Studies on the early development of Carmyerius marchandi Seck & Ba, 2007 from Guinea-Bissau (Paramphistomoidea, Gastrothylacidae)

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Abstract
As a contribution to the indepth of the biology of Carmyerius marchandi Seck & Ba, 2007 (Paramphistomoidea; Gastrothylacidae), a rumen trematode of cattle from Guinea Bissau (West Africa), studies on eggs morphological and biometrical along with the embryo formation process were performed and the parasite/intermediate host compatibility was analysed. In laboratory conditions, eggs hatched to miracidium in 10-12 days. The embryo development was similar to other amphistomes described. Four species of local freshwater snails were infected with miracidia, but none shed cercaria in a 45 days period.

INTRODUCTION
Amphistomes are the most common cattle trematode worldwide and the immature flukes cause heavy morbidity and mortality among livestock, especially in calves. In Guinea Bissau these infections are of great concern because they affect livestock with prevalence almost reaching 100% (Crespo et al., 2011), impairing animal health and causing important productivity and economic losses. Carmyerius marchandi (Paramphistomata; Gastrothylacidae) was recently described from cattle at Senegal (Seck & Ba, 2007) and referred to infect cattle at Guinea Bissau as well (unpublished data). Their intermediate hosts are still unknown. This short note aims to contribute to the indepth of C. marchandi biology by studying the eggs morphology and biometric along with the embryo formation process and the miracidium compatibility to some sympatric freshwater snails.

MATERIAL AND METHODS
Adults were collected from one naturally infected bovine slaughtered at Bafatá (Guinea Bissau) abattoir, which showed a single infection by Carmyerius marchandi. The parasitized rumen was introduced in a cold saline and in laboratory the adults were removed, washed and replaced in Petri dishes with saline. The eggs were obtained by adult oviposition overnight at room temperature. Adults were preserved in alcohol 70º and 1000 eggs were washed twice in tap water and incubated at 25º C in Petri dishes with daily changed tap water. Biometrical studies on eggs were performed daily from the 3rd to the 16th days after oviposition, in a total of 260. After hatching, miracidia morphology was studied in specimens stained by methylene blue and neutral red. Freshwater snails Bulinus truncatus (n=10), B. forskalii (n=10), Lymnaea natalensis (n=10) and Biomphalaria pfeifferi (n=10) from Guinea Bissau were individually infected with three miracidia, during 12 hours (overnight) at 25º C. From the 8th day post infection cercaria shedding were daily searched for a 45 days period.

RESULTS
The C. marchandi eggs showed a variable shape: oval, elliptical, attenuated towards the opercular pole end, or rarely pyriform; the average length was 120.6 µm (min 104.9/ max 164.6 µm) and the width 70.3 µm (min 61.1/ max 79.8 µm); white-grey in colour; some evidenced a small projection suggesting a terminal bent spine at the posterior pole (fig. 1). At 3rd day the early segmentation of the embryo was seen with two or four large central cells (average 26.1/26.1 µm) (fig. 2); at 5th day, the embryo centrally positioned and consisted in a rounded cellular mass surrounded by vitelline cells (average 37.0/33.0 µm) (fig. 3); at 6th and 7th day the embryo appears as an irregular central mass of cells (average 43.6/37.0 µm) (fig. 4); at 8th day, an irregular elongated outline of the miracidium can already be seen with trhebatorium already outlined (average 51.1/42.2 µm) (fig. 5). The primordial gut is seen, flame cells are active and some movements are noticeable; at 9th day miracidium is elongated (average 84.7/42.2 µm); at 10th a complete and active miracidium (133.1/39.8 µm) was observed, although the most of them still have slow movements (fig. 6). A reduction in the vitelline cells was visible at this stage and a few miracidia successfully hatched, after light exposure; at 12th day an active miracidium was observed, occupying the great majority

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of the egg cavity, displaced to one side by the remaining yolk cells, which coalesced to form three large cells (fig. 7). It showed rapid movements, contracting forward and pushing against the operculum. Periods of activity are followed by rest periods. All these contraction/elongation movements contribute for the release of the enzymes from the penetration glands which will help in the hatching process. About 80% of miracidia hatched by this time (fig 8); at the 16th day the last eggs with active and viable miracidia were seen to hatch. The miracidium evidenced a phototropism positive, since they hatched in the first hours after light exposure. When swimming straight ahead the body is covered with a ciliated ectoderm; it has a pyriform shape (145,5/47, 6µm), broad anteriorly and narrow posteriorly (fig. 9); the anterior cavity of the body has a visible central primordial gut (g), extending posteriorly in the first quarter; two pairs of bulky penetrating glands (pg) positioned laterally to the primordial gut; numerous germ cells attached to the wall cavity posteriorly and germinal tissue (gt) occupying the cavity centre; vibrating flame cells are seen laterally in the first third and in the second one; epidermal plates are quite distinct. At 30th days after infection, the freshwater snail mortality rate was low (10% at B. forskalii and L. natantisa), but none of the infected snails shed cercaria in a period of 45 days.

DISCUSSION AND CONCLUSIONS

These results support that the chronology of miracidium development in the egg of this trematode is identical to that described for other species (Bennett, 1936; Sey, 1972; Eduardo & Kaw, 1986; Kumar & Lal, 2015). The C. marchandi eggs evidenced a greater variability in length/width measures than those described for C. spatiosus (115/125 µm length; 60.0/68.0 µm width) (Kumar, 1999). The variability of amphistomes eggs measures is so large that it is quite difficult to identify species, especially in areas where sympatric species occurred, as is the case of Guinea Bissau (Crespo et al., 2011). The hatching period depends on the species and laboratory conditions, namely temperature: C. cotylophorum took from 15 to 29 days to hatch (Bennett, 1936); Paramphistomum daubneyi began to hatch at 9th day (Sey, 1972); O. scolicoloeiium hatching process varies between 14 to 19 days (Eduardo & Kaw, 1986); Ceylonotyle chauhani start hatching at the 10th day (Kumar & Lal, 2015). According to these variability, C. marchandi has a hatching period from the 10th to the 16th day. In nature, it will depend on local environmental conditions, where temperature and water characteristics seem to assume an important role. The failure to infect the freshwater snails could be related to the fact that none of those were the natural intermediate host (from the known freshwater snails at Guinea Bissau, B. senegalensis and B. umbilicatus were not tested) or as described for other trematoda species, freshwater snails should be previously infected by another trematode to be receptive to a second infection (Southgate et al., 1985).

REFERENCES

Southgate, VR; Brown, DS; Rollinson, D; Ross, DC; Knowles, R.J (1985) - Bulinus tropicus from central Kenya acting as a host for Schistosoma bovis. Z. Parasitenkd., 71, 61-69.