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ORIGINAL PAPER

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Fasciola hepatica and *Fasciola gigantica*: comparative morphometric studies on the redial stage of both species

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Abstract Experimental infections of *Galba truncatula* with *Fasciola gigantica* or *F. hepatica* were carried out under laboratory conditions (20°C) to determine the characteristics of rediae of both species via their morphometry and to find reliable measurements that might be efficiently used to discriminate between the rediae of both species of *Fasciola*. These results were compared to those of another snail: *Radix natalensis*, infected with either *F. gigantica* or *F. hepatica* under the same protocol. At day 28 post-exposure, abortive infections with *F. hepatica* were found in a group of *R. natalensis*. By contrast, live rediae were observed in the other three groups. The group of infected snails and the redial category significantly influenced the mean values of the seven measurements studied and those of three indices. Using the PSLD Fisher test, it was found that the index, distance from the anterior end of the body to the collar/length of the body, was an efficient means of distinguishing the rediae of *F. hepatica* from those of *F. gigantica* [second-appearing mother rediae (R1b) of the first generation, 0.14 instead of 0.22; daughter rediae (R2a) produced by the first mother rediae, 0.19 instead of 0.24]. Another index, distance from the anterior end of the body to the collar/diameter of the collar, could also be used to discriminate between rediae (R1b, 0.80 for *F. hepatica* instead of 1.09 for *F. gigantica*; R2a, 0.90 instead of 1.26, respectively). Compared to measurements recorded for the rediae of *F. hepatica*, rediae of *F. gigantica* can be characterized by the following measurements: the diameter of the pharyngeal lumen and the distance from the anterior end of the body to the collar for larvae developed in *R. natalensis*, and the length of the body and the distance from the posterior end of the body to lateral projections for those found in *G. truncatula*. The species of snail host and, consequently, its growth, as well as the species of *Fasciola*, had a significant influence on the morphometric characters of the redial stage.

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Introduction

In Egypt, the two species of *Fasciola*, *F. gigantica* and *F. hepatica*, are found in locally bred ruminants. This has been proven by morphoanatomical studies on adult flukes and confirmed by isoelectric focusing of soluble proteins of fluke (Lotfy et al. 2002). As both trematode species may infect the same local intermediate hosts, *Radix natalensis* and *Galba truncatula* (Farag 1998; El-Shazly et al. 2002), this situation leads to the question of whether the larval forms of both species may be identified in naturally infected snails and, particularly, in those which are dually infected by these trematodes. Most tools of molecular biology, such as isoenzyme patterns or sequence analysis (e.g. Hashimoto et al. 1997; Panaccio and Trudgett 1999) may be used to identify the species of *Fasciola* within snails. However, these methods are too sophisticated to be routinely applied during the dissection of each snail and it is useful to have a simpler technique, such as morphometry, to identify the species of *Fasciola* when larval forms are used. Despite several differences between the mean sizes of full-grown sporocysts or between those of cercariae given by Dinnik and Dinnik (1956) for *F. gigantica*, and Thomas (1883a, 1883b) for *F. hepatica*, the values reported by these authors cannot be easily used to identify the larvae of each digenean species in snails, as the shapes of each larval stage are similar in both species of *Fasciola*. The only possible means of identifying their larvae is to study the rediae but the morphology of these larvae greatly varies during snail infections (their size increases up to the differentiation of cercariae and decreases afterwards) and there are several redial generations. Thus, comparative morphometric studies on these larvae are necessary to determine their specific characteristics and find criteria which allow one to differentiate between these digenean species.

Many measures concerning the length of redial bodies, their width, and the dimensions of their pharynxes had already been reported by Dinnik and Dinnik (1956) for the first- and second-generation rediae of *F. gigantica*. In the case of *F. hepatica*, morphometric studies on the body and cephalic region of rediae were carried out by Augot et al. (1998, 1999) to identify their different generations. However, the values given for the rediae of both *Fasciola* in the previous reports are difficult to compare, as each study was performed under different experimental conditions (temperature, miracidial dose, or shell height of snails at exposure, for example). According to Ollerenshaw and Graham (1986), the length of the intestine in cercariae-containing rediae allows the identification of *Fasciola* species (it was significantly greater in the case of *F. gigantica*) but this parameter cannot be used for immature rediae. In view of the previous findings, the following two questions arise: (1) what are the reliable measurements that should be used to efficiently discriminate between the rediae, especially the immature ones, of both species of *Fasciola*; and (2) does the snail species have an effect on the morphometry of these rediae, as the maximum shell height of *G. truncatula* greatly differs from that of *R. natalensis*. To answer these questions, experimental infections of *R. natalensis* and *G. truncatula* with *F. gigantica* and *F. hepatica* were carried out to measure different dimensions on live rediae at day 28 post-exposure (p.e.). This time was chosen in the present study, as all rediae of the first generation and numerous daughter rediae produced by the first rediae of the first generation were mature at that time and still contained their cercariae.

Materials and methods

The population of *G. truncatula* was living on the banks of a river, at Saint Priest Taurion, department of Haute Vienne (central France). Snails from this population are known to be devoid of any natural digenean infections proved by repeated collections of adult snails over the previous 6 months and the absence of trematode larval forms in dissected snails. A total of 50 snails, measuring 4 mm in height, were collected from this site in November, transported to the laboratory, and placed in breeding aquaria at 20°C. The population of *R. natalensis* was living in the El-Mansoria river, Giza governorate, Egypt. Adult snails were reared under laboratory conditions to provide the 4-mm-high snails used in this experiment. Eggs of *F. gigantica* originated from the abattoir of Tanta, Gharbia governorate, Egypt; those of *F. hepatica* came from the abattoir of Limoges, Haute Vienne. Eggs were collected from the gall-bladders of naturally infected cattle and were incubated at 20°C for 20 days in the dark according to the method described by Ollerenshaw (1971) for the eggs of *F. hepatica*.

Four groups of 25 snails each were used in this experiment. The first two groups of *G. truncatula* were individually subjected to bimiracidial infections with *F. hepatica* for the first group, and with *F. gigantica* for the other. The same protocol was used for the other two groups of *R. natalensis*. These snails were then raised in open

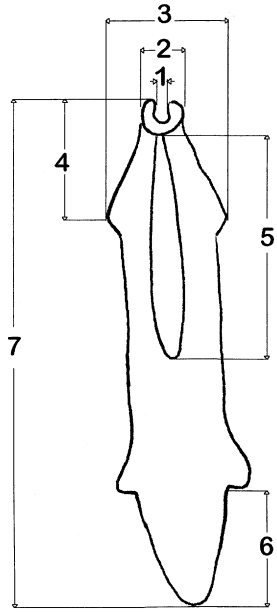

Fig. 1 Morphometric measurements on live rediae of *Fasciola* sp.: maximal width of pharyngeal lumen (*1*), width of the pharynx (*2*), diameter of the collar (*3*), distance from the anterior end of the body to the collar (*4*), length of the intestine (*5*), distance from the posterior end of the body to lateral projections (*6*), and length of the body (*7*)

aerated boxes (25 snails/box) at 20°C for 28 days and were fed with lettuce ad libitum. At day 28 p.e., the surviving snails from each group were dissected in tap water under a stereomicroscope to detect live rediae. These larvae were then transferred using a pipette to a single-cavity slide containing spring water and their measurements were taken using an image-processor (Aries; Prolabo, France). Because of the frequent movements of live rediae, the dimensions were only determined when the larvae were completely relaxed. Three categories of rediae for each species of *Fasciola* were considered in the present study according to the criteria used by Dinnik and Dinnik (1956), or by Rakotondravao et al. (1992) to identify the rediae of *F. gigantica*, and by Augot et al. (1998, 1999) for those of *F. hepatica*. These categories are: (1) mother rediae appearing from the sporocyst first (R1a), (2) mother rediae appearing from the sporocyst second (R1b), and (3) daughter rediae from R1a (R2a).

Seven measurements (Fig. 1) were determined for each live redia: (1) the length of the body, (2) the width of the pharynx, (3) the maximum diameter of the pharyngeal lumen, (4) the distance from the anterior end of the body to the collar, (5) the diameter of the collar, (6) the length of intestine, and (7) the distance from the posterior end of the body to lateral projections. The following indices: distance from the anterior end of the body to the collar/length of the body (AC/LB), distance from the anterior end of the body to the collar/diameter of the collar (AC/DC), length of the intestine/length of the body (LI/LB), and distance from the posterior end of the body to lateral projections/length of the body (PP/LB), were also considered. Mean values and SDs were calculated for each measurement or each index, taking into account the redial category. A two-way ANOVA was used to determine levels of significance.

Results

The survival rates of *G. truncatula* at day 28 p.e. were 32% in the *F. hepatica* group and 84% in snails infected

Table 1 Mean values and SDs for seven measurements and four indices of the live rediae of *Fasciola hepatica* in six *Galba truncatula*. *R1a* First mother rediae appearing from the sporocyst,*R1b* second mother rediae appearing from the sporocyst, *R2a* daughter rediae from R1a

Measurements	Mean ± SD (μm) for each redial category		
	R1a (6) [a]	R1b (34)	R2a (43)
Length of the body (LB)	1,400.3 ± 324.2	1,255.6 ± 272.3	688.6 ± 239.4
Width of the pharynx (WP)	99.1 ± 9.1	79.4 ± 12.0	64.2 ± 7.6
Pharyngeal lumen width (PL)	43.8 ± 3.6	35.6 ± 7.8	26.3 ± 4.5
Distance from the anterior end to the collar (AC)	203.7 ± 80.0	176.9 ± 48.4	127.9 ± 36.0
Diameter of the collar (DC)	270.6 ± 43.8	221.8 ± 38.4	142.7 ± 27.8
Length of the intestine (LI)	486.6 ± 103.1	415.1 ± 91.4	270.3 ± 70.0
Distance from the posterior end to lateral projections (PP)	546.8 ± 218.4	337.0 ± 111.0	139.4 ± 83.0
AC/LB ratio	0.14 ± 0.07	0.14 ± 0.04	0.19 ± 0.04
LI/LB ratio	0.32 ± 0.07	0.34 ± 0.06	0.40 ± 0.08
PP/LB ratio	0.34 ± 0.08	0.26 ± 0.07	0.27 ± 0.06
AC/DC ratio	0.75 ± 0.26	0.80 ± 0.20	0.90 ± 0.21

[a]No. of rediae in each category

with *F. gigantica*. In *R. natalensis*, the rates were 96% for the former parasite and 56% for the latter. The prevalences of experimental infections were 75.0%, 24.0%, 0%, and 64.0%, respectively (data not shown).

The values recorded for the measurements and indices are listed in Table 1 for the *G. truncatula* infected with *F. hepatica*, in Table 2 for those harbouring larval forms of *F. gigantica*, and in Table 3 for *R. natalensis* infected with *F. gigantica*. The results recorded together with ANOVA are given in Table 4. The group of infected snails and the redial category significantly influenced the mean values of the seven measurements studied and those of three indices: AC/LB, LI/LB, and AC/DC (Table 4). Using the PSLD Fisher test, it was found that the AC/LB and AC/DC ratios were the most appropriate measurements to differentiate the rediae of *F. hepatica* from those of *F. gigantica*. Compared to *F. hepatica* rediae, those of *F. gigantica* could be characterized by the width of the pharyngeal lumina and the distance from the anterior end of the body to the collar (for larvae developed in *R. natalensis*), whereas for those found in *G. truncatula* they were distinguished by the length of the body and the distance from the posterior end of the body to lateral projections. However, it was useful to identify the redial category which could be used to determine the values of these measurements or ratios. Under these conditions, the AC/LB index used in the case of *F. hepatica* must be calculated on the R1b and R2a rediae because their values were lower than those of *F. gigantica* (0.14 instead of 0.22 in the case of R1b rediae, and 0.19 instead of 0.24 for R2a rediae, respectively). A similar finding could be noted for the AC/DC index, which showed lower values in the case of *F. hepatica* (R1b, 0.80 instead of 1.09 for *F. gigantica*; R2a, 0.9 instead of 1.26). In the case of *F. gigantica* in *R. natalensis*, the width of the pharyngeal lumen and the distance from the anterior end of the body to the collar must be measured on R1a rediae, as their mean values were higher than those noted in *G. truncatula* (73.7 and 318.8 μm, respectively, instead of 47.2 and 209.1 μm). By contrast, in *G. truncatula*, the length of the body and the distance from the posterior end of the body to lateral projections can be determined, for example, on R1b rediae because their values were lower than those noted for the corresponding rediae in *R. natalensis* (a mean of

Table 2 Mean values and SDs for seven measurements and four indices on the live rediae of *Fasciola gigantica* in five *G. truncatula*. For abbreviations, see Table 1

Measurements	Mean ± SD (μm) for each redial category		
	R1a (5) [a]	R1b (7)	R2a (8)
LB	1,097.3 ± 332.9	662.5 ± 103.8	591.7 ± 86.3
WP	103.3 ± 7.6	70.0 ± 8.0	58.4 ± 4.8
PL	47.2 ± 5.5	34.9 ± 4.9	23.7 ± 4.2
AC	209.1 ± 51.3	148.6 ± 33.9	146.7 ± 26.7
DC	2,14.0 ± 27.8	138.8 ± 16.4	116.8 ± 9.6
LI	569.5 ± 128.1	360.0 ± 60.5	353.8 ± 49.6
PP	310.5 ± 112.8	178.2 ± 54.9	146.0 ± 17.3
AC/LB ratio	0.19 ± 0.03	0.22 ± 0.05	0.24 ± 0.04
LI/LB ratio	0.53 ± 0.08	0.54 ± 0.07	0.60 ± 0.07
PP/LB ratio	0.27 ± 0.03	0.26 ± 0.05	0.24 ± 0.02
AC/DC ratio	0.97 ± 0.21	1.09 ± 0.31	1.26 ± 0.24

[a]No. of rediae in each category

Table 3 Mean values and SDs for seven measurements and four indices on the live rediae of *F. gigantica* in nine *Radix natalensis*. For abbreviations, see Table 1

Measurements	Mean ± SD (μm) for each redial category		
	R1a (11)[a]	R1b (68)	R2a (95)
LB	1,417.0 ± 186.6	1,120.3 ± 237.9	788.6 ± 158.1
WP	156.5 ± 23.2	82.1 ± 14.0	66.6 ± 6.9
PL	73.7 ± 14.0	42.8 ± 5.7	30.6 ± 5.2
AC	318.8 ± 78.5	192.4 ± 50.6	168.8 ± 43.0
DC	301.2 ± 51.6	185.8 ± 44.6	141.5 ± 26.1
LI	752.9 ± 131.4	581.8 ± 134.2	408.5 ± 92.5
PP	414.8 ± 113.6	319.1 ± 106.2	220.5 ± 78.3
AC/LB ratio	0.22 ± 0.05	0.17 ± 0.03	0.22 ± 0.07
LI/LB ratio	0.53 ± 0.07	0.52 ± 0.07	0.52 ± 0.08
PP/LB ratio	0.29 ± 0.07	0.28 ± 0.06	0.27 ± 0.07
AC/DC ratio	1.06 ± 0.20	1.04 ± 0.21	1.20 ± 0.30

[a]No. of rediae in each category

Table 4 Results of two-way ANOVA on the seven measurements and four indices of *Fasciola* sp. rediae, showing the *F* -values and levels of significance. *NS* Not significant; for other abbreviations, see Table 1

Measurements	*F* -values and levels of significance		
	Group	Redial categories	Interaction between the two factors
LB	20.39***	68.96***	5.89***
WP	65.34***	182.16***	24.20***
PL	78.74***	146.38***	15.51***
AC	23.25***	27.87***	4.63**
DC	19.28***	96.02***	6.22***
LI	47.52***	45.90***	3.29*
PP	14.52***	42.95***	3.08*
AC/LB ratio	11.08***	6.88**	1.63 (NS)
LI/LB ratio	67.39***	4.59*	3.33*
PP/LB ratio	1.28 (NS)	1.69 (NS)	0.62 (NS)
AC/DC ratio	16.75***	6.28**	0.39 (NS)

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

662.5 and 178.2 µm, respectively, instead of 1,120.3 and 319.1 µm).

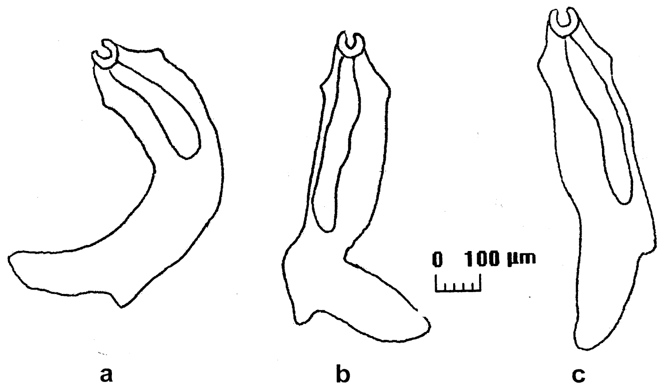

Fig. 2 Schematic diagrams showing the general morphology of R1b rediae: *Fasciola hepatica* in *Galba truncatula* (**a**), *Fasciola gigantica* in *G. truncatula* (**b**), and *F. gigantica* in *Radix natalensis* (**c**)

Discussion

To explain the previous results, two points should be taken into account. The first concerns the morphometric differences between the rediae of both *Fasciola* species when each develops in the same snail host: *G. truncatula*. The second point is the role of the two snail species used in the present study on the normal larval development of *F. gigantica*.

Regarding the first point, the AC/LB and AC/DC indices could be used to identify *F. hepatica* from *F. gigantica* in *G. truncatula*, as the redial categories of the former parasite had lower mean values than those noted for *F. gigantica* rediae. These differences in the morphological characters of both *Fasciola* rediae may be due to the differences in their development and larval productivity when each develops in the same snail host, as demonstrated by Dreyfuss and Rondelaud (1995, 1997). In our study, the length of the intestine might not be selected as an efficient parameter to discriminate the rediae of *F. hepatica* from those of *F. gigantica*. Nevertheless, in most rediae of *F. hepatica*, the length of the intestine comprised one third of that of the redial body, while it reached nearly halfway along the body of *F. gigantica* rediae (Fig. 2). This last finding was in concord with Ollerenshaw and Graham (1986) who stated that the intestine was longer in the case of cercaria-containing rediae of *F. gigantica*. As this was also found in immature rediae, it could be used together with the AC/LB and AC/DC indices to differentiate the rediae of *F. hepatica* from those of *F. gigantica* and, consequently, might be applied to identify these digenean species in Egypt where *G. truncatula* and *R. natalensis* are found naturally infected with either or both *Fasciola* (Farag 1998; El-Shazly et al. 2002).

Secondly, the values recorded for the four measurements (diameter of the pharyngeal lumen, distance from the anterior end of the body to the collar, distance from the posterior end of the body to lateral projections, and length of the body) for the rediae of *F. gigantica* in *G. truncatula* significantly differed from those noted in the *F. gigantica* - *R. natalensis* group. This last finding indicates an effect of snail species on the morphometric measurements of *F. gigantica* rediae. Two perhaps complementary hypotheses may be proposed to explain these differences. The first is that snail size would have a direct effect on the growth of rediae, as the shell height of *G. truncatula* adults was less than that of *R. natalensis*, which in turn grew rapidly in volume (Hubendick 1951) and, consequently, provided a suitable internal environment for the normal development of rediae. An argument in support of this first assumption was the effect of snail size on the redial production of *F. hepatica*, as noted by Rondelaud and Barthe (1987) in *G. truncatula* of different shell heights. The second hypothesis is that the effect of snail species on the growth of rediae would result in an individual susceptibility of each lymnaeid species to *Fasciola* infections. Indeed, the ability of the intermediate host to sustain the larval development of *F. hepatica* or that of *F. gigantica* greatly varied with the species of snails used for experiments (Boray 1978). According to Vignoles et al. (2002), when young lymnaeid snails were infected with *F. hepatica*, the species of snail had an effect not only on the body length of redial generations but also on their larval productivity. As the species of the definitive host had an influence on the morphology of adult flukes [Kendall (1965) for *F. hepatica*, or Al-Kubaisee and Altaif (1989) for *F. gigantica*, for example], the species of the intermediate host might also have an effect on the morphometry of the redial stage.

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