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Molecular cloning and production of type III Hsp40 protein co-chaperone PfZRF1 of human malaria parasite Plasmodium falciparum

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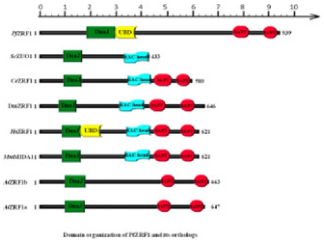
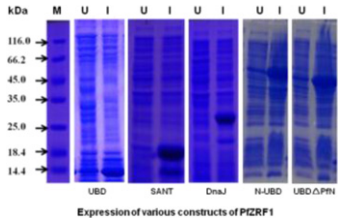
Background: Despite of remarkable progress in combating malaria, this deadly disease still accounts for more than a half million deaths annually. In the light of growing incidences of drug resistance, an understanding of parasite biology is necessary for the development of new antimalarials. During life cycle in two different hosts *Plasmodium falciparum* experiences frequent thermal variations and physiological stress. Heat shock proteins (Hsps) are key players for its survival and making them attractive drug targets. Hence, present research emphasizes on *in silico* analysis, cloning and production of a type III Hsp40 protein, PfZRF1.

Methods & Materials: Orthologs of PfZRF1 were assigned by BLASTp and literature search. Domain architecture was drawn by SMART and Pfam. ClustalW and T-coffee were employed for multiple sequence alignment analysis. Phylogenetic tree was generated by NJ method using Phylip-3.695 for evolutionary relationship analysis. PfZRF1 and its domain constructs were amplified and cloned in pETM11 vector between NcoI, XhoI and Kpn1 restriction sites. His-tagged PfZRF1 and other domain constructs were expressed in *E. coli* B834.

Results: PfZRF1 orthologs were identified in 31 eukaryotes however found to be absent in prokaryotes. PfZRF1 is composed of a Hsp70-binding DnaJ domain and two DNA-binding SANT domains. An ubiquitinated histone H2A binding UBD domain was also identified based on human ortholog HsZRF1. As compared to HsZRF1, DnaJ domain has a 50 aas long parasite-specific insertion in loop region between helix II and III near Hsp70-binding HPD motif. Additionally, ribosome associating RAC_head domain was found less conserved with many insertions in PfZRF1. Phylogenetic analysis depicted PfZRF1 to be closer to unicellular eukaryotes. Full ORF (2820 bp) and several constructs covering different domains were amplified and cloned in pETM11. Different constructs were expressed after inducing with 0.5 mM IPTG at 16 °C in *E. coli* B834 cells for soluble expression.



Evolutionary relationship of PfZRF1 and its orthologs.



Expression of various constructs of PfZRF1

Conclusion: This study presents preliminary characterization of a type III Hsp40 co-chaperone of the parasite. Further functional characterization of this putative multifunctional chaperone would undoubtedly provide an insight in parasite molecular biology for the development of novel antimalarials.

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Molecular evidence of Bothriocephalus acheilognathi (Cestoda: Bothriocephalidea) from India

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Background: *Bothriocephalus acheilognathi*, a tapeworm that infects a variety of fishes worldwide. This worm is native to East Asia and spread throughout the world due to aquaculture trade of ornamental fishes. Although, this worm has not been parasitize to any mammals, but recently, a study from Saint Laurent du Maroni (French Guiana) describes the first report of the egg isolation of *B. acheilognathi* from human stool, a case of accidental infection. However, this worm is also described from Northern part of India but currently there are no reliable data is available based on molecular studies in India. This study aimed to assess the molecular phylogenetic analyses of *B. acheilognathi* based on sequences of 18S and 28S ribosomal DNA and its distribution in India.

Methods & Materials: Specimens of *Bothriocephalus* were collected from *Xiphophorus hellerii*, a native of North and Central America. For morphology, worms were washed in saline and were fixed in 70% ethanol for further processing. For molecular study, a small fragment from the strobila was cut and stored in 95% ethanol until DNA extraction. Genomic DNA was extracted and ribosomal 18S and 28S were amplified and sequenced. The data were then

analyzed using the Maximum Likelihood (ML) and Bayesian Inference (BI) algorithms.

Results: Data obtained from 28S and 18S gene shows close relationships with all the sequences of *B. acheilognathi* reported from other isolates of the same species available on the database. Although in both gene sequences, 28S shows more conserved in isolates of *B. acheilognathi*. In comparing to 28S, 18S gene shows deep phylogenetic relationships in *B. acheilognathi* sequences. In two different phylogenetic methods used for analyses of 28S gene, all the *B. acheilognathi* isolates were divided into three clades with the Indian isolate showed a close relationship with an isolate from South Korea along with other isolates of the same species from different geographical regions.

Conclusion: This study describes the molecular identification of *B. acheilognathi* from India. This study also highlights that low specificity of this cestode for a host can affect the native fish resources of India and can be a problem for adversely affecting a number of wild fish species.

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Parasitic infections in children presenting with acute diarrhea in Mozambique: National surveillance data (2013 – 2015)

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Background: Acute diarrhea remains a public health problem with a major cause of morbidity and mortality in children. The low middle countries continue to be the most affected areas in worldwide. The determination of the cause is the first step in right treatment management. Parasitic infections are common in children and are related to gastrointestinal disorders. Altogether, it has been established National Surveillance in Acute Diarrhea in children in Mozambique with the aim to determine the pathogens prevalent in this health condition. In this data we will only present information related to the parasitic infections.

Methods & Materials: From 2013 to 2015, a hospital-based surveillance was conducted in 5 hospitals of Mozambique. The surveillance is being conducted in children younger than 14-years, with acute diarrhea defined as three or more stools per day of decreased form from the normal, lasting for less than 14 days. Stool samples were examined for the presence of parasites using formol-ether concentration method and the Modified Ziehl-Neelsen staining technique. Laboratory results from children with information of age (in months) and gender were selected for this analysis. Statistics analysis were made using STATA version 12.1 package in 95% of confidence interval.

Results: A total of 597 children with acute diarrhea provided stool sample. Overall 64% (n = 383) had completed information regarding age and gender. The median age of the children was 11 months (IQR: 8 to 16 months). Intestinal parasites species were prevalent, with *Ascaris lumbricoides* 18% (22), *Trichuris trichiura* 16% (20), *Entamoeba histolytica/dispar* 12% (15), *Endolimax nana* 11% (13), *Entamoeba coli* 6% (8), *Giardia intestinalis* 2% (2) and hookworm 1% (1). *Cryptosporidium* sp. was the only opportunistic parasite detected in 34% (42) children. Infection with *Cryptosporidium* sp. was significantly associated with the presence of *E. histolytica/dispar* ($\rho = 0{,}04$).

Conclusion: Soil-transmitted helminthes, *A. lumbricoides* and *T. trichiura*, are an important public health issue related to acute diarrhea in children. Detection of *Cryptosporidium* sp. was high and with the high rate of HIV infection in Mozambique, it can be consider important pathogen to take in account in diagnosis routine.

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Trypanosoma cruzi infection in the heart of Colombian wild bats

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Background: *Trypanosoma cruzi* is a parasite protozoa that infects mammalians and in the human cause Chagas' disease, which represent a major health problem in Colombia where an estimated of 436,000 individuals are infected, with 11% of the population at risk for contracting the disease. Moreover, the potential epidemiological significance of bats as possible reservoir hosts for *T. cruzi*, has been previously remarked. Different neotropical bats species have been reported to be susceptible to *T. cruzi* infection. They participate in important ecological processes and because of its ability to fly can spread infectious diseases from the natural environment to the homes of people. In Colombia, few studies on bats in endemic areas for Chagas' disease have been performed. Thus, we evaluated the presence of *T. cruzi* in heart tissue taken from bats Cordoba department (northern Colombia), considered an endemic area for this infection.

Methods & Materials: 30 hearts of bats were collected in four rural localities from Cordoba department. The DNA was purified using a commercial high-purity PCR template preparation kit (Roche, Mannheim, Germany). The integrity of the purified DNA was analyzed through PCR amplification of the bat *cyt b* gene. PCR tests based on the TcH2AF-R and S35-S36 primers which amplify a fragment of SIRE element and a conserved region of minicircles from *T. cruzi* respectively, were evaluated for the detection of parasite in batsheart tissue

Results: A total of 11 samples (36.6%) of three localities were positives for both PCR. Three species were positive for the presence of *T. cruzi*: *Carolia perspicillata* and *Dermanura phaeotis* (frugivorous) and *Molossus molossus* (insectivore).

Conclusion: This is the first report of *T. cruzi* in the heart of naturally infected bats in Colombia. These findings imply that there