The current study investigated the possible role of CUBN in evolution using phylogenetic testing. A total of 588 BLAST hits were found for the cubilin query sequence and these hits showed putative conserved domain, CUB superfamily (as on 27th Nov 2012). A first-pass phylogenetic tree was constructed to identify the taxa which most often contained the CUBN sequences. Following this, we narrowed down the search by manually deleting sequences which were not CUBN. A repeat phylogenetic analysis of 25 taxa was performed using PhyML, RAxML and TreeDyn softwares to confirm that CUBN is a conserved protein emphasizing its importance as an extracellular domain and being present in proteins mostly known to be involved in development in many chordate taxa but not found in prokaryotes, plants and yeast.. No horizontal gene transfers have been found between different taxa.

Key words: Cubilin, CUBN, Amino acid sequences, Phylogeny, Sequence alignment.

Background:

Cubilin (CUBN, also known as intestinal intrinsic factor receptor or intrinsic factor-cobalamin receptor or intrinsic factor-vitamin B12 receptor), acts as a co-transporter and helps in the uptake of lipoprotein, vitamin and iron. It functions as a transporter in many absorptive epithelia (intestine, renal proximal tubules and embryonic yolk sac) [1]. A potential role of mutations in CUBN gene has been hypothesized to play a role in the etiology of autosomal recessive megaloblastic anemia [2]. This hereditary condition is also known as MGA1 Norwegian type or Imerslund-Grasbeck syndrome. The disease is characterized by defective absorption of vitamin B12 and impaired function of the enzyme thymidine synthase; therefore, DNA synthesis, particularly during erythropoiesis is affected [1, 3]. Cubilin interacts with megalin in a calcium dependent manner by forming a dual-receptor complex called cubam which facilitates the uptake of specific ligands like hemoglobin, uteroglobin etc. [4]. In addition, CUBN also controls and

facilitates endocytosis of various ligands [5, 6]. In the proximal tubule cells, cubilin helps in reabsorption of vitamin D binding protein from glomerular filtrates and assists in the synthesis of 1 α ,25-dihydroxyvitamin D(3) [7, 8]. In addition, a missense mutation in CUBN gene was found to alter the levels of [9].

The cubilin protein has 27 CUB domains and 7 EGF-like domains and is coded by the human CUBN gene is located on chromosome 10 (10p12.31; 11933 bp mRNA; Entrez PubMed ref NM_001081.3; NG_008967.1; GI:119606627) and expressed as a 3623 amino acid, 460 kDa receptor [10]. The acquisition of CUBN in eukaryotes indicates one of the important events in evolution of cells. Because of the inherent role of CUBN as a transporter in many epithelia and its ability to facilitate absorption of hemoglobin and other important ligands, we aimed to understand if variance in this gene exists among in various organisms using a phylogenetic analysis of published protein sequences.

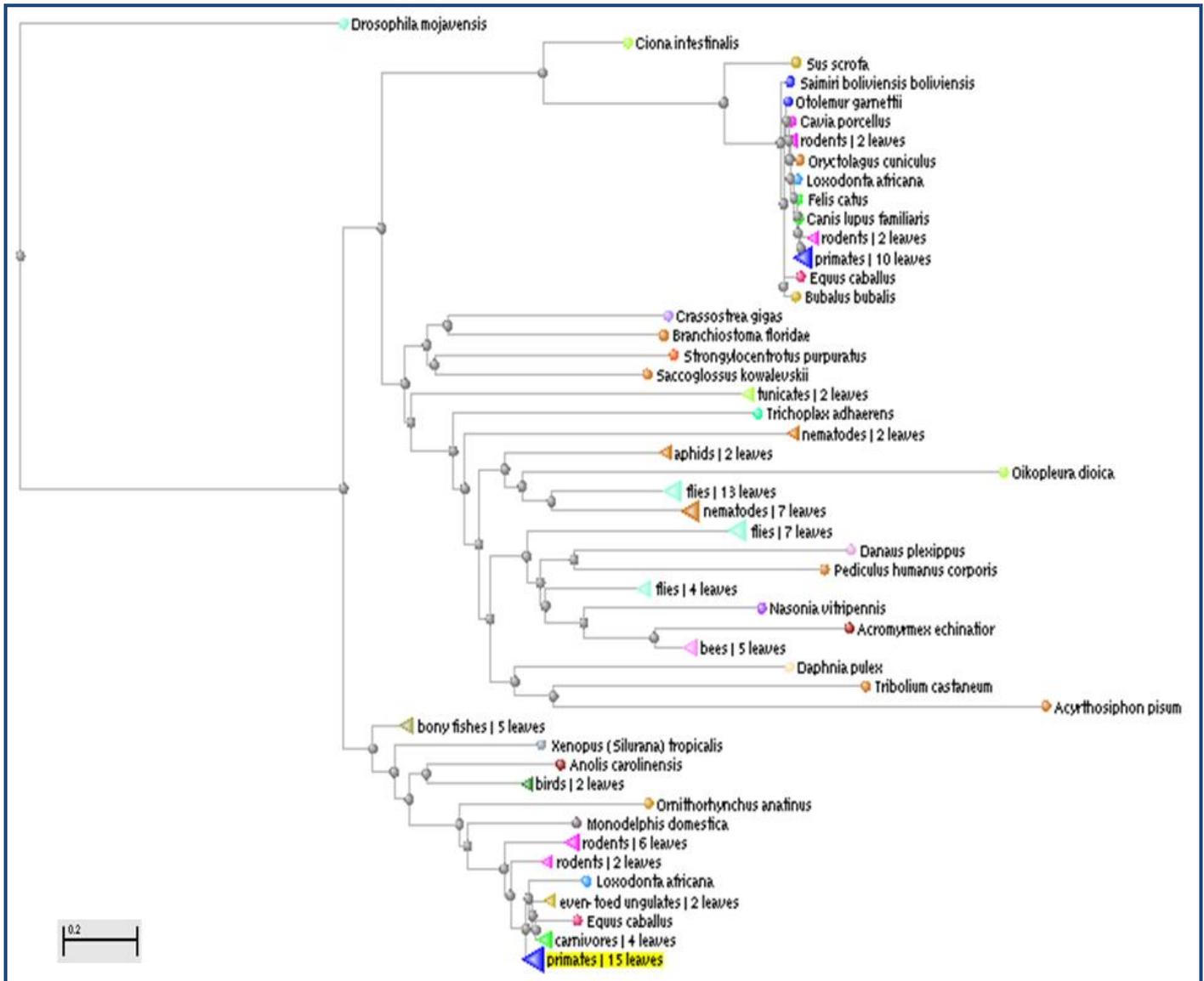


Figure 1: First pass phylogenetic tree constructed by multiple alignment using BLAST pair wise alignments: Results presented using Taxonomic name. First pass phylogenetic tree constructed showed that the CUBN sequences were mostly from arthropods, placozoans, nematodes, tunicates, rabbits, hares, primates, rodents, placentals, odd toed ungulates, even toed ungulates, bivalves, lancelets, hemichordates, sea urchins, bony fishes, amphibians, birds, lizards, marsupials and monotremes.

Methodology:

Data Set, Sequence Alignment and Construction of Phylogenetic Tree

We queried the GenBank database [11] for all available protein sequences of the CUBN. The retrieved sequences were saved in FASTA format. An initial first-pass phylogenetic tree was constructed using Neighbour Joining method [12] (maximum sequence difference of 0.85) using Domain Enhanced Lookup Time Accelerated Basic Local Alignment Search Tool [DELTA BLAST] pairwise alignments between the query and the database sequences searched [13]. Grishin computation was used by the software to calculate the evolutionary distance between any two sequences. This was modeled as expected fraction of amino acid substitutions per site [14].

The list of sequences obtained after DELTA BLAST were narrowed down to delete sequences which are not CUBN and

which were hypothetical. The concerned retrieved sequences were then aligned using Kalign software 2.03 version [15] with the results being produced in the Clustal W [16] format and employing a gap extension penalty of 0.85. The phylogenetic tree was reconstructed using the maximum likelihood method implemented in the PhyML program (v3.0 aLRT) [17, 18]. The Jones-Taylor-Thornton (JTT) substitution model was selected along with gamma-distributed rate categories to account for rate heterogeneity across sites. The reliability for internal branch was assessed using the aLRT test (SH-Like). The graphical representation and edition of the phylogenetic tree were performed with TreeDyn (v198.3) [19]. The RAxML (Randomized Axelerated Maximum Likelihood) program version 7.2.8 was then used for sequential and parallel Maximum Likelihood of the sequences [20]. An additional analysis of the presence of horizontal gene transfer was performed using the T-REX online software [21].

have evaluated horizontal gene transfers in the selected organisms. It is well understood that this involves a transfer of genetic material from one lineage to another commonly found in prokaryotes as an adaptation mechanism to environment [21]. However, the selected organisms did not show the presence of this phenomenon. The final phylogenetic tree indicated that CUBN is a relatively new protein having evolved very late in evolution (Figures 2 & 3). Also, no homologous gene transfer has been observed among the short-listed organisms which are indicative of the largely conserved domains within the amino acid sequences.

Conclusion:

Acquisition of CUBN in relatively evolved organisms indicates its crucial role in the physiology of cells. A higher conservation at CUB domains indicates preserved function. The phylogenetic tree for CUBN revealed the presence of this gene in eukaryotes and indicated its importance in the biochemical pathways related to absorption of many ligands from the epithelial linings in cells re-emphasizing the importance of certain proteins in evolution.

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Supplementary material:

Table 1: Accession details of short-listed Cubilin (CUBN) sequences

Accession	Description
EAW86221.1	Homo sapiens
XP_003831226.1	Pan paniscus – Predicted
XP_003257731.1	Nomascus leucogenys– Predicted
XP_003930687.1	Saimiri boliviensis boliviensis– Predicted
XP_003919387.1	Papio anubis– Predicted
XP_003786834.1	Otolemur garnettii– Predicted
XP_003988178.1	Felis catus– Predicted
EHB08007.1	Heterocephalus glaber
EDL78718.1	Rattus norvegicus– isoform CRA_b
XP_002666724.2	Danio rerio– Predicted
XP_001235156.2	Gallus gallus– Predicted
EKC18187.1	Crassostrea gigas
XP_003455793.1	Oreochromis niloticus– Predicted
XP_002806994.1	Callithrix jacchus– Predicted
EGI60892.1	Acromyrmex echinator
EFN89302.1	Harpegnathos saltator
XP_394526.3	Apis mellifera – Predicted
XP_001843996.1	Culex quinquefasciatus
EFN66210.1	Camponotus floridanus
XP_001655059.1	Aedes aegypti
EFX86741.1	Daphnia pulex
EHJ68165.1	Danaus plexippus
XP_003381549.1	Trichinella spiralis
EGW02057.1	Cricetulus griseus
ABV24853.1	Xenopus (Silurana) tropicalis
AAF61487.1	Mus musculus

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Pan	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Oreochromis	ATWSSPQG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID	Cricetulus	-----GRLFWTS-----
Nomacrus	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Callithrix	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID	Xenopus	VALFS--GRLFWT-----
Saimiri	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Acromyrmex	I1YTTDAGRGCGRIENYGRPTSELPTNYNNTCTMWSVYVGGKILQLGLVFDIG	Mus	ATVSS--GRLFWSS-----
Papio	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Harpegnathos	I1YTTDAGRGCGRIENYGRPTSELPTNYNNTCTMWSVYVGGKILQLGLVFDIG		
Otolemur	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Apis	I1YTTDAGRGCGRIENYGRPTSELPTNYNNTCTMWSVYVGGKILQLGLVFDIG		
Felis	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Culex	ATYLAQDRGCGRIENYGRPTSELPTNYNNTCTMWSVYVGGKILQLGLVFDIG		
Heterocephalus	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Camponotus	I1YTTDAGRGCGRIENYGRPTSELPTNYNNTCTMWSVYVGGKILQLGLVFDIG		
Rattus	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Aedes	ASVYLAQDRGCGRIENYGRPTSELPTNYNNTCTMWSVYVGGKILQLGLVFDIG		
Danio	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Daphnia	I1YTTDAGRGCGRIENYGRPTSELPTNYNNTCTMWSVYVGGKILQLGLVFDIG		
Gallus	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Danaus	I1YTTDAGRGCGRIENYGRPTSELPTNYNNTCTMWSVYVGGKILQLGLVFDIG		
Crasostrea	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Rattus	I1YTTDAGRGCGRIENYGRPTSELPTNYNNTCTMWSVYVGGKILQLGLVFDIG		
Oreochromis	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Trichinella	I1YTTDAGRGCGRIENYGRPTSELPTNYNNTCTMWSVYVGGKILQLGLVFDIG		
Callithrix	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Cricetulus	I1YTTDAGRGCGRIENYGRPTSELPTNYNNTCTMWSVYVGGKILQLGLVFDIG		
Acromyrmex	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Xenopus	I1YTTDAGRGCGRIENYGRPTSELPTNYNNTCTMWSVYVGGKILQLGLVFDIG		
Harpegnathos	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Mus	I1YTTDAGRGCGRIENYGRPTSELPTNYNNTCTMWSVYVGGKILQLGLVFDIG		
Apis	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Homo	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
Culex	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Pan	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
Camponotus	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Pan	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
Aedes	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Nomacrus	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
Daphnia	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Saimiri	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
Danaus	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Papio	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
Rattus	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Otolemur	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
Trichinella	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Felis	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
Cricetulus	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Heterocephalus	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
Xenopus	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Rattus	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
Mus	-CRDCHAFQGLS-----PQFQNYDMQCTVTLPAQPHNTISLFFHSFG-IE-NS	Danio	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Gallus	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Crasostrea	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Oreochromis	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Callithrix	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Acromyrmex	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Harpegnathos	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Apis	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Culex	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Camponotus	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Aedes	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Daphnia	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Danaus	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Rattus	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Trichinella	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Cricetulus	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Xenopus	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
		Mus	DRDCCQNY--L1YDGNAS--SFSBP-----YCGDTSIAFFVASSNQLVFKF-HAY		
Homo	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Pan	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Fan	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Nomacrus	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Saimiri	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Papio	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Otolemur	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Felis	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Heterocephalus	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Rattus	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Danio	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Gallus	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Crasostrea	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Oreochromis	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Callithrix	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Acromyrmex	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Harpegnathos	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Apis	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Culex	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Camponotus	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Aedes	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Daphnia	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Danaus	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Rattus	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Trichinella	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Cricetulus	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Xenopus	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Mus	VECHDFLEVRHNSNSFLKRYCOTLL--NFVFSQNELVLAH-KSD-SVYRNGOYE				
Homo	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Pan	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Fan	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Nomacrus	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Saimiri	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Papio	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Otolemur	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Felis	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Heterocephalus	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Rattus	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Danio	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Gallus	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Crasostrea	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Oreochromis	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Callithrix	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Acromyrmex	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Harpegnathos	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Apis	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Culex	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Camponotus	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Aedes	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Daphnia	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Danaus	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Rattus	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				
Trichinella	I1WSSPFG--CGTLYGDRGSPFSFY POTY PNNTCHDMVLAFAGRVLYTNYFVLSID				

Similar residues are colored as the most conserved one (according to BLOSUM62).