

# **IN SILICO PHYLOGENETIC STUDY ON ANCYROCEPHALIDAE (CLASS: MONOGENEA) USING 28 S RIBOSOMAL RNA EXTENDING GEO-MAPPING IN SEARCH OF EVOLUTIONARY CUES**

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**ABSTRACT :** Class Monogenea attracted lot of attention for phylogenetic study in recent past. Family Ancyrocephalidae present a good material to employ *in silico* tools. Present study is an attempt to employ 28S rRNA information in predicting phylogeny of the different members. Another approach of integrating geo-mapping is also attempted for understanding diversity, distribution and relatedness among the various members of the family under the study.

**Key words:** Monogenea, Ancyrocephalidae, phylogeny 28SrRNA, geo-mapping.

## **INTRODUCTION**

Monogenea is the class of parasitic Platyhelminthes being extensively studied over the past many decades (Littlewood, 2007). Being a diversity rich class among lower parasite, there have been a chance to determine the ancient evolutionary clue (Chaudhary *et al*, 2013), with approximately 35 families, 220 genus and 1850 species (Poulin, 2002). Ancyrocephalidae, subjected to the present study, has 24 genus and 218 species (Gillardin *et al*, 2012) and represents two kinds of evolutionary descendents; monophyletic and paraphyletic descendents (Kritsky and Boeger, 1989). Monophyletic species- one that includes the most recent common ancestor of a group of organisms, and all of its descendents, whereas paraphyletic- includes the most recent common ancestor, but not all of its descendents (Hörandl and Stuessy, 2010). Diversity and molecular distinction are greatly raised by speciation of organism (Hunter, 2007), higher the level longer the time to diversification and *vice versa* (Rabosky, 2009). Simply lower organisms are needed lesser time to evolve and diversify at a faster rate (Mittelbach *et al*, 2007). Above all, unique genomic composition has significant effect over physiological behavior, determining the intrinsic tendency of organism (Milne *et al*, 2011). In ancient time, organism from the same family or genera were distributed in different geographical zones and exposed to varied environment and habitat that drastically modified genetic composition to the extent of speciation (Golestani *et al*, 2012). In order to evaluate the phylogenetic relationship among species of different genus and/or species from a particular family with their zoogeographic distribution, evaluation of

zoogeographical distribution together with molecular clue may present evolutionary history including probable origin of the organisms (Lomolino and Brown, 2009). New evidences may present new avenues for the study of evolutionary aspects, such as a picture of present and ancient history of organism that can be possessed by Zoogeographical distribution (Boero and Bouillon, 1993). Monogeneans have been used as one such tool for indirect study of their host, distribution, migration, zoogeographical diversity and settlement over period of time. Reasons behind great speciation and high diversity in family Ancyrocephalidae is that species represent either monophyletic or paraphyletic pattern of evolution (Pariselle *et al*, 2011). In both of the cases, species descendent is shown from nearby ancestors that indicate close chance of speciation at any time (Struck *et al*, 2014). Also, it is said to follow a faster route of evolution (Teeling *et al*, 2002).

Molecular phylogeny and its correlation with zoogeographical distribution are to measure the structural parameters of 28S rRNA (Amit Roy, 2014) (Chaudhary and Singh, 2012). Phylogenetic characters of ribosomal RNA basically include loops, bulges, helices and separation of single strands since they have been conserved throughout the evolution (Mathews *et al*, 2010). The Secondary structure of ribosomal RNA also provides satisfying information about evolutionary relationship that cannot be simply inferred from phylogenetic tree analyses using simple RNA/DNA sequences (Fozail *et al*, 2015) (Chen *et al*, 1999). Information regarding development of biomarker can also be obtained from ribosomal RNA for each species (Adams *et al*, 2013). In past, exhaustive

phylogenetic analyses have been performed on various family of monogenea. With data available in National Center for Biotechnology Information (NCBI), it is worth analyzing the phylogenetic relationships and re-setting the evolutionary relations in context of zoogeographical distribution. A general trend among Monogenean parasites Ancyrocephalidae is that almost all of them exhibit monophyletic and paraphyletic feature of evolution, therefore, understanding the molecular trends and utilizing 28S rRNA would be useful in comprehending and tracking ancient lineage of this family.

## MATERIALS AND METHODS

### Selection of species and genus

A total of 71 species from 12 genus of the family Ancyrocephalidae were selected based on upon confirmation of geographical distribution and the availability of 28S rRNA data.

### Molecular Phylogenetic Analysis

Sequences were aligned by ClustalW, analysis and optimization of MSA was performed in the same program. MEGA 6 was used for construction of phylogenetic tree using Neighbor joining method. Most parsimonious tree was obtained using the close-neighbor-interchange algorithm. Bootstrap procedure with 1000 replications for every species. The optimized phylogenetic tree was represented with 12 and many sister clades for further analysis.

### Inferring Secondary Structure of 28SrRNAs

Every cluster in the tree was given with two or more than two sequences, so they were aligned using ClustalW multiple sequence program and the sequence with the highest alignment score was obtained for inferring secondary structure. Multiple sequence alignment of each cluster provided the most conserved sequence based on score generated by ClustalW. Secondary structure of RNA was predicted using Mfold (URL <http://mfold.rna.albany.edu>); at a fixed temperature of 37°C. Formed structure was analyzed for unique structural patterns like bulges, stems, loops and negative free energy ( $\Delta G$ ). The procedure was repeated for all clusters that resulted in the formation of seven different structures of RNA molecule. Every cluster in the tree had been associated with its common ribosomal RNA that averaged out the evolutionary commonalities between the species of a particular cluster. This procedure facilitated the cladistic analysis more precise than the traditional comparison of clusters with bootstrap values only.

### Geo mapping

For global scenario of the species relatedness and

diversity, all the selected species as per table 1 were marked on simple world map manually (Fig. 5) and joined with reference to their respective cluster for inferring molecular relatedness.

## RESULTS

### Construction of Phylogenetic Tree

MEGA 6 constructed the phylogenetic tree are presented with bootstrap values (1000 replicates) forming 12 clusters, consisting of two or more than two species showing evolutionary cross relationship (fig-1).

Bootstrap values below 50% were removed from the tree. In the tree, Cluster A, B, C, D, E, F, G, H, I, J, K and L had 3, 3, 12, 3, 8, 5, 2, 10, 5, 2, 5 and 6 species respectively. Cluster A with three species exhibited strong (99%) bootstrap value and all species in the cluster belong to the same genus (*Enterogyrus*). This cluster showed the evolutionary distinction from other genus as represented in the crosses diagram with phylogenetic tree. Cluster B presented 99% bootstrap value but unlike A, clustered for two different genus (*Ancyrocephalus* & *Actinocleidus*), confirming their ancestral relationship and earlier divergence from each other. Cluster C was represented with two clades in which first clade expressed inter genus relationship between *Haliotrema* and *Pseudohaliotrema* whereas second clade expressed pure lineage of *Bravohalisa* with significant bootstrap values. Cluster D was shown with species from two genus (*Lethrinitrema* and *Haliotrema*). Cluster E was shown with pure lineage of *Haliotrema* with considerable bootstrap values of 60-70%. Cluster F was represented with species from two genus (*Metahaliotrema* and *Haliotrema*) with significant bootstrap values of 80-90%. Cluster G showed strong relatedness between the members of *Scutogyrus* with significant bootstrap value of 99%. Cluster H showed a unique pattern of cladistic relationship in which two clades were formed for the same genus, *Ligophorus*.

Both of the clades showed poor but considerable bootstrap values. Bootstrap for the second clade is 79% which is better than previous one with 62% only. The anomaly can be further accounted for the course of speciation and genus diversification. It has indicated that over the period of time cladistic relationship between members of the same the genus would follow a new route of lineage since mutation in genetic composition is more than enough to go through a different path of diversity.

Few of the species from the genus like *Onchobdella*, *Haliotrema* & *Ancyrocephalus* did not show cladistic relationship and hence were not counted as clade. For, four species of *Ligophorus* expressed strong values of

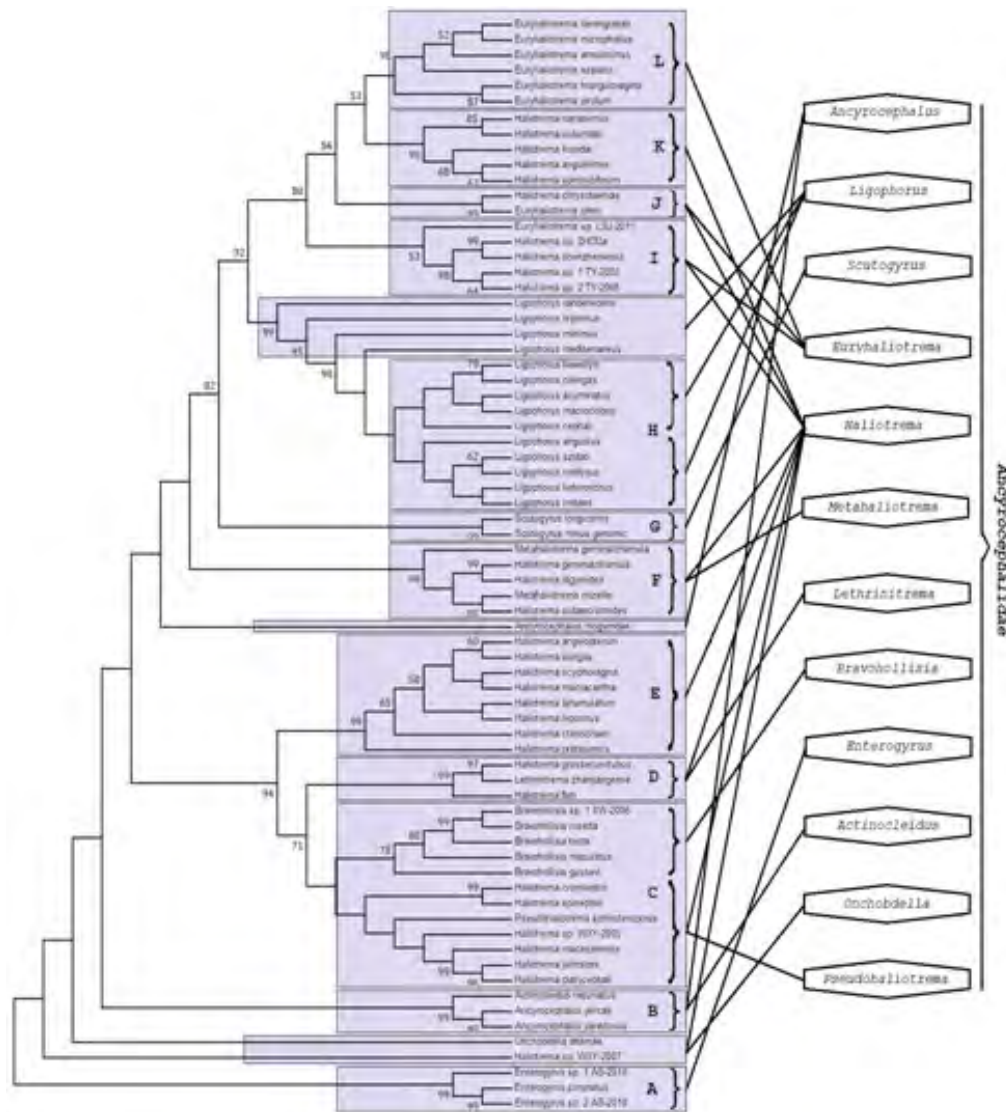


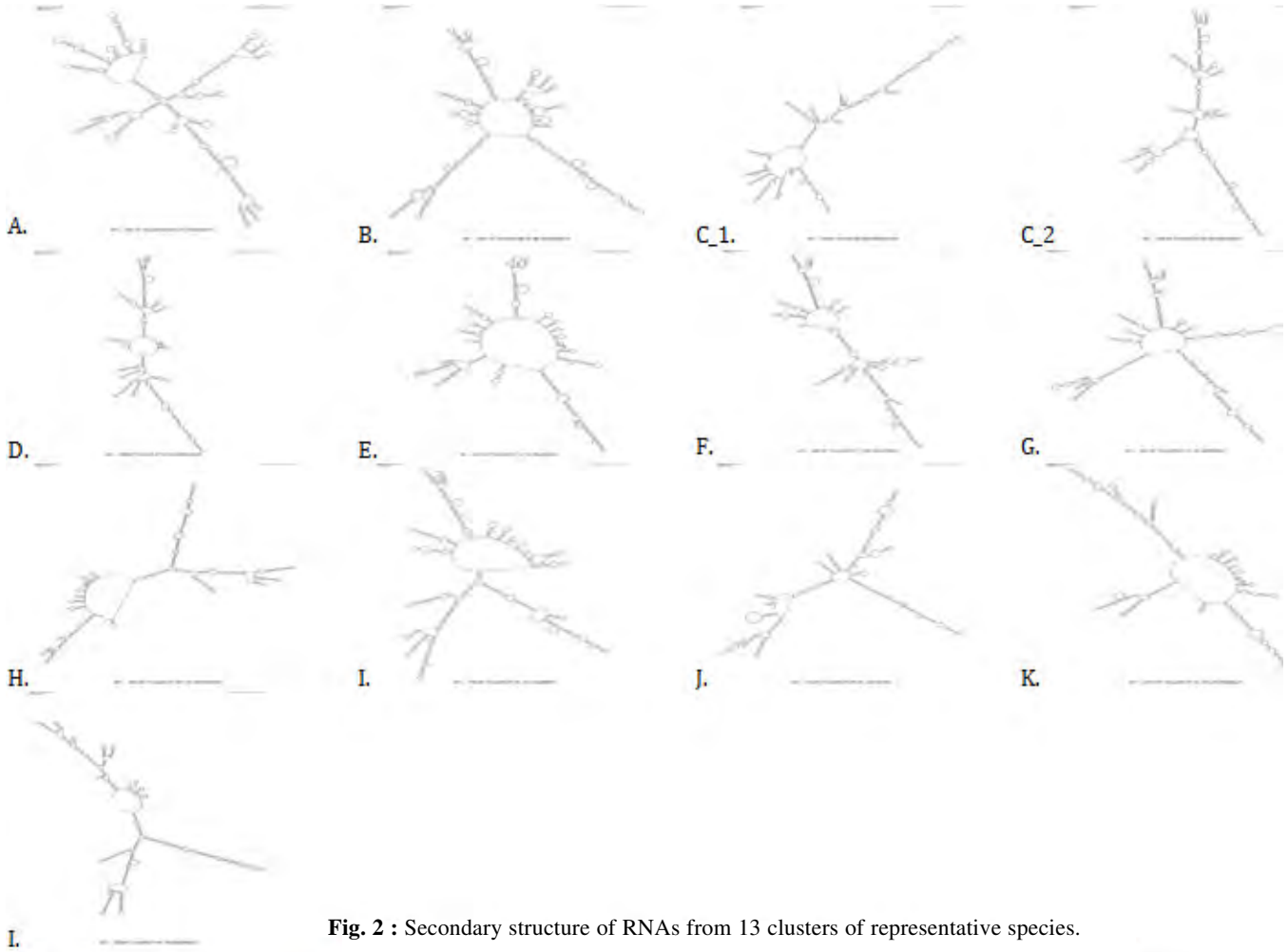
Fig. 1 : Phylogenetic tree of 71 species from family Ancyrocephalidae (Neighbor joining method).

90-99%, but fail to represent relatedness with others. Cluster I and J showed the inter genus relatedness *i.e.* species from two different genus whereas K and L showed pure lineage of the clusters with significant bootstrap values.

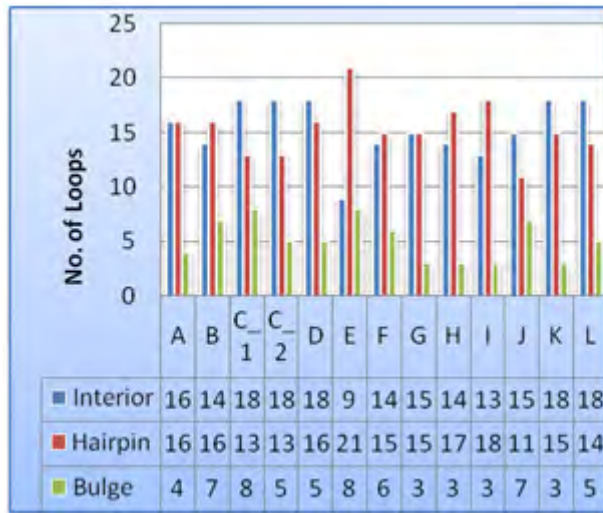
### Secondary Structure Analysis

The inferred secondary structure of 28S rRNA by Mfold of representative species from 13 clusters exhibited the evolutionary distinction between species and clusters as well (Fig. 2) also provided the stability of molecules in terms of negative free energy ( $\Delta G$ ). Representative species were selected by multiple sequence alignment of species from each cluster, and the most conserved sequence of the species was considered based on alignment score given by ClustalW. Formation of secondary structure is characterized by bulge loops, interior loops and hairpin loops conferred by negative free

energy of molecule. Higher the negative free energy ( $\Delta G$ ), more stable the molecule. Negative free energy of cluster A, B, C\_1, C\_2, D, E, F, G, H, I, J, K and L (rRNA from species) were predicted to be -242.70 kcal/mol, -243.10 kcal/mol, -219.40 kcal/mol, -222.20 kcal/mol, -208.60 kcal/mol, -231.40 kcal/mol, -202.20 kcal/mol, -243.40 kcal/mol, -284.40 kcal/mol, 244.60 kcal/mol, -218.20 kcal/mol, -235.10 kcal/mol and -220.70 kcal/mol respectively (Fig-3). The negative free energies of clusters A, B, G and I fall in the range of -243 kcal/mol and discrete by -2.0 kcal/mol approximately, representing that species from these groups had followed similar pattern of evolution. Anomaly to this observation can be accounted as the varying number of different loops directly affects stability of molecule. Cluster C\_1 and cluster J had an average negative free energy of -218.5 kcal/mol (discrete by approximately  $\Delta G = -0.5$  kcal/mol), shown to be correlating each other and representing evolutionary



**Fig. 2 :** Secondary structure of RNAs from 13 clusters of representative species.



**Fig. 3 :** Number of loops form Representative RNA secondary structure for each cluster.

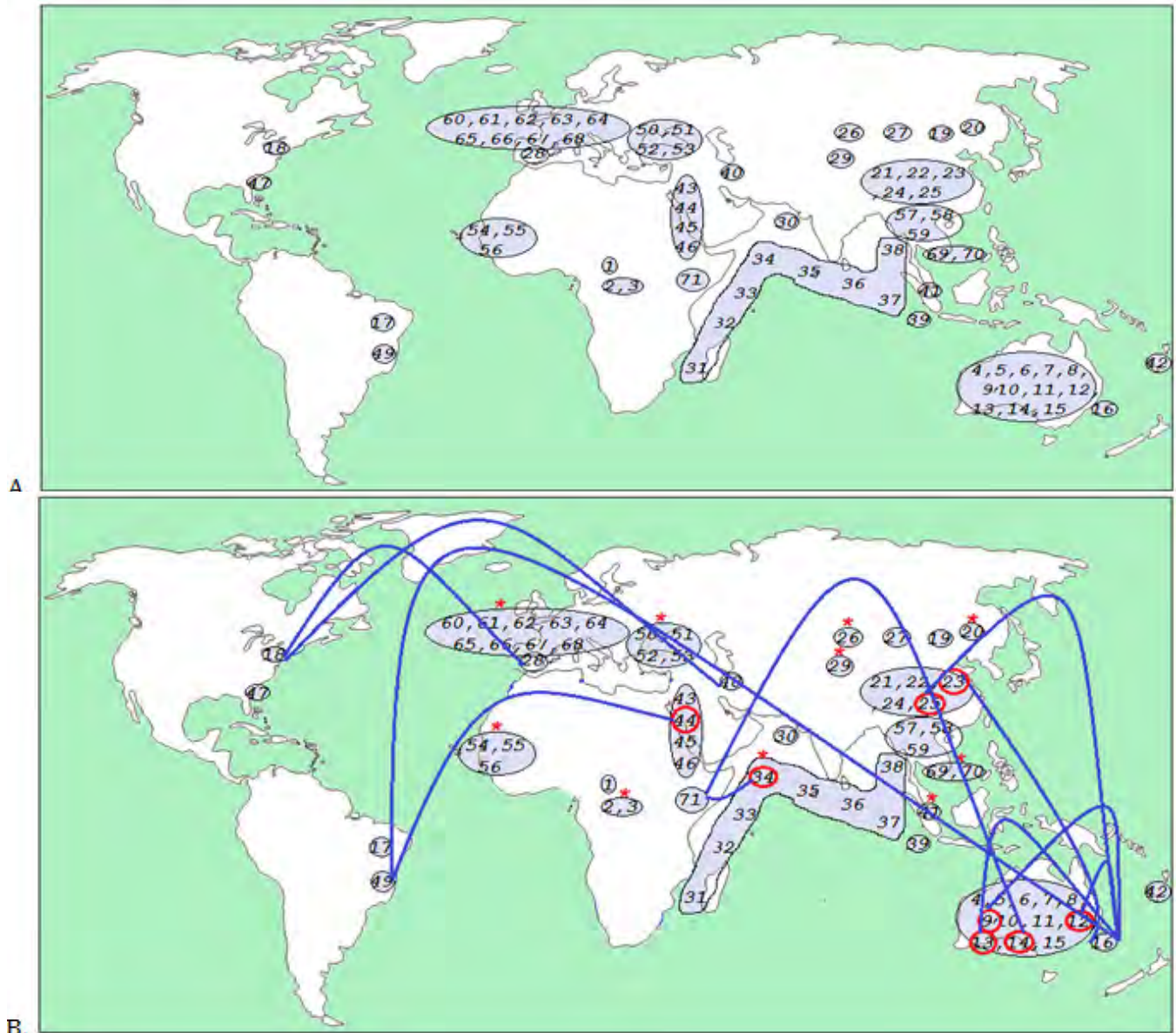


**Fig. 4 :** Negative free energy of the representative RNA structure for each cluster.

relatedness. Cluster D and F showed considerable difference of -6.0 kcal /mol, moreover they have been given the least negative free energies. As mentioned before, higher the  $\Delta G$  more stable the molecule. It also comprehends that more stable ribosomal RNA in the

species would represent lesser mutation and hence lesser speciation events over the period of time. These two clusters belong to three *Haliotrema*, *Metahaliotrema lithrinotrema*. Species from the genus exhibit faster rate of divergence. On the contrary, Species of the cluster H

## Geomapping



**Fig. 5 (A & B) :** Geo-mapping of 71 species, A- Manual plotting; B-Clade connections [\* represents group of species from same genus that did not relate with others and circle represents that species belong to the same genus].

with the genus *Ligophorus* ( $\Delta G = -284.40$  kcal/ mol) will exhibit gradual rate of divergence. Clusters C\_1, J and L represented almost equal amount of  $\Delta G$ , showing similar pattern of evolutionary conservation. Those of moderate  $\Delta G$  for the clusters would follow a general trend of speciation. The higher distinctions in negative free energy of some cluster also indicate that over the period of time they will soon tend to follow a different route of evolution. Since current study has considered family Ancyrocephalidae, genus with such significant features will represent a new family over the period of time. Three types of loops are formed in the secondary structure of RNA molecule (cluster/representative species) with unique pattern of occurrence (Fig. 2). The formation of

loops, as mentioned earlier, is almost conferred by negative free energy, resolves stability and constancy of the entire molecule.

The present secondary structure is characterized by number and pattern of secondary structure in RNA molecule. Number of loops varied for individual cluster species (Fig. 3). Number of hairpin loops (11-21) was highest whereas bulge loops (3-8) were found to be least in number. In this section of result, the most important analysis to be inferred was to find out the coincidences between number of loops and negative free energies. Sum of number of loops had fallen 33 to 39 did not show greater distinctions among clusters. Cluster C\_1 and D

**Table 1** : Genus with respective species (family Ancyrocephalidae) investigated in the study.

Genus	Species	Host	Locality	Env.	Acc. ID
<i>Actinocleidus</i>	<i>A. recurvatus</i> Mizelle & Donahue, 1944	<i>Lepomis gibbosus</i>	Canada	M	AJ969951
<i>Ancyrocephalus</i>	<i>A. mogurndae</i> Gussev, 1955	<i>Siniperca chuatsi</i>	China	F	DQ157667
	<i>A. paradoxus</i> Creplin, 1839	<i>Sander lucioperca</i>	Kurish Gulf	M	AJ969952
	<i>A. percae</i> Ergens, 1966	<i>Perca fluviatilis</i>	Germany	M	KF499080
<i>Bravohollisia</i>	<i>B. tecta</i> Venkatanarasaiah, 1984	<i>Pampus argenteus</i>	Hainan	M	KJ571012
	<i>B. gussevi</i> Lim, 1995	<i>Pomadasyss hasta</i>	Sungai Buloh	M	KJ571007
	<i>B. sp. Malaysia</i> Priesner, 1933		Malaysia	M	AF387509
	<i>B. maculates</i> Venkatanarasaiah, 1984		China	M	KJ571008
	<i>B. rosetta</i> Venkatanarasaiah, 1984	<i>Pomadasyss hasta</i>	Sungai Buloh	M	DQ537364
	<i>B. sp. 1 XW-2006</i> Priesner, 1933		Malaysia	M	DQ537365
<i>Enterogyrus</i>	<i>E. coronatus</i> Pariselle <i>et al</i> , 1991	<i>Tilapia dageti</i>	Senegal	M	HQ010030
	<i>E. sp. 1 AS-2010</i>	<i>Sarotherodon galilaeus</i>	Senegal	M	HQ010032
	<i>E. sp. 2 AS-2010</i>	<i>Sarotherodon galilaeus</i>	Senegal	M	HQ010031
<i>Euryhaliotrema</i>	<i>E. annulocirrus</i> Yamaguti, 1968	<i>Pachyurus junki</i>	I-W P. Ocean	M	EU836195
	<i>E. mehen</i> Solar <i>et al</i> , 2012	<i>Pachyurus junki</i>	I-W P. Ocean	M	HQ615997
	<i>E. aspistis</i> Plaisance & Kritsky, 2004	<i>Pachyurus junki</i>	I-W P. Ocean	M	AY820614
	<i>E. berenguelae</i> Plaisance & Kritsky, 2004	<i>Pachyurus junki</i>	I-W P. Ocean	M	AY820615
	<i>E. johni</i> Tripathi, 1959	<i>Pachyurus junki</i>	I-W P. Ocean	M	EU836193
	<i>E. microphallus</i> Yamaguti, 1968	<i>Pachyurus junki</i>	I-W P. Ocean	M	AY820617
	<i>E. pirulum</i> Plaisance & Kritsky, 2004	<i>Pachyurus junki</i>	I-W P. Ocean	M	AY820618
	<i>E. triangulovagina</i> Yamaguti, 1968	<i>Pachyurus junki</i>	I-W P. Ocean	M	AY820619
	<i>E. sp LSJ-2011</i>		I-W P. Ocean	M	HQ615997
<i>Haliotrema</i>	<i>H. angeloferum</i> Johnston & Tiegs, 1922	<i>Chaetodontidae</i>	I-W Islands	M	AY820620
	<i>H. aurigae</i> Yamaguti, 1968	<i>Chaetodontidae</i>	S W Parite	M	EU836198
	<i>H. bihamulatum</i> Zhang, 2001		China	M	DQ537378
	<i>H. chrysotaeniae</i> Young, 1968		Brazil	M	AF026115
	<i>H. cromileptis</i> Young, 1968	<i>Epinephelus coioides</i>	Australia	M	EU523146
	<i>H. ctenochaeti</i> Young, 1968		China	M	EU836199
	<i>H. digyroides</i> Zhang, 2001	<i>Epinephelus coioides</i>	China	M	DQ537377
	<i>H. epinepheli</i> Young, 1968	<i>Pinephelus fasciatus</i>	Australia	M	EU836201
	<i>H. fleti</i> Young, 1968	<i>Pinephelus fasciatus</i>	Australia	M	DQ157661
	<i>H. geminatohamula</i> Bychowsky & Nagibina, 1970	<i>Pinephelus fasciatus</i>	Australia	M	DQ157649
	<i>H. grossecurvitubus</i> Li & Chen, 2005		China	M	EU836204
	<i>H. johnstoni</i> Bychowsky & Nagibina, 1970	<i>Pinephelus fasciatus</i>	Australia	M	DQ157664
	<i>H. kurodai</i> Ogawa & Egusa, 1978	<i>Pinephelus fasciatus</i>	Australia	M	DQ537376
	<i>H. leporinus</i> Johnston & Tiegs, 1922	<i>Acanthurus nigrofuscus</i>	South China	M	EU836206
	<i>H. macasarensis</i> Yamaguti, 1963	<i>Platycephalus indicus</i>	China	M	EU836207
	<i>H. macracantha</i> Yamaguti, 1968		N. Caledonia	M	EU836208
	<i>H. nanaensis</i> Pan & Zhang, 2000	<i>Epinephelus coioides</i>	Australia	M	DQ537373
	<i>H. platycephali</i> Yin & Sproston, 1948	<i>Epinephelus coioides</i>	Australia	M	FJ767866
	<i>H. pratasensis</i> Sun <i>et al</i> , 2007	<i>Acanthurus nigrofuscus</i>	South China	M	EU836209
	<i>H. scyphovagina</i> Yamaguti, 1968		I-W P. Ocean	M	AY820622
<i>H. shenzhenensis</i> Wang <i>et al</i> , 2003	<i>Sciaenops ocellatus</i>	South China	M	DQ537372	
<i>H. spirothubiforum</i> Zhang, 2001	<i>Lutjanus vita</i>	Red Sea	M	DQ157656	

Table 1 continued....

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	<i>H. subancistroides</i> Zhang, 2001	<i>Gerres lucidus</i>	Red Sea	M	EU836210
	<i>H. sp. 1 TY-2005</i>		Red Sea	M	DQ058213
	<i>H. sp. 2 TY-2005</i>		Red Sea	M	DQ058214
	<i>H. sp. WXY-2005</i>	<i>pinephelus fasciatus</i>	Australia	M	DQ157663
	<i>H. sp. WXY-2007</i>	<i>pinephelus fasciatus</i>	Australia	M	EF437158
	<i>H. sp. ZHDDa</i>	<i>pinephelus fasciatus</i>	Australia	M	DQ157658
<i>Lethrinitrema</i>	<i>L. zhanjiangense</i> Sun et al, 2014	<i>Lethrinus nebulosus</i>	S. China Sea	M	KJ571017
<i>Ligophorus</i>	<i>L. acuminatus</i> Euzet & Suriano, 1977	<i>Liza saliens</i>	Spain	M	JN996816
	<i>L. angustus</i> Euzet & Suriano, 1977	<i>Chelon labrosus</i>	Spain	M	JN996816
	<i>L. cephalic</i> Euzet & Suriano, 1977	<i>Liza cephalus</i>	Spain	M	JN996830
	<i>L. confuses</i> Gil Corrado, 1936	<i>Liza ramada</i>	Spain	M	JN996807
	<i>L. heteronchus</i> Euzet & Suriano, 1977	<i>Liza saliens</i>	Spain	M	JN996812
	<i>L. imitansn</i> Euzet & Suriano, 1977	<i>Liza ramada</i>	Spain	M	JN996815
	<i>L. leporinus</i> Zhang & Ji, 1981	<i>Liza cephalus</i>	China	M	DQ537380
	<i>L. llewellyni</i> Dmitrieva et al, 2007	<i>Liza haematocheila</i>	Sea of Azov	M	JN996823
	<i>L. macrocolpos</i> Euzet & Suriano, 1977	<i>Liza saliens</i>	Spain	M	JN996819
	<i>L. mediterraneus</i> Hargis, 1955	<i>Liza cephalus</i>	Spain	M	JN996827
	<i>L. minimus</i> Euzet & Suriano, 1977	<i>Liza saliens</i>	Spain	M	JN996817
	<i>L. pilengas</i> Sarabeev & Balbuena, 2004	<i>Mugil soiuu</i>	Sea of Azov	M	JN996824
	<i>L. szidati</i> Euzet & Suriano, 1977	<i>Mugil soiuu</i>	Sea of Azov	M	JN996806
	<i>L. vanbenedenii</i> Parona & Perugia, 1890	<i>Mugil soiuu</i>	Sea of Azov	M	DQ157655
<i>Metahaliotrema</i>	<i>M. geminatohamula</i> Bychowsky & Nagibina, 1970	<i>Sphyrna argus</i>	S. Brazil	M	DQ157646
	<i>M. Mizellei</i> Yamaguti, 1953	<i>Sphyrna argus</i>	China	M	DQ157647
<i>Onchobdella</i>	<i>O. atramae</i> Peters, 1857	<i>Hemichromis fasciatus</i>	Africa	M	HQ010034
<i>Pseudohaliotrema</i>	<i>P. Sphincteroporos</i> Yamaguti, 1953	<i>Siganus doliatus</i>	Australia	F	AF382058
<i>Scutogyrus</i>	<i>S. longicornis</i> Paperna & Thurston, 1969	<i>African Cichlids</i>	Africa	M	HQ010035
	<i>S. minus</i> Pariselle & Euzet, 1995	<i>Sarotherodon</i>	Africa	M	HE792779

**Note :** IWP = Indo West Pacific, N = North, S = South, W = West, M = Marine, F = Freshwater

All the sequences of present study was taken from NCBI database. Acknowledgement is due to all the contributors.

with highest number of loops (39) (Fig. 4) were contended with lower negative free energies, showing an anomalous behavior of the molecules.

Second highest number of loops were contended by E, B, A, C\_2, K and L (38, 37, 36 & 36, 36 & 37 respectively) as a group. Their negative free energies did not coincide for number of loops. Clusters F, G, H, I and J had 34, 33, 34, 34 and 33 loops respectively with highly varied negative free energies. The anomalies between loops and  $\Delta G$  are occurred due size of hairpin, bulge and interior loops and unique pattern of nitrogenous bases in RNA. It also was confirmed that all cluster were evolutionary distinct. The great anomalous behavior of species representing cluster confirmed that individuals tend to evolve at faster rate than those of lesser coincidences with loops and  $\Delta G$ . This finding confirmed

that species belonging to different genus had a specific pattern of evolution and later on distributed in different regions. Apart from  $\Delta G$ , the equal number of loops of D and C\_1 showed that member belonging to these cluster had been following the same pattern of evolutions. The same hypothesis have been imposed for A & C\_2, B & E, and F, G, H, I & J with almost equal number of loops, representing strong ancestral relatedness among species. Though  $\Delta G$  was not very well peered for the clusters, it had been due to size and number of nitrogenous bases of RNA molecules. Only  $\Delta G$  does not account for relatedness of species in a clade, loops also insight into evolutionary relationship. Comparatively, three types of loops represented uniqueness, stability, conservation pattern, evolutionary relatedness and range of ancestral lineage.

## DISCUSSION

The molecular distinction among species from different genus provided an understanding over evolutionary process and expressed ancestral lineage to the origin of a new species. The phylogenetic tree with 13 clusters demonstrated the inter-genus relationship as few of them like *Haliotrema* was distributed into eight clades, showing the genus richness and high evolution in the family Ancyrocephalidae. Some of them remains highly conserved like *Ligophorus*, *Scutogyrus* & *Enterogyrus* and were not distributed in any clades. Distribution was further supported by RNA secondary structure of representative species in the folded form showed paired and unpaired (loops) bases. Number of different loops and negative free energies coincide the relatedness between genus. *Haliotrema*, *Ancyrocephalus*, *Ligophorus* & *Euryhaliotrema* were all found to relate with other clades. It was strengthened further by structural analyses of RNA molecule. Qualitatively, bases which are bonded tend to stabilize molecule due to higher negative free energy whereas unpaired bases tend to destabilize the molecule due to lesser negative free energy (Svoboda and Di Cara, 2006). Quantitatively, loop that are more in number destabilize the secondary structure because they require more positive free energy (Ding, 2006). Therefore, cluster A, B, and H are the most stable and cluster C\_1, C\_2, D, E, F, G, I, J, K and L are lesser stable structure, signifying that organisms belonging to the particular cluster will be of equal stability in terms of negative free energy of their RNA molecules, and hence, will follow the same pattern of origin and evolution (Shabalina, 2006 and Schuster, 2006). Although, negative free energy and number of loops showed noticeable variations within all clusters but a correlation between the two parameters have been established. Except cluster D, F, E, K and H, remaining eight clusters (cluster A, B, G & I), (cluster C\_1, C\_2, J and L) represented equal stability, conservation pattern and sympatric speciation events (Fig. 4). This was further strengthened by equal number of loops developed in the representative molecule. Loops more in number but smaller in size are formed with less negative free energy whereas loops less in number but larger in size require more negative free energies (Aalberts and Nandagopal, 2010). Evidently, both, size and number of loops are accounted for estimating out the stability of a molecule (Zhang *et al*, 2008). The pattern of evolution and relatedness among species is reflected by the development of loops and their sizes which in turn account for the overall stability of RNA (Petrillo *et al*, 2006). Evolution, most of the time, rises the level of complexity that is strictly coincided with

the necessities of situations (Stewart, 2014). RNA having more complex secondary structure presents with more loops and small sizes whereas molecule with lesser loops and large sizes shows lower level of complexity (Adami *et al*, 2000). Cladistic analysis corroborated that even after great speciation events, molecular information were maintained by species as two different species from two different genus, represented the cladistic relationship and had fallen in the same cluster (Fig. 1). Although, genus were distributed in different geographical zones (Fig. 6) but represented a particular group in the same family from different genus, indicating a common ancestral lineage, so their evolutionary history can be traced back to thousands of year (Fozail *et al*, 2015).

## CONCLUSION

The family Ancyrocephalidae shows species richness due to having dual evolutionary features in the family. Phylogenetic study confirmed the monophyletic and paraphyletic feature which was further supported by secondary structure analyses of representative species. Cladistic analysis giving strong clues about ancient lineage, origin and range of similarity was comprehended by secondary structure of 28S rRNA. Species distribution strengthened intra genus relationship, divergence, and migration over period of times. In the phylogenetic tree, clustering and cladistic hypothesis was supported by zoogeographical (geo-mapping) distribution in different zones of the world.

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