Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v39i3.35615

Detection of Anisakidae larvae parasitizing *Plagioscion squamosissimus* and *Pellona castelnaeana* in the State of Pará, Brazil

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ABSTRACT. Five specimens of *Plagioscion squamosissimus* from Xingu River and ten specimens of *Pellona castelnaeana* from Mosqueiro Island, both in the State of Pará, Brazil, were examined to investigate the presence of anisakid nematodes, due to their zoonotic potential. Their parasitism indices and sites of infection were also determined. This is the first record of Anisakidae parasitizing *Pellona castelnaeana*. Four-hundred and eighty-four third-stage larvae (L_3) of Anisakidae were found, of which 42 were found in *P. squamosissimus* and 442 in *P. castelnaeana*. The parasitism indices of the anisakid collected from the *P. squamosissimus* comprised prevalence (P) of 100%, mean infection intensity (MI) of 8.4, range of infection (RI) of 1-13, mean abundance (MA) of 8.4, and infection site (IS) in the abdominal cavity. *P. castelnaeana* showed P = 100%, MI = 44.2, RI = 10-114, MA = 44.2, and IS = abdominal cavity, cecum and stomach. The life cycle of these nematodes can be completed in the Amazon basin, since the two hosts fish are part of the cetacean diet of the region, which participate as final hosts. These findings have an important consequence on epidemiology of anisakiasis, so attention should be extended to human protection against this public health risk.

Keywords: freshwater fish, zoonosis, Anisakidae, Amazon basin.

Detecção de larvas de Anisakidae parasitando *Plagioscion squamosissimus* e *Pellona castelnaeana* no Estado do Pará, Brasil

RESUMO. Cinco espécimes de *Plagioscion squamosissimus* do rio Xingu e dez espécimes de *Pellona castelnaeana* da Ilha do Mosqueiro, ambos no Estado do Pará, Brasil, foram examinados para investigar a presença de nematodas Anisakidae, devido ao seu potencial zoonótico. Também foram determinados seus índices de parasitismo e sítios de infestação. Esse é o primeiro registro de Anisakidae parasitando *Pellona castelnaeana*. Foram encontradas 484 larvas de Anisakidae de terceiro estágio (L_3), sendo 42 em *P. squamosissimus* e 442 em *P. castelnaeana.* Os índices parasitários de anisaquídeos coletados de *P. squamosissimus* compreenderam prevalência (P) de 100%, intensidade de infecção média (IM) de 8.4, amplitude de infecção (AI) de 1-13, abundância média (AM) de 8,4 e local de infecção (LI) na cavidade abdominal. *P. castelnaeana* apresentou P = 100%, IM = 44,2, AI = 10-114, AM = 44,2 e LI = cavidade abdominal, ceco e estômago. O ciclo de vida desses nematodas pode ser completo na bacia Amazônica, já que os dois hospedeiros fazem parte da dieta de cetáceos da região, que atuam como hospedeiros finais. Esses achados têm consequência importante na epidemiologia de anisaquíases, devendo haver mais atenção em relação à proteção humana contra esse problema de saúde pública.

Palavras-chave: peixes de água doce, zoonoses, Anisakidae, Bacia Amazônica.

Introduction

The presence of parasites in fish is relatively frequent and has many consequences related mainly with economic and health aspects. Some parasites generate high mortality in fish and also cause tissue damage leading to economic loss (Ferre, 2001). Like other vertebrate hosts, fish have their own parasitic fauna with numerous parasitic species distributed and classified in major taxonomic groups (Luque, 2004). The fauna of freshwater parasites may have different compositions, depending on the host species, trophic level occupied by the host, age, size, sex, and other biotic and abiotic factors. Moreover, the fish can harbor adult worms and their larvae (Takemoto, Lizama, Guidelli, & Pavanelli, 2004).

Nematodes might occur in fish, both in adult and larval forms. Larvae are frequently found

encysted in the muscles, liver, visceral surfaces and cavities, intestines and, rarely, in the integument (Dick & Choudhury, 1995). Its life cycle is heteroxenic and complex; moreover, six stages of development are recognized: egg, four larval stages, and adulthood. Generally, the cycle involves intermediate hosts, sometimes paratenic hosts. In adulthood, the site of infection is, for most species, the gastrointestinal tract (Takemoto et al., 2004).

Included in the Phylum Nematoda, the family Anisakidae contains 24 genera and its representatives have great zoonotic potential (Martins, Onaka, & Fenerick, 2005; Fontenelle, Knoff, Felizardo, Lopes, & São Clemente, 2013). In the larval stage, nematodes of this family are common parasites of fishes, cephalopods and prawns while the adults are parasites of fish, reptiles, birds and mammals (Moravec, 1998; Klimpel & Palm, 2011). Accidental ingestion of these larvae by humans can cause the gastrointestinal infection, anisakiasis, and a series of allergic reactions. However, in Brazil, there is a lack of information about the anisakiasis (Iñiguez, Carvalho, Motta, Pinheiro, & Vicente, 2011). A single case of anisakiasis has been reported in Brazil, in a man in the State of Mato Grosso, with the larvae located in the mucosa of the duodenum (Cruz, Souto, Ferrari, Allegretti, & Arrais-Silva, 2010).

Plagioscions quamosissimus (Heckel, 1840) commonly called "corvina" or "pescada branca", is a sciaenid fish mainly found in large rivers and constitute an important resource for commercial and sport fishing (Casatti, 2003). This species is a carnivorous freshwater fish restricted to South America and originally from the Amazon basin with a wide distribution in Brazil (Rocha et al., 2016).

Pellona castelnaeana Valenciennes, 1847, commonly called "apapá amarelo" or "sarda", is considered the largest sardine of South America, and one of the largest species known from the order Clupeiformes (Queiroz et. al., 2013). It is a piscivorous species, inhabit lakes and rivers and can also be found in flooded forest. *Pellona castelnaeana* is a pelagic migratory species, with full spawn and external fertilization (Freitas & Siqueira-Souza, 2009).

The present study aimed to report the presence of larval stages of anisakid nematodes in *Plagioscion squamosissimus* from Xingu River, near the municipality of Altamira, and *Pellona castelnaeana* from Mosqueiro Island, in the municipality of Belém, both in the State of Pará, Brazil, and to determine their parasitism indices and infection sites.

Material and methods

Between September and October 2013, five specimens of the fish species *P. squamosissimus* (with 46.8 ± 4.9 cm mean length and 437.60 ± 57.17 g mean weight) were purchased in Xingu River near Altamira (coordinates: 3° 12′ S and 52° 12′ W), Pará, Brazil. Between May and July 2015, ten specimens of the fish species *P. castelnaeana* (with 45.36 ± 4.79 cm mean length and 779.72 ± 317.80 g mean weight) were purchased in warehouse fishing "Cajueiro", located on the Mosqueiro Island (coordinates: 1º 10' S and 48º 28' W), Pará, Brazil.

Fishes were transported in isothermal boxes with ice to the Laboratory of Histology and Embryology, Institute of Animal Health and Production, *Universidade Federal Rural da Amazonia*, Belém, State of Pará, Brazil, and identified in accordance with Reis, Kullander, & Ferraris (2003). The content of the visceral cavity was separated in Petri dishes with physiological saline for parasitological analysis as described by Fontenelle et al. (2015).

Anisakidae larvae were fixed in AFA and stored in glycerin-alcohol in proportion 1:1 and examined with the aid of a magnifying glass. The material was cleaned and clarified with Amman's lactophenol, as described by Knoff and Gomes (2012). Posteriorly, the parasites were placed between two glass slides and observed under an Olympus CX41 light microscope.

The taxonomic classification of nematode larvae was made in accordance with Moravec (1998) and Fontenelle et al. (2013). The parasitism indices of prevalence, mean intensity and mean abundance were obtained as described by Bush, Lafferty, Lotz, and Shostak (1997).

Morphometrical analysis was performed on ten larval samples obtained from *Pellona castelnaeana*. A stereomicroscope Leica DM 2500 coupled with a camera lucida was used. For topographic characterization of the cuticular surface, the material was processed as described by Giese, Furtado, Lanfredi, and Santos (2010): third-stage larvae were washed in phosphate-buffered saline (pH 7.0), post-fixed in 1% osmium tetroxide, dehydrated to the CO_2 critical point, metalized with gold-palladium, and analyzed with a scanning electron microscope VEGA 3 LMU/TESCAN in the Laboratory of Histology and Animal Embryology at the Institute of Animal Health and Production, *Universidade Federal Rural da Amazônia* (UFRA), Campus Belém, State of Pará, Brazil.

One exemplar of each fish species was deposited in the Ichthyological Collection of the Fishery and Agribusiness Resource Coordination of *Instituto Federal do Pará* (IFPA). The parasites found were deposited in the Helminthological Collection of Laboratory of Histology and Animal Embryology, UFRA, Belém, State of Pará, Brazil.

Results

All fish specimens investigated were infested with living third-stage larvae (L_3) of Anisakidae (prevalence = 100%): five specimens of *P. squamosissimus* containing 42 L_3; and ten specimens of *P. castelnaeana* containing 442 L_3. In both host species, the parasite intensity was not correlated with the length and weight of fish. Table 1 lists the prevalence (P), mean infection intensity (MI), range of infection intensity (RI), mean abundance (MA), and infection site (IS).

Table 1. Prevalence (P), mean intensity (MI), range of infection (RI), mean abundance (MA), infection/infestation site (IS) and Identification code (IC) of anisakid third-stage larvae collected in *Plagioscion squamosissimus* from Xingu River, municipality of Altamira, and *Pellona castelnaeana* from Mosqueiro Island, municipality of Belém; both in the State of Pará, Brazil.

Hosts	P (%)	MI	RI	MA	IS	IC
P. squamosissimus	100	8.4	1-13	8.4	AC	APVII-048
P. castelnaeana	100	44.2	10-114	44.2	AC, C, S	APIII-006

(AC) Abdominal Cavity, (C) Cecum, (S) Stomach.

Measurements were taken on ten anisakid L_3 collected from *P. castelnaeana*: body length 11.8-15.14 (13.04 ± 1.43); body width 0.186-0.266 (0.217 ± 0.025); nerve ring 0.067-0.210 (0.164 ± 0.043); esophagus length 0.673-1.026 (0.867 ± 0.095); esophagus width 0.060-0.106 (0.080 ± 0.015); ventriculus length 0.266-0.433 (0.354 ± 0.057); ventriculus width 0.086-0.160 (0.132 ± 0.024); cloaca 0.060-0.210 (0.102 ± 0.047).

Phylum Nematoda (Rudolphi, 1808)
Class Secernentea von Linstow, 1905
Subclass Rhabditia Inglis, 1983
Order Ascaridida Skrajabin & Schulz, 1940
Family Anisakidae Skrjabin & Karokhin, 1945
(larvae) (Figures 1, 2)

Description of the main morphological features observed in L_3: cuticle with crosscutting narrow striations most evident on the posterior body portion, conical tail and mucron present (Figures 1b, 1d, 2d); anterior end with a dorsal lip and two ventrolateral lips, all of them poorly developed; six cephalic papillae, one pair in the dorsal lip and a pair in each ventrolateral lip; boring tooth below the oral aperture, between the two ventrolateral lips; excretory pore opening beneath the boring tooth (Figures 1a, 1c, 2a, 2b, 2c).

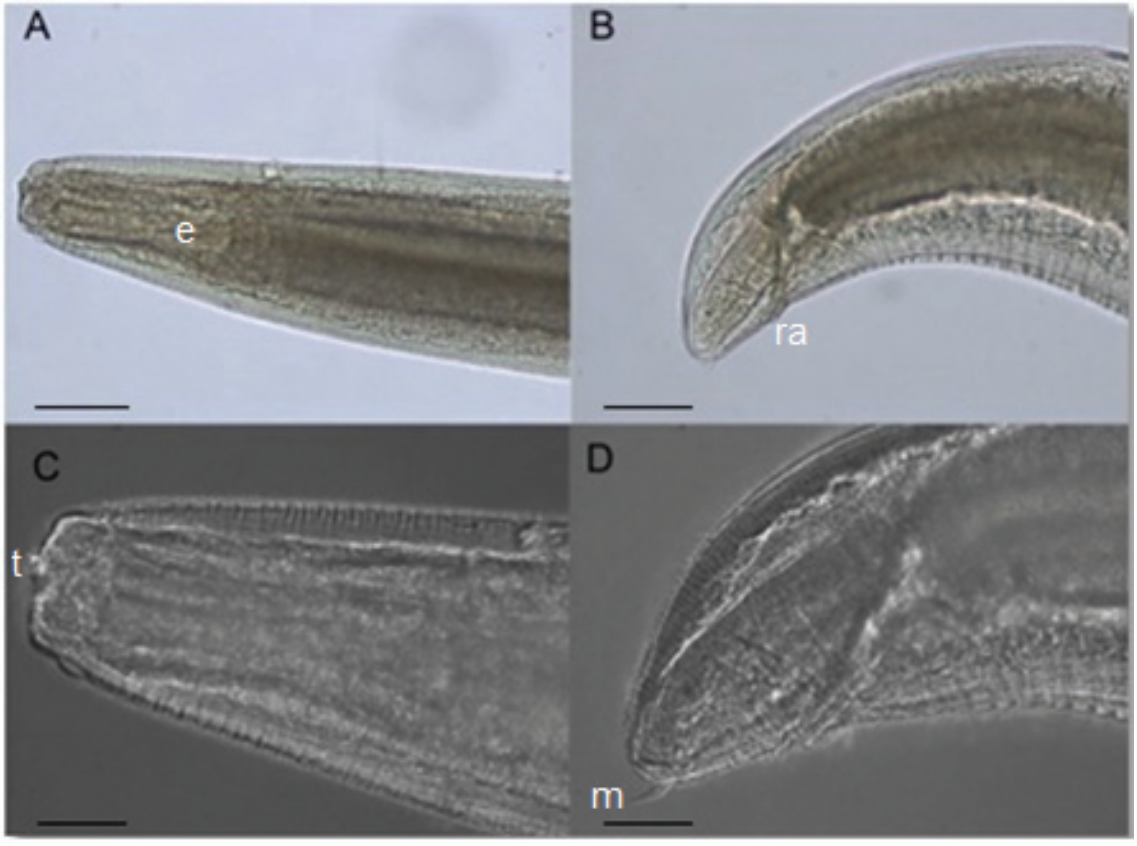

Figure 1. Anisakidae (L_3) from *Pellona castelnaeana* observed by differential interference contrast. A and C - Anterior portion showing esophagus (e) and boring tooth (t). B and D - Posterior portion showing rectal ampoule (ra) and tail with terminal mucron (m). Scale bars: A and B = 100 μm, C and D = 50 μm.

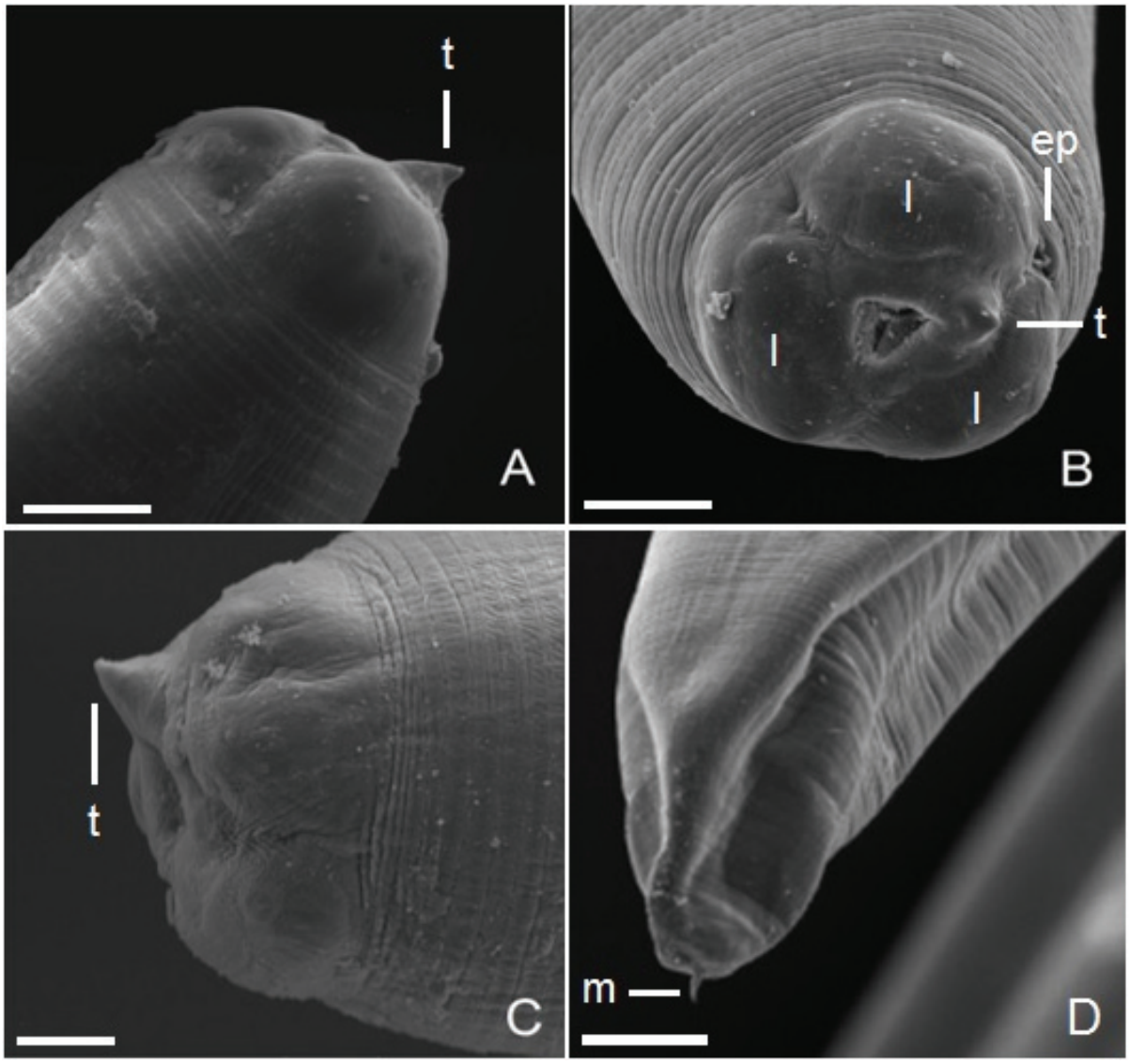

Figure 2. Anisakidae (L_3) from *Pellona castelnaeana* observed by scanning electron microscopy. A, B, and C – anterior portion showing three lips (l), boring tooth (t) and excretory pore (ep). D - posterior portion showing striated tail with terminal mucron (m). Scale bars: A and B = 20 μm, C = 10 μm, and D = 50 μm.

Discussion

The prevalence of anisakid larvae in fish can vary greatly. Among the most recent studies, low prevalence rates were found in *Selene setapinnis* = 13.3% (Fontenelle et al., 2015), *Sandelia capensis* = 23% (Moravec, van Rensburg, & Van As, 2016) and *Plagioscion squamosissimus* = 23.3% (Fontenelle et al., 2016); high prevalence rates were recorded in *Hoplias malabaricus* = 100% and *Hoplerythrinus unitaeniatus* = 80% (Martins et al., 2005), *Dicentrarchus labrax* = 95% (Bernardi et al., 2011) and *Arapaima gigas* = 98% (Andrade-Porto et al., 2015). In the present study, in *Plagioscion squamosissimus* and *Pellona castelnaeana*, the prevalence was 100%, and the highest average intensity and abundance values were observed in *P. castelnaeana* from the Mosqueiro Island (Table 1).

Plagioscion squamosissimus showed a carnivorous diet with preference for fish, but also including prawns and insects (Odonata and Ephemeroptera) in areas with low fish density (Barros, Santos, Zanuncio, & Dergam, 2012); *Pellona castelnaeana* feeds mainly on small Characiformes and Perciformes, however, prawns and other aquatic invertebrates can be part of the diet (Freitas & Siqueira-Souza, 2009). Since the two host fish occupy the same trophic level and their specimens in the samples were adults, with similar sizes, the difference in parasite intensity is due to other biotic factors and most probable to seasonal changes.

The life cycle stages of anisakid nematodes include four larval stages (L_1–L_4), within the eggs (L_1–L_3) and subsequently in the intermediate or paratenic hosts (L_3), and as preadults (L_4) and adults in the cetacean definitive hosts (Klimpel & Palm, 2011). In the present study, Anisakidae larvae were collected in two municipalities, Altamira and Belém, in the Amazon hydrographic basin. The presence of third-stage larvae (L_3) of Anisakidae parasitizing *Plagioscion squamosissimus* and *Pellona castelnaeana* suggests the participation of these fish as intermediate or paratenic hosts in said area.

The final hosts of Anisakidae in the Amazon basin are cetaceans widely distributed in the fluvial waters of the region, particularly the tucuxi dolphin (*Sotalia fluviatilis*) and the pink river dolphin (*Inia geoffrensis*).

Sotalia fluviatilis is endemic to the Amazon basin, with records for almost all major tributaries, lakes and smaller rivers. Essentially piscivorous, it feeds mainly on pelagic fish that form shoals, with a maximum length of 37 cm. *Plagioscion squamosissimus* is the species most consumed by *S. fluviatilis* in Central Amazon and also appears among the most consumed in the Amazonian Estuary (Beltrán-Pedreros & Pantoja, 2006; Rosas, Marigo, Laeta, & Rossi-Santos, 2010). In addition, Borobia and Barros (1989) mentioned fish of the genus *Pellona* among the species predated by the tucuxi. *Inia geoffrensis*, essentially fluvial, is the largest river dolphin, being endemic to the Amazon and Orinoco river basins. Adult females and pups occupy flooded areas, while adult males prefer to feed on the river channels. This piscivorous species uses dozens of fish species in their diet, although there are records of ingestion of crabs and turtles (Rocha-Campos, Câmara, & Pretto, 2010). *Plagioscion* spp. and *Pellona* spp. appear among the food items of the pink river dolphin (Best & da Silva, 1993).

Anisakid L_3 collected from *P. castelnaeana* were morphometrically similar to those collected by Fontenelle et al. (2016) in *P. squamosissimus* from the State of Pará (12.90 length x 0.33 width). Notwithstanding, they showed larger body size than the ones collected by Andrade-Porto et al. (2015) in *Arapaima gigas* from the State of Amazonas (2.53 length x 0.10 width) and smaller than the ones collected by Felizardo, Knoff, Pinto, and Gomes (2009) in *Paralichthys isosceles* from the State of Rio de Janeiro (15.60 length x 0.36 width), Fontenelle et al. (2013) in *Cynoscion guatucupa* from the State of Rio de Janeiro (22.33 length x 0.44 width) and Moravec et al. (2016) in *Sandelia capensis* (32.48 length x 1.39 width) from South Africa. These larvae may be different anisakid species. Besides that, according to Fontenelle et al. (2016), it is possible that these differences in length and width of Anisakidae larvae are also related to different hosts or the influences of the different ecoregions in which the fish examined were collected.

Vanzolini (2004) reports that some species of the family Anisakidae were described from specimens collected in *Sotalia fluviatilis* and *Inia geoffrensis* from the Brazilian Amazon, yet in the 19th century. Therefore, the life cycle of these nematodes can be completed in the Amazon basin, since their biodiversity houses cetaceans that participate as final hosts, as well as fish (*Plagioscion squamosissimus* and *Pellona castelnaeana*, in the present study) that can host larvae (L_3) until they are predated by the dolphins.

Conclusion

We have now witnessed a rapid increase in the number of publications on the presence of anisakid larvae in various intermediate hosts in Brazil and in other parts of the world, so attention should be extended to human protection against anisakiasis. Members of the family Anisakidae find in Amazonia the necessary conditions to fully complete their heteroxenous life cycle. Among the possible intermediate hosts, *Plagioscion squamosissimus* and *Pellona castelnaeana* are presented. As far as we know, this is the first record of anisakid parasitizing *Pellona castelnaeana*.

Acknowledgements

The authors are grateful to the Laboratory of Scanning Electron Microscopy and Laboratory of Histology and Animal Embryology-LHEA/UFRA for putting at our disposal the scanning electron microscope (VEGA 3).

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Received on February 24, 2017.
Accepted on May 16, 2017.