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ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

FIRST COMPARATIVE MORPHOLOGICAL STUDY OF *FASCIOLA HEPATICA* (LINNAEUS, 1758) FROM BRAZIL AND ARGENTINA

PRIMER ESTUDIO MORFOLÓGICO COMPARATIVO ENTRE *FASCIOLA HEPATICA* (LINNAEUS, 1758) PROVENIENTE DE BRASIL Y ARGENTINA

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Abstract

Fascioliasis is an infection posing a substantial threat to public health, besides causing economic impacts due to its increasing prevalence in recent years. It is considered endemic in Peru and Bolivia and in Brazil's southern state of Rio Grande do Sul. Studies conducted with specimens of *Fasciola hepatica* from different geographic regions have shown that the characteristics of the adult worms and eggs vary according to host's distribution. In this study, we evaluated whether these variations also occur in specimens obtained from cattle bred in Brazil (Cordeiro in Rio de Janeiro state and Lavras in Minas Gerais) and Argentina (Catamarca and Salta provinces), using light microscopy and light microscopy with differential interference contrast. The results demonstrated differences in the specimens from each locale, mainly in the distribution and quantity of spines in the tegument. The integrated use of various microscopic techniques is necessary to gain a better understanding of the morphology of this parasite.

Keywords: Argentina **-** Brazil **-** *Fasciola hepatica* – light microscopy - morphological study.

Resumen

La fasciolosis ha sido considerada una infección de gran importancia para la salud pública, además de causar impactos en la economía debido al aumento del número de casos en los últimos años. Es considerada endémica en Perú y Bolivia. En Brasil, la enfermedad es endémica en el estado de Río Grande do Sul. Estudios realizados con especímenes de *Fasciola hepatica* de diferentes regiones geográficas demuestran que las características de vermes adultos y huevos pueden variar de acuerdo con la localización de origen del hospedero. En este estudio evaluamos si estas variaciones también ocurren en especímenes obtenidos de bovinos provenientes de Brasil (Cordeiro/ Rio de Janeiro y Lavras/ Minas Gerais) y Argentina (Catamarca y Salta), utilizando microscopia de campo claro y microscopia de campo claro con interferencia diferencial. Los resultados demonstraron diferencias en las muestras de cada localidad, principalmente en la distribution y cantidad de espinas en el tegumento. El uso integrado de diversas técnicas microscópicas es necesario para obtener una mejor comprensión de la morfología de este parásito.

Palabras clave: Argentina – Brazil – estudio morfológico - *Fasciola hepatica* – microscopía de luz.

INTRODUCTION

Fasciola hepatica (Linnaeus, 1758) is a flatworm that causes the zoonosis fascioliasis. Its occurrence is mainly associated with areas of cattle and sheep breeding. These animals are its most frequent final hosts, with freshwater snails in nearby water bodies serving as intermediate hosts (Bargues *et al*., 2012). Infection in water buffaloes, goats, horses (Pile *et al*., 2001), pigs (Araújo *et al*., 1995), various wild animals and humans (Apt *et al*., 1993) is less frequent. The disease, which affects the liver, is of great economic and veterinary importance due to the losses caused by unfitness of liver for consumption, reduced wool, milk and meat production, secondary infections, weight loss, interference in fertility and even death of animals, besides the high cost of anthelmintic treatment (Queiroz *et al.*, 2002). Also, fascioliasis is one of the main food-borne parasitic diseases in humans (Gulsen *et al.*, 2006).

The disease has wide global distribution (Gulsen *et al.*, 2006). In Europe, cases have been reported in France, Portugal, Spain and the United Kingdom (Esteban *et al*., 1998), while it has also been reported with rising frequency in many countries in Africa, Asia and Oceania (Kimura *et al.*, 1984; Şakru *et al*., 2011).

In South America the disease is common in Bolivia (Fuentes, 2006), Peru (Espinosa *et al.*, 2010, González *et al.*, 2011) and Argentina (Mera y Sierra *et al.*, 2009). In Argentina specifically, human cases of fascioliasis have been reported in the provinces of Buenos Aires, Córdoba, Catamarca, San Luis and Mendoza (Mera y Sierra *et al.*, 2011).

In Brazil, cases have been confirmed in animals and humans in the states of Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Rio de Janeiro and Minas Gerais (Gomes *et al*., 2002). In Rio Grande do Sul, the disease is considered endemic (Cunha *et al*., 2007). A study by Dutra *et al.* (2010) confirmed that this zoonosis occurs most often in southern Brazil, although there have also been cases in the southeastern region, in Espírito Santo and Rio de Janeiro (Alves *et al.*, 2011, Bennema *et al.*, 2014).

While molecular techniques have been applied successfully for genotypic characterization (Ai *et al.*, 2011), phenotypic analysis is also important in systematic studies of the genus *Fasciola*. Studies applying light microscopy have demonstrated morphological variations between adult specimens from Bolivia, Spain and Corsica (Valero *et al.*, 2005; Periago *et al.*, 2006) and eggs of *F. hepatica* from Bolivia (Valero *et al.*, 2005), Vietnam, Georgia and Egypt (Valero *et al*., 2009). On the other hand, light microscopy with differential interference contrast (DIC), or Nomarski microscopy, has been applied to obtain detailed knowledge of the morphological structures of trematodes and especially for taxonomic purposes (Moravec *et al.*, 2002; Gomes *et al.*, 2002; Singh *et al*., 2014). However, investigations of possible phenotypical variations and techniques that can identify new morphological traits of *F. hepatica* have not been comprehensively employed in Brazil. This article compares the phenotypical traits of isolates of *F. hepatica* from different regions of Brazil and Argentina by means of light microscopy with and without DIC.

MATERIALS AND METHODS

The adult specimens were collected from naturally infected cattle at abattoirs in the Brazilian municipalities of Cordeiro, Rio de Janeiro (22°01'43"S - 42°21'33"W) and Lavras, Minas Gerais (21°14'S - 45°00'W) and in the Argentine provinces of Salta (24°47'00"S - 65°25'00"W) and Catamarca (29°16'00"S – 65°3'34"W).

The study method applied was based on the protocol proposed by Valero *et al.* (2005). The adult specimens were fixed in 70% ethanol, pressed between glass slides to facilitate visualization of the structures and stained with alcoholic chlorhydric carmine (Langeron, 1949). Immediately thereafter, the samples were dehydrated in an ascending alcohol series followed by clarification with a 1:1 solution of methyl salicylate and Canada balsam. The material was then mounted on slides, which were filled with Canada balsam. Photomicrographs were obtained with a light microscope (Olympus BX51). For the same samples, photomicrographs were also taken using differential interference contrast (DIC), to enable observing the spines on the tegument more clearly. The study was conducted with material collected from slaughtered cattle. So it is not applicable to an assessment of the ethics committee.

RESULTS

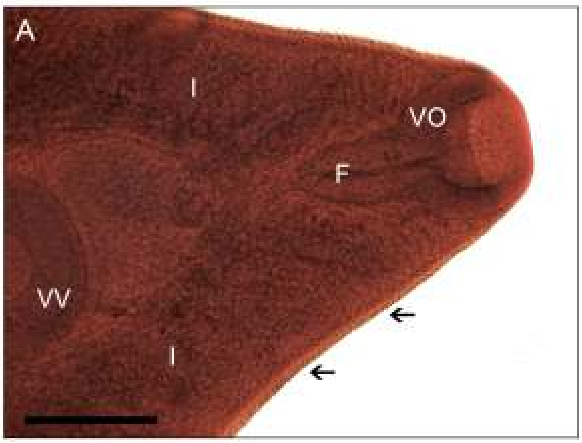
Light microscopy

One hundred and two specimens were analyzed. Forty-six specimens from Cordeiro- Rio de Janeiro, eighteen specimens from Lavras- Minas Gerais, twenty from Catamarca and fifteen from Salta, Argentina. Regardless of geographic origin, all the specimens presented a dorso-ventrally flattened, leaf-shaped body, with a very evident cephalic cone located in the anterior part of the body (Figure 1a).

Besides these features, a rounded oral sucker was observed at the apex of the cone, followed by a muscular pharynx and stomach. The esophagus bifurcated into two highly dendritic intestinal ceca, from which secondary branches emerged (Figure 2a). The intestinal ceca extended to the anterior end of the body (Figure 2b). The ventral sucker was located in the middle region of the body, near the reproductive apparatus, and was larger than the oral sucker.

The male reproductive system contained branched testes located one after the other, without reaching the hind region or the sides of the body, having the vitelline glands as limits. A well-formed cirrus pouch was observed near the ventral sucker. The female reproductive system was formed by a branched ovary, oviduct and uterus, which was filled with eggs in the specimens analyzed (Figure 2c – 1b). The vitelline glands were positioned on the side of the body and were highly branched, extending from the region of the ventral sucker to the posterior body end. The Mehlis gland, located near the ovary in the middle part of the body, was easily observed.

All the specimens from Cordeiro, Catamarca and Salta presented tegument covered with spines (Figures 1b; 2b; 3b). These spines were pointed toward the hind part of the body. All the specimens from Lavras, it was not possible to see spines on the tegument (Figure 4).



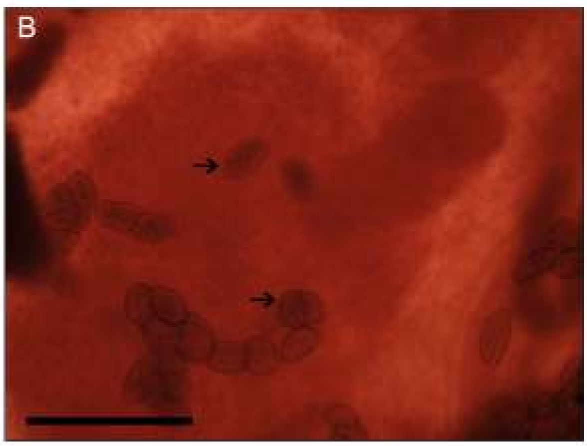


Figure 1. Morphological structures of *Fasciola hepatica* from cattle in the municipality of Cordeiro, RJ, Brazil revealed by light microscopy. (A) Cephalic cone. OS, oral sucker; I, intestinal ceca; PH, pharynx; VS, ventral sucker; spines (arrows). Magnification 4x. (B) Uterus filled with eggs (arrows). Magnification 10x.

Light microscopy with differential interference contrast

This technique was applied to observe 23 specimens. Eight specimens from Cordeiro, Rio de Janeiro, seven specimens from Lavras, Minas Gerais, four specimens from Catamarca and four specimens from Salta, Argentina. It was possible to visualize spines on the tegument of specimens from all the collection sites. On the specimens from Cordeiro and Salta (Figures 5 and 6), these spines were in larger quantities, with a different pattern of distribution, but with the same aspect, than the specimens from Catamarca and Lavras, on which very few spines were observed (Figures 7 and 8).

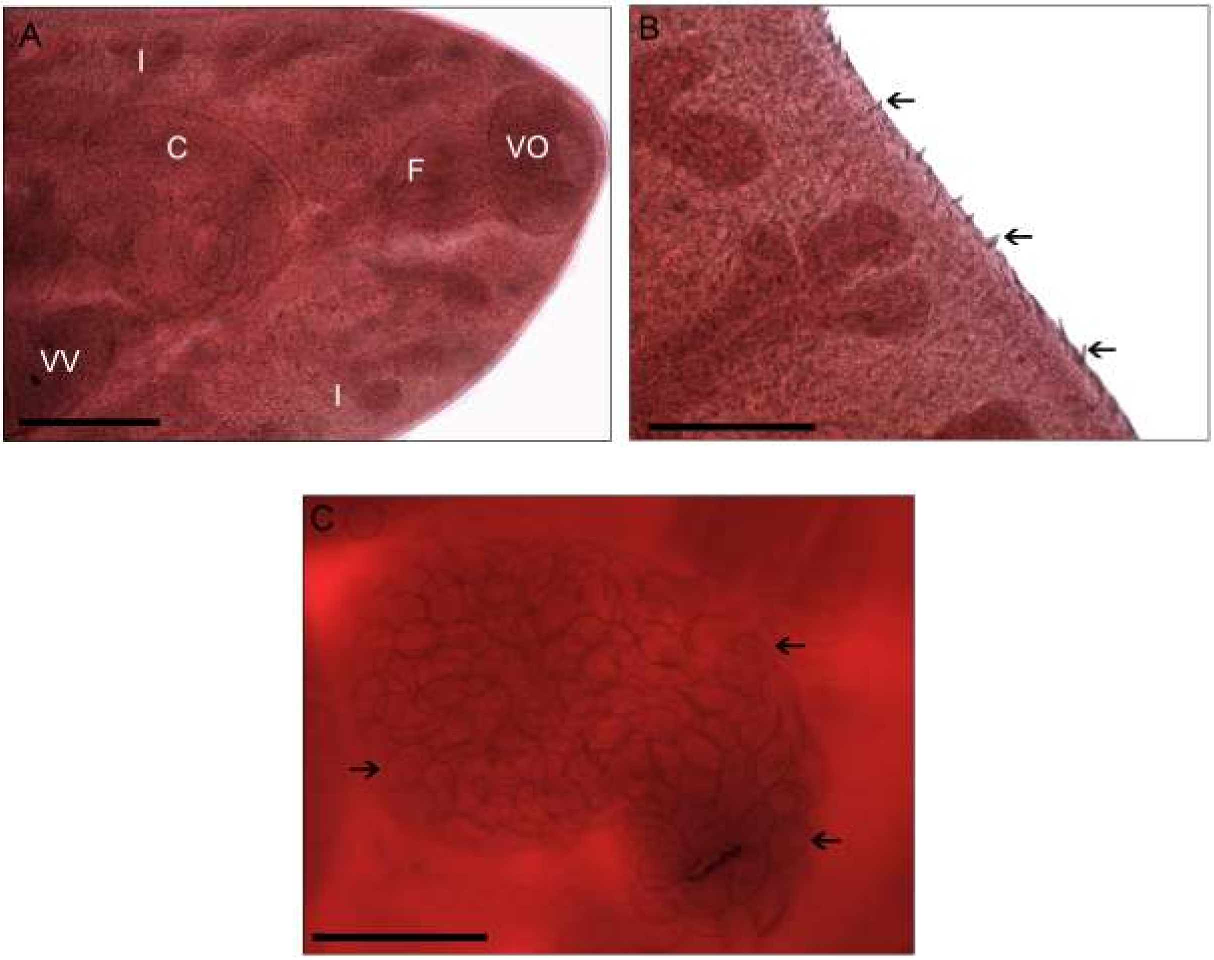


Figure 2. Morphological structures of *Fasciola hepatica* from cattle in the province of Catamarca, Argentina revealed by light microscopy. (A) cephalic cone. OS, oral sucker; I, intestinal ceca; PH, pharynx; C, cirrus; VS, ventral sucker. Magnification 4x. (B) Uterus filled with eggs (arrows). (C) Detail of the cephalic cone showing the spines (arrows); I, intestinal ceca. Magnification 10x.

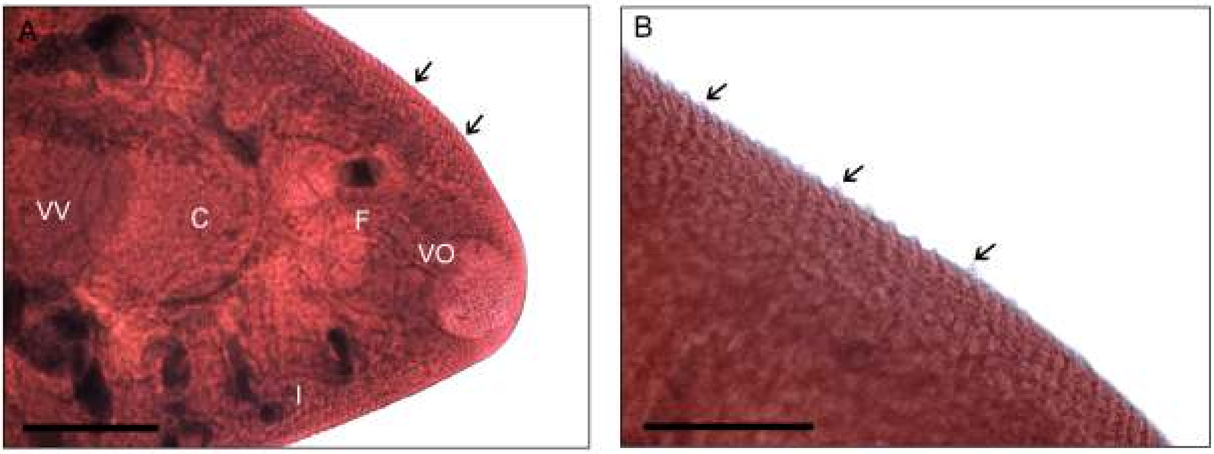


Figure 3. Morphological structures of *Fasciola hepatica* from cattle in the province of Salta, Argentina revealed by light microscopy. (A) cephalic cone. OS, oral sucker; I, intestinal ceca; PH, pharynx; C, cirrus; VS, ventral sucker; spines at the edge of the tegument (arrows). Magnification 4x. (B) Detail of the cephalic cone showing the spines (arrows). Magnification 10x.

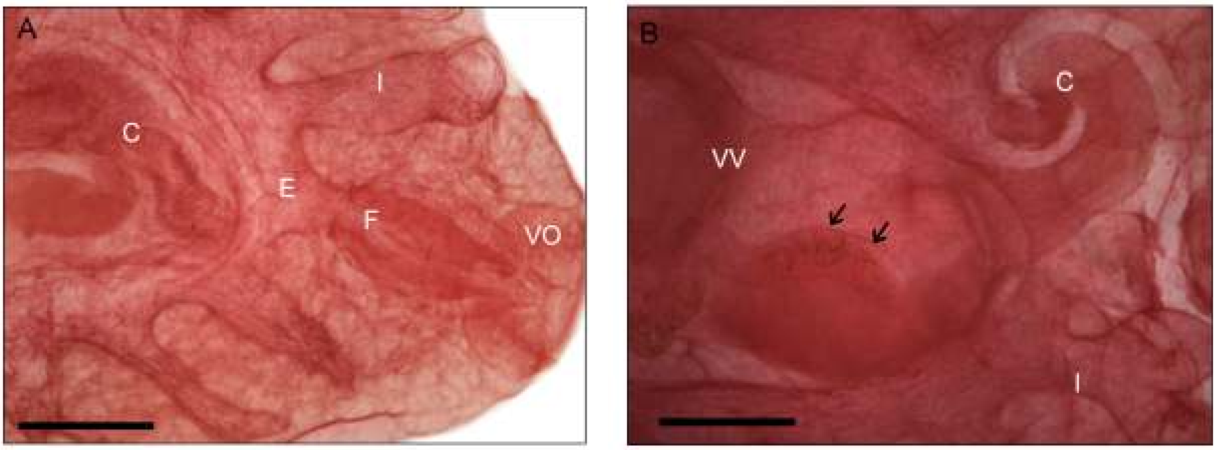


Figure 4. Morphological structures of *Fasciola hepatica* from cattle in the municipality of Lavras, MG, Brazil revealed by light microscopy. (A) cephalic cone. OS, oral sucker; I, intestinal ceca; PH, pharynx; E, esophagus; C, cirrus pouch. Magnification 4x. (B) Uterus filled with eggs (arrows); C, Cirrus; I, intestinal ceca; VS, ventral sucker. Magnification 10x.

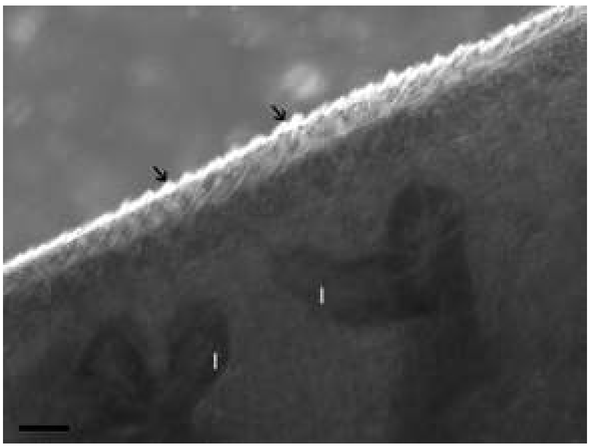


Figure 5. Morphological structures of *Fasciola hepatica* from cattle in the municipality of Cordeiro, RJ, Brazil revealed by light microscopy with differential interference contrast. Final portion of the cephalic cone, showing the spines (arrows) and I, intestinal ceca.

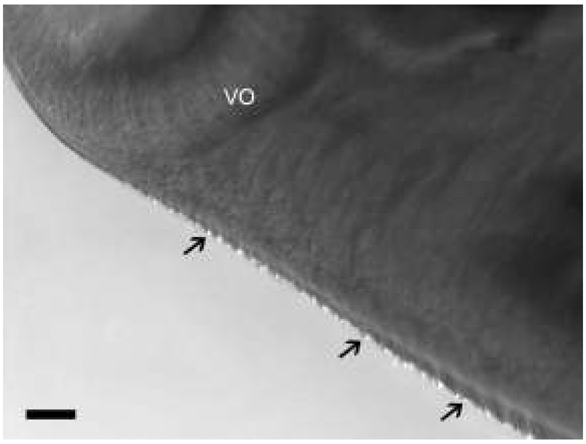


Figure 6. Morphological structures of *Fasciola hepatica* from cattle in the province of Salta, Argentina revealed by light microscopy with differential interference contrast. Final portion of the cephalic cone, showing the spines (arrows); OS, oral sucker.

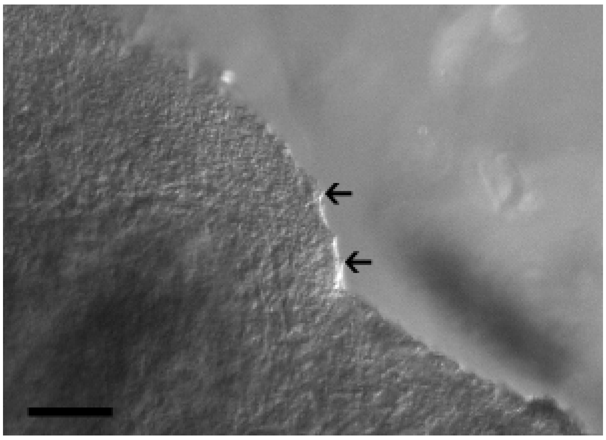


Figure 7. Morphological structures of *Fasciola hepatica* from cattle in the province of Catamarca, Argentina revealed by light microscopy with differential interference contrast. Final portion of the cephalic cone, showing the spines (arrows).

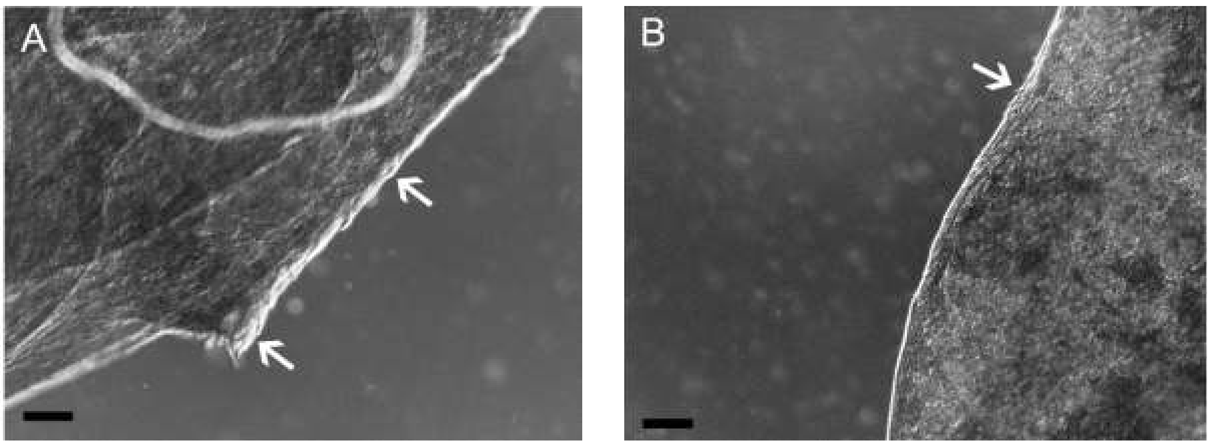


Figure 8. Morphological structures of *Fasciola hepatica* from cattle in the municipality of Lavras, MG, Brazil revealed by light microscopy with differential interference contrast. (A, B) with differential interference contrast. Final portion of the cephalic cone, showing the spines (arrows); and I, intestinal ceca.

DISCUSSION

Light microscopy has been widely used to study the morphology of different developmental phases of trematodes, to establish the taxonomy or provide more information on their structures. Over the past decade, this technique has made important contributions to the study of adult worms and eggs of *F. hepatica* (Valero *et al.*, 2005; Periago *et al.*, 2008; Valero *et al.*, 2012). The appearance of the eggs in this study did not differ from other descriptions in the literature (Hussein *et al.*, 2010a,b). We found morphological similarities of the adult specimens from Cordeiro, Salta and Catamarca, which did not differ from reports on Brazilian specimens from the city of Porto Alegre (Marques, 2002), Goiás state (Araújo *et al.*, 1995), and the cities of Careaçu and Itajubá (Oliveira, 2008). Even populations from geographic areas that are far apart (Spain and Bolivia) can present only small allometric differences (Valero *et al.*, 1999).

By means of light microscopy it was not possible to observe the presence of spines on the tegument of the specimens from Lavras, although the other structures analyzed of these specimens were similar to those of specimens from the other geographic regions. The presence of spines on the tegument helps the helminth's fixation in its habitat. Besides this, the tegument is the region of interaction between the parasite and host, where the parasite both incorporates its nutrients (Oliveira, 2008) and develops strategies to overcome the host's immune system (Moreau & Chauvin, 2010). It is speculated that adult parasites have limited interaction with the host, but further research is necessary to demonstrate whether this is a phenotypic trait.

The bright field microscope with differential interference contrast is widely used in studies helminths, especially nematode. According to Szmygiel *et al.* (2014).The technique can be used to visualize structures that are not possible with scanning electron microscopy, demonstrating the need for integrated use of such microscopy techniques (Bennet, 1975).

Despite the many advantages of light microscopy in helminthology studies, the characterization of certain morphological details requires the use of other techniques, such as differential interference contrast (Singh *et al.*, 2014; Szmygiel *et al.,* 2014). With this technique was possible visualize the difference in the distribution of spines in the tegument of *Fasciola hepatica*. To the best of our knowledge, this paper is the first to report the use of this technique on *F. hepatica*. With the integrated use of the two techniques, it was possible to observe spines also on the specimens from Lavras, evidencing the need for integration of microscopic techniques to obtain reliable results. While most of the specimens of *F. hepatica* examined here presented morphological similarity when analyzed by light microscopy alone, the integrated use of various microscopic techniques is necessary to gain a better understanding of the morphology of this parasite.

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