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Molecular and Phylogenetic Analysis of Genus *Bychowskyella*, using the 18S rDNA/18S rRNA for Studying Conserveness in the Genome for Species- Level Differentiations and Uses for the Taxonomy

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Abstract: **The Current Research Deals with the Molecular Characterization of Genus *Bychowskyella* and Comparison among its Species based on 18S rDNA/rRNA, as earlier studies were only focused on Morphological and Morphometric Features which fails to differentiate accurately the closely related species. Phylogenetic Studies have been made based on Construction of Neighbour Joining and Maximum Parsimony Methods, using the MEGA Software. Vectorbuilder tool used for the calculation of Percentage of GC Content in the Genome. Using the ExpaRNA tool Conserveness in the loops of Secondary Structure are made, and MEME Suite Software is used Lastly for Motif Predictions and Confirmation of the Molecular based Studies. This Study further opens the door for many future Researches in the Areas of Parasitology, which will help in combating many Unknown Diseases possibly linked to the Genus *Bychowskyella*, Studying Host Parasite Interactions. Thus the entire Research bridges the gap between Morphological Based Studies and Molecular Based Studies, Helping in Accurate and Reliable way to differentiate between the Species within the Genus Bychowskyella with that of other Genus in Monogenea.**

Keywords: **Genus *Bychowskyella*, Monogenea, 18S rDNA/rRNA, Phylogenetic Analysis, Secondary Structure, Motif Predictions.**

I. INTRODUCTION

Monogenean are a special class of parasitic flatworms of the Phylum Platyhelminths [1]. This organisms are known for their ectoparasitic nature on fishes, commonly found on their gills, skin and fins feeding on the gill mucus and skin tissues or epidermis [2]. Opisthaptor (main adhesive apparatus in hind part) is the Key Feature for this Group equipped with hard sclerotinized structures(clamps,suckers and hooks) (cabi.org). Also the fore part of the worm has attaching capacities (adhesive pads, cephalic openings) and is referred to as the prohaptor [3]. *Bychowskyella* has been associated with infection in the gills of Siluriformes fishes, commonly called catfish which are consumed widely. Till Date 32 species has been

attributed to *Bychowskyella*, Of which 24 species are valid [4]. Members of *Bychowskyella* have well developed dorsal anchors without roots, with long recurved patches; dorsal connecting bar wide and fenestrated; onchium absent; ventral anchors small, without roots, without patches and similar types of sclerotized reproductive structures [5].

Earlier Researches were based majorly on only Morphological and Morphometrics features of Genus *Bychowskyella* like: Haptor, Anchors structures, Number of Hooks, Copulatory Organs. Although this parameters can give detailed morphological and morphometry data but is not absolutely a reliable indicator for Phylogenetic analysis and Taxonomy studies, As there still existing species in the Genus *Bychowskyella* which have almost same resemblance according to their morphological features, and there is very slight variations among them. Thus to tackle this, There was a need for Molecular Based Studies. 18S rDNA/18S rRNA are studied and analysed for Phylogenetic relationships and Motif Predictions. RNA Polymerase is responsible for the formation of RNA from the DNA, Which is a part of the Framework in Central Dogma.18S rRNA is component of small ribosomal subunit 40S, present in eukaryotic genome studied largely for its high stability and high degree of conserveness. Motifs are regularly occurring short stretch of nucleotide bases which have common biological functions and with the help of this variability across species are studied. Short nucleotide sequences acts as perfect molecular markers for species level differentiation in Genus *Bychowskyella* as this sequences remain conserved and combining this molecular features with morphological features helps us in establishing a accurate phylogenetic position to a particular species and eventually studying the Evolutionary relationships. ExpaRNA Software is used for generation of Secondary Structures for comparison of Sequence Conserveness. Reference to Secondary structure can improve the assignment of the positional homology and also finds out highly conserved elements in the Structure of the *Bychowskyella* [6].

II. MATERIALS AND METHODS

Method of Phylogenetic Relationships and Evolutionary Analysis

The genomic sequences of all the species coming under *Bychowskyella* genus were retrieved from the NCBI Database. Using ClustalW all sequences were aligned and after that manually adjusted. BLAST was run to identify most similar reference sequences. After aligning the genomic sequences using ClustalW, the 18S sequences data was entered in the MEGA5.0 software[7] and Phylogenetic trees were constructed. The Phylogenetic trees[8] were constructed based on neighbour-joining NJ(Distance based method) and Maximum Parsimony MP(Character State method). Distance matrix and neighbour joining tree were based on Kimura-2-Parameter model. Using 1000 bootstrap replicates further remaining analysis was done and executed (trjfas.org). MFold was used to predict the Secondary structures of 28S RNA Sequences and are MFold is basically based on the concepts of minimal free energy state. MEME Software was used for motif prediction and their regular expressions. Expa RNA[9] was used to measure conserveness in loops of RNA Secondary structure. Lastly Percentage of Guanine and Cytosine calculated using GC Calculator, vectorbuilder.com.

III. RESULTS AND DISCUSSIONS

Bychowskyella sp. Were compared based on the 18S rDNA molecular marker by Construction of two Important Phylogenetic trees which are: Neighbour Joining and Maximum Parsimony. The 18S rDNA Sequence of *Bychowskyella fossilisi* (KT852454.1:32-1142) has high similarity with 18S rDNA of *Bychowskyella tchangi* (852455.1:1-1027), and both are part of Ingroups comprising a Monophyletic taxon. Both of this species have evolved from a Single Ancestor and evolutionary forming 2 unique species with minor molecular variations. *Bychowskyella wallagonia* (KT852456.1:1-1120) have Polyphyletic relationship with *Bychowskyella fossilisi* and *Bychowskyella tchangi*, as it has evolved into a separate taxon with a different latest ancestor, although all of these species had same initial ancestor. Due to Evolutionary Pressures *Bychowskyella wallagonia* has evolved into a different branch thus having less similarities with all other species. *Bychowskyella pseudobagri* (KY680236.1:42-1175) has paraphyletic relationship with these groups and is a total outgroup, having negligible similarities with the 3 other Known Species of *Bychowskyella*. It does not shares ancestors with the remaining species. Both the Neighbour Joining and Maximum Parsimony Tree inferred from 18S rDNA sequences gives good results showing variations among the *Bychowskyella* Species for Species-Level Differentiations, *Bychowskyella fossilisi* (KT852454.1:32-1142) and *Bychowskyella tchangi*(852455.1:1-1027) shows the maximum similarity among all the species, followed by *Bychowskyella wallagonia* (KT852456.1:1-1120)which have intermediate similarity among all the species followed by *Bychowskyella pseudobagri* (KY680236.1:42-1175) which has the least similarity among all the Species. Based on this Inferences, Identification of *Bychowskyella* Species will be easier among the Parasite Species found globally.

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A. Neighbour Joining Tree

B. Maximum Parsimony Tree

Secondary Structures Prediction

The Percentage of Guanine(G) and Cytosine(C) was calculated using VectorBuilder GC Content Calculator. It was found to be 49.5% in *Bychowskyella fossilisi* (KT852454.1:32-1142), 49.07% in *Bychowskyella tchangi* (852455.1:1-1027), 48.66% in *Bychowskyella wallagonia* (KT852456.1:1-1120) and 47.27% in *Bychowskyella pseudobagri* (KY680236.1:42-1175). Higher GC Content in a Structured RNA will give more Stable Secondary Structure.

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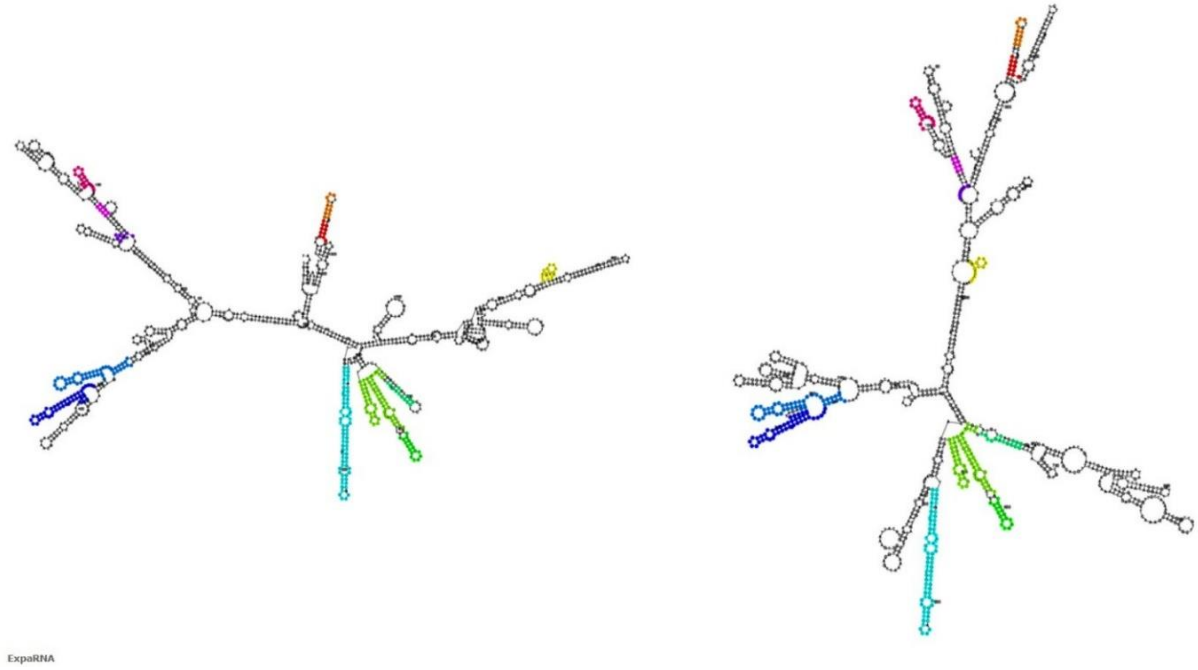
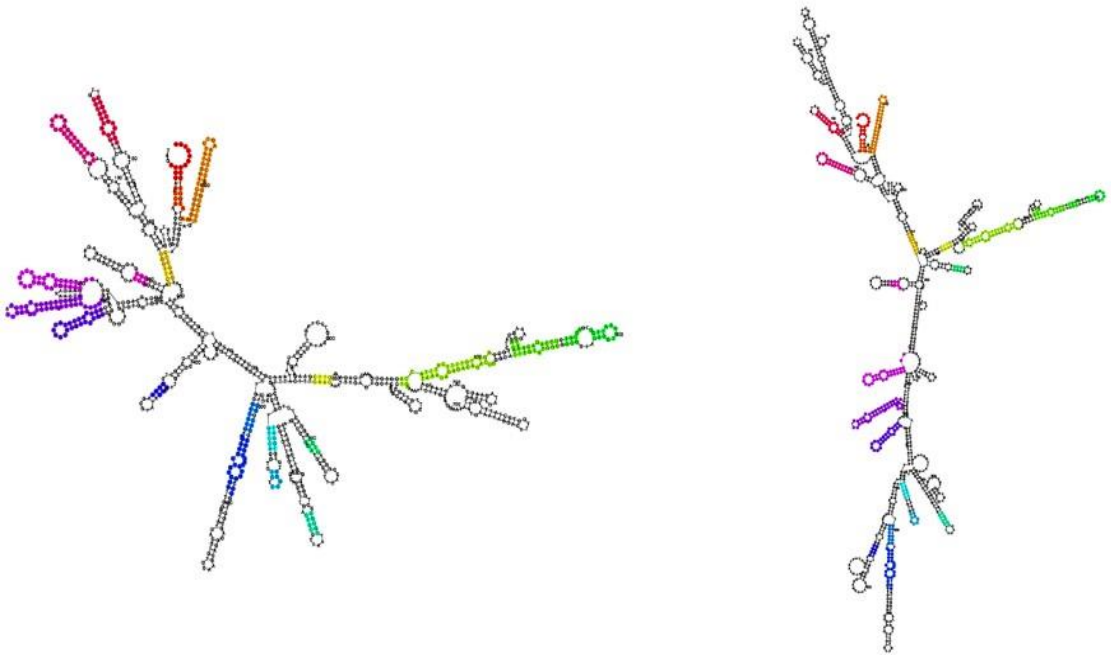
ExpaRNA Software used for the Prediction of RNA Secondary Structure [10], and the structure of RNA Secondary Structure by confirming the Short Conserved Sequences and by analysing various types of loops in the secondary structure like: Bulging, Hairpin like, Interior, Curved, Multi and Exterior loop appearance. Colour Code Patterns present in the generated Secondary Structure further confirms the Presence/Absence of Particular Conserved Sequences in a Particular Location. Although the sequences in the Secondary Structure are conserved across the Species in the Genus *Bychowskyella*, but this are present at various different locations which can be identified easily with the help of Colour Gradients. Light Green, Sky Blue, Violet, Yellow, Deep Blue, Pink, Red, Orange Colours particularly denote the Location of Conserved Sequences in the loops of Secondary Structure RNA, and these colours are variably located in the *Bychowskyella* species, Although all the Sequences are present in the all of its Species.



A. Bychowskyella wallagonia(On the Left) and B. Bychowskyella fossilisi(On the Right)



A. Bychowskyella tchangi(On the Left) and B. Bychowskyella pseudobagri(On the Right)

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Motif Predictions

MEME Suit Software[11] is used for the Identification Short Conserved Sequences across the Species. The data generated in this Software usually shows heighted alphabetical nucleotide bases, more is the height of the Nucleotide bases more is the base conserved. The Motifs generated confirms and validates the conserved regions in the Species under the Genus *Bychowskyella*. Three different types of Motifs are generated with the help of MEME Suit Software, The width length of the three Motifs are of 50 bases. Motif 1 is of Sky Blue Colour, Motif 2 Red Colour and Motif 3 of Light Green Colour. Motifs contains AAAA or other bases which means they contain repetitive sequences. In our Current Study, the colourful bars also represent the degree of conserveness across the species within the genus. Example- The violet bar, representing a particular sequence which is getting repeated across the species and at a particular location. The prediction is also done by observing the height of the nitrogenous bases generated, if the height of the sequences generated are higher means the sequences is conserved across generations and if its height is with decreasing intensity it means that it is less conserved among the species of the Genus *Bychowskyella*. It is compared along with the Phylogenetic tree. The P-Values for the Motifs of Particular Species are: 4.77e-249(KT852456.1:1-1120), 4.38e-249 (KT852454.1:32-1142), 1.17e-247 (KT852455.1:1-1027), 1.39e-244 (KY680236.1:42-1175) for all the Sequences.

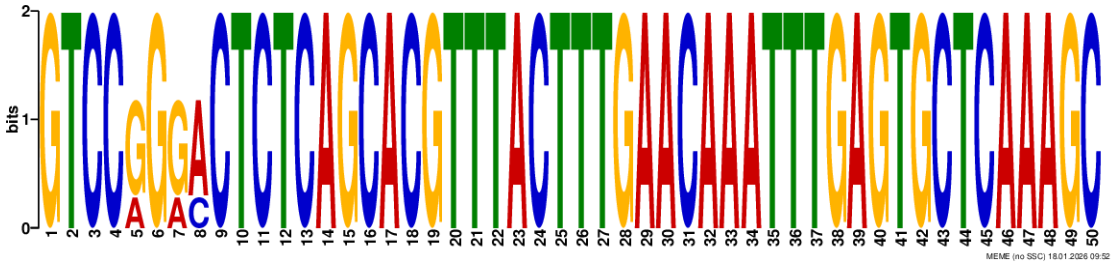


Fig. One Motif of 50 base pairs

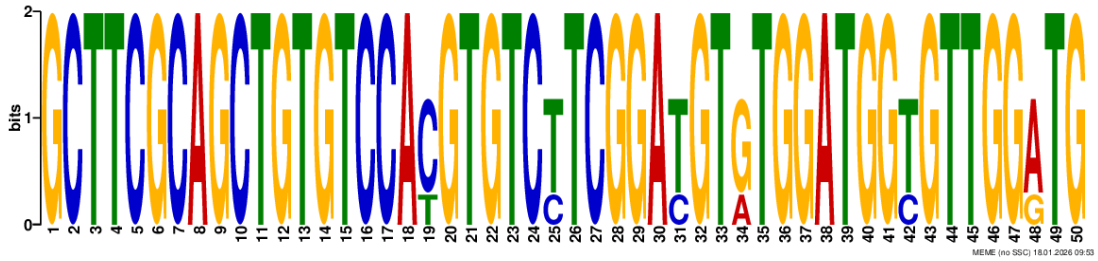


Fig. Second Motif of 50 base pairs

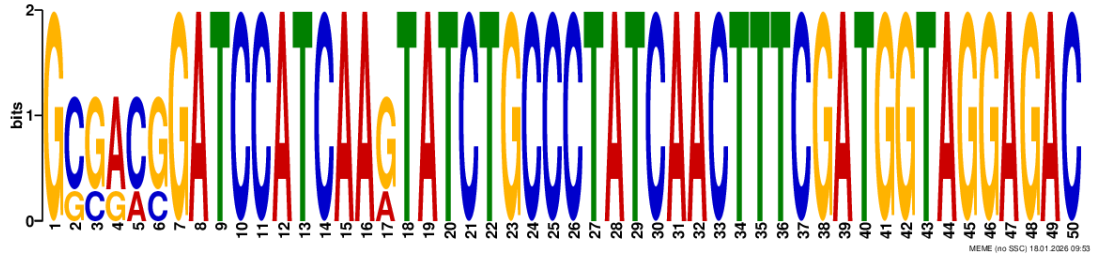
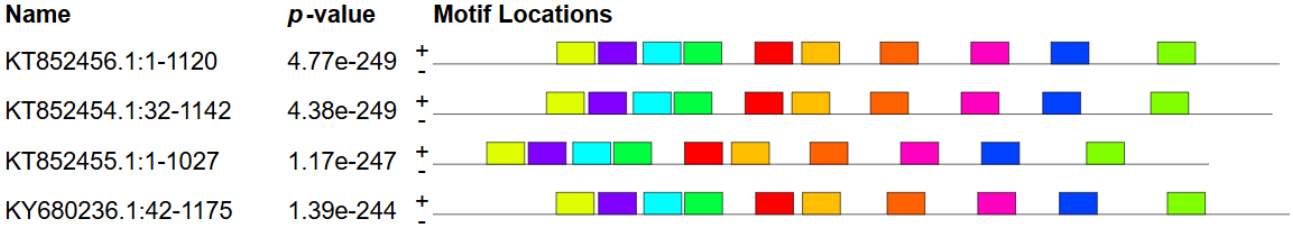


Fig. Third Motif of 50 base pairs



IV. CONCLUSION

The Genomic and Molecular Analysis of the above Species helps us in Distinction between the *Bychowskyella* Species very easily, as earlier studies on *Bychowskyella* were focused only on the Morphological and Morphometric features of *Bychowskyella*. Combining the current Molecular Studies along with Morphological Features will be very useful for easy Identification, Separation and Characterization of Individual *Bychowskyella* Species. In earlier studies, there was lot of confusion between Dactylogyrus and Bychowskyella Species leading to Species Inquirenda, But now though our current research, Molecular Based Studies along with Morphological Studies solves the Problems upto 99.5% accurately by precisely identifying the exact Species. The Molecular Level Studies gives very accurate results. The Phylogenetic and Evolutionary Studies using the 18S rDNA/18S rRNA is done by the use of Software like MEGA, ExpaRNA, MEME Suite Software which gives Gold Standard Results for Species Level Differentiations and Uses for the Taxonomy.

The Variations between the species can be possibly due to their nature of occurrence in a particular region, Hosts Organisms, Feeding Patterns etc. The Prediction of RNA Secondary Structure along with Motifs Prediction will guide future Researchers in the areas of Parasitology for Nobel Discoveries like Treatment of Diseases, Combating Parasitic Diseases, Studying Host Parasite Interactions etc.

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