

IN-SILICO PHYLOGENETIC TOOLS EMPLOYED ON SOME MEMBERS OF FIVE MAJOR FAMILIES OF MONOGENEA VIZ., MONOCOTYLIDAE, ANCYLODISCOIDIDAE, ANCYROCEPHALIDAE, CICHLIDOGYRIDAE AND POLYSTOMATIDAE FOR INVESTIGATING THEIR RELATEDNESS AND GLOBAL DIVERSITY DISTRIBUTION

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ABSTRACT : The global diversity and the relatedness among the members of the same group remain a key attraction for evolutionary diversity. Members of parasitic Class Monogenea is extensively investigated during recent past. Present paper is an attempt to explore the concept of relatedness and global diversity evolution in five major families of this class using various *in-silico* tools. Study involve investigations on 227 species using 28S rRNA data and its geomapping co relations.

Key words : Geomapping, phylogeny, evolution, Monogenea.

INTRODUCTION

Enumerating the present time diversity of lower organisms and comprehending how they diversified in ancient time, are the points of milestones in evolutionary biology, ecology and conservation biology (Pariselle *et al*, 2011). The estimation of past parasitic biodiversity and present diversification is remained in its initial stage (Dobson *et al*, 2008). Efforts with multiple approaches have been carried out to present a convincing answer to these questions. Being an ideal taxon for investigation of past diversifications and present diversity, monogeneans have been extensively studied for number of important reasons (Poulin, 2002). Monogenea form a diverse group with thousands of species (Cribb, 2002). They don't show diversifications in numbers only but are the group among flatworms to have undergone an adaptive radiation, ecological adaptation, parasitism, multiple host relationship, adaptation from being external to internal parasite on the same host and morphological versatility (de León *et al*, 2010; Karvonen *et al*, 2012 and Vanhove *et al*, 2013). Apart from these features, host switching is a common phenomena in monogeneans at all the branches of its phylogeny making analysis easier to explore for a link between ecological characteristics of host and diversity of parasites, and to control for the phylogenetic history of their associations (Bakke *et al*, 2002; Badrane *et al*, 2001 and Reeves *et al*, 2015). As a whole it is quite difficult to estimate species and parasitic diversity, still there is a chance with good range of possibility of analyzing into families and subfamilies (Gerasev, 2004).

For all (approximately 4000) species, a total of 35 families have been classified followed by 250 genus designated in the literature and at various databases (Türkay Öztürk *et al*, 2014). Out of these families, Gyrodactylidae, Monocotylidae, Ancyrocephalidae, Capsilidae, Cichlidogyridae, Polystomatidae and Diplectanidae are among constantly studied and providing a novel hypothesis of evolutionary relatedness of their member species (Williams, 1991). Each of them possesses distinct features in terms of morphology, physiology, host specification, co-evolution and ecological patterns (Mladineo *et al*, 2013). Families like Ancylodiscooididae and Polystomatidae and members of Dactylogyrids are afforded with the members of fresh water bodies, making a geographic linking among those of other fresh water species across the globe (Vanhove *et al*, 2014). Incorporation of information into family analysis have been paid attention due to encompassing a range of diversity richness in monogeneans with a vital understanding over all aspects of parasitism, making evolutionary study more interesting and easier at the secondary stage of analyses (Cribb *et al*, 2002 and Fozail *et al*, 2015a-c).

Geographical study on monogeneans does not exactly show their origin and hence it needs to be strengthened further, since their distribution merely demonstrates a clue to the root of diversification (Badets *et al*, 2009 and Fozail *et al*, 2015a-c). Together with molecular phylogeny and zoogeographical tracking as a combinatorial approach to the ancient history may provide an insight to common origin and diversification of this taxon (Poisot *et al*, 2011).

Phylogeny itself is not capable of resolving this problem, however a molecular pattern among members of the group can be established in order to understand parasitic diversity with all due consideration of features mentioned above (Telford, 2006).

In present study, we intend to present the prevalence of major families in different geographical zones and their evolutionary relatedness using molecular data in order to understand their possible pattern of occurrence/diversification/relatedness.

MATERIALS AND METHODS

Selection of families

Selection of families (Table 1) is based upon diversity of family and the previous phylogenetic analyses being performed by us and genomic data of species exists in NCBI (National Center for Biotechnology Information).

Table 1 : Summary about families selected for the study.

Sl.	Family	Total genus	Total species	rRNA type
1.	Ancylodiscoididae	6	27	28S
2.	Ancyrocephalidae	12	72	28S
3.	Cichlidogyridae	1	23	28S
4.	Monocotylidae	12	39	28S
5.	Polystomatidae	15	44	28S

Molecular Phylogenetic Analysis

Initially nucleotide sequences of all species for all families were retrieved from NCBI. The sequences for separate family were aligned using Multiple Sequence Alignment (MSA) program with clustalW. Subsequently, each MSA was subjected to MEGA6 for inferring phylogenetic tree. The average pathway method was used to calculate the branch length depicted in the number of variations all over the sequences. Resultantly, the most parsimonious tree was chosen by the close-neighbor-interchange algorithm by keeping bootstrap value of 1000 replication.

Geomapping and Cladistic Comparison of families

Geomapping of each family was done on physical world map. Later on occupied positions by species on the map were connected to infer their geographical pattern and parasitic diversity. Phylogenetic tree for each species were represented with clades/cluster so as to determine intra genus relationship and to strengthen geographical occurrence.

RESULTS

Construction of Phylogenetic Tree

After MSA sequences were processed for tree

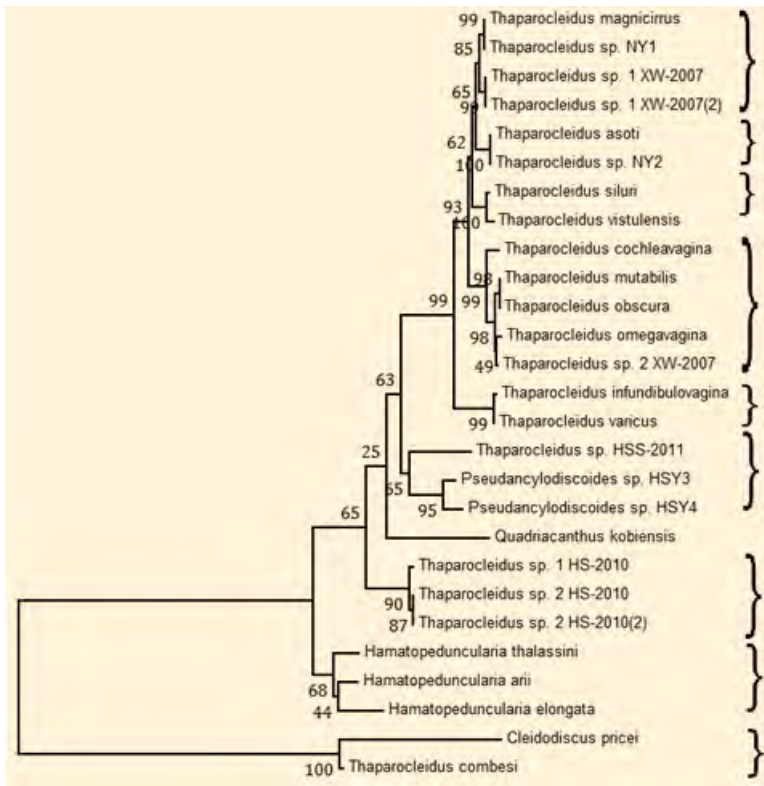
construction, five trees were constructed using MEGA6 for each family (fig. 1). Number of species for each family in the phylogenetic tree varied due to unavailability of molecular (rDNA) data in NCBI. Later on trees were grouped into clades/cluster. Number of clades in each tree differed because number of species was not equal for all families. Possible error was minimised by focusing onto the geographical distribution of members into families and not clades (later section). Bootstrap values exhibited significant variations over branches and rendered to be 70% as standard value to significance.

The family Ancylo-discoididae (fig. 1 A) gave a total of nine cluster wherein, many sister clades were present. Evolutionarily, species followed distinctive root of diversity as shown by branch length of its phylogenetic tree. Although, members of this family are less in number, approximately 27, but formation of nine clades signifies that parasitic diversity has deep root so far as evolution is concerned. They have been evolving at a much faster rate than the members of other families in the study.

The family Ancyrocephalidae (fig. 1 B) with highest number of species formed highest number of clades that has been coincided with its length of phylogenetic tree. Family Cichlidogyridae (fig. 1 C) with 23 species had five clades that followed a conserved root of evolution. Family Polystomatidae and Monocotylidae (fig. 1 D & E) with 44 and 39 species respectively had showed equal number of clades, following almost adequate pattern of evolution.

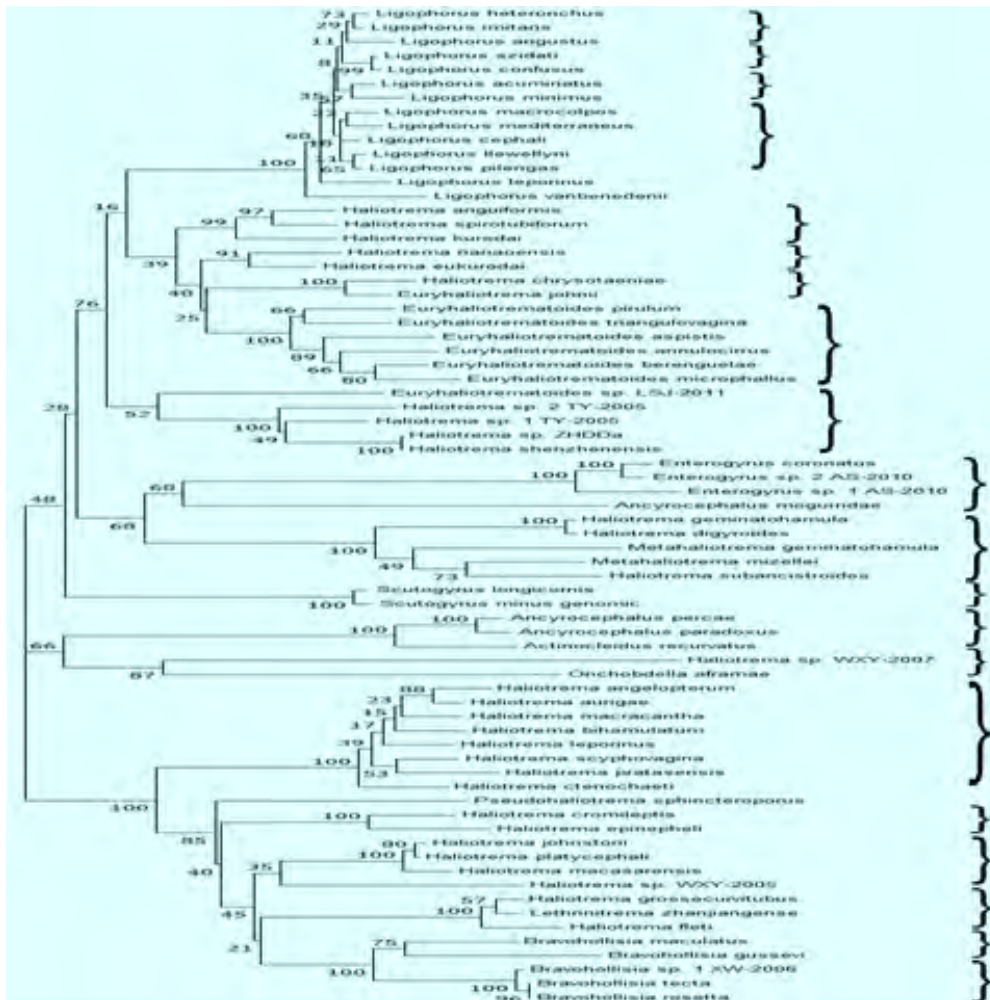
Clade versus Geomapping

Family distribution was not bound to a specific location except certain families. Ancyrocephalidae with highest number of species and clades found to be distributed in all sub-continent. This family was more related with Australian zones and less propagated in other zones. Phylogenetic patterns, although, did not reveal that which group of species was more prevalent still smaller number of clades reflected rapid pace of variability among members of this family. China in parallel to Australia displayed a thorough distribution along with Indo-west Pacific Ocean (fig. 2). Members of Ancyrocephalidae were distributed over all geographic zones including Africa, Europe, and North & South America (fig. 2). This was pretty agreeable to the pattern of formation of cluster in the phylogenetic tree but it had deviated from the number and geographical distribution that most of the species should not have been found in confined in the specific locations rather it should have been equally dispersed. Therefore, it has been confirmed that reason behind high number of cluster in the phylogenetic tree is



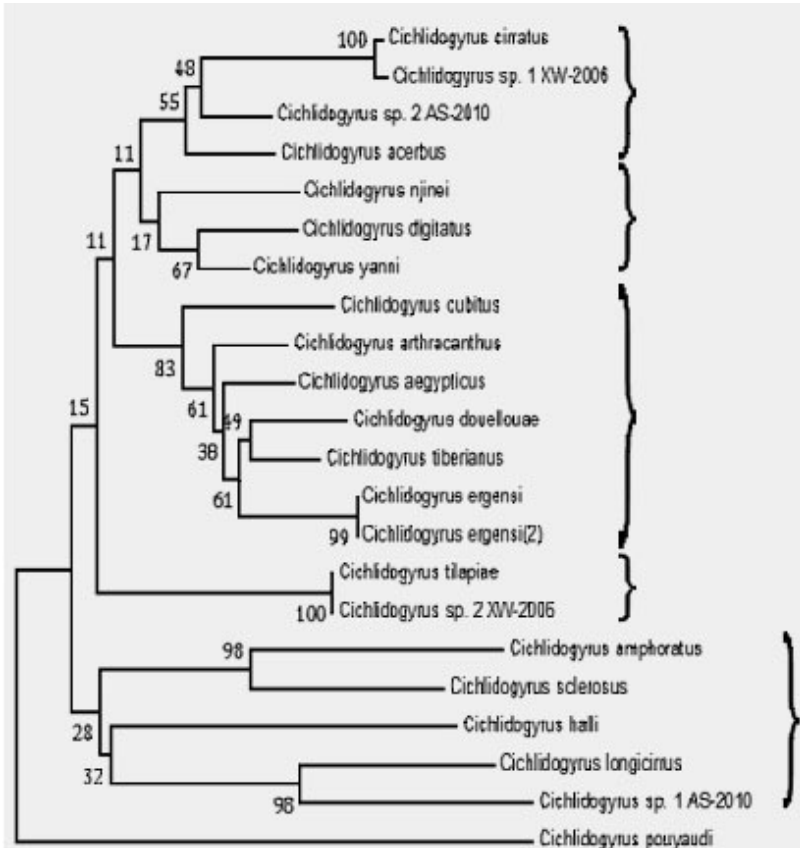
vast geographical distribution as environmental and ecological factors have caused over all changes in members including molecular and physiological variations. Clades presented in this study have shown that if more species fall in the same cluster then rate of diversification is less and if clades are formed with less species then rate of diversification is higher in that particular taxon. On the other hand, highly diversified taxon greatly distributed across the globe, expected to be found in all regions of the world. Majority of members of the family Ancylo-discoididae were falling into Indian water bodies and few of them were distributed into other sub-continent. Importantly, other aspect of the information could be comprehended as they have been widely found in Indian zones; they would have followed a route back to fresh water lineage. Their origin would have aroused through river systems and then turned into brackish and marine organism at lateral stage of evolution, resolving a clue

A



B

Fig. 1 continued...



C



D

Fig. 1 continued...

towards tracing of ancestral lineage and ancient history as well. Moreover, confinement in a particular location would decrease down the variability factor due to environmental and ecological constancy. Here number of clades did not matter efficaciously but prevalence did for Ancylo-discoididae. Most African and South African countries afforded the family Cichlidogyridae with least number of species in the study. According to the number of clades in its phylogenetic tree, distribution was shown to be normal. Out of 23 members only three from non African regions, showing a lesser variability among genus and good compatibility in molecular pattern of species. Reason behind lesser number of species in the family could be hypothesized by ecological and environmental features of a particular place. Besides this limited dispersion and geographical separation could have been one of the reasons leading to minor variability among members. Family Monocotylidae had a better coincidence between number of clades and geographical spots, it contained 11 clusters and distributed in all regions except China and Europe. Australian and American zones afforded more species than any other part. Out of these geographies, maximum members were confined to Australian regions representing a higher frequency of conservation as a group among all others. Although, clusters had varied a bit from dispersion but it totally depends upon number of species in a clade. Apart from Australian zones, North & South America regions also kept significant number of Monocotylidae along with North Atlantic Ocean. Even after confining in a specific location, species represent wide molecular pattern, signifying that all of the individuals in that particular region would show great variability in their nucleic acid composition. It has been supported by the distribution of families Monocotylidae and Ancyrocephalidae itself as both of them have been found in specific zones but molecularly represent higher diversity as far as evolutionary relatedness is

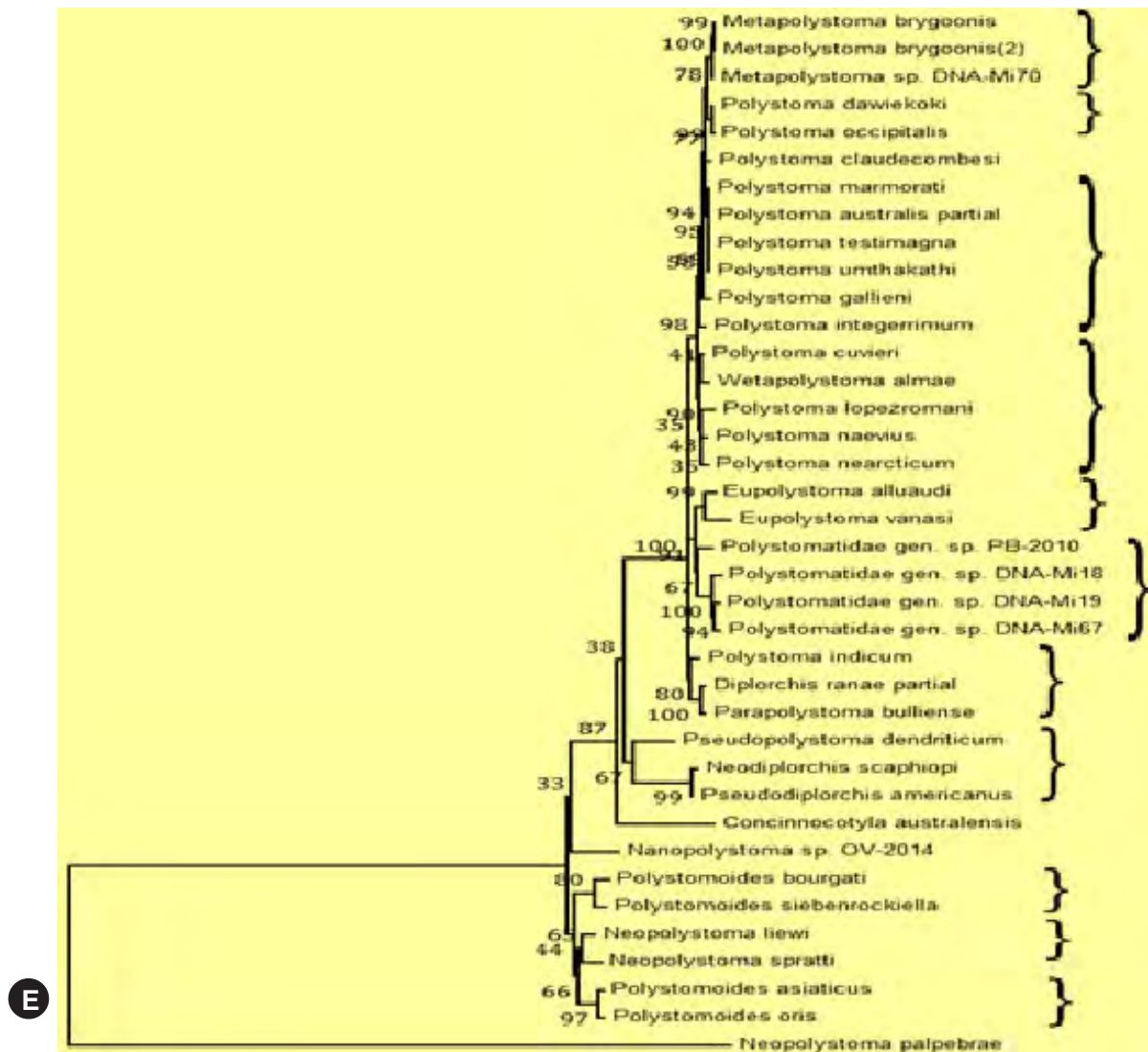


Fig. 1 : Phylogenetic tree representing of five different families-

A. Ancylo-discoididae : In all 27 species from 6 different genus studied; **B.** Ancyrocephalidae : In all 72 species from 12 different genus studied; **C.** Cichlidogyridae : In all 23 species from 1 genus studied; **D.** Polystomatidae : In all 44 species from 15 different genus studied & **E.** Monocotylidae : In all 39 species from 12 different genus studied.

concerned. Ecological and environmental elements would have definitely caused such anomalies in individuals. Therefore, it is not limited to Monogeneans only but other member from different class would face the same environmental attributes. Family Polystomatidae represented the best coincidence between number of clades and geographical patterns. Eleven clusters with 44 species were given to this family and their distribution came out to be equal in all regions of the world. No sub-continent was left unoccupied from Polystomatidae. Resultantly, such expression strengthen the fact about molecular conservation and parallel evolution and show that even after being exposed to various environmental and ecological conditions, individual were not much affected to the extent of totally different route of diversity

and evolution. On the other hand they possess the magnificent tendency to conserve their molecular composition for a longer period of time.

DISCUSSION AND CONCLUSION

Among all family Ancyrocephalidae showed the greater prevalence followed by family Polystomatidae (fig. 2), confirming that these two families are the most diversified among others in the study. It was supported by cladistic analyses wherein species were clustered with two or three members. This finding coincides well with evolutionary relatedness among species of the same families that more the clades more the distribution/diversification. Other families did not represent similar pattern of diversification as they showed conserved or confined origin to a specific location. Ancylo-discoididae

Table 2 : Summary of 227 species studied.

Family : Ancylo-discoididae		
Sl.	Species	Location
1.	<i>Hamatopeduncularia arii</i>	India
2.	<i>Hamatopeduncularia thalassini</i>	India
3.	<i>Hamatopeduncularia elongata</i>	India
4.	<i>Cleidodiscus pricei</i>	Lake Norman
5.	<i>Notopterodiscoides notopterus</i>	India
6.	<i>Pseudancylo-discoides</i> sp. HSY3	India
7.	<i>Pseudancylo-discoides</i> sp. HSY4	India
8.	<i>Quadriacanthus kobienis</i>	India
9.	<i>Thaparocleidus asoti</i>	India
10.	<i>Thaparocleidus caecus</i>	Southeast Asia
11.	<i>Thaparocleidus cochleavagina</i>	India
12.	<i>Thaparocleidus combesi</i>	India
13.	<i>Thaparocleidus infundibulovagina</i>	India
14.	<i>Thaparocleidus magnicirrus</i>	India
15.	<i>Thaparocleidus mutabilis</i>	India
16.	<i>Thaparocleidus obscura</i>	India
17.	<i>Thaparocleidus omegavagina</i>	India
18.	<i>Thaparocleidus siluri</i>	India
19.	<i>Thaparocleidus varicus</i>	India
20.	<i>Thaparocleidus vistulensis</i>	India
21.	<i>Thaparocleidus</i> sp. 1 HS-2010	India
22.	<i>Thaparocleidus</i> sp. 1 XW-2007	India
23.	<i>Thaparocleidus</i> sp. 2 HS-2010	India
24.	<i>Thaparocleidus</i> sp. 2 XW-2007	India
25.	<i>Thaparocleidus</i> sp. HSS-2011	India
26.	<i>Thaparocleidus</i> sp. NY1	India
27.	<i>Thaparocleidus</i> sp. NY2	India
28.	<i>Hamatopeduncularia laria arii</i>	India
29.	<i>Hamatopeduncularia thalassini</i>	India
Family: Ancyrocephalidae		
30.	<i>Actinocleidus recurvatus</i>	Canada
31.	<i>Ancyrocephalus mogurndae</i>	China
32.	<i>Ancyrocephalus paradoxus</i>	Kurish Gulf
33.	<i>Ancyrocephalus percae</i>	Germany
34.	<i>Bravohollisia tecta</i>	Hainan
35.	<i>Bravohollisia gussevi</i>	Sungai Buloh
36.	<i>Bravohollisia</i> sp. Malaysia	Malaysia
37.	<i>Bravohollisia maculates</i>	China
38.	<i>Bravohollisia rosetta</i>	Sungai Buloh
39.	<i>Bravohollisia</i> sp. 1 XW-2006	Malaysia
40.	<i>Enterogyrus coronatus</i>	Senegal
41.	<i>Enterogyrus</i> sp. 1 AS-2010	Senegal
42.	<i>Enterogyrus</i> sp. 2 AS-2010	Senegal
43.	<i>Euryhaliotrema annulocirrus</i>	I-W P. Ocean
44.	<i>Euryhaliotrema mehen</i>	I-W P. Ocean
45.	<i>Euryhaliotrema aspistis</i>	I-W P. Ocean
46.	<i>Euryhaliotrema berenguelae</i>	I-W P. Ocean
47.	<i>Euryhaliotrema johni</i>	I-W P. Ocean
48.	<i>Euryhaliotrema microphallus</i>	I-W P. Ocean
49.	<i>Euryhaliotrema pirulum</i>	I-W P. Ocean
50.	<i>Euryhaliotrema triangulovagina</i>	I-W P. Ocean
51.	<i>Euryhaliotrema</i> sp. LSJ-2011	I-W P. Ocean

Table 2 continued...

Table 2 continued...

52.	<i>Haliotrema angelopterus</i>	I-W Islands
53.	<i>Haliotrema aurigae</i>	S W Parite
54.	<i>Haliotrema bihamulatum</i>	China
55.	<i>Haliotrema chrysoaeniae</i>	Brazil
56.	<i>Haliotrema cromileptis</i>	Australia
57.	<i>Haliotrema ctenochaeti</i>	China
58.	<i>Haliotrema digyroides</i>	China
59.	<i>Haliotrema epinepheli</i>	Australia
60.	<i>Haliotrema fleti</i>	Australia
61.	<i>Haliotrema geminatohamula</i>	Australia
62.	<i>Haliotrema grossecurvitubus</i>	China
63.	<i>Haliotrema johnstoni</i>	Australia
64.	<i>Haliotrema kurodai</i>	Australia
65.	<i>Haliotrema leporinus</i>	South China
67.	<i>Haliotrema macasarensis</i>	China
68.	<i>Haliotrema macracantha</i>	N. Caledonia
69.	<i>Haliotrema nanaoensis</i>	Australia
70.	<i>Haliotrema platycephali</i>	Australia
71.	<i>Haliotrema pratasensis</i>	South China
72.	<i>Haliotrema scyphovagina</i>	I-W P. Ocean
73.	<i>Haliotrema shenzhenensis</i>	South China
74.	<i>Haliotrema spiro-tubiforum</i>	Red Sea
75.	<i>Haliotrema subancistroides</i>	Red Sea
76.	<i>Haliotrema</i> sp. 1 TY-2005	Red Sea
77.	<i>Haliotrema</i> sp. 2 TY-2005	Red Sea
78.	<i>Haliotrema</i> sp. WXY-2005	Australia
79.	<i>Haliotrema</i> sp. WXY-2007	Australia
80.	<i>Haliotrema</i> sp. ZHDDa	Australia
81.	<i>Lethrinotrema zhanjiangense</i>	S. China Sea
82.	<i>Ligophorus acuminatus</i>	Spain
83.	<i>Ligophorus angustus</i>	Spain
84.	<i>Ligophorus cephalic</i>	Spain
85.	<i>Ligophorus confuses</i>	Spain
86.	<i>Ligophorus heteronchus</i>	Spain
87.	<i>Ligophorus imitansn</i>	Spain
88.	<i>Ligophorus leporinus</i>	China
89.	<i>Ligophorus llewellyni</i>	Sea of Azov
90.	<i>Ligophorus macrocolpos</i>	Spain
91.	<i>Ligophorus mediterraneus</i>	Spain
92.	<i>Ligophorus minimus</i>	Spain
93.	<i>Ligophorus pilengas</i>	Sea of Azov
94.	<i>Ligophorus szidati</i>	Sea of Azov
95.	<i>Ligophorus vanbenedenii</i>	Sea of Azov
96.	<i>Metahaliotrema geminatohamula</i>	S. Brazil
97.	<i>Metahaliotrema Mizellei</i>	China
98.	<i>Onchobdella atramae</i>	Africa
99.	<i>Pseudohaliotrema Sphincteroporos</i>	Australia
100.	<i>Scutogyrus longicornis</i>	Africa
101.	<i>Scutogyrus minus</i>	Africa
Family: Cichlidogyridae		
102.	<i>Cichlidogyrus amphoratus</i>	Africa
103.	<i>Cichlidogyrus falcifer</i>	Africa
104.	<i>Cichlidogyrus sclerosus</i>	Uganda
105.	<i>Cichlidogyrus</i> sp. 1 AS-2010	

Table 2 continued...

Table 2 continued...

106.	<i>Cichlidogyrus</i> sp. 1 XW-2006	
107.	<i>Cichlidogyrus</i> sp. 2 AS-2010	
108.	<i>Cichlidogyrus</i> sp. 2 XW-2006	
109.	<i>Cichlidogyrus amphoratus</i>	Africa
110.	<i>Cichlidogyrus acerbus</i>	Africa
111.	<i>Cichlidogyrus aegypticus</i>	Africa
112.	<i>Cichlidogyrus digitatus</i>	Africa
113.	<i>Cichlidogyrus acerbus</i>	Africa
114.	<i>Cichlidogyrus aegypticus</i>	Africa
115.	<i>Cichlidogyrus arthracanthus</i>	Africa
116.	<i>Cichlidogyrus arthracanthus</i>	Africa
117.	<i>Cichlidogyrus cubitus</i>	Benin
118.	<i>Cichlidogyrus ergensi</i>	Benin
119.	<i>Cichlidogyrus cubitus</i>	Benin
120.	<i>Cichlidogyrus njinei</i>	Cameroon
121.	<i>Cichlidogyrus cirratus</i>	Israel
122.	<i>Cichlidogyrus cirratus</i>	Israel
123.	<i>Cichlidogyrus tiberianus</i>	Israel
124.	<i>Cichlidogyrus pouyaudi</i>	Kogon River
125.	<i>Cichlidogyrus yanni</i>	Kogon
126.	<i>Cichlidogyrus douellouae</i>	Mékrou Rive
127.	<i>Cichlidogyrus halli</i>	Phongolo
128.	<i>Cichlidogyrus tilapiae</i>	South Africa
129.	<i>Cichlidogyrus longicirrus</i>	Ghana
Family: Monocotylidae		
130.	<i>Caliocotyle affinis</i>	N. A. Ocean
131.	<i>Caliocotyle japonica</i>	Japan
132.	<i>Caliocotyle kroyeri</i>	Mexico
133.	<i>Caliocotyle palombi</i>	N. A. Ocean
134.	<i>Caliocotyle stossichi</i>	Mexico
135.	<i>Caliocotyle urolophi</i>	Australia
136.	<i>Caliocotyle</i> sp. CWAI	
137.	<i>Caliocotyle</i> sp. EMP	
138.	<i>Clemacotyle australis</i>	Australia
139.	<i>Decacotyle floridana</i>	Mexico
140.	<i>Decacotyle tetrakordyle</i>	Australia
141.	<i>Dendrocotyle ardea</i>	Australia
142.	<i>Dendrocotyle bradsmithi</i>	Australia
143.	<i>Dendrocotyle octodiscus</i>	N. A. Ocean
144.	<i>Dictyocotyle coeliaca</i>	N. A. Ocean
145.	<i>Empruthotrema dasyatidis</i>	Queensland
146.	<i>Empruthotrema quindecima</i>	Australia
147.	<i>Heterocotyle capricornensis</i>	Australia
148.	<i>Merizocotyle australensis</i>	Australia
149.	<i>Merizocotyle icopae</i>	Australia
150.	<i>Merizocotyle sinensis</i>	Taiwan
151.	<i>Merizocotyle urolophi</i>	Tasmania
152.	<i>Monocotyle corali</i>	Australia
153.	<i>Monocotyle helicophallus</i>	Australia
154.	<i>Monocotyle multiparous</i>	Australia
155.	<i>Monocotyle spiremae</i>	Australia
156.	<i>Monocotyle</i> sp. Tunisia	Tunisia
157.	<i>Neoheterocotyle hinobatidis</i>	Australia
158.	<i>Neoheterocotyle rhinobatis</i>	Australia

Table 2 continued...

Table 2 continued...

159.	<i>Neoheterocotyle rhynchobatis</i>	Australia
160.	<i>Potamostrygonocotyle aramasae</i>	Brazil
161.	<i>Potamostrygonocotyle chisholmae</i>	River basin (USA)
162.	<i>Potamostrygonocotyle dromedarius</i>	Brazil
163.	<i>Potamostrygonocotyle quadracotyle</i>	Brazil
164.	<i>Potamostrygonocotyle rarum</i>	Brazil
165.	<i>Potamostrygonocotyle rionegrense</i>	Brazil
166.	<i>Potamostrygonocotyle tsalickisi</i>	River basin (USA)
167.	<i>Potamostrygonocotyle umbella</i>	Brazil
168.	<i>Trogocephalus rhinobatidis</i>	Australia
Family: Polystomatidae		
169.	<i>Diplorchis ranae</i>	
170.	<i>Madapolystoma</i> sp. DNA-Mi18	
171.	<i>Madapolystoma</i> sp. DNA-Mi19	
172.	<i>Madapolystoma</i> sp. DNA-Mi67	
173.	<i>Metapolystoma</i> sp. DNA-Mi70	
174.	<i>Nanopolystoma</i> sp. OV-2014	
175.	<i>Neodiplorchis scaphiopi</i>	
176.	<i>Polystomoides oris</i>	
177.	<i>Polystomatidae</i> gen. sp. PB-2010	
178.	<i>Diplorchis ranae</i>	
179.	<i>Polystomoides asiaticus</i>	Africa
180.	<i>Polystoma claudecombesi</i>	Africa
181.	<i>Polystoma dawiekoki</i>	Africa
182.	<i>Concinnocotyla australensis</i>	Australia
183.	<i>Neopolystoma palpebrae</i>	Australia
184.	<i>Concinnocotyla australensis</i>	Australia
185.	<i>Polystoma integerrimum</i>	Europe
186.	<i>Polystoma indicum</i>	India
187.	<i>Polystoma occipitalis</i>	Ivory Cost
188.	<i>Pseudopolystoma dendriticum</i>	Japan
189.	<i>Metapolystoma cachani</i>	Madagascar
190.	<i>Metapolystoma brygoonis</i>	Malagasy
191.	<i>Diplorchis ranae</i>	Africa
192.	<i>Madapolystoma</i> sp. DNA-Mi18	Africa
193.	<i>Madapolystoma</i> sp. DNA-Mi19	Africa
194.	<i>Madapolystoma</i> sp. DNA-Mi67	Australia
195.	<i>Metapolystoma</i> sp. DNA-Mi70	Australia
196.	<i>Nanopolystoma</i> sp. OV-2014	Australia
197.	<i>Neodiplorchis scaphiopi</i>	Europe
198.	<i>Polystomoides oris</i>	India
199.	<i>Neopolystoma spratti</i>	Malaysia
200.	<i>Neopolystoma liewi</i>	Malaysia
201.	<i>Polystomoides siebenrockiella</i>	Malaysia
202.	<i>Polystoma naevius</i>	Mexico
203.	<i>Polystoma gallieni</i>	Morocco
204.	<i>Polystomoides bourgati</i>	Nigeria
205.	<i>Parapolystoma bulliense</i>	Northern Queensland
206.	<i>Neopolystoma orbiculare</i>	Palaeartic region
207.	<i>Polystoma cuvieri</i>	Paraguay
208.	<i>Polystoma lopezromani</i>	Paraguay
209.	<i>Eupolystoma vanasi</i>	South Africa
210.	<i>Polystoma australis</i>	South Africa
211.	<i>Polystoma marmorati</i>	South Africa

Table 2 continued...

Table 2 continued...

212.	<i>Polystoma testimagna</i>	South Africa
213.	<i>Polystoma umthakathi</i>	South Africa
214.	<i>Eupolystoma alluaudi</i>	Togo
215.	<i>Wetapolystoma almae</i>	Tropical Peru
216.	<i>Pseudodiplorchis americanus</i>	USA
217.	<i>Polystoma nearcticum</i>	USA
218.	<i>Neopolystoma spratti</i>	Malaysia
219.	<i>Neopolystoma liewi</i>	Malaysia

Table 2 continued...

220.	<i>Polystomoides siebenrockiella</i>	Malaysia
221.	<i>Polystoma naevius</i>	Mexico
222.	<i>Polystoma gallieni</i>	Morocco
223.	<i>Polystomoides bourgati</i>	Nigeria
224.	<i>Parapolystoma bulliense</i>	Northern Queensland
225.	<i>Neopolystoma orbiculare</i>	Palaeartic region
226.	<i>Polystoma cuvieri</i>	Paraguay
227.	<i>Polystoma lopezromani</i>	Paraguay

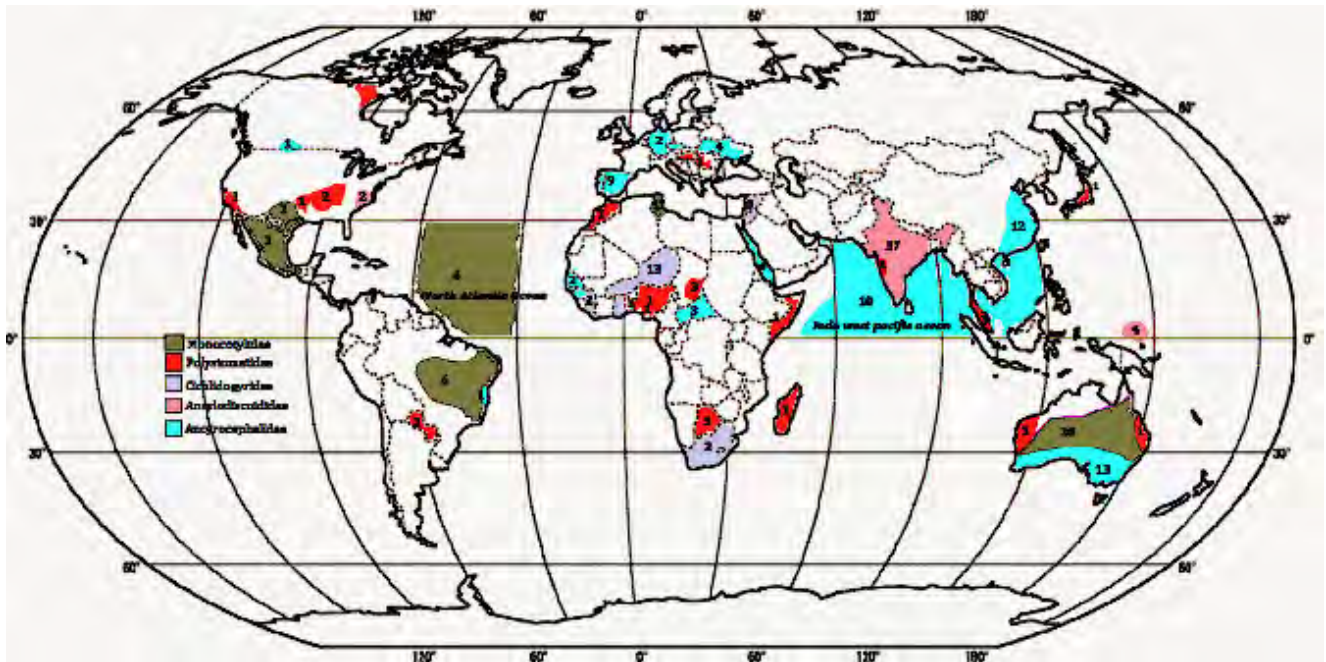


Fig. 2 : Geomapping of species from five major families (numbers representing number of species in the respective region).

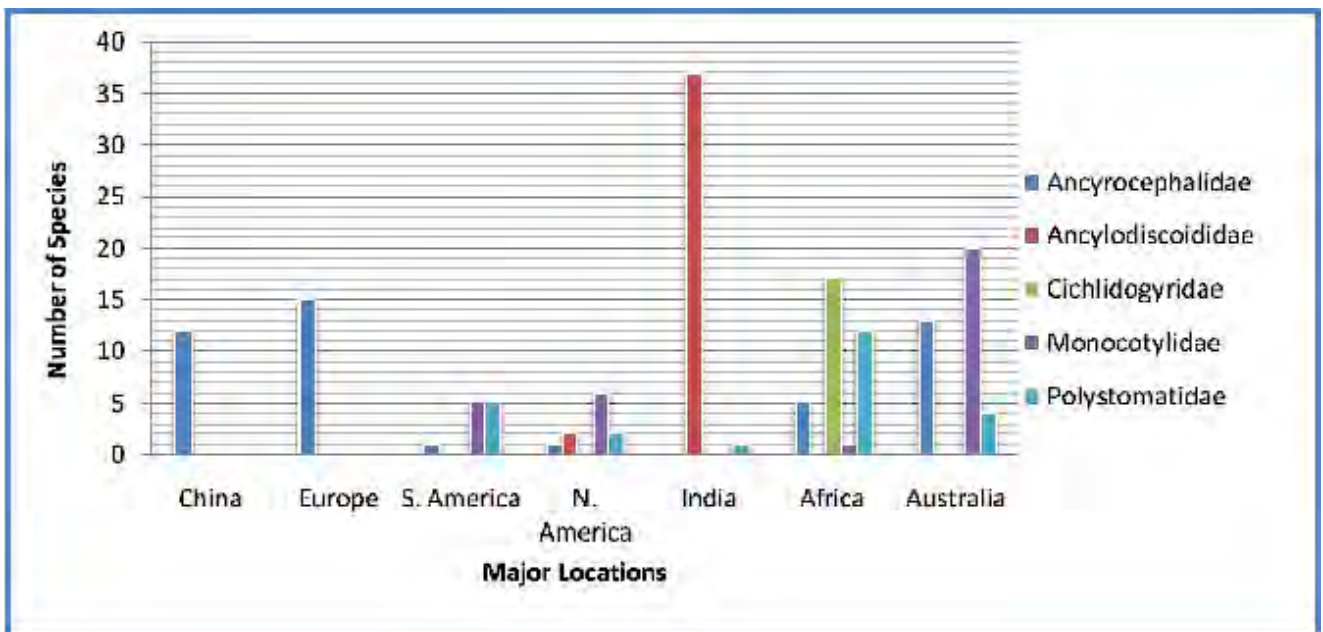


Fig. 3 : Families showing zoogeographical distribution of selected five families in major zones of the world.

and Cichlidoxyridae represented significant level of conservation being confirmed by both geomapping and clustering as well. Another aspect of this conservatory point could be accounted as the robustness of the species, genus or families as they possessed the potential to confront the changing environmental and ecological conditions. This finding provides a range of enumerations that how species went prevalent into specific geographical zones of the world and what was the amount of change that caused their migration to other corner of the globe. Monogeneans have versatile nature to switch from one place to another and rapidly change morphology and become adapted, suggesting that families are specific to their member species and allow evolving when exposed to suitable environmental conditions.

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