

# ***Diphyllobothrium latum* AND *Diphyllobothrium* SP. AS THE AGENTS OF DIPHYLLOBOTHRIASIS IN BRAZIL: MORPHOLOGICAL ANALYSIS AND OF TWO NEW CASE REPORTS\***

## ***Diphyllobothrium latum* E *Diphyllobothrium* SP. COMO AGENTES DA DIPHYLLOBOTRIOSES NO BRASIL: ANÁLISE MORFOLÓGICA E RELATO DE DOIS NOVOS CASOS**

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**ABSTRACT.** Knoff M., Pinto R.M., de São Clemente S.C., Fonseca M.C.G. & Gomes D.C. *Diphyllobothrium latum* and *Diphyllobothrium* sp. as the agents of diphyllobothriasis in Brazil: morphological analysis and two new case reports. [*Diphyllobothrium latum* and *Diphyllobothrium* sp. como agentes da diphyllobotrioses no Brasil: análise morfológica e relato de dois novos casos]. *Revista Brasileira de Medicina Veterinária*, 33(3):159-164, 2011. Laboratório de Helmintos Parasitos de Vertebrados, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Avenida Brasil 4365, Manguinhos, RJ 21045-900, Brasil. E-mail: knoffm@ioc.fiocruz.br

Between August 2004 and April, 2005 two adults, a man and a woman, living in Rio de Janeiro, frequently feeding on raw fish (sushi and sashimi) without a recent historical of being abroad, presented similar symptoms of abdominal pain. Fecal samples with the presence of eggs recovered from both patients with together a strobila found in the stools of the woman were sent to the Laboratório de Helmintos Parasitos de Vertebrados, Instituto Oswaldo Cruz for examination. In order to avoid the accurate identification of parasites, eggs and proglottids were measured and analyzed; transversal and sagittal sections of the proglottids were also obtained. Results showed the presence of *Diphyllobothrium latum* and *Diphyllobothrium* sp. in the two infected individuals, man and in the woman, respectively. A survey of other human cases of diphyllobothriasis occurring in Brazil is presented.

**KEY WORDS.** Diphyllobothriasis, *Diphyllobothrium latum*, *Diphyllobothrium* sp., humans, Zoonosis, Brazil.

**RESUMO.** Entre agosto de 2004 e abril de 2005, dois adultos, um homem e uma mulher residentes na cidade do Rio de Janeiro e consumidores habituais de pescado cru (sushi e sashimi) sem histórico recente de viagem internacional, apresentavam sintomas similares de dores abdominais. As análises

das amostras de fezes com ovos dos pacientes e uma porção do estróbilo provenientes da mulher foram enviados ao Laboratório de Helmintos Parasitos de Vertebrados, Instituto Oswaldo Cruz. Com objetivo de identificar os parasitos, os ovos e as proglotes foram medidos e analisados; nas proglotes foram re-

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alizados cortes histológicos transversais e sagitais. Após análise taxonômica dos ovos e proglotes grávidas foi possível a determinação específica como *Diphyllobothrium* sp. para a amostra proveniente do homem e para aquela proveniente da mulher como *D. latum*. É apresentado o levantamento dos casos ocorridos no Brasil.

PALAVRAS-CHAVE. Diphyllbothriose, *Diphyllobothrium latum*, *Diphyllobothrium* sp., humanos, zoonose, Brasil.

## INTRODUCTION

The identification of species of *Diphyllobothrium* (Cobbold 1858), has been reported from different geographical regions, on the basis of the morphological characters of adults or parts of the strobila (Torres 1982). The proper identification of species included in *Diphyllobothrium* is to be achieved more by means of reliable morphological characters than those only related to the size of eggs. Thus, according to Torres (1982), the host species, intensity of infection and age of the parasite can interfere in the dimension of eggs that has not to be considered as the best method for the specific diagnosis.

The present investigation deals with the results obtained with the study of the eggs, transversal and sagittal sections of the proglottids of *Diphyllobothrium* specimens by means of bright-field and confocal scanning laser microscopies.

Taking into account the fact that several studies related to these infections have increased during the last years in Brazil, a survey of the previously reported cases is also presented.

## HISTORICAL FINDINGS

Between August, 2004 and April, 2005, two adults (a man and a woman), both living in the municipality of Rio de Janeiro, State of Rio de Janeiro, Brazil, often feeding on raw imported salmon meat and on other species of native fishes (served as sushi and sashimi) without references to trips abroad in the last five years, presented symptoms of parasitism. The man (47 years old) complained of severe abdominal pain, nausea and vomiting sensation. In August, 2004, the patient was admitted in a clinical center, attended and conducted to a private laboratory for stool examination. The analysis revealed an infection by an uncommon helminth. In order to confirm the diagnostic, another stool sample with parasite eggs was sent to the Laboratório de Helminthos Parasitos de Vertebrados (LHPV),

Instituto Oswaldo Cruz/Fiocruz. The woman (53 years old) presented mild abdominal pain, frequent flatulence and nocturnal hypoglycemia and in November, 2004 expelled part of a strobila in the feces. In April, 2005, the patient, expelled parts of the parasite, together with the feces. The material was collected and sent to the LHPV for examination. The sample consisted of eggs and parts of strobila, without the scolex. A portion of strobila was stained with Langeron's carmine and mounted in Canada balsam whereas another was prepared for paraffin embedding, sectioned (7 micrometers thick) and stained with hematoxylin-eosin (HE), for histological examination of the internal structures of taxonomic interest, by means of light and confocal laser microscopies. The sedimentation method of Lutz was performed for the analysis of eggs in the fecal samples of both patients. Analysis and measurements were based on 50 eggs obtained from each patient. The adopted methodology for the proper diagnosis related to the different species of *Diphyllobothrium*, the measurement of eggs, proglottids and terminology, follows several authors (Baer et al. 1967, Rausch & Hilliard 1970, Torres et al. 1989, Andersen et al. 1987, Sampaio et al. 2005) whereas the classification is in accordance with Kuchta et al. (2007). In the description, measurements of proglottids are in millimeters, that of the eggs in micrometers, using an ocular micrometer connected to a bright-field BX-41 Olympus microscope, except when otherwise indicated, and means are in parentheses. Studied specimens are deposited in the Coleção Helminológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, RJ, Brazil. The general survey on the Brazilian cases of diphyllbothriasis was based on previously published data.

Examination of eggs present in the fecal samples obtained from both patients, together with the strobila expelled by the woman, revealed the presence of a cestode species, included in Pseudophyllidea Carus, 1863, Diphyllbothriidae Luhe, 1910 and *Diphyllobothrium* Cobbold, 1858. Eggs present in feces of the man were identified to *Diphyllobothrium* sp., whereas eggs and parts of the strobila obtained from the woman could be related to *D. latum* (Linnaeus, 1758).

Description of eggs present in the fecal sample from the man (Figure. 1a): eggs non-embryonated, operculated and an abopercular knob, 50.9-56.4 (52.8) long, 38.2-45.5 (40.8) wide, not collapsed. Material deposited: CHIOC no. 35582.

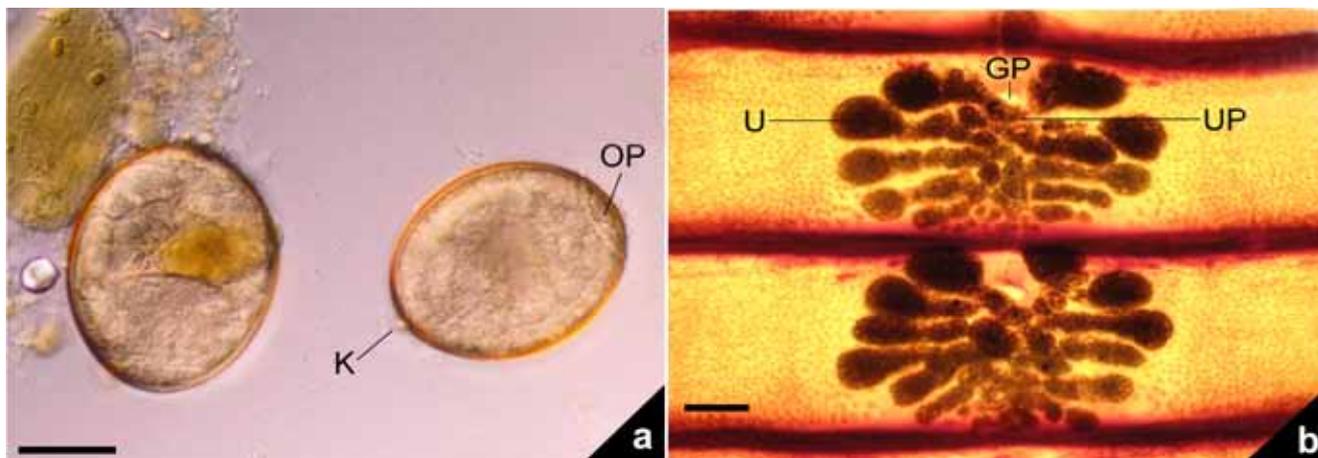


Figure 1. *Diphyllobothrium latum*. Eggs elliptical, non-embryonated, operculum (OP) in one of the extremities and abopercular knob (K) (a) (Bar = 20 µm). Gravid proglottids, uterus (U) rosette-shaped, occupying the total extension of the segment, uterine pore (UP) below to the common genital pore (GP), median-ventral, in the anterior third of the proglottid, testes not convergent in the anterior portion of the cirrus pouch, ventral view (b) (Bar = 0.5 mm).

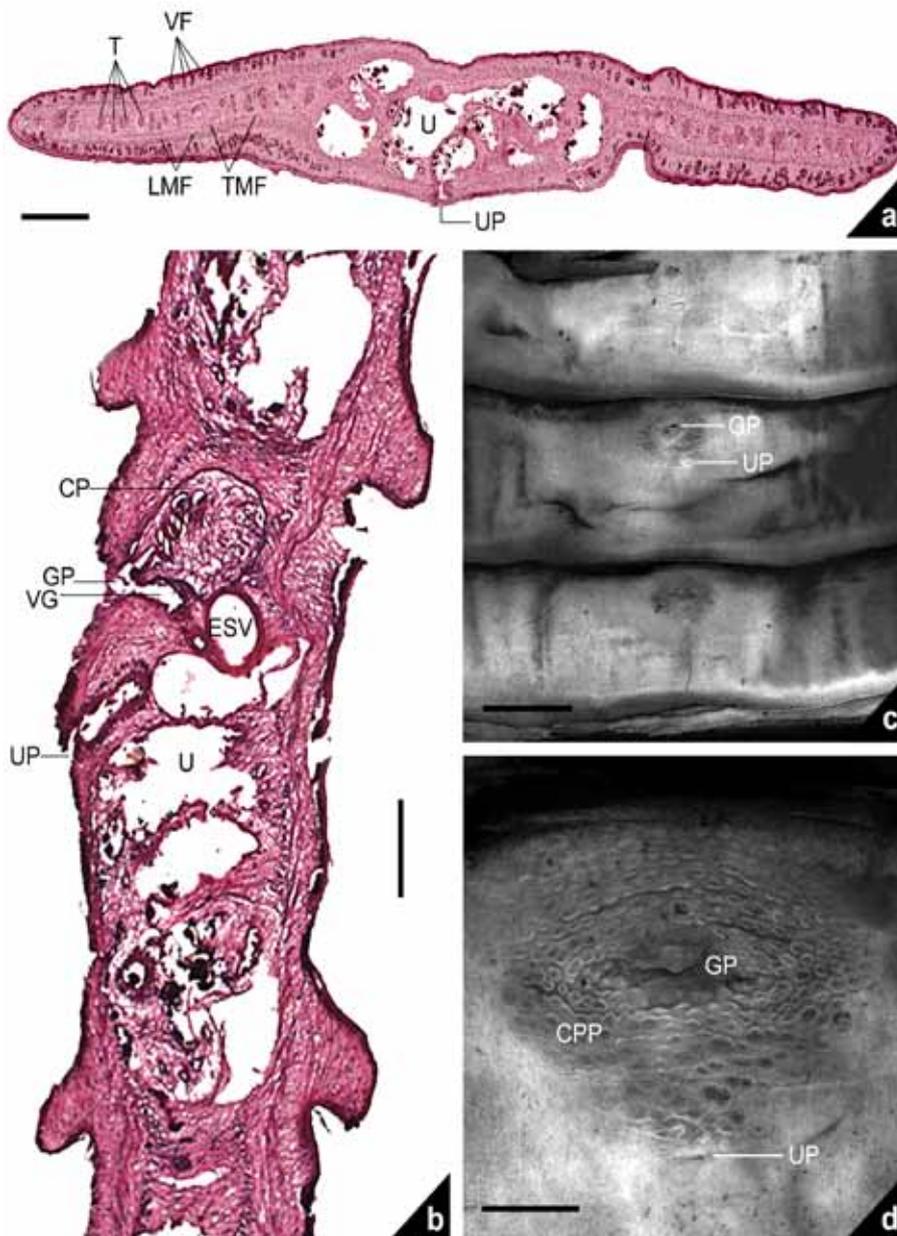


Figure 2. *Diphyllobothrium latum*. **Transverse and sagittal sections of the proglottids** - Transverse section passing through the uterine pore (UP). Margins of the segments rounded, medular testes (T) and vitelline follicles (VF) in a single layer, distributed laterally in two fields, without connection, separated by transversal and longitudinal muscular fibers (TMF and LMF). Uterus (U) central (a) (Bar = 0.5 mm). Sagittal section passing to the common genital pore (GP). Cirrus pouch (CP) oriented horizontally in relation to the segment and situated in the anterior portion of the proglottid, external seminal vesicle (ESV), dorsocaudal in relation to the cirrus pouch. Vagina (VG) opening posterior to the cirrus pouch. Uterine pore located below the common genital pore (b) (Bar = 0.25 mm). **Proglottids ventral surface by confocal laser microscopy** - Common genital pore central, uterine pore slightly on the left of the common genital pore, from the anterior end (c) (Bar = 1 mm). **Detail of c** - Showing the circular papillae (CPP) around the common genital pore and above of the uterine pore (d) (Bar = 0.2 mm).

Description of the part of the strobila and eggs in fecal sample from the woman (Figure 1b) and Figure 2a,b,c,d): strobila, with 64 gravid proglottids, separated by constrictions, 12 cm long. Proglottids 1.5-1.9 (1.7) long by 7.2-8.2 (7.8) wide. Ovary ventral, bilateral in posterior portion of segment, lobes similar extending to the lateral portion, 0.84-0.93 (0.84) long, 0.15-0.26 (0.23) wide in each lobe. Ootype inconspicuous. Mehlis gland oval-shaped in posterior portion of proglottid, located between the ovarian lobes, 0.16-0.26 (0.19) long, 0.25-0.55 (0.40) wide. Uterus central, rosette-shaped, median, extending from the anterior region of ovary towards the uterine pore, occupying the total extension of the segment, 1.47-1.87 (1.65) long, 2.40-2.90 (2.67) wide. Uterine pore located bellow the common genital pore, median-ventral in anterior third of proglottid, on ventral surface, slightly on the left of the genital pore, 0.73-0.83 (0.79) from the anterior end. The genital pore is central with the opening of masculine and feminine apertures median in the anterior third of proglottid, 0.18-0.48 (0.30) from its anterior end. The genital pore is encircled, on ventral surface, by circular papillae that extend from the anterior region of segment to uterine pore. Vagina running from oviduct to genital pore, and opening posterior to cirrus pouch. Distance of genital pore to uterine pore is of 0.23-0.35 (0.28). Testes about 1,200 sepa-

rated in two lateral fields, distributed among the uterine branches. Vitelline follicles abundant, separated in lateral fields, overlapped at the end of the gravid uterine branches and in the lateral margins of ovary. Testes and vitelline follicles not convergent in the anterior portion of cirrus pouch. Transversal sections of proglottids: margins of segments rounded, medullar testes and vitelline follicles in a single layer, distributed laterally in two fields, without connection, separated by transversal and longitudinal muscular fibers. In each side of proglottid there are 18-20 testes and 60-80 vitelline follicles. Sagittal sections show an elongate cirrus pouch oriented horizontally in relation to the segment and situated in anterior portion of proglottid, 0.40-0.45 (0.42) long, 0.31-0.41 wide. The cirrus occupies almost half of pouch; external elliptical seminal vesicle, dorsocaudal in relation to cirrus pouch, 0.18-0.27 (0.23) long, 0.16-0.24 (0.18) wide. Eggs non-embryonated, operculated and an abopercular knob, 65-70 (67.5) long, 55-60 (57.5) wide, non-collapsed.

Material deposited: CHIOC no. 37003 a-e.

## DISCUSSION

The species *D. latum* and *Diphyllobothrium* sp. were referred by several authors as the agents of human diphyllobothriasis in Brazil (Table 1), are commonly related to the ingestion of raw fish meat.

Table 1. Cases of human diphyllobothriasis previously reported in Brazil, due to *Diphyllobothrium* species.

Cases	States	Species	Reference
NA	Bahia São Paulo	<i>Diphyllobothrium latum</i>	Coutinho (1957) <sup>a</sup>
NA	São Paulo	<i>D. latum</i>	Castilho et al. (2001) <sup>b</sup>
A	São Paulo	<i>Diphyllobothrium</i> sp. <i>D. latum</i>	Eduardo et al. (2005a,b) <sup>c</sup> Sampaio et al. (2005) <sup>d</sup>
A	São Paulo	<i>Diphyllobothrium</i> sp.	Capuano et al. (2007) <sup>b</sup>
A	Rio de Janeiro	<i>Diphyllobothrium</i> sp.	Tavares et al. (2005) <sup>b</sup>
A	Bahia	<i>D. latum</i>	Santos & Faro (2005) <sup>b</sup>
A	Rio Grande do Sul	<i>D. latum</i>	Emmel et al. (2006) <sup>e</sup> Mezzari & Wiebbelling (2008) <sup>b</sup>
A	Paraíba	<i>D. latum</i>	Lacerda et al. (2007) <sup>b</sup>

A – Autochthonous

NA - non-autochthonous

a- Specific identification, methodology not informed.

b- Specific identification on the basis of morphology and morphometrics of eggs.

c- Specific identification on the basis of morphology and morphometrics of eggs and proglottids. Methodology adopted for the examination of proglottids was not available.

d- Specific identification based on morphological and morphometrics data of eggs and proglottids with the examination of longitudinal and transversal sections and also on the observation of the proglottids by means of scanning electron microscopy.

e- Specific identification on the basis of morphology and morphometry of eggs and analysis of the proglottids clarified with glacial acetic acid.

Biological data as well as the availability of entire cestode specimens are necessary to a proper specific diagnosis. The specific identification of *Diphyllobothrium* spp. has to be based on several characters, such as the scolex form, neck length, external and internal morphology of proglottids, including maximum strobila width, limits type of segments, ovary form, number of uterine coils in gravid proglottids filled with eggs, position of the genital atrium, cirrus morphology, cirrus pouch location, seminal vesicle, vagina and uterine pore, the presence or absence of convergence of vitelline follicles and testes in the region anterior to the cirrus pouch (Andersen et al. 1987, Torres et al. 1989). The minimal characteristics to allow the differentiation of *D. latum* from the others species are related to the testes and vitelline follicles not convergent in the anterior portion of cirrus pouch. Uterine and genital pores apertures are median in the anterior third of the proglottid, medullar testes and vitelline follicles in a single layer. External elliptical seminal vesicle dorsocaudal in relation to the cirrus pouch. Thus, eggs size alone is not considered taxonomically significant in the identification of species of *Diphyllobothrium* (Andersen & Halvorsen 1978), since the host species, intensity of infection and age can interfere in the dimension of eggs (Torres 1982). The measurements of eggs of different species can be overlapped and the greater values were those referred for *D. latum* (Baer et al. 1967, Andersen & Halvorsen 1978, Torres et al. 1989). In South America *Diphyllobothrium latum* (L., 1758), *D. dendriticum* (Nitzsch, 1824) and *D. pacificum* (Nybelin, 1931) have been referred parasitizing humans (Acha & Szyfres 2003). In Brazil, since 1915 cases of human diphyllbothriasis were reported on eggs basis and proglottids examination and related to *Diphyllobothrium* sp. and *D. latum*. Most of the specific identifications was accomplished by means of eggs measurements and of a superficial proglottids examination; in some cases, however, the presence of genital papilla, seminal vesicle position in relation to cirrus pouch, uterus form together with the eggs size, have been considered (Table 1).

Morphological and morphometric data on eggs found in the feces from the male patient were in accordance with the range of variation reported for several species of *Diphyllobothrium*. Nevertheless, eggs alone, do not provide the specific identification, that is to be achieved on the basis of eggs and proglottids that were found in the fecal sample from the

woman; differential specific characters are in accordance with thus confirming the inadequacy of this character for the specific diagnosis, and the identification of *D. latum* on the basis of eggs and proglottids in the fecal sample from the woman, agrees with the differential specific characters (Rausch & Hilliard 1970, Andersen & Halvorsen 1978, Torres et al. 1989, Andersen et al. 1987, Sampaio et al. 2005).

Taking into account the methodologies adopted for specific identification in previous reports related to diphyllbothriasis in Brazil in comparison with the present data, it is suggested that in some of the cases, the species should be referred to as *Diphyllobothrium* sp., instead of *D. latum*. These reports indicate that the principal via of transmission for diphyllbothriasis is the ingestion of raw, poorly cooked or smoked fish meat and incriminate as the major responsible for human infection the imported salmon. In the present paper, patients informed about the ingestion of this and other native fishes and it is known that certain species of Brazilian fishes captured in hinterland waters and mainly in the littoral have been utilized in the preparation of exotic food (Eduardo et al. 2005b) and thus, the salmon should not be considered the only agent of this zoonosis. Recently, the presence of larval *Diphyllobothrium* sp., was detected in specimens of *Genypterus brasiliensis* Regan, 1903 a fish species commercialized in the State of Rio de Janeiro, indicating the necessity of the study of other teleosts that occur in Brazil (Knoff et al. 2008).

In accordance with Scholz et al. (2009), the detection of sources of *Diphyllobothrium* species and their identification are of great importance with respect to epidemiology. Although the techniques of molecular identification of *D. latum* are presently well developed and properly interpreted (Nakao et al. 2007), they are of restrict access thus justifying, by the moment, the species identification, on the basis of gravid proglottids. The present paper presents morphological data on *D. latum* in order to suggest the specific identification should not be based on the finding of eggs alone, since they are of generic value, only.

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