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Review

Genetic and morphological characterization of *Thysaniezia* tapeworms from cattle and sheep in Senegal

Mallé Ndom[a,*], Tetsuya Yanagida[b], Gora Diop[a], Yann Quilichini[d], Aïssatou Ba[a], Yasuhito Sako[c], Minoru Nakao[c], Bernard Marchand[d], Alioune Dieye[e], Cheikh Tidiane Ba[a], Akira Ito[c]

[a] Université Cheikh Anta Diop de Dakar, Laboratoire de Biologie Évolutive, d'Écologie et Gestion des Écosystèmes, Faculté des Sciences et Techniques, BP 5005 Dakar, Senegal
[b] Laboratory of Veterinary Parasitology, Joint Faculty of Veterinary Medicine, Yamaguchi University, Yoshida 1677-1, Yamaguchi 753-8515, Japan
[c] Department of Parasitology, Asahikawa Medical University, Asahikawa 078-8510, Japan
[d] CNRS, Université de Corse, UMR SPE 6134, Service d'Étude et de Recherche en Microscopie Électronique, Campus Grimaldi, BP 52, 20250 Corte, Corse, France
[e] Unité d'Immunogénétique, Institut Pasteur de Dakar, Dakar, Senegal

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ABSTRACT

Genetic and morphological diversity of *Thysaniezia* tapeworms from cattle and sheep in Senegal was investigated using light and scanning microscopic observations and molecular analysis based on mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) and nuclear small subunit ribosomal RNA gene (SSU rDNA). A total of 52 adult tapeworms were collected from sheep and cattle. Although the tapeworms of the two hosts were morphologically very close, phylogenetic analysis based on *cox1* and SSU rDNA gene sequences showed that they were divided into two clades corresponding each to a host. The maximum pairwise divergence between the clades were 12.1% in *cox1* and 2.9% in SSU rDNA, indicating they are distinct species. The tapeworms collected from sheep were morphologically identified as *Thysaniezia ovilla*, a cosmopolitan species in domestic ruminants. Detailed morphological observations revealed a consistent difference between the tapeworms obtained from sheep and those from cattle. The latter were identified as *Thysaniezia connochaeti*. The present study highlights presence of two species of *Thysaniezia* among domestic ruminants in Senegal: *T. ovilla* specific to sheep and *T. connochaeti* specific to cattle. Our work is the first report of *T. connochaeti* from domestic animals.

1. Introduction

Thysaniezia tapeworms are cestodes belonging to the family Anoplocephalidae. They are cosmopolitan parasites (Soulsby, 1968), and the adult tapeworms inhabit the small intestine of ruminants, mainly cattle and sheep (Morel, 1959; Vassiliades, 1981; Narsapur, 1988). Their life cycle includes oribatid mites as intermediate hosts (Denegri et al., 1983; Denegri et al., 1998). After ingestion of infected mites, hatched larvae actively move to the small intestine of definitive hosts, attach with their suckers and become mature (Denegri et al., 1998). Signs such as loss of body weight are often visible when heavily infected (Euzéby, 1966). Prevalence of *Thysaniezia* spp. is generally low to moderate among ruminants; it was 3% among goats in Mongolia (Sharkhuu, 2001) and 2.7% among sheep in Egypt (Bashtar et al., 2011). In Senegal, prevalence of *Thysaniezia* spp. among ruminants was reported as 13% among cattle, 2–6% among sheep and 0.4% among goats (Ba et al., 1994; Ndom et al., 2016). Despite the veterinary importance of *Thysaniezia* tapeworms, the taxonomy of the genus remains controversial. A number of *Thysaniezia* species have been described from domestic and wild ruminants in different localities of the world, but only few are currently considered valid (Spasskii, 1951; Yamaguti, 1959; Schmidt, 1986). *Thysaniezia ovilla* (Rivolta, 1878) Skrjabin, 1926 is the type species of the genus, having a worldwide distribution. It was originally described in sheep, then it was found in a variety of ruminants including cattle. Another valid species of the genus is *Thysaniezia connochaeti* (Fuhrmann, 1943), which was described from the wild blue wildebeest *Connochaetes taurinus* in Angola (Fuhrmann, 1943).

Previous studies only found *T. ovilla* in domestic herbivores in Senegal, however identification was based on morphology (Morel, 1959; Vassiliades, 1981; Nadège, 2006). A multilocus isoenzyme electrophoresis study suggested that *Thysaniezia* tapeworms collected from domestic ruminants in Senegal are a species complex (Ba et al., 1994). Therefore, investigation on the genetic diversity of *Thysaniezia* tapeworms from domestic ruminants in Senegal is needed, for the establishment of reliable identification and the reevaluation of conventional taxonomy, as the molecular biology has become a key voice in

* Corresponding author.
E-mail address: mall.ndom@ucad.edu.sn (M. Ndom).

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systematic of helminths (Blaxter et al., 2000).

In this study, characterization of *Thysaniezia* tapeworms collected from cattle and sheep in Senegal was conducted using morphological identification characters and molecular analysis based on mitochondrial DNA (mtDNA) and ribosomal RNA gene (rDNA).

2. Materials and methods

2.1. Cestodes sampling and morphological identification characters

Cattle (n = 99) and sheep (n = 462) were examined for the presence of intestinal tapeworms in the main slaughterhouse of Dakar, Senegal. Samples came from ruminants of two to five years old that were bred in different localities throughout Senegal. Following collection, parasites were kept alive in physiological salt solution and classified after staining, using the following keys of helminth identification (Spasskii, 1951; Yamaguti, 1959; Schmidt, 1986; Khalil et al., 1994). For light microscopy, mature proglottids were fixed and washed first in 70% ethanol. Then, they were stained with iron hydrochloric carmine, destained in acid ethanol (100 mL of 70% ethanol + 2 mL of concentrated HCl), dehydrated in a gradual ethanol series, cleared with eugenol (clove oil) and finally mounted in Canada balsam. Stained specimens were observed and photographed with the SMZ-168 Series Stereo Zoom Microscope model (Motic) and Motic Camera "Moticam 2300, 3.0M Pixel, USB 2.0" model. For SEM, living tapeworms were placed into a small amount of saline buffer. Then, scolex, mature and gravid proglottids were fixed overnight in cold 2.5% glutaraldehyde in a 0.1 M sodium cacodylate buffer at pH 7.4, dehydrated in a gradual ethanol series and dried using CO_2 in an Emitech K850 critical point dryer. After being mounted on metal stubs, specimens were coated with gold/palladium in a Quorum Technologies SC7640 sputter coater and examined with a Hitachi S-3400 N scanning electron microscope at acceleration voltages between 3 and 20 kV at the "Service d'Etude et de Recherche en Microscopie Electronique de l'Université de Corse". For molecular analysis, five or six proglottids of each parasite were kept in 70% ethanol.

2.2. DNA analysis

Genomic DNA was extracted from tapeworms using the DNA Mini Kit (Qiagen) following the tissue extraction protocol, and used as the template for polymerase chain reaction (PCR). The fragment of mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) gene was amplified using forward and reverse primers *MoCox1F* (5′-CTG AGT GTT TTC AAA ACA TTT AG-3′) and *MoCox1R* (5′-AAG CAT GAT GCA AAA GGC A-3′) as described previously (Diop et al., 2015). A partial small subunit ribosomal RNA gene (SSU rDNA) was amplified for a subset of the parasite isolates using primers SSU rDNA *PF*: 5′-CTA TGG TTT ATT GGA TCA TCT C-3′ and SSU rDNA *PR*: 5′-TCT AAA TGA TCA AGT TTG GTC GT-3′. These primers were designed using Primer 3 software (Untergasser et al., 2012) to amplify partial SSU rDNA (≈ 2000 bp) of *Thysaniezia* spp. based on the SSU rDNA sequences of *Thysaniezia giardi* (= *T. ovilla*) available in GenBank (JQ609341 and JQ609342). PCR and sequencing were carried out as previously described (Diop et al., 2015), but only the reverse primer was used for sequencing of SSU rDNA.

Both mtDNA and rDNA sequences were edited using Geneious Pro software ver. 7.0.4 (created by Biomatters, available from http://www.geneious.com). Nucleotide sequences of *cox1* and SSU rDNA were aligned by Clustal W 2.0 (Larkin et al., 2007) and MAFFT (Katoh and Standley, 2013), respectively. Haplotypes were determined with DnaSPv5 (Librado and Rozas, 2009) and only unique haplotype sequences were used for the phylogenetic analyses. The phylogenetic trees were inferred using the Maximum Likelihood (ML) method under Tamura-Nei model with Gamma distribution (Nei and Kumar, 2000) in MEGA5 (Tamura et al., 2007). The robustness of the trees was tested by bootstrapping method with 1000 replicates. Anoplocephalid cestodes *Moniezia expansa* and *Moniezia benedeni* were used as the outgroup root of the phylogenetic tree. The pairwise nucleotide divergence (%) of *cox1* and SSU rDNA sequences were calculated based on the alignments of each gene sequence with the removal of gaps and ambiguous sites.

3. Results

3.1. Prevalence of infection and morphological identification characters

A total of 52 adult tapeworms were collected from cattle (48) and sheep (4), with some individual hosts having multiple tapeworms. Prevalence of animals with one or more tapeworms was 17% (17/99) in cattle and 0.4% (2/462) in sheep. Using light microscopy for morphological examination, the tapeworms collected from sheep were identified as *T. ovilla*. Indeed, they showed: craspedote strobila, proglottids wider than longer, irregularly alternating genital pores, convoluted vas deferens located at the anterior part of cirrus pouch, ovary poral, vitelline gland compact postovarian, vagina posterior to cirrus pouch and testes numerous into two lateral fields outside the excretory vessels. The poral field of testes lying behind the cirrus pouch and vas deferens. The aporal field of testes extending along the whole length of the proglottid (Fig. 1 a–c). *Thysaniezia* tapeworms from cattle also showed craspedote strobila, proglottids wider than longer, irregular alterning of genital pores, convoluted vas deferens anterior to cirrus pouch, ovary poral, vitelline gland compact postovarian, vagina posterior to cirrus pouch and testes into two lateral extravascular fields, but both the poral and aporal groups extended along the whole length of the lateral field (Fig. 2 a–c).

SEM analysis was performed on 10 specimens gathered from cattle (6 specimens) and sheep (4 specimens). In general tapeworms from cattle and sheep exhibited similar morphological characteristics. Scolex was unarmed and had a globular appearance, measuring 300 to 450 µm of diameter with four suckers (Fig. 3 a, b). The tegument of suckers were covered with acicular and capilliform filitriches (Fig. 3 c, d) and the scolex with lineate spinitriches (Fig. 3 e, f). The morphological characteristics of specimens are summarized in Table 1.

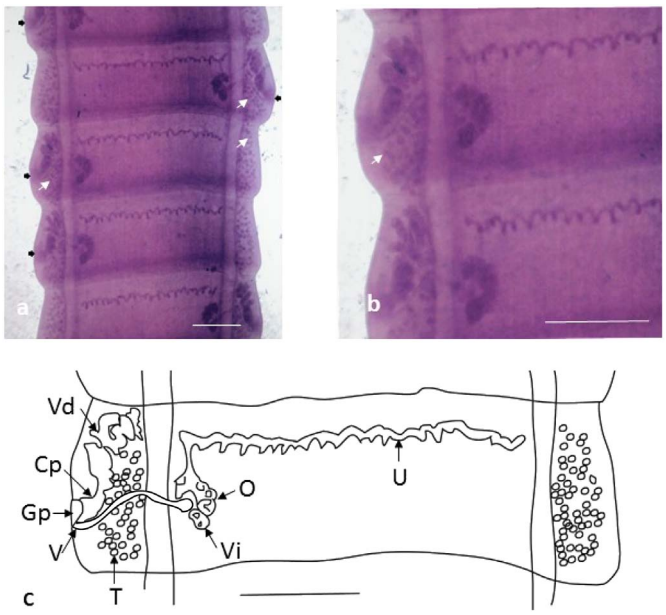

Fig. 1. Proglottids of *Thysaniezia* from sheep. (a) Portion of strobila. White arrows show testes located behind the cirrus pouch and vas deferens. Black arrows show genital pores irregularly alternating. (b) Higher magnification of poral side of the proglottids. White arrows show testes located behind the cirrus pouch and vas deferens. (c). Internal organization of a mature proglottid.
Abbreviations: Cp: Cirrus pouch, Gp: Genital pore, O: Ovary, T: Testes, U: Uterus, V: Vagina, Vd: Vas deferens, Vi: Vitellarium. Scale bar = 1 mm.