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Molecular analyses reveal two geographic and genetic lineages for tapeworms, *Taenia solium* and *Taenia saginata*, from Ecuador using mitochondrial DNA

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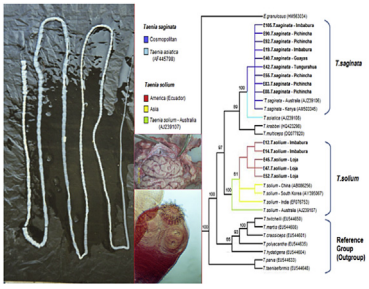
HIGHLIGHTS

- *Taenia solium* and *Taenia saginata* cause taeniasis/cysticercosis, a NTD in Ecuador.
- Maximum Parsimony analyses in *Taenia solium* revealed greater geographic structure.
- COI haplotype networks suggest two geographical events in the introduction of T. solium in Ecuador.
- Two NDI geographical lineages in *T. solium* derivate from a common Indian ancestor.

GRAPHICAL ABSTRACT



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ABSTRACT

Tapeworms *Taenia solium* and *Taenia saginata* are the causative agents of taeniasis/cysticercosis. These are diseases with high medical and veterinary importance due to their impact on public health and rural economy in tropical countries. The re-emergence of *T. solium* as a result of human migration, the economic burden affecting livestock industry, and the large variability of symptoms in several human cysticercosis, encourage studies on genetic diversity, and the identification of these parasites with molecular phylogenetic tools. Samples collected from the Ecuadorian provinces: Loja, Guayas, Manabí, Tungurahua (South), and Imbabura, Pichincha (North) from 2000 to 2012 were performed under Maximum Parsimony analyses and haplotype networks using partial sequences of mitochondrial DNA, cytochrome oxidase subunit I (COI) and NADH subunit I (NDI), from Genbank and own sequences of *Taenia solium* and *Taenia saginata* from Ecuador. Both species have shown reciprocal monophyly, which confirms its molecular taxonomic identity. The COI and NDI genes results suggest phylogenetic structure for both parasite species from south and north of Ecuador. In *T. solium*, both genes gene revealed greater geographic structure, whereas in *T. saginata*, the variability for both genes was low. In conclusion, COI haplotype networks of *T. solium* suggest two geographical events in the introduction of this species in Ecuador (African and Asian lineages) and occurring sympatric, probably through the most common

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routes of maritime trade between the XV-XIX centuries. Moreover, the evidence of two NDI geographical lineages in *T. solium* from the north (province of Imbabura) and the south (province of Loja) of Ecuador derivate from a common Indian ancestor open new approaches for studies on genetic populations and eco-epidemiology.

1. Introduction

Infestations by *Taenia* spp., and *Echinococcus* spp., are common, not only in developing countries but also in industrialized countries, where apart from being a threat to health, they represent a socio-economic impact. Cysticercosis causes a debilitating disease in humans as well as losses in the meat industry due to the condemnation of meat from infected animals (Jiménez et al., 2002; Raether and Hanel, 2003; Rodriguez Hidalgo, 2007; Schantz, 1999; Tsai et al., 2013).

The Andean region of Ecuador was described as hyperendemic for taeniasis/cysticercosis, with prevalences in rural communities up to 1.60% for taeniasis and 14.4% for *T. solium*-cysticercosis by. The prevalence of *T. saginata* in Ecuador is not well established, however, a moderate prevalence of bovine cysticercosis with 0.37% on veterinary inspection and 4.03% by serological techniques has been reported (Cayo-Rojas et al., 2011; Cruz et al., 1989; Rodriguez-Hidalgo et al., 2006; Rodríguez-Hidalgo et al., 2010, 2003; Rodriguez Hidalgo, 2007).

As a result of their great importance in Ecuador, these two cestodes species have been extensively studied. However, the strategies aiming to control these parasitoses have major limitations i.a. it has not been possible to accurately identify the prevalence of *Taenia* spp. and the determination of strains by means of ecological, biological or morphological criteria is difficult (Ito et al., 2003). The efficiency of control depends on detailed epidemiological information including identification and precise characterization of the causative agent in each endemic area (Gasser et al., 1999; Jia et al., 2010; McManus, 1990).

The genus *Taenia* has been successfully identified using, enzyme electrophoresis and mitochondrial molecular markers to differentiate between species and also to infer phylogenies (Hoberg et al., 2000; Nakao et al., 2010; Queiroz and Alkire, 1998). However, little is known about the genetic intra-specific variation in cestodes (Pawlowsky Zbigniew, 2002) and considerable research indicates a great heterogeneity in pathology caused by *Taenia solium* but there is a misunderstanding about the role of genetic diversity and adaptability of species (Del Brutto, 2013; Finsterer and Auer, 2013; Marquez and Arauz, 2012; Román, 2014; Sotelo, 2011). Based on this issue, mitochondrial DNA analysis provides complementary tools for characterization of a population. Gene fragments or complete genome of mitochondrial DNA have been successfully used in population genetics, ecology and identification of tapeworms (Jia et al., 2010). Genetic variation associated with different hosts is a well-known fact in several cestodes species e.g. *Echinococcus granulosus* and Ito et al (Ito et al., 2003) suspected that for *Taenia saginata* and *Taenia solium* it may well be equally the case.

Nakao et al., (Nakao et al., 2002), using the complete genes Cytochrome Oxidase subunit I and Cytochrome *b* of the mitochondrial DNA, showed the existence of two lineages of *Taenia solium* worldwide: an Asian group and an African and Latin-American group of strains. A sequence from Ecuador was included in these studies, showing minimal variation with the rest of the sequences in the analysis, albeit this sequence represents only a small portion of the Ecuadorian gene pool. Yanagida et al (Yanagida et al., 2014) using different mitochondrial genes, report two genetic sympatric lineages of *T. solium* in Madagascar close related with Asia and Africa/Latin America as consequence of historical human migration. These authors shown the Africa/Latin America lineage connected by haplotypes network with the Ecuadorian haplotype AB066491 here also used.

It is important to complete more detailed variability studies for this species, even more so when genetic variation of tapeworms from various geographic locations might be linked to clinical and pathological differences found in human cysticercosis (Maravilla et al., 2003; Vega et al., 2003).

The aim of this research is to determine the genetic diversity within populations of *Taenia solium* and *Taenia saginata* collected in six locations, from the north and the south of Ecuador. We also hypothesized that different geographical origins or introductions, can be assessed by means of presence of geographic phylogenetic structuration within sequences-populations and/or haplotypes networks analysis. The results in this study can underpin epidemiological research and the control of these parasites. Furthermore, it provides a great source of information and support for new diagnostic methods and reinforces vaccines developing in the fight against these important diseases (Assana et al., 2010; Fernadez et al., 2006; Maravilla et al., 2008; Sciutto et al., 2013; Tsai et al., 2013).

2. Materials and methods

2.1. Source of Taenia spp. specimens

Specimens used in this research belong to the biological bank of the Centro Internacional de Zoonosis (CIZ) at Universidad Central del Ecuador from localities of a former study and control program. Samples have been conserved in ethanol solution 70%, and kept frozen at −20 °C. Samples were collected from the Ecuadorian provinces of: Loja, Guayas, Manabí, Tungurahua (South), and Imbabura, Pichincha (North) from 2000 to 2012 (Supplementary data 1). Specimens were isolated form human faecal material after anthelmintic treatment. Patients became from both urban and rural areas. The vouchers of remains specimens are deposited in the bank of CIZ.

2.2. DNA extraction, amplification and sequencing of COI and NDI genes

For DNA extraction of *Taenia*'s proglottids, the Wizard Genomic DNA Purification of Promega® commercial kit was used following the manufacturer's protocol. The DNA samples obtained from proglottids of *Taenia* spp. were analysed in agarose gel 0.8% to ascertain presence, quality and size of the extracted material. The quantification of genomic DNA extracted from *Taenia* spp. was performed using the Invitrogen fluorometer QUBIT®. We used the Quant-iT™ Broad-Range DNA Assay Kit according to the manufacturer's instructions (Data not shown).

The reactions were performed in 25 μL, using a thermo cycler TECHNE TC-412, using the primers for NDI sequence (Bowles and