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Phylogenetic Analysis of Voltage Gated Ion Channels

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ABSTRACT

Voltage-gated ion channels (VGICs) are among the most fascinating proteins because of their function to generate electrical activity in cells and are responsible for many of the most overt manifestations of life. Although VGICs are seen as being critical to animals, particularly those with complex nervous systems, they are relatively old proteins, some of which are well represented in diverse prokaryotes. The present investigation was carried out to highlight the utility of using an evolutionary approach to glean useful information about ion channel function and, by extension, about the properties of other types of proteins. A total of 8 common organism's protein sequence for VGKC (Voltage-gated potassium channel), VGCC (Voltage-gated calcium channel), and VGSC (Voltage-gated sodium channel) were obtained from Uniprot and subjected to multiple sequence alignment using Praline & ClustalW. The phylogenetic trees were constructed using different methods in MEGA v5.05. The sequence alignment of VGSC proteins of different species revealed no consensus residue. In the sequence alignment of VGKC proteins, five residues (Isoleucine395, Arginine 400, Aspartic Acid 490, Cysteine 502 and Valine 519) were observed to have 70% conservation across different species, while Cysteine 489 was found to be 80% conserved across the species. The sequence alignment of VGCC proteins of different species revealed very little (~50%) conservation across the species. The nature of residue conservation in VGKC reflects that the conservation is majorly for larger amino-acids that help the protein to form channels. The trees obtained for VGKC and VGCC had a remarkable similarity of forming a monophyletic group which was shared by *Xenopus* or *Rattus* and *Nocardioidaceae* or *Streptomyces*. Contrary to the results of individual trees obtained for VGSC proteins by different methods, the consensus tree generated had a monophyletic group of *Homo sapiens* and *A. gambiae* and the group was found to be again very near to prokaryotic VGSC of *Streptomyces*. The present study is very much of clinical significance because it has revealed that ion channels also exist in lower organisms which are very much related to higher biological systems.

1. INTRODUCTION

Voltage gated ion channels are a class of transmembrane ion channels that are activated by changes in electric potential difference near the channel; these types of ion channels are especially critical in neurons, but are common in many types of cells. They have a crucial role in excitable neuronal and muscle tissues, allowing a rapid and co-ordinated depolarization in response to triggering voltage change. Found along the axon and at the synapse, voltage-gated ion channels directionally propagate electrical signals [1]. They generally are composed of several subunits arranged in such a way that there is a central pore through which ions can travel down their electrochemical gradients. The channels tend to be ion-specific, although similarly sized and charged ions may sometimes travel through them [2]. Voltage gated ions channels, on the basis of ions transported across the channel are of various types like, Na^+, K^+, and Ca^{2+} channels. Although voltage-gated ion channels are seen as being critical to animals, particularly those with complex nervous systems, they are relatively old proteins, some of which are well represented in diverse prokaryotes. As a result of major genetic events such as gene duplications and more minor ones involving numerous single base mutations, a plethora of channel types has evolved from what was, presumably, a single or limited number of precursors [3]. Voltage-gated sodium channels (VGSC) are multi-molecular protein complexes expressed in both excitable and non-excitable cells. They are primarily formed by a pore-forming multi-spanning integral membrane glycoprotein (α-subunit) that can be associated with one or more regulatory β-subunits [4]. Voltage-gated calcium channels (VGCC) are large, transmembrane multiprotein complexes that couple membrane depolarization to cellular calcium entry. These channels are central to cardiac action potential propagation, neurotransmitter and hormone release, muscle contraction and calcium-dependent gene transcription [5-7]. Voltage-gated potassium channels (Kv) or Voltage Gated Potassium Channels (VGKC) are a group of membrane proteins that regulate the flow of potassium ions into and out of cells in response to changes in the membrane potential. Kv channels are found throughout the body in different cell types [8-10].

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The more important purpose of this investigation was to highlight the utility of using an evolutionary approach to glean useful information about ion channel function and, by extension, about the properties of other types of proteins [11,12]. The present study is very much of clinical significance because it has revealed that ion channels also exist in lower organisms which are very much related to higher biological systems. Indeed, as the search for other ion channels in prokaryotes and lower eukaryotes continues, aided by various genome projects, it will be very useful to generate models for other types of ion channels in other organisms too.

2. MATERIALS AND METHODS

2.1 Sequence retrieval and Multiple Sequence Alignment

The protein sequences of different voltage gated ion channels (VGSC, VGCC and VGKC) of various species were retrieved from Uniprot [13] in FASTA format. The retrieved sequences of each VGICs were subjected to multiple sequence alignment (MSA) using PRALINE [14]. BLOSSUM62 was selected as the substitution matrix with 12 as the gap opening and 1 as gap extension penalties for the alignment. The sequences were also subjected to ClustalW for MSA and the results were generated with three output formats i.e. .aln, .phy, .dnd.

2.2 Generation of Phylogenetic Trees

The .aln file of ClustalW result was used as input to MEGA v5.1software [15] and different phylogenetic trees were generated using MEGA software for VGSC, VGKC and VGCC. The trees were generated using Neighbour-Joining (NJ), Maximum Parsimony (MP), Minimum Evolution (ME), UPGMA and Maximum Likelihood (ML) methods.

2.3 Generation of Consensus Trees

The consensus tree of Maximum Parsimony method was generated from the distance matrix by PROTPARS module of PHYLIP v3.695 [16]. The .phy file of Clustal W results for all organisms was exported to exe folder of PHYLIP software and outfile containing distance matrix was generated in DNAPARS and PROTPARS. Thereafter the distance matrix was the input for generating tree. Two results were obtained viz., outfile containing phylogenetic tree and outtree containing New Hampshire (nh) format. The 'nh' format for all proteins and nucleotide sequences were saved in notepad and consensus tree were generated from the CONSENSUS program of PHYLIP.

3. RESULTS AND DISCUSSION

3.1 Sequence retrieval and Multiple Sequence Alignment

The protein sequences of VGSC, VGKC and VGCC of different organisms (Prokaryotes, Lower Invertebrates, Vertebrates and Plants) were retrieved from Uniprot. A total of 8 common organism's protein sequence for VGKC, VGCC, and VGSC were obtained as given in Table 1. The MSA of retrieved sequence data was carried out using Praline and ClustalW. The .aln file of clustalW result were saved in notepad and further used for generation of phylogenetic tree in MEGA. The sequence alignment of VGSC proteins of different species revealed no consensus residue (Figure 1). In the sequence alignment of VGKC proteins, five residues (Isoleucine395, Arginine 400, Aspartic Acid 490, Cysteine 502 and Valine 519) were observed to have 70% conservation across different species, while Cysteine 489 was found to be 80% conserved across the species (Figure 2). The sequence alignment of VGCC proteins of different species revealed very little (~50%) conservation across the species (Figure 3). The nature of residue conservation in VGKC reflects that the conservation is majorly for larger amino-acids that help the protein to form channels.

3.2 Generation of phylogenetic trees

The phylogenetic trees were constructed for the sequence alignments obtained for all the three voltage gated ion channels, using Minimum Evolution, Maximum parsimony, Maximum Likelihood, UPGMA, Neighbor- joining, method in MEGA 5.05 (Figure 1a-e to 3a-e). While analyzing the phylogenetic trees of VGKC protein (Figure 1a-e), a total of 6 monophyletic groups were observed. *Homo sapiens* and *Rattus norvegicus* always formed one group and is well justified as they are evolutionarily closer to each other. To a surprise, *Streptomyces* VGKC was observed closer to the other clades than *A. thaliana* and *Anopheles gambiae*. This observation needs more of phylogentic analysis but prima facie, it reflects that either VGKC evolved from other voltage gated ion channels or has mutated in bacterial system. The results needs to be validated further by boot-strapping and determining the origin.

During the analysis of phylogenetic trees of VGCC protein (Figure 2a-e), a total of 7 monophyletic groups were observed. *G. gallus* and *X. laevis* always formed one group and are evolutionarily closer to each other being avian and amphibians. The bacterial VGKC of *Nocardioidaceae* was always observed to have a separate lineage during evolution; however the root of the tree remains to be identified. To a surprise, *Homo sapiens* and *Anopheles gambiae* VGKCs were observed closer to each other than *R. norvegicus*. Thus, the VGKC acting insecticidal agents can be predicted to be toxic to humans also. The structural verification of the same is the next target being investigated in our lab.

After analyzing the phylogenetic trees of VGSC protein (Figure 3a-e), a total of 7 monophyletic groups were observed in general for all the methods adopted except for NJ-method, which predicted 6 monophyletic group. *G. gallus* and *R. norvegicus* always formed one group while Homo *sapiens* and *X. laevis* were found to be evolutionarily closer as far as VGSC proteins are concerned. But, the bacterial VGSC of *Streptomyces* observed closer to the other clades than *A. thaliana*, similar to VGKC. This reflects that voltage gated ion channels (VGIC) evolved separately at different time intervals and have very different lineage in plant systems. The evolution of VGIC in plants is another exciting field of study that needs to be investigated.

Table. 1: Protein sequence of VGCC, VGKC and VGSC of different organism.

Voltage Gated Calcium Channel		Voltage Gated Potassium Channel		Voltage Gated Sodium Channel	
Organism name	**gi number**	**Organism name**	**gi number**	**Organism name**	**gi number**
Gallus gallus	sp|O73700	*Gallus gallus*	sp|O73606	*Gallus gallus*	tr|F9W2Y0
Xenopus laevis	tr|C5MKI6	*Xenopus laevis*	sp|P70057	*Xenopus laevis*	sp|P51167
Rattus norvegicus	sp|Q62897	*Rattus norvegicus*	tr|Q6I9B6	*Rattus norvegicus*	sp|Q62968
Anopheles gambiae	tr|A5YW88	*Anopheles gambiae*	tr|Q7PKK8	*Anopheles gambiae*	tr|Q5PTB3
Nocardioidaceae bacterium	gi|325951161	*Streptomyces coelicolor*	sp|P0A333	*Streptomyces sp.*	tr|B4UY60
Homo sapiens	sp|O00555	*Homo sapiens*	sp|Q9NZV8	*Homo sapiens*	sp|Q07699
Arabidopsis thaliana	sp|Q94KI8	*Arabidopsis thaliana*	sp|O23016	*Arabidopsis thaliana*	sp|O65718
Danio rerio	tr|Q90ZA7	*Danio rerio*	tr|B3DJD2	*Danio rerio*	sp|Q2XVR3

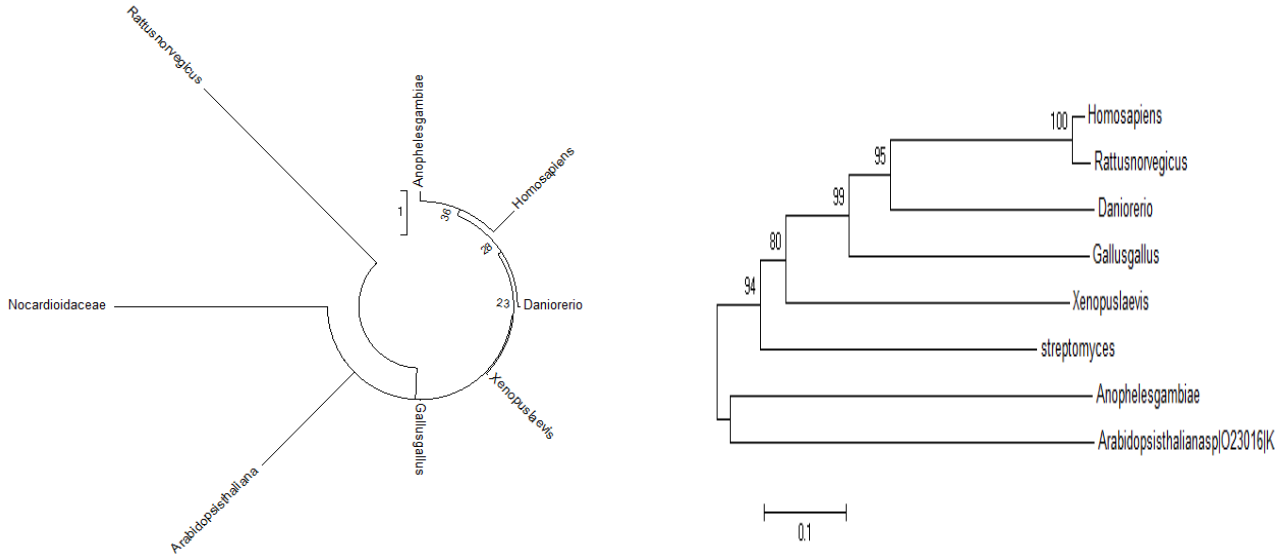

Fig. 1a: Maximum Likelihood & **Fig. 1b:** Neighbor Joining.

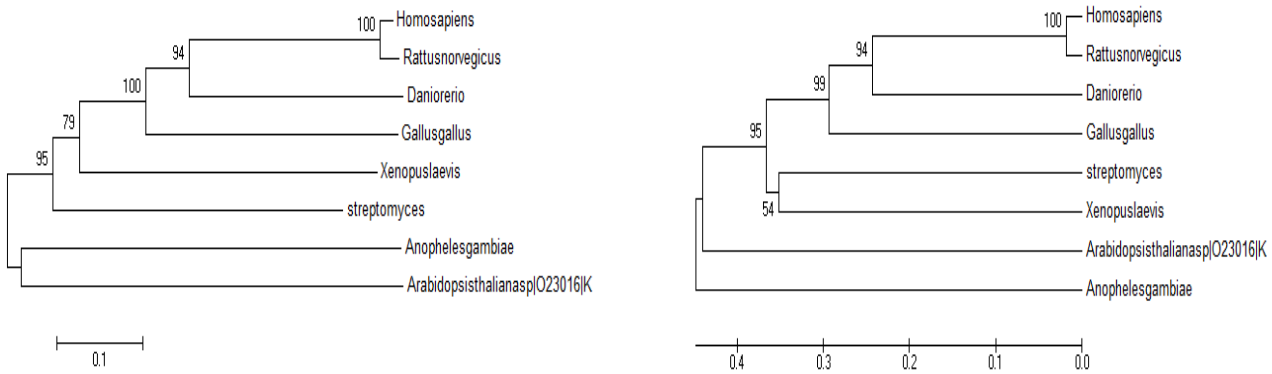

Fig. 1c: Minimum Evolution & **Fig. 1d:** UPGMA

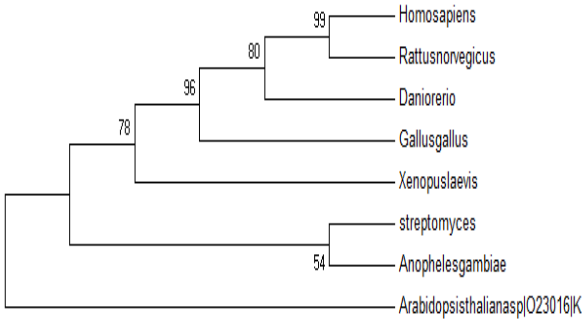

Fig. 1: Maximum Parsimony

Fig. 1a-e: Phylogenetic tree of VGKC Protein of different species by different methods.

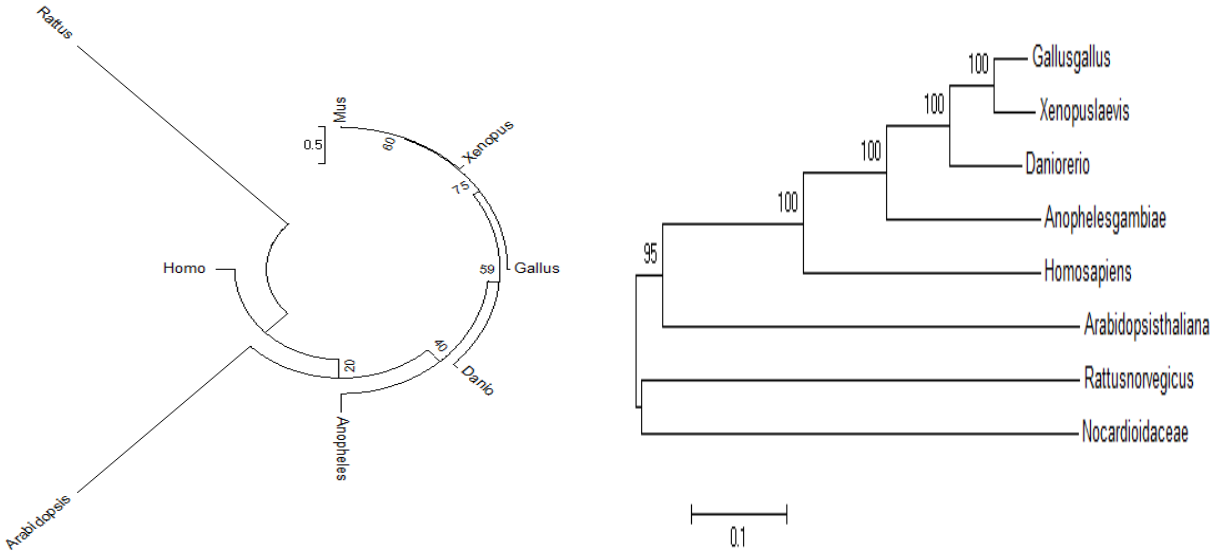

Fig. 2a: Maximum Likelihood Method & **Fig. 2b:** Neighbor Joining Method

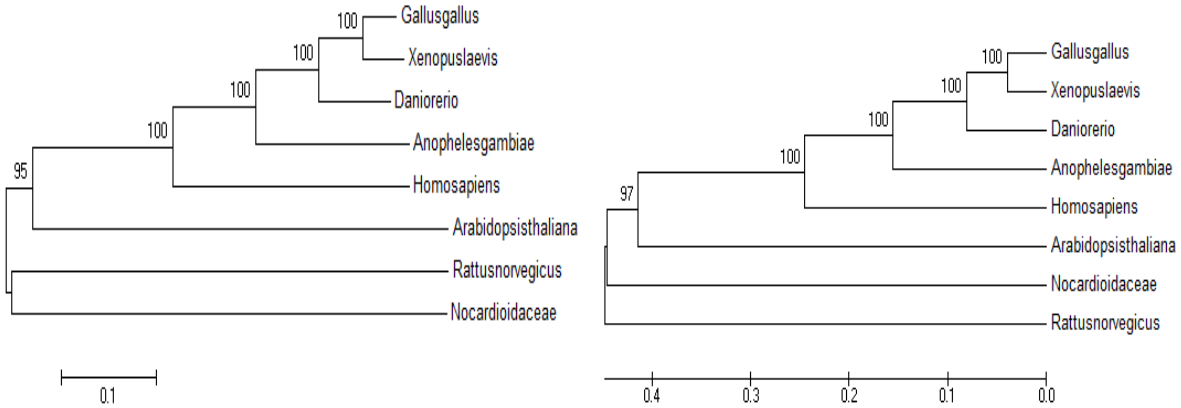

Fig. 2c: Minimum Evolution Method & **Fig. 2d:** UPGMA Method.

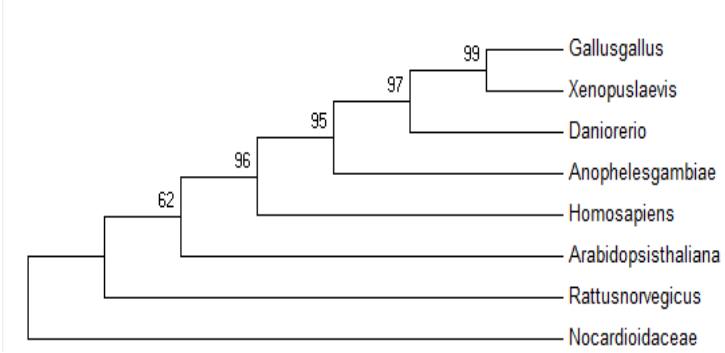

Fig 2e: Maximum Parsimony Method.

Fig. 2a-e: Phylogenetic tree of VGCC Protein of different species by different methods.

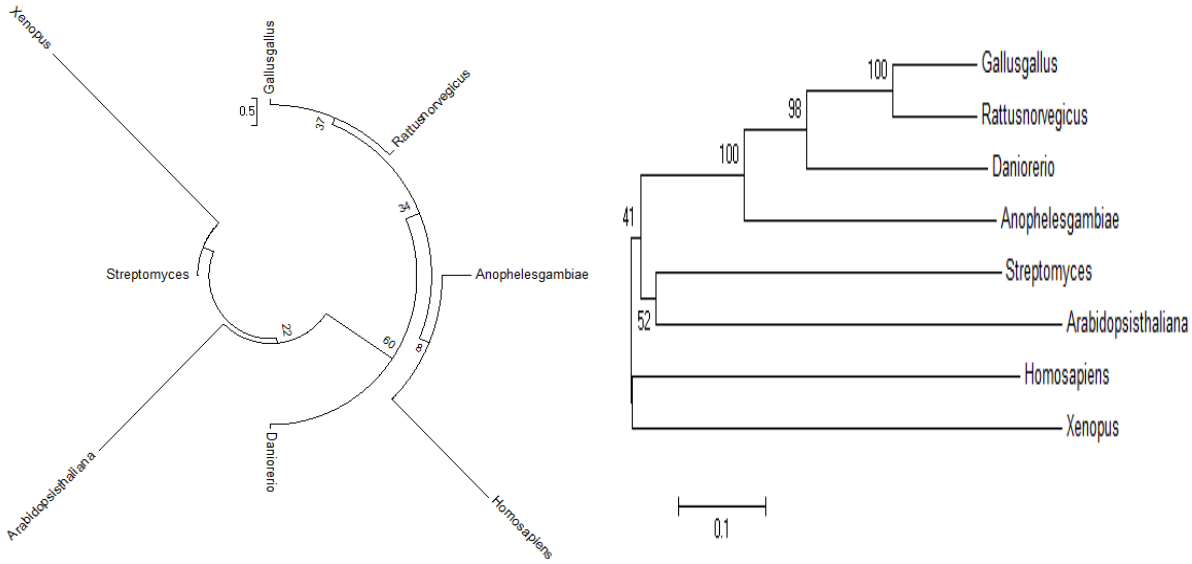

Fig. 3a: Maximum Likelihood Method & **Fig. 3b:** Neighbor Joining Method

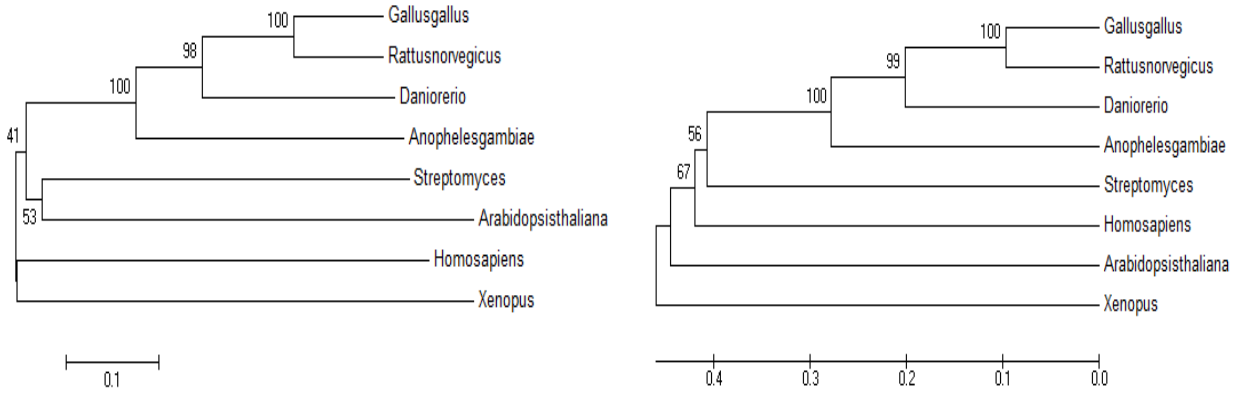

Fig. 3c: Minimum Evolution Method & **Fig. 3d**: UPGMA Method

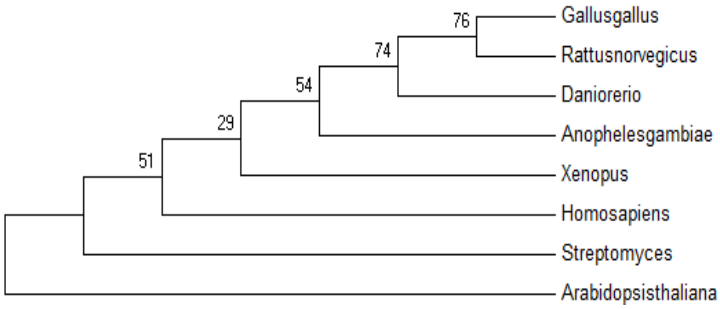

Fig 3e: MaximumParsimony Method.

Fig. 3a-e: Phylogenetic tree of VGSC Protein of different species by different methods.

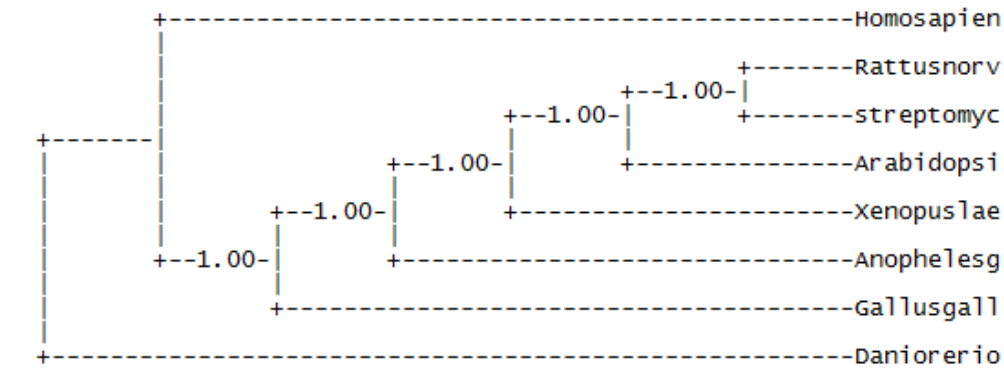

Fig. 4a: Consensus Phylogenetic tree of VGKC Protein of different species.

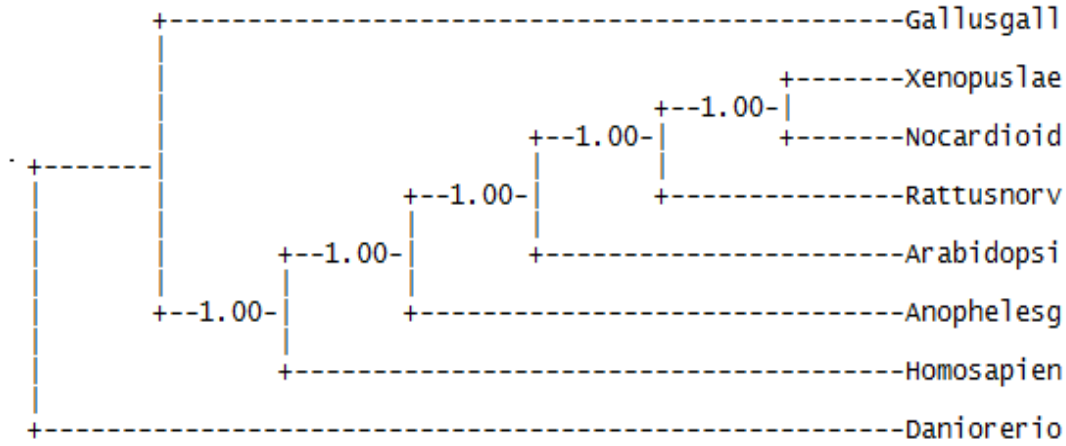

Fig. 4b: Consensus Phylogenetic tree of VGCC Protein of different species.

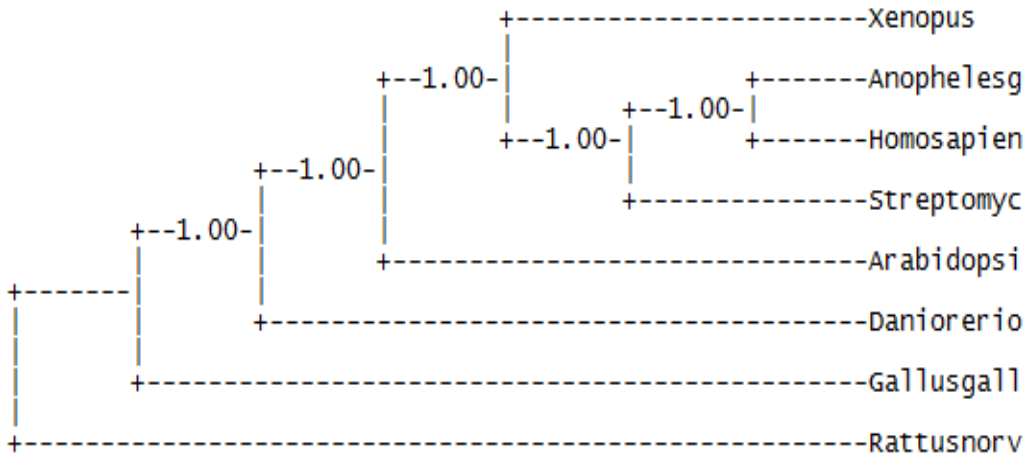

Fig. 4c: Consensus Phylogenetic tree of VGSC Protein of different species.

3.3 Generation of consensus tree

The consensus tree obtained for VGKC (Figure 4a) and VGCC (Figure 4b) had a remarkable similarity of forming a monophyletic group which was shared by *Xenopus* or *Rattus* and *Nocardioidaceae* or *Streptomyces*. These observations are in accordance with the trees obtained by different methods for VGKC and VGCC proteins, as discussed above. Contrary to the results of individual trees obtained for VGSC proteins by different methods in MEGA, the consensus tree generated had a monophyletic group of *Homo sapiens* and *Anopheles gambiae* and the group was found to be again very near to prokaryotic VGSC of *Streptomyces* (Figure 4c).

Therefore, it was concluded that the evolutionary relationship of VGICs of prokaryotes and higher vertebrates needs to be investigated further to arrive at some logical view about their origin.

4. CONCLUSION

The phylogenetic tree obtained for VGKC and VGCC had a remarkable similarity of forming a monophyletic group which was shared by *Xenopus* or *Rattus* and *Nocardioidaceae* or *Streptomyces*. These observations are in accordance with the trees obtained by different methods for VGKC and VGCC proteins. Contrary to the results of individual trees obtained for VGSC proteins by different methods in MEGA, the consensus tree generated had a monophyletic group of *Homo sapiens* and *A. gambiae* and the group was found to be again very near to prokaryotic VGSC of *Streptomyces*. Therefore, the evolutionary relationship of VGICs of prokaryotes and higher vertebrates needs to be investigated further to arrive at some logical view about their origin. The comparison between phylogenetically distinct ion channels has yield useful information that would have been otherwise difficult to obtain.

5. ACKNOWLEDGEMENT

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