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SHORT COMMUNICATION

HOST-PARASITES RELATIONSHIP FOR LAKE VICTORIA CLARIID FISHES AND THEIR PARASITE FAUNA

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ABSTRACT

Host-parasite co-evolutionary relationships for the clariid fish species of the Lake Victoria basin and their parasite fauna was inferred by cytochrome b gene and the 18S rDNA using 'a strict coevolutionary' constrained tree method, the results of which revealed a phylogenetic incongruence between the fish hosts and their parasite fauna. The present study further indicated that the sympatric association between the clariid hosts and non-clariid fishes in Lake Victoria makes it possible for the parasites to switch from one host to another and hence the large number of shared parasites observed. The specificity of some of the parasites, e.g. Diplostomum mashonense *and* Tylodelphys species *1 & 2 observed is most likely a result of a strong host-habitat association, such that the position of infective stages (cercariae) in the water column might preclude infection of diplostomids to other clariids. However, this ecological factor, together with the possibility of immuno-physiological barrier requires further investigation.*

Key words: Host-parasites coevolution, Lake Victoria.

INTRODUCTION

Phylogenetic studies of interacting organisms have often revealed congruence between the phylogenies of the interacting taxa (Hafner and Nadler, 1990). This congruence is ascribed to concurrent speciation (cospeciation) of the taxa involved in the interaction (Johnson et al., 2003). Sometimes however, the congruence between the interacting taxa is not perfect (Desdevises *et al*. 2002). Most studies in which host-parasite associations were found to be congruent involved particular groups in which biological characteristics made parasite host switching highly improbable (Desdevises *et al*. 2002). Cospeciation of parasites with their hosts happens therefore, when the hosts are allopatric to one another.

The precise reconstruction of a hypothetical coevolutionary scenario between hosts and their parasites on the other hand, is not always straightforward. A host-parasite association whose phylogenetic trees are not congruent can closely coevolve, likewise the absence of congruence may not always signify lack of historical association between the two components (Desdevises *et al*. 2002).

[1] Nucleotide sequence data reported in this paper are available in the GenBank database under the accession numbers, DQ813444 – DQ813465 and DQ646345 – DQ646374.

The host-parasite interactions analysed in the present study were composed of twenty two most common parasites including the nematodes, cestodes, trematodes and crustaceans, and five clariid fish species one of which was obtained from the Malagarasi River system, and an outgroup *B. docmac* (Claroteidae). Despite the number of clariid species investigated, certain parasite species were found to be specific to certain fish hosts. However, the majority of the parasites are generalists known to infect the Clariidae and other fish families in the lake.

The strict specialist parasites make it possible to study the influence of tight host-specificity on cospeciation. In addition, the high number of parasites and host fish species living sympatrically in the lake make the potential hosts to always be available to the parasites. The present study utilised 'a strict coevolution' constraint tree to evaluate coevolutionary events in the association between the clariids and their most common parasites. The objective of the present study was to construct the molecular phylogenies of the clariid fish species and their associated parasite fauna, subsequently assess the extent of cospeciation between the interacting hosts and parasites.

STUDY AREA

Lake Victoria, the largest tropical lake in the world, is shared between Tanzania, Uganda and Kenya. The lake lies in a shallow continental sag between the two arms of the Great Rift Valley, 1170 m above sea level. The lake has a maximum depth of 84 m, a volume of 2750 km3, and a surface area of 68,800 km2. Primary inflows to the basin include rivers such as the Kagera in the west and the Mara in the east. All outflows are to the north along the Nile through Lake Kyoga. The mean surface temperature is about 25 oC while the temperature of deeper layers is about 1 to 2 degrees lower (Witte and Van Densen, 1995). The present study therefore, covered the surroundings of Bukoba town, the Mwanza Gulf, Speke Gulf, parts of Ukerewe Island and the delta of Mara River (Fig. 1)

MATERIALS AND METHODS

Field sampling, examination of fish for parasites and collection of samples for DNA extraction, PCR amplification and sequencing

Field sampling and examination of fish for parasites is as elaborated in Mwita and Nkwengulila (2008a). DNA analysis for fish and parasites are reported in Mwita and Nkwengulila (2008b; 2010). DNA extraction for parasites was performed using the Qiagen DNA extraction kit and the manufacturer's instructions. The 18S rDNA gene sequences for the parasites were amplified using primers JLR24 and JLR25 as described by Campos *et al.* (1998). PCR products purification was achieved via the Qiaquick purification kit and protocol whereas DNA sequencing was done by the dideoxy-termination method using an ABI 377 Prism automated DNA sequencer utilizing the respective primers for parasites.

Phylogenetic and coevolutionary analyses

Phylogenetic relationships were inferred independently for fish (Mwita and Nkwengulila 2008b) and parasites (Mwita and Nkwengulila 2010) using PAUP* v.4.b10 for Macintosh (Swofford, 2000). For tree reconstruction, parsimony searches were performed with all characters equally weighted and unordered and then tree-bisection-reconnection (TBR) branch swapping with 100 heuristic random addition replicates. Reliability of each tree was assessed statistically using 1000 replicates of bootstrap resampling (Felsenstein 1985). To compare host and parasite trees, 'a strict coevolution' constraint tree, as implemented in PAUP* v.4b10 for Macintosh (Swofford 2000), was constructed to group the parasites according to their host affiliation, such that all parasites infecting a particular clariid host fish form monophyletic groups. In order to test the strict coevolution hypothesis, the best tree consistent with this topology was

compared with unconstrained optimal tree using the Kishino and Hasegawa test under the maximum likelihood criterion (Kishino and Hasegawa 1989).

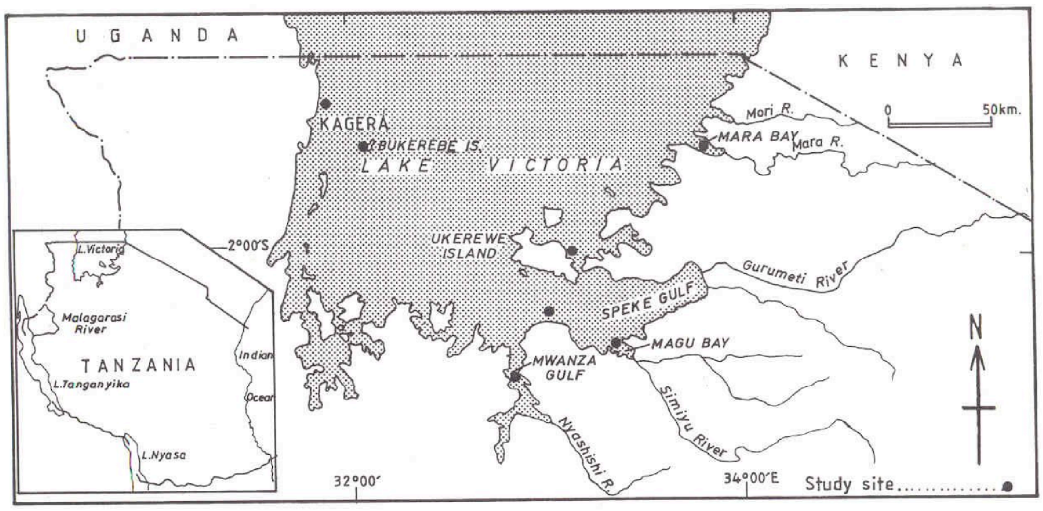

Figure 1: Study sites

RESULTS

A total of 1122 bp segment of cytochrome b sequences for clariid fishes were analysed of which 814 positions were constant and 300bp were phylogenetically informative. The HKY + G + I (Hasegawa *et al*. 1985) model corresponded to the settings of this test with the following parameter estimates: Ti / Tv = 1.52942, gamma = 3.0324, pinvar = 0.8950, base frequencies A = 0.28318, C = 0.31447, G = 0.13147, T = 0.27088. Uncorrected sequence divergences ranged from 0.3% to 11.5% among the different clariid species. The 18S rDNA fragments for the parasites were analysed and consisted of 530 bp of which 133 characters were constant. 180 variable characters were parsimonious uninformative and 217 characters were parsimonious informative. The HKY + G + I model (Hasegawa et al., 1985) was selected as the best fit for the 18S rDNA data set. The parameters estimated for this model were: Ti/ Tv = 0.80709, gamma = 1.60185, pinvar = 0.42878, base frequencies A = 0.25138, C = 0.22034, G = 0.28570, T = 0.24258. Maximum percentage sequence difference among the parasites was 49% and the minimum was 0.9%.

The topology of the ML trees for the clariid hosts and their parasites are shown in Fig. 2. The monophyly of most species in both the fish hosts and their parasites was supported by bootstrap resampling analysis. Segregation of the parasites by their clariid host species was not supported by phylogenetic analysis as seen in Fig. 2. Enforcing the strict coevolution constraint tree on the original data set resulted in an ML tree with –ln L = 1979.8799 (unconstrained –ln L = 1968.20902). The likelihood ratio test rejected the constrained tree (p = 0.001) in favour of the best tree (Fig. 2) that indicates a no relationship between the parasites and their affiliated clariid host fishes.

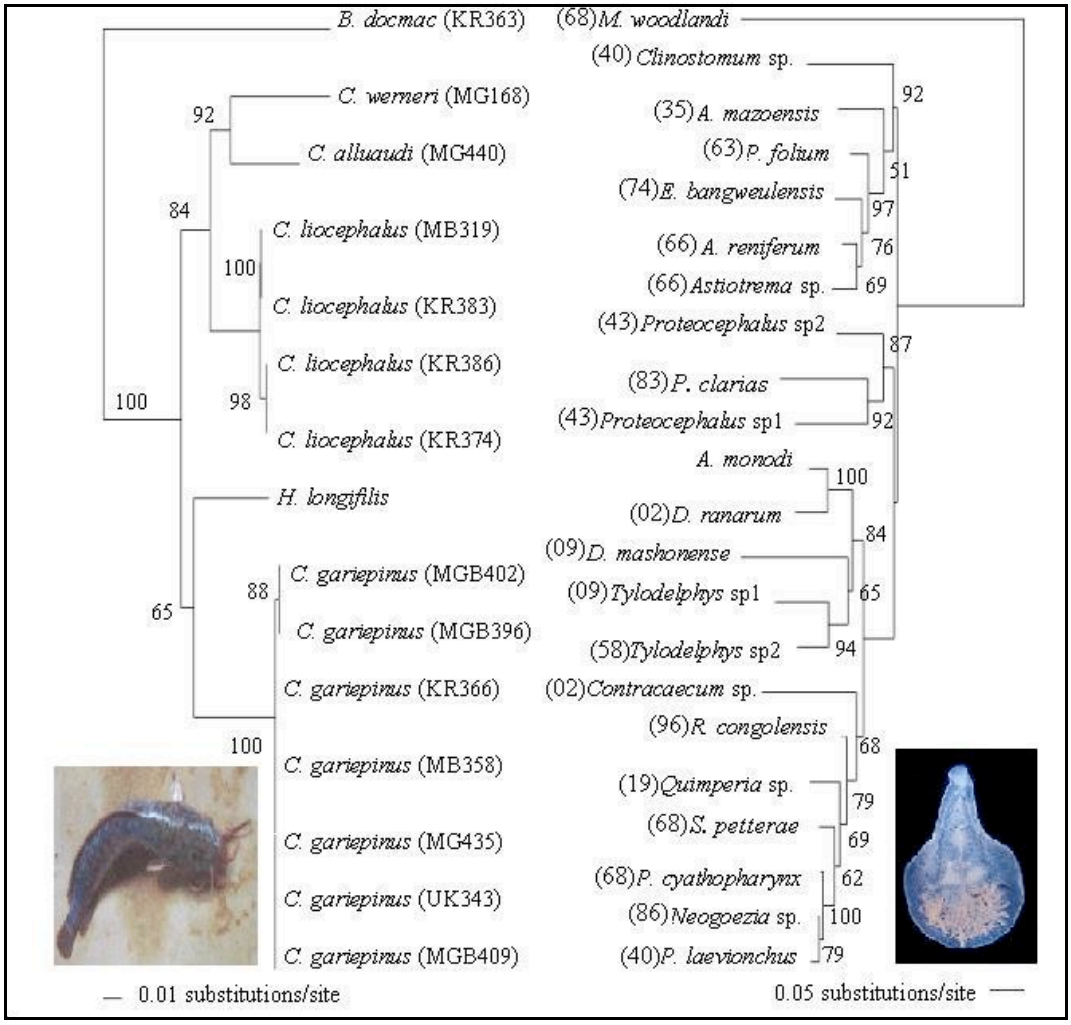

Figure 2: The maximum likelihood tree for (a) clariid fish hosts and (b) their parasites based on the HKY + G + I model. Bootstrap values are shown on the branches. Numbers before the parasites correspond to the last two digits in the serial numbers of their respective clariid hosts [e.g. (**40**) *P. laevionchus* = *C. alluaudi* (MG4**40**)]

DISCUSSION

Phylogenetic analyses of cytochrome b gene fragment for the clariid fish hosts and the 18S Rdna for the parasites yielded well-resolved trees. Comparison of these trees found no evidence for cospeciation between the clariids and their most common parasites. This is an indication that a combination of events such as duplication, sorting and host-switching is likely to be involved in the evolutionary processes of the clariid fish and their respective parasites.

The host-parasite relationships between the clariid fishes and their parasites observed in the present study can be explained in various ways. Most of the clariid species investigated share the same ecological niche; for instance, *Clarias gariepinus* was

collected from all the five localities sampled, *C. liocephalus* from three localities (Kagera, Mwanza Gulf and Mara Bay), and *C. werneri* and *C. alluaudi* from two (Mwanza Gulf and Mara Bay). The fore going observation indicates that the clariid fish species live in sympatric association in the Lake Victoria basin, a scenario that provides chances for their parasites to switch from one host to another, and not only among the clariids but to other fish families as well. The parasites therefore have many opportunities to switch and this happens in nature and the choice of hosts and the resulting specialization are not driven by evolutionary factors, and thus cospeciation may be a by-product of host separation either, geographically or behaviourally (Desdevises *et al*. 2002).

Table 1: Parasite species analysed in the present study.

Family/Species	Acc.no	Host
Paracamallanus cyathopharynx	DQ813445	*C. werneri*
Neogoezia sp.	DQ813444	*C. liocephalus*
Procamallanus laevionchus	DQ813446	*C. alluaudi*
Spinitectus petterae	DQ813447	*C. werneri*
Quimperia sp.	DQ813448	*C. liocephalus*
Rhabdochona congolensis	DQ813457	*C. gariepinus*
Contracaecum sp.	DQ813456	*C. gariepinus*
Diplostomum mashonense	DQ813458	*C. gariepinus*
Tylodelphys sp.1	DQ813454	*C. gariepinus*
Tylodelphys sp.2	DQ813455	*C. gariepinus*
Eumasenia bangweulensis	DQ813461	*C. liocephalus*
Astiotrema reniferum	DQ813459	*C. gariepinus*
Astiotrema sp.	DQ813460	*C. gariepinus*
Phylodistomum folium	DQ813462	*B. docmac*
Allocredium mazoensis	DQ813450	*C. gariepinus*
Clinostomum sp.	DQ813463	*C. alluaudi*
Proteocephalus sp.1	DQ813465	*C. gariepinus*
Proteocephalus sp.2	DQ813451	*H. longifilis*
Polyonchobothrium clarias	DQ813464	*C. liocephalus*
Argulus monodi	DQ813452	*H. longifilis*
Dolops ranarum	DQ813453	*C. gariepinus*
Monobothrioides woodlandi	DQ813449	*C. werneri*

The above observation is further supported by the fact that most parasites reported in the present study were generalists (Mwita and Nkwengulila 2008a) that have also been recorded from non clariid fishes in Lake Victoria (Khalil and Thurston 1973, Paperna 1980, Mbahinzireki 1984). Furthermore, most of these parasites are polyxenous, and some have mobile intermediate hosts such as copepods and snails, and vagile final hosts capable of spreading the parasites' infective stages to distant geographical areas

within the lake. Still, certain clariid species such as *C. gariepinus* can traverse long distances and hence serve as parasite dissemination agents from one part of the lake to the other. This is supported by the fact that *C. gariepinus* was collected from all five localities sampled, and harboured the highest parasite species richness among the clariids investigated (Mwita and Nkwengulila, 2008a). As well, most parasites infecting *C. gariepinus* were shared with the other clariid species. Given the nature of the host-parasite relationships observed, it seems most plausible that the shared parasites among the clariid species studied were not a result of cospeciation.

Poulin (1992) suggested that tight host specificity is linked to cospeciation processes. In the present study three digenetic parasites namely *Diplostomum mashonense*, *Tylodelphys* sp. 1 and 2 were considered to be specific parasites of *Clarias gariepinus*. However, these parasites did not exhibit any cospeciation patterns with their *C. gariepinus* host. As suggested by Desdevises et al. (2002), processes leading to parasite specificity do not necessarily lead to cospeciation. The absence of cospeciation between specialist parasites and their hosts represents a strong ecological host-parasite association as might be the case in the present study (Brooks 1979, Desdevises *et al*. 2002). However, considering the geological history of the lake (Johnson *et al*. 1996), it is possible that the association between the clariids and their parasites is relatively recent in evolutionary terms and perhaps there has not been enough time to co-evolve.

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