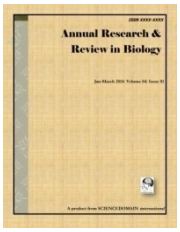

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Understanding the Phylogenetics and Evolution of Genus *Schistosoma*- Africa and Asia Stand Point

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Authors' contributions

Author OSO designed the study, performed the software analysis, wrote the protocol and wrote the first draft of the manuscript. Author BO help in the design of methodology and also provide some technical assistance during analysis. Author CIA conducted the literature searches, make final review of the analysis and study for correction and provide us with further professional information. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The evolutionary spread of schistosomes infection was reportedly prominent more in Africa and Asia continents. This study therefore examined the evolutionary trend of this parasite while limiting the investigation to *Schistosoma species* peculiar to this region of the world. The evolutionary history of this species group was inferred using DNA sequences from NCBI Genbank database and Maximum likelihood, Ancestral inference; Neighbor-Joining method analysis was employed in this study. All members of this species complex were AT rich, with *S. mekongi* and *S. malayensis* having the highest AT nucleotide composition. The smallest evolutionary divergence was also observed in *S. curassoni* and *S. bovis*. The finding of this study slightly contradict previous report on ancestral prediction of Schistosomes.

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1. INTRODUCTION

Human schistosomiasis is endemic in large areas of the sub-tropics. Over 700 million people in 74 countries have been estimated to be exposed to the risk of *Schistosoma* infection and almost 200 million were estimated to be infected in 2003 [1], of which 85% in sub-SaharanAfrica [21]. Schistosomiasis is a disease affecting agricultural communities, particularly those dependent upon irrigation to support their agriculture. Evolutionary history of the parasite *Schistosoma* has generated much interest especially with regard to understanding the precise roles that particular species have in causing human disease [2,3,21]. *Schistosoma species* as decribed by Lawton *et al*., were categorised into four major groups based on their distribution, host specificity and egg morphology, this include the *S. japonicum*(found throughout eastern and southern Asia), *S. indicum* (inhabits the western and southern regions including India, Thailand and Sri Lanka), *S. mansoni* (Africa) and *S. haematobium* (throughout Africa) group [4]. Phylogenetic and phylogeographic studies can identify historically and evolutionary independent groups of populations and can also identify natural or anthropogenic colonization routes [5]. Analyses of genetic structure use patterns of neutral genetic diversity to reveal how populations are structured across geography, within and among hosts on a local scale. This approach can provide information about pathogen transmission that is difficult to ascertain otherwise [6]. Genetic structure analyses are powerful tools for inferring epidemiological and evolutionary processes in schistosomes. They indicate that transmission foci are structured by watershed boundaries, habitat types, and host species. Recently molecular markers have been used for a variety of genomic-based taxonomic, phylogenetic, population and evolutionary investigations in animal species [7,8]. One of these DNA markers is the Mitochondrial DNA (mtDNA) sequence. Although mtDNA-sequence data have proved valuable in phylogenetic analysis, the selection of the appropriate gene for analysis is important. Among the coding genes in the mitochondrion genome, subunit I of the cytochrome oxidase (COI) gene possesses features suitable for evolutionary studies [9,10]. MtDNApossess a relatively fast mutation rate, which results in significant variation in mtDNA sequences between species providing an ample within species variance useful for phylogenetic investigations [11,12].The present study, explored by retrieving the cytochrome oxidase subunits I (COI) of mitochondrial DNA of the 16 member of the *Schistosomaspecies* to infer the evolution and phylogeny of this group while considering Africa and Asia continent perspective.

2. MATERIALS AND METHODS

2.1 Schistosomes Sequence Retrieval

16 nucleotide sequences of individual mtDNA gene sequences of *COI* and an outgroup *ssrDNA* sequence, of members of the *Schistosoma species* from the GenBank database (www.ncbi.nim.nih.gov) was retrieved (see Table 1). These were chosen based on their classification as African and Asian oriented schistosoma species [3]. Sequence alignment was performed by using Clustal Omega software (http://www.ebi.ac.uk/Tools/msa/clustalo), through elimination of all positional gaps and missing data. The sequences were then trimmed to get their equal lengths for all the species.

2.2 Inferring Evolutionary History

The evolutionary history was inferred using Maximum Likelihood method based on the Tamura-Nei model [13], Neighbor-Joining method [14] and Minimum Evolution method [15]. Allthe phylogenetic analyses were conducted in MEGA software (http://www.megasoftware.net), version 5.0 [16]. Ancestral states were inferred using the Maximum Likelihood method [17], under the General Time Reversible model [17]. All the positions containing gaps or missing data were eliminated (complete deletion) from the dataset prior to analysis. However, MEGA software also provides option to retain all such sites initially and excluding them as necessary in the pair-wise distance estimation (pair-wise deletion option) or to use the partial deletion (site coverage) as a percentage. Evolutionary analyses were conducted in MEGA 5 [16].

2.3 Prediction of Evolutionary Divergence

Evolutionary divergence between and overall Sequences pairs of the *Schistosoma species* were achieved by using the Maximum Composite Likelihood model [17] of the MEGA 5 software.

3. RESULTS AND DISCUSSION

The nucleotide composition of each taxon in the examined family as presented in Fig. 1, shows that all members of this family were all AT rich, which could possibly imply that AT (Adenine-thymine) rich environment in schistosome COI genes revealing long (dA).(dT) tracts that are found preferentially in introns and flanking gene regions, and thus pose an advantage for evolutionary study. *S. mekongi* and *S. malayensis* had the highest AT composition with both having 47.8% thymine(T)/21.8% Adenine(A) and 47.6% thymine (T)/21.8% Adenine (A) respectively, while *S. mansoni* had the least among the family with 41.2% thymine (T) and 27.1% Adenine (A).The nucleotide frequencies are 21.74% (A), 46.78% (T), 21.47% (C) and 10.01% (G). There is more of thymine in the sequences and less of guanine. The estimated evolutionary distance of divergence between sequences of *Schistosoma species* is presented in table 3, with the smallest evolutionary divergence found between *S. curassoni* and *S. bovis*(0.053),which is followed by *S. intercalatum*/*S. curassoni* and *S. intercalatum*/*S. curassoni* (with both groups at evolutionary divergence of 0.064 respectively). The predicted evolutionary divergence is observed to closely related to the outcome of phylogenetic analysis conducted in this study (Figs. 2-5). This result slightly differs from phylogenetic report where this species were duly considered (3). The divergence between other paired species was quite greater. It could be inferred from this result that *S. curassoni* and *S. bovis*are the most closely related taxa. The overall transition/transverse bias is R=0.823, where R=[A*G*k_1+T*C*k_2]/ [(A+G)*(T+C)]. The study shows that transverse mutational frequencies were more than that of transitional mutation (Table 2). Transitional mutation occurs more in adenine (16.75), guanine (16.56) and far less occur in cytosine (3.51). The ancestral state was inferred using Maximum Likelihood method [16] and is presented in Fig. 2. It could be incidental that the ancestral origin from which other members of this complex was predicted is *S. mansoni*, being the species with the longest estimated ancestral inference. *S. bovis* and *S. nasale* are the most recently evolved taxa among the *Schistosomaspecies*, which is followed by *S. mattheei* from where they possibly share similar ancestors. The ancestral node from which *S. indicum* and *S. intercalatum* evolved from was inferred to be *S. malayensis*, which share a very close ancestral linkage with *S. mansoni*. *S. haematobium* and *S. incognitum* also share a close ancestral connection with *S.*

margrebowiei and *S. spindale*. The ancestral prediction of this study shows a migration initiated from Africa via *S. mansoni* through the central and South East Asia (India) and then back to Africa (Figs. 2,3,4,5). To forster our undertsnading about the ancestral prediction of schistosoma species, an outgroup maximum likelihood phylogenetics analysis was also conducted, using a Schistosoma species ssrDNA sequence (Fig. 5), the outcome of this analysis also correspond with other related analysis and thus further buttress the evolutionary migration of Schistosomiasis from Africa to Asia and back to Africa. It could be deduced that lack of records of infection in Africa and cultural challenges while considering the symptoms of this infection may be a challenge in proper report of this infection. Many studies have reported the geographical evolution of this infection [3,19,20] and have reported the Asian ancestry of the schistosomes, and also gave weight to the concept of the reinvasion back from Africa. Evolutionary history inferred using the Maximum likelihood and Neighbour-Joining tree methods are both presented in figure 3 and 4 respectively. It was inferred that the topology of the two phylograms show some level of similarities, as *S. bovis* and *S. curassoni*[18] with *S. intercalatum* being their closest relative, *S. haematobium* and *S. leiperi*, also *S. malayensis* and *S. mekongi* are all closely related as appeared in both phylograms. The result of these phylograms could help our understanding in the pattern of infection of closely related members of the parasite, having considered the phylogenetic relationship in this complex and also attest useful in vector control.

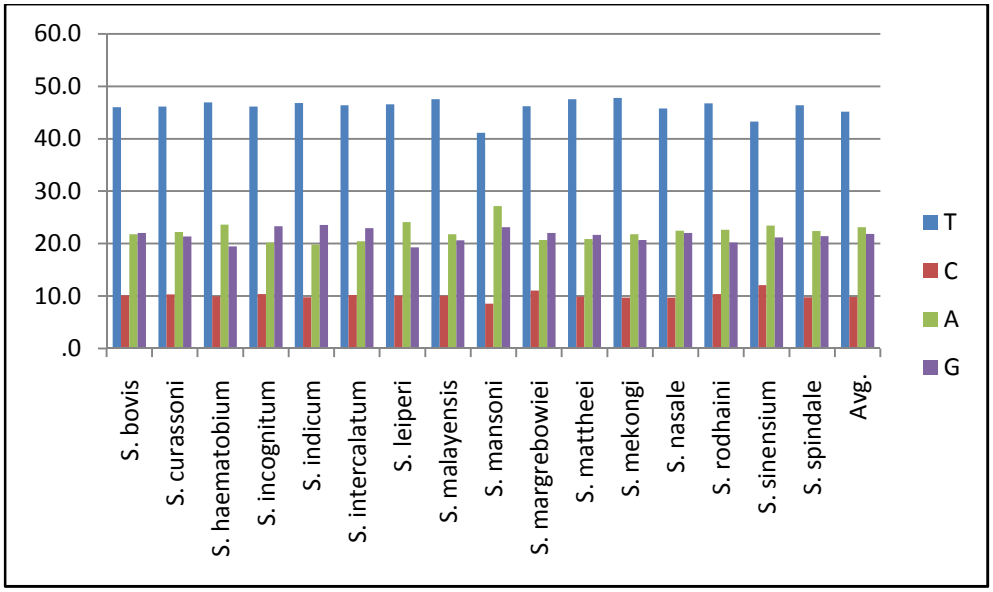

Fig. 1. Nucleic acid composition of the taxa of *Schistosoma species*

** Avg. represent average nuceic acid composition, T (thymine), C (cytosine), A (adenine) and G (Guanine)*

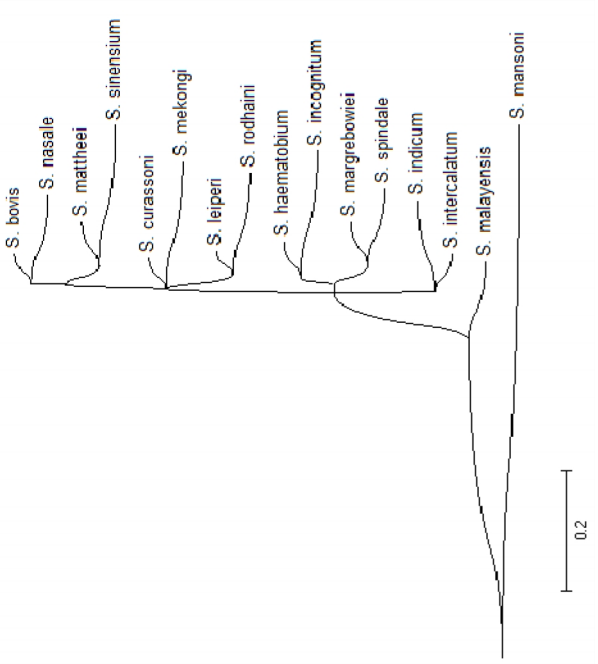

Fig. 2. Inferred Ancestral Sequences of the *Schistosoma species* taxa

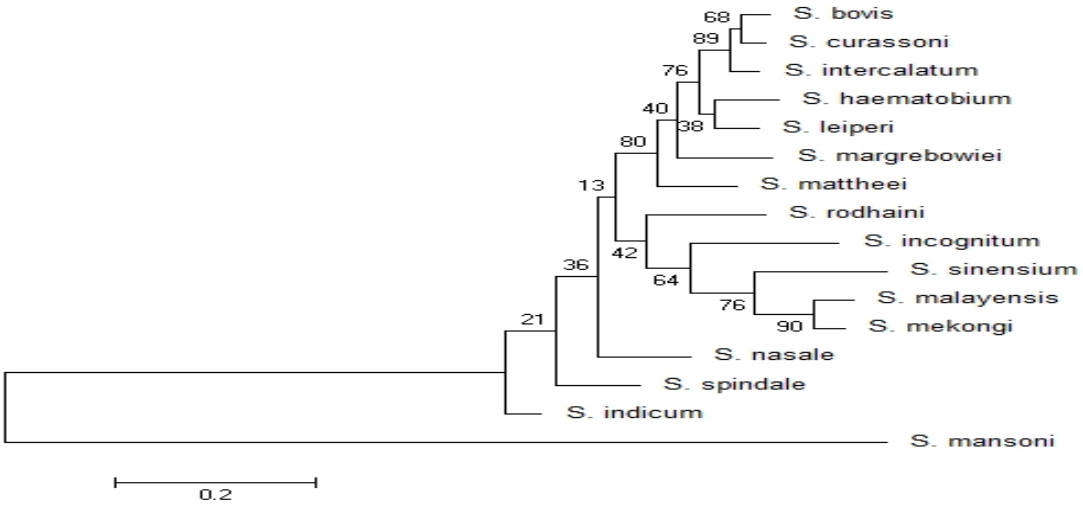

Fig. 3. Phylogenetic analysis by maximum likelihood and bootstrap percentages (n=3000)

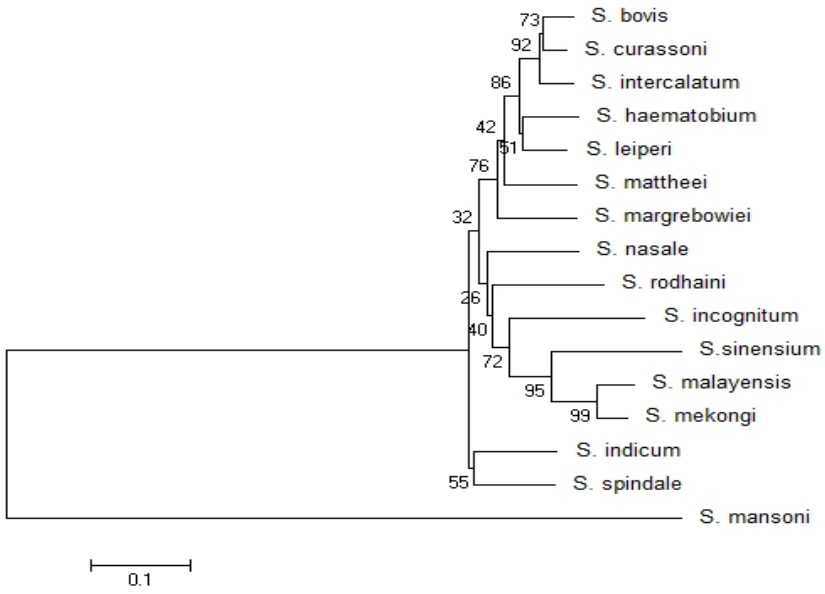

Fig. 4. Phylogenetic analysis by Neigbouring-Joining tree and bootstrap percentages (n=3000)

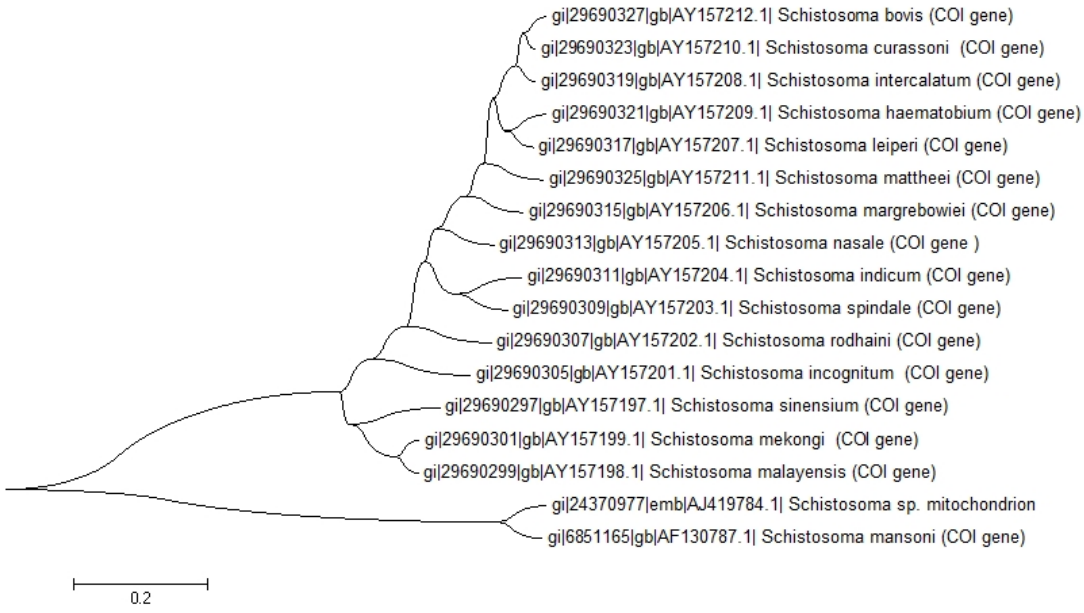

Fig. 5. An outgroup based phylogenetic analysis by maximum likelihood showing Schistosomes evolutionary trend

Table 1. Sequences of retrieved COI genes in *Schistosoma species*

No.	Species	Accession No.	Size (bp)	Continent
1	*S. mansoni*	AF130787.1	5,190	Africa
2	*S. bovis*	AY157212.1	1,125	Africa
3	*S. mattheei*	AY157211.1	1,125	Africa
4	*S. curassoni*	AY157210.1	1,125	Africa
5	*S. haematobium*	AY157209.1	1,125	Africa
6	*S. intercalatum*	AY157208.1	1,125	Africa
7	*S. leiperi*	AY157207.1	1,125	Africa
8	*S. margrebowiei*	AY157206.1	1,125	Africa
9	*S. nasale*	AY157205.1	1,125	India & S. Asia
10	*S. indicum*	AY157204.1	1,125	India & S. Asia
11	*S. spindale*	AY157203.1	1,125	India & S. Asia
12	*S. rodhaini*	AY157202.1	1,125	Africa
13	*S. incognitum*	AY157201.1	1,125	Central Asia
14	*S. sinensium*	AY157197.1	1,125	Central & S. E. Asia
15	*S. malayensis*	AY157198.1	1,125	Central & S. E. Asia
16	*S. mekongi*	AY157199.1	1,125	Central & S. E. Asia
17	*Schistosoma sp*	AJ419784.1	754	-

*S.E- South-East, S- South

Table 2. Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution

	A	T	C	G
A	-	*10.94*	*2.34*	16.56
T	*5.08*	-	3.51	*5.02*
C	*5.08*	16.41	-	*5.02*
G	16.76	*10.94*	*2.34*	-

**Each entry shows the probability of substitution (r) from one base (row) to another base (column). For simplicity, the sum of r values is made equal to 100. Rates of different transitional substitutions are shown in bold and those of transverse substitutions are shown in italics*

Table 3. Estimated evolutionary divergence among species

Schistosoma Sp.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
S. mansoni															
S. bovis	0.990														
S. mattheei	1.021	0.135													
S. curassoni	1.012	0.053*	0.137												
S. haematobium	1.001	0.110	0.158	0.113											
S. intercalatum	1.013	0.064*	0.139	0.064*	0.114										
S. leiperi	1.008	0.105	0.137	0.097*	0.103	0.098*									
S. margrebowiei	1.024	0.149	0.170	0.141	0.157	0.148	0.140								
S. nasale	1.019	0.193	0.188	0.194	0.171	0.196	0.181	0.204							
S. indicum	0.969	0.197	0.202	0.197	0.205	0.202	0.189	0.209	0.200						
S. spindale	1.042	0.203	0.206	0.207	0.200	0.210	0.190	0.195	0.194	0.166					
S. rodhaini	0.993	0.218	0.220	0.223	0.203	0.230	0.203	0.237	0.210	0.245	0.219				
S. incognitum	1.065	0.257	0.270	0.251	0.263	0.253	0.258	0.264	0.254	0.253	0.271	0.279			
S. mekongi	1.027	0.260	0.251	0.261	0.241	0.268	0.241	0.265	0.257	0.253	0.266	0.243	0.267		
S. malayensis	1.025	0.256	0.259	0.261	0.254	0.258	0.260	0.262	0.261	0.258	0.256	0.247	0.259	0.067	
S. sinensium	1.102	0.302	0.307	0.312	0.302	0.328	0.311	0.317	0.275	0.292	0.294	0.302	0.313	0.205	0.218

* Areas identified with smallest evolutionary divergence

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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