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# Invasive slug *Meghimatium pictum* (Stoliczka, 1873) infected by *Angiostrongylus costaricensis* Morera & Céspedes, 1971, and the possible risk of human infection associated with grape consumption

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**Abstract**

Many molluscs may be infected with angiostrongylid larvae. Following the histopathological diagnosis of abdominal angiostrongyliasis in a grape farmer from southern Brazil, molluscs in the area were investigated. During a nocturnal search, 245 specimens of slugs were collected and identified as the invasive Chinese slug *Meghimatium pictum*. *Angiostrongylus costaricensis* worms were recovered from mice that were experimentally infected with larvae obtained from 11 (4.5%) of the molluscs. This study presents the first report of *M. pictum* being identified as an intermediate host for *A. costaricensis*. Most of the slugs were collected from grape plants, which suggests that transmission may be associated with grape consumption.

## Introduction

Abdominal angiostrongyliasis (AA) is an infection caused by *Angiostrongylus costaricensis* Morera & Cespedes, 1971, a metastrongylid nematode that lives inside mesenteric arteries (Morera, 1973). Infection with *A. costaricensis* occurs via ingestion of raw molluscs that harbour third-stage larvae (L3) or their mucus containing larvae released on fruit and vegetables (Bonetti and Graeff-Teixeira, 1998). L3 are able to penetrate the intestinal wall and migrate through blood or lymphatic vessels to become adult worms inside mesenteric arteries. First-stage larvae (L1) are released with rodent faeces, and may infect molluscs by penetrating their tegument or after ingestion (Morera, 1973; Mota and Lenzi, 2005). The L1 of molluscs molt twice to evolve to L3, with L3 representing the infective stage for vertebrate hosts (Morera, 1973; Mota and Lenzi, 2005).

Here we report the identification of a new mollusc host, the Chinese slug *Meghimatium pictum* (Stoliczka, 1873), which is a pest of vineyards in southern Brazil and is associated with human abdominal angiostrongyliasis, and we raise the hypothesis that *A. costaricensis* may infect humans through grape consumption.

## Materials and methods

A 69-year-old female grape farmer living in a rural area of Marau (28°26′42″S, 52°12′35″W), Rio Grande do Sul, Brazil, presented with liver nodules and intestinal lesions. Following a histopathological examination, the farmer was diagnosed with abdominal angiostrongyliasis. When the area surrounding the farmer's house was searched at night, a large number of slugs with the same external characteristics were found climbing on grape plants in her backyard. Seven of these slugs were collected and digested with pepsin solution according to the method of Wallace and Rosen (1969) to recover larvae.

Larvae with a subterminal notch (suggestive of metastrongylid larvae) were subsequently inoculated *per os* in Swiss mice, which are susceptible to *A. costaricensis* infection (Mentz and Graeff-Teixeira, 2010). The experimental infection of mice was conducted under ethical clearance, CEUA 15/00443. After 30 days, adult worms were obtained and identified based on their morphology and their localization in the mesenteric arteries (Morera, 1973; Mota and Lenzi, 2005; Spratt, 2015). After the initial identification of *M. pictum* infection, we surveyed the area around the farm to estimate the prevalence of infection in the slug population.



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Two slugs with lengths/widths of 40.12/7.90 mm and 42.59/7.57 mm were deposited in the malacological collection of the Institute Oswaldo Cruz (CMIOC), Rio de Janeiro, Brazil (CMIOC 9.997). The slugs were dissected and examined under a stereomicroscope for morphological analysis.

Partial sequencing of the 5′ region of the cytochrome *c* oxidase I (COI) gene from one slug specimen was generated and deposited in GenBank (accession number: KX781994). In brief, fragments of COI were polymerase chain reaction (PCR) amplified with the primers LCO-1490 (5′GGTCAACAAATCATAAAGATATTGG) and HCO-2198 (5′TAAACTTCAGGGTGACCAAAAAATCA) (Folmer *et al.*, 1994) according to the protocol of Hayes *et al.* (2009) at the Laboratório de Referência Nacional em Esquistossomose – Malacologia (LRNEM) at IOC/FIOCRUZ. DNA sequencing was carried out at the Genomic Platform - RPT01A (Rede de Plataformas Tecnológicas Fiocruz).

## Results and discussion

The bodies of the slugs were characterized by a beige background colour on the sides and dorsum, with two dark brown to black lateral stripes, and one medial stripe that was generally lighter than the lateral stripes (fig. 1). Below the lateral stripes and surrounding the central medial stripe there was a scattering of dark brown irregular spots or short lines. The foot of these slugs occupied the entire ventral extension and was cream coloured. The penis was claviform, short, thick, and had a constriction halfway along its length. The vas deferens was slightly longer than the penis. The bursa copulatrix had a spherical to oval form and both the penis and the bursa copulatrix opened in a large, barrel-shaped atrium. These external and internal characteristics are consistent with those previously described for *Meghimatium pictum* (Stoliczka, 1873) according to Tsai *et al.* (2011) and Gomes *et al.* (2011).

A total of 655 base pairs were amplified and this DNA sequence was identical to the COI sequence deposited in GenBank (JQ712572) from an *M. pictum* collected in southern Brazil (Gregoric *et al.*, 2013).

After 30 days inoculating the larvae in Swiss mice, adult nematodes were found and collected from the mesenteric arteries of the mice. Based on the morphology and the localization of adult worms in the mesenteric arteries the adult worms were identified as *A. costaricensis*. The morphology and length of copulatory bursa rays were in accordance with the updated description of *A. costaricensis* (Morera, 1973). Also, there is only one other species in the genus *Angiostrongylus* known to exhibit this type of localization, namely *A. siamensis*, which occurs in Asia (Spratt, 2015).

A total of 245 specimens of *M. pictum* (ranging from 0.03 g to 2.25 g in weight) were collected from the same area to estimate prevalence. Eleven slugs were infected with metastrongylid larvae, producing a prevalence of 4.5%. Two, three, four and eight larvae were detected in four individually digested molluscs. Another seven slugs were pooled for digestion, from which 200 L3 were recovered.

Many species of terrestrial molluscs have been reported as successful hosts of *Angiostrongylus* spp. larvae in various countries of Central and South America. Slugs from the Veronicellidae family are the best-adapted hosts in the southern region of South America, especially species belonging to the genera *Phyllocaulis*, *Sarasinula* and *Belocaulus* (Bonetti and Graeff-Teixeira, 1998). However, several other families of terrestrial molluscs are also naturally infected with *A. costaricensis* (Graeff-Teixeira *et al.*, 1993; Maurer *et al.*, 2002; Ohlweiler *et al.*, 2010).

**Fig. 1.** The invasive slug *Meghimatium pictum* (Stoliczka, 1873).

*Meghimatium pictum* is a Stylommatophora slug belonging to the Philomycidae family. Stylommatophora encompass *c.* 95% of all terrestrial gastropods, whereas Systellommatophora, which contains Veronicellidae slugs, encompass < 1% (Thomé *et al.*, 2006; Ponder and Lindberg, 2008). Both groups (Stylommatophora and Systellommatophora) are traditionally distinguished in orders or clades (Bouchet *et al.*, 2005; Ponder and Lindberg, 2008).

The slug *M. pictum* is considered endemic to China. However, it has also been found in Brazil and Argentina (Gomes *et al.*, 2011; Gregoric *et al.*, 2013). The global dispersal of terrestrial gastropods has been facilitated by an expanding global economy and associated transportation opportunities (Robinson, 1999). In 2011, this slug was recorded for the first time in Brazil and quickly emerged as an agricultural pest in vineyards (Baronio *et al.*, 2014). Terrestrial molluscs are considered one of the most significant and intractable threats to sustainable agriculture worldwide (Barker, 2002).

*Meghimatium pictum* is found mainly in the south-eastern regions of China (Li *et al.*, 2006). This region is also inhabited by *M. bilineatum*, which is a phylogenetically sister-species of *M. pictum* that is a confirmed carrier of another species of *Angiostrongylus*, *A. cantonensis*. This species is the primary causative agent of eosinophilic meningoencephalitis (EoM) in humans (Wang *et al.*, 2008).

Cases of EoM caused by *A. cantonensis* in Brazil have only recently been documented, with the parasite being detected in several mollusc species along the coast, including *Achatina fulica*, *Bradybaena similaris*, *Sarasinula* spp. and *Subulina octona* (Graeff-Teixeira *et al.*, 1993; Morassutti *et al.*, 2014), but not in *M. pictum*. To our knowledge, there is also no previous report of *M. pictum* being infected with *A. costaricensis*.

The infective stage of angiostrongylid worms may be carried by the slime of molluscs (Bonetti and Graeff-Teixeira, 1998). Consequently, the risk of angiostrongyliasis infection may be associated with the consumption of vegetables and fruit, especially grapes, because molluscs can come in contact with the external surfaces of this fruit that is often consumed in nature. Furthermore, grape harvesters and garden keepers may be at a higher risk of infection, because their hands could be contaminated with L3 released from snails, with L3 remaining infective

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in the environment for three to 17 days (Richinitti *et al.*, 1999). The low parasitic burden that was observed in this case (4.5%) supported previous findings that the natural infection of molluscs with *A. costaricensis* is characterized by very low parasitic burden (Rambo *et al.*, 1997; Laitano *et al.*, 2001).

This study confirmed *M. pictum* as a new intermediate host of *A. costaricensis*. We also suggest there is a risk of human infection through consuming grapes, because *M. pictum* slugs were frequently found on the fruit, both during our surveys and based on information provided by the infected grape farmer. Considering the low specificity of angiostrongylid worms for their intermediate hosts and the documented natural infection of *M. bilineatum* by *A. cantonensis*, which is a sister-species of *M. pictum* in China (Li *et al.*, 2006), the present report should be considered an alert of the high potential risk for both abdominal and cerebral angiostrongyliasis transmission to humans in Brazil.

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**Conflict of interest.** None.

**Ethical standards.** Ethical standards are in accordance with Brazilian regulations.

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