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Some Aspects of the Anatomy and Histology of Digestive Tracts in Two Sympatric Species of Freshwater Fishes

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بعض الجوانب التشريحية والنسيجية للجهاز الهضمي لنوعين من أسماك المياه العذبة

طاهر بن عبدالرحمن باعمر، ريجنالد فيكتور و دانيال توباز

خلاصة : دراسة مقارنة للجهاز الهضمي لنوعين من أسماك المياه العذبة *Garra barreimiae* and *Aphanius dispar* من منظور تشريحي ونسيجي. تم قياس كل من طول الأسماك والقناة الهضمية وعدد عكنات المعدة وارتفاعها. كما تم تقييم العلاقة بين طول هاتين السمكتين والأجزاء المشار إليها أعلاه. كذلك تم توصيف ومقارنة أنسجة الجهاز الهضمي لكلا السمكتين. سوف تستخدم نتائج هذه الدراسة لمناقشة ما إذا كانت هذه الأسماك تملك معدة حقيقية.

ABSTRACT: Comparative anatomy and histology of the digestive tracts of two sympatric species of freshwater fish, *Aphanius dispar* (Cyprinodontidae) and *Garra barreimiae* (Cyprinidae) are studied. Morphometric measurements of alimentary canal such as length and the number and height of rugae in sections have been made for both species. Relationships between these morphometric characters and the total length of fish have been evaluated. The ratio between the length of alimentary canal and total length of fish in both species reflects their feeding habits. Histology of the 'stomach' and 'intestine' of these two species as shown by light microscopy has been described and compared. Results of this study are used to discuss the query whether these species have true stomachs.

KEYWORDS: *Aphanius*; *Garra*; Fish; Anatomy; Histology; Stomach; Intestine

1. Introduction

Several studies have been carried out on the digestive tracts of teleost fishes (Grau *et al*. 1992; Osman and Caceci 1991; Gargiulo *et al*. 1997;1998). Recently, we have studied the structure and ultrastructure of the stomach of *Aphanius dispar* (Rüppell 1828), a cyprinodont fish with special reference to stress from starvation (Ba-Omar *et al.* 1998; Ba-Omar and Victor, 2000). What is referred to as 'stomach' in these studies is the morphologically distinct, enlarged sac-like portion of the gut separated from intestine by a constriction (Ba-Omar and Victor, 2000). However, it is well known that many fish including cyprinids and cyprinodonts lack a 'true' stomach, that is a portion of the digestive tube with a typically acid secretion and a distinctive epithelial lining different from that of the intestine (Lagler *et al*. 1977). From our work so far on *A. dispar*, the 'stomach' we describe could well be a 'stomach-like' pouch, a hollow organ covered by an intestinal mucosa. Despite being different in gross anatomy, this portion of the alimentary canal could well be the same as that of 'true' intestine both histologically and functionally. Therefore, it is necessary to describe and compare sections of alimentary canal above and below the constriction that seemingly separates 'stomach' from that of 'intestine' in *A. dispar*.

In this study, we also compare the anatomy and histology of the alimentary canal of another cyprinid, *Garra barreimiae* that is sympatric with *A. dispar* in the freshwaters of Oman. These two species occupy different feeding niches. The cyprinid, is a benthic herbivore, while *A. dispar* is an ubiquitous omnivore. Both species have 'stomachs' and 'intestines' *sensu* Ba-Omar and Victor (2000). The descriptions of alimentary canals provided here are of value in their own right because very little is known about the biology of these species. However, we also use the results to address the existence of 'stomachs' in these species.

2. Materials and methods

Specimens of *Aphanius dispar* and *Garra barreimiae* were collected from Wadi Al-Khod near Sultan Qaboos University, Sultanate of Oman (Lat. 23° 30' N; Long. 58° 40' E). Both species are sympatric in the temporary and permanent pools of the same *wadi* (i.e. seasonal river) system. Most often they are mutually exclusive in the same pools. *G. barreimiae*, however, regularly shares its habitat with another cyprinid *Cyprinion microphthalmum*. All fishes for this study were collected in freshwater, although *A. dispar* also lives in brackish waters of the coastal lagoons and estuaries.

The fishes were kept in an aerated holding tank in the laboratory and were fed with the commercial Tetramin flakes *ad libitum* daily. For each species 11 specimens irrespective of sex were killed and the entire alimentary canal was removed from each fish. The total length of the fish and the entire gut length were measured in mm. The size ranges of *A. dispar* and *G. barreimiae* were 30 – 48 and 29 – 69 mm, respectively. Ten percent buffered formalin (pH 7.3) at room temperature was used to fix the gut. The entire gut was processed, embedded in paraffin with an anterior-posterior orientation and serially sectioned at 5 µm. Serially numbered paraffin sections were stained using Mayer's hematoxylin – eosin for examination under routine light microhoscopy.

The histological topography was studied separately for the 'stomach' and 'intestine' regions under low and high magnifications (100 – 400 x). All measurements of rugae (i.e. internal folds) were made on 25 randomly chosen representative sections. In each section five randomly chosen rugae were measured making up a total of 125 measurements for each region. Measurements in µm were made using an ocular meter calibrated with a stage micrometer. Data transformations were made wherever necessary. All statistical analyses were performed using SYSTAT (Version 7.0) and all graphs were generated using SYGRAPH (Version 7.0).

3. Results

3.1 *Aphanius dispar*

In the alimentary canal of *Aphanius dispar* two distinct parts, an anterior dilated segment, presumably the 'stomach', and a posterior tubular segment, presumably the 'intestine', are recognized (Figure 1A). The anterior segment is shorter than the posterior segment, which is approximately five times longer.

The mean alimentary canal length of *A. dispar* is approximately twice the total length (TL) of the fish (Table 1). Figure 2 shows the positive relationship between the total length (TL) and the length of alimentary canal in *A. dispar* irrespective of sex. The regression equation describing this relationship, length of alimentary canal = - 58.483 (TL) is significant ($n = 11$; ANOVA , $F = 10.609$; $r = 0.736$; $P < 0.01$).

Figure 3 shows the histological organization of the 'stomach'. It is composed of four different layers, mucosa, submucosa, muscularis and serosa. The four layers differ in their thickness and the mucosa is thrown into rugae. These rugae vary in length and width. They are made of columnar epithelial cells with the nuclei located at the basal side.

Figure 4 presents the histology of the 'intestine'. It is not different from that of the 'stomach'. Part of the mucosa is thrown into rugae while the rest is flat. The heights of the rugae are shorter than those found in the 'stomach'. Table 1 shows the number and the mean heights of rugae in the 'stomach' and in the 'intestine'. The number of rugae in the 'stomach' was significantly higher than those in the intestine (t-test, $t = 4.912$; $p < 0.001$). There is no correlation between the number of rugae in the 'stomach' and in the 'intestine'($n = 11$; $r = - 0.148$; $p > 0.05$). Similarly, the height of rugae in the 'stomach' (Figure 5) is significantly greater than that in the intestine (paired t-test, $t = 19.162$; $p < 0.001$). There is no correlation between rugae heights of the 'stomach' and those of the 'intestine'($n = 125$; $r = -0.157$; $p > 0.05$).

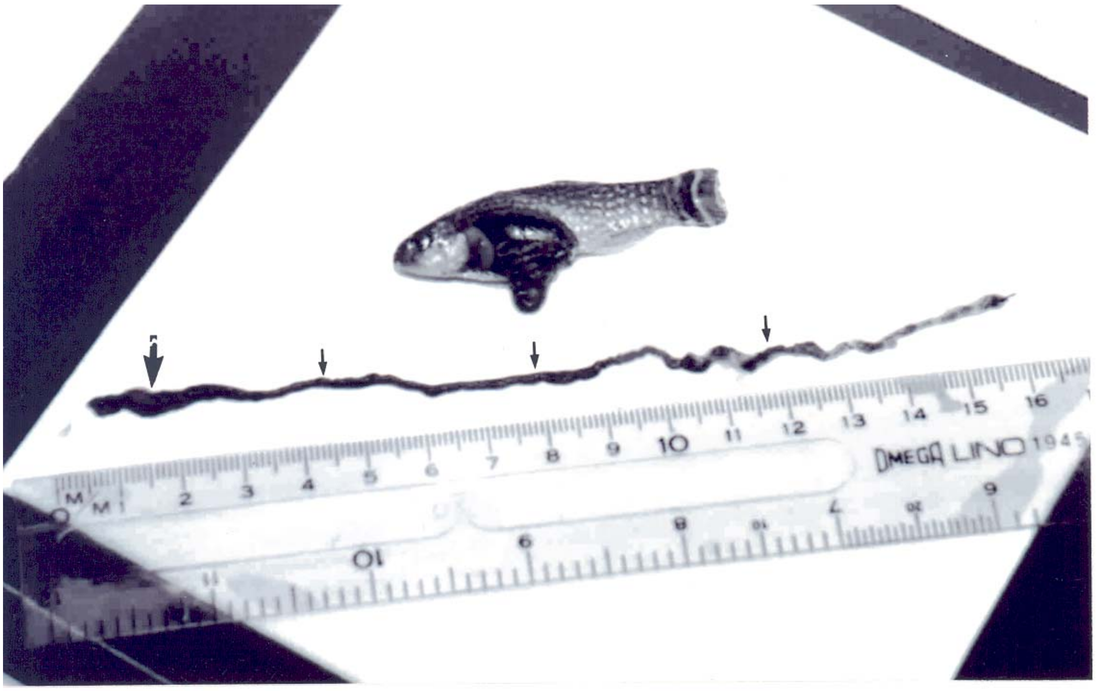

Figure 1A. *Aphanius dispar* and its alimentary canal; stomach (large arrow) and intestine (small arrow).

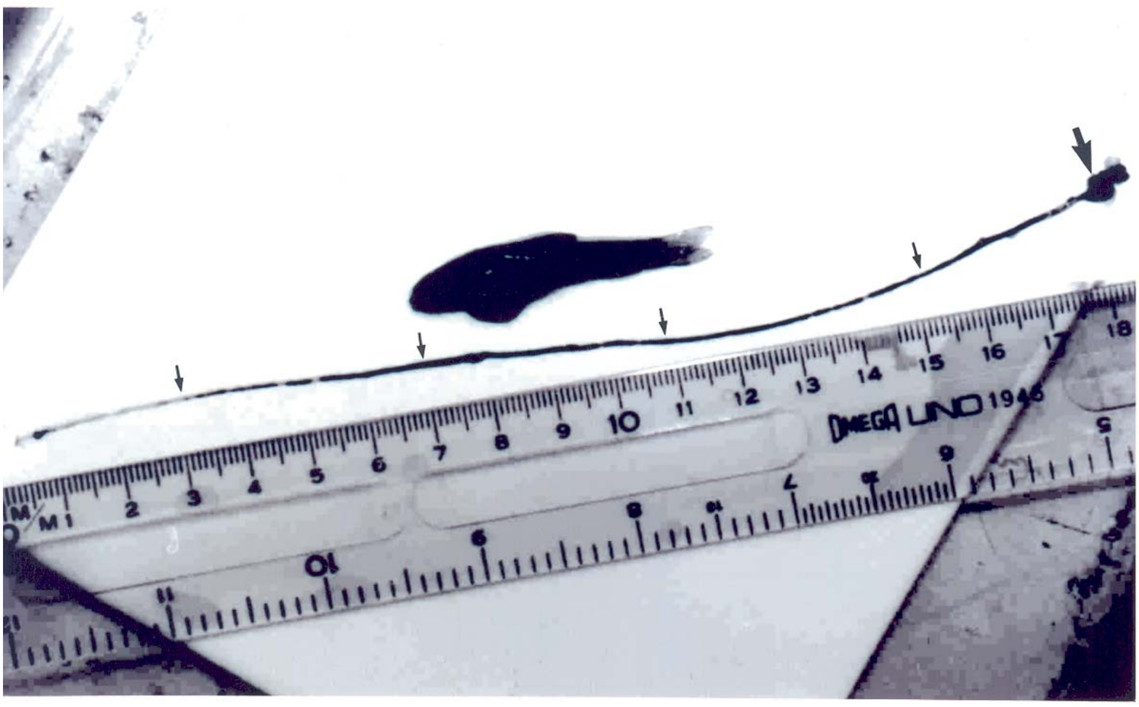

Figure 1B. *Garra barriemiae* and its alimentary canal; stomach (large arrow) and intestine (small arrow).

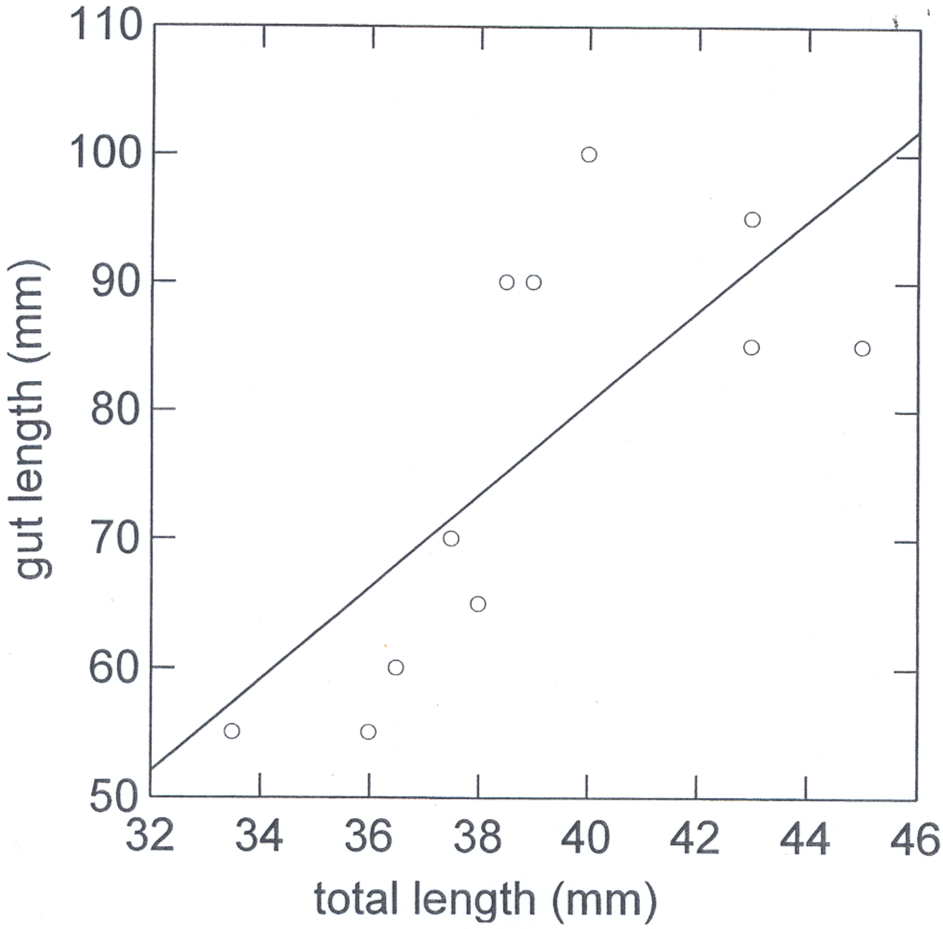

Figure 2. Relationship between total length and alimentary canal length in *Aphanius dispar.*

Table 1: Morphometric measurements comparing alimentary canals of *Aphanius dispar* and *Garra barreimiae*; n = number of measurements; values given or mean ± standard deviation; * indicates significant differences within species at P<0.001.

Character	n	*A. dispar*	*G. barriemiae*
Total length (mm)	11	39.1 ± 3.4	49.6 ± 10.7
Canal length (mm)	11	77.3 ± 16.6	371.1 ± 134.3
Stomach – No. of rugae	62	32 ± 6*	15 ± 4*
Intestine – No. of rugae	62	25 ± 6*	12 ± 2*
Stomach – rugae height (μm)	125	21.1 ± 9.3*	10.3 ± 3.0*
Intestine – rugae height (μm)	125	4.3 ± 2.0*	1.4 ± 0.7*

3.2 *Garra barreimiae*

The external morphology of the alimentary canal is characterized by a ‘stomach’ and an ‘intestine’. The ‘stomach’ is short and poorly dilated. (Figure 1B). The alimentary canal of *Garra barreimiae* is a long tube with a narrow lumen. Its length is nearly 7.5 times the total length (TL) of the fish (Table 1). The positive relationship between the total length (TL) and the length of the alimentary canal (Figure 6) described by the equation, length of alimentary canal = -210.46 (TL) is significant (n = 11; ANOVA, F =83.492; r = 0.950; p < 0.001).

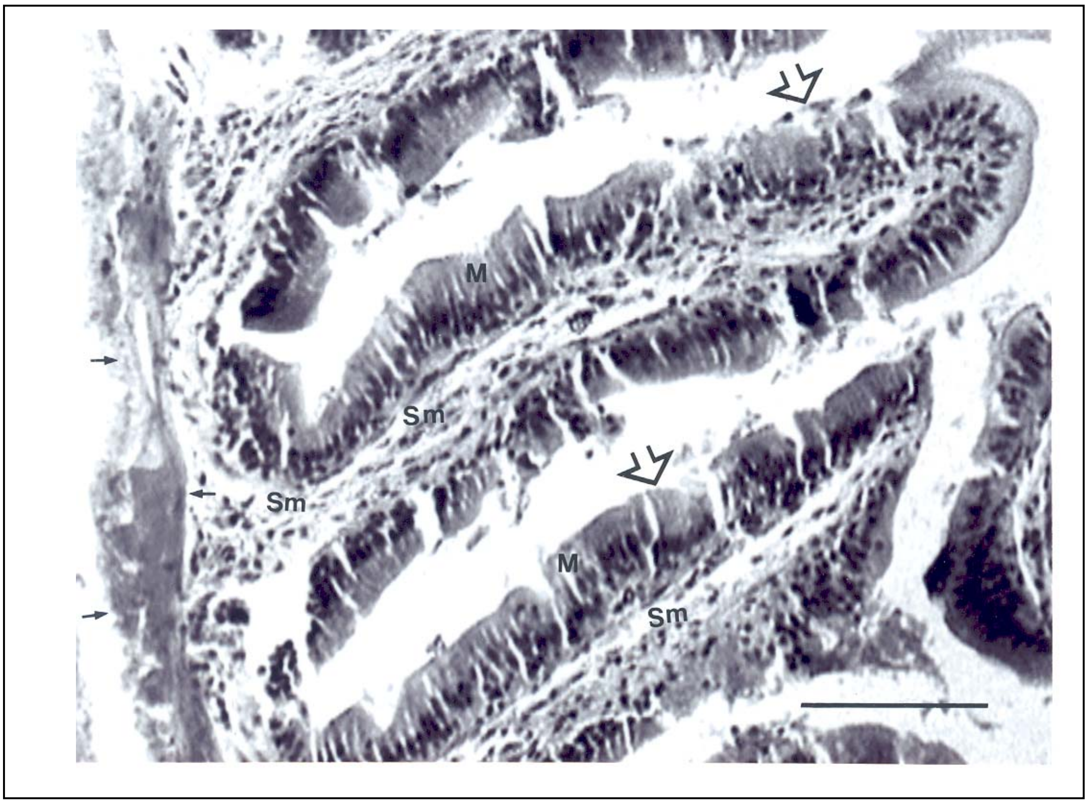

Figure 3. Light micrograph of a transverse section showing histological organization of the 'stomach' of *Aphanius dispar* fish; folds (rugae) (Large open arrowheads), mucosa (M), submucosa (Sm), muscularis (small arrow). Bar = 25 μm.

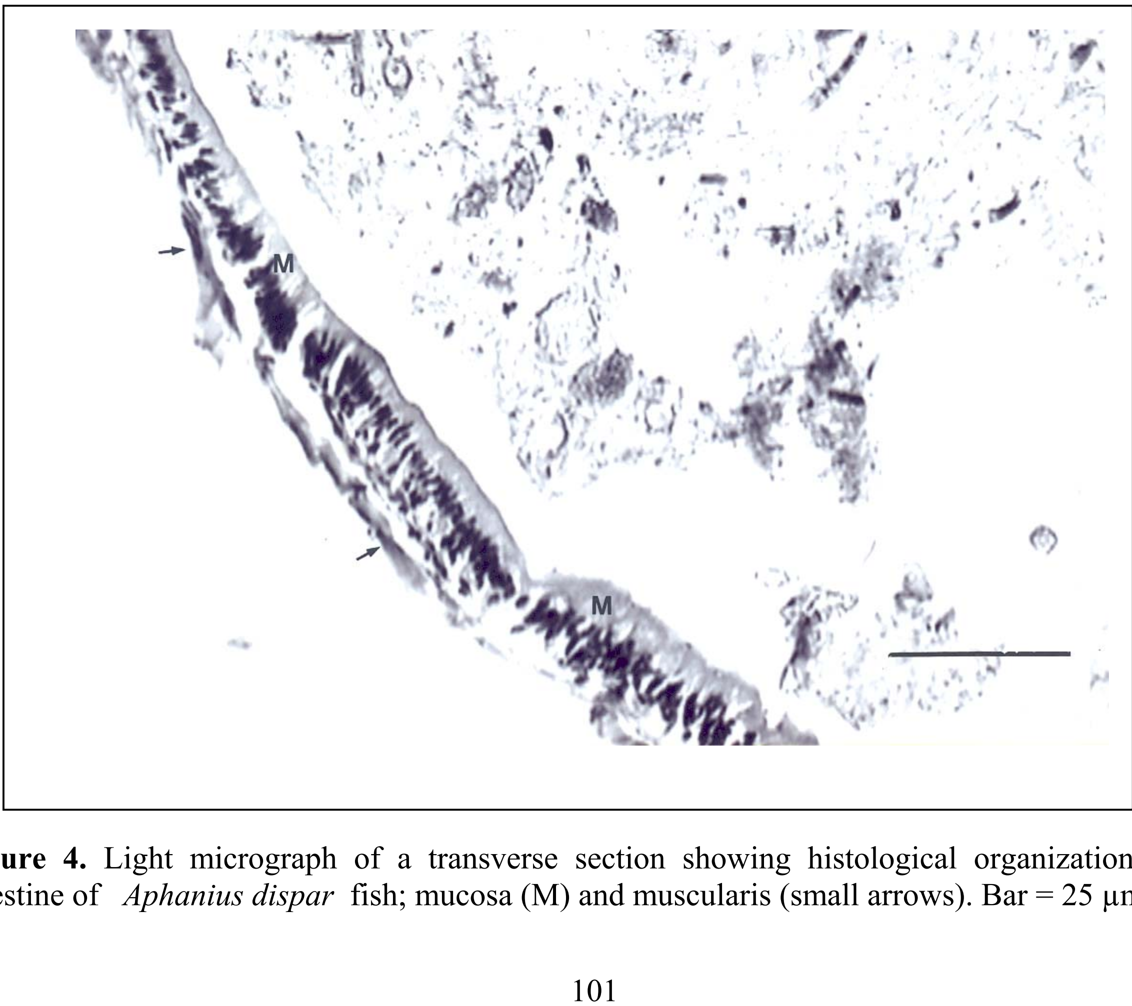

Figure 4. Light micrograph of a transverse section showing histological organization of the intestine of *Aphanius dispar* fish; mucosa (M) and muscularis (small arrows). Bar = 25 µm.

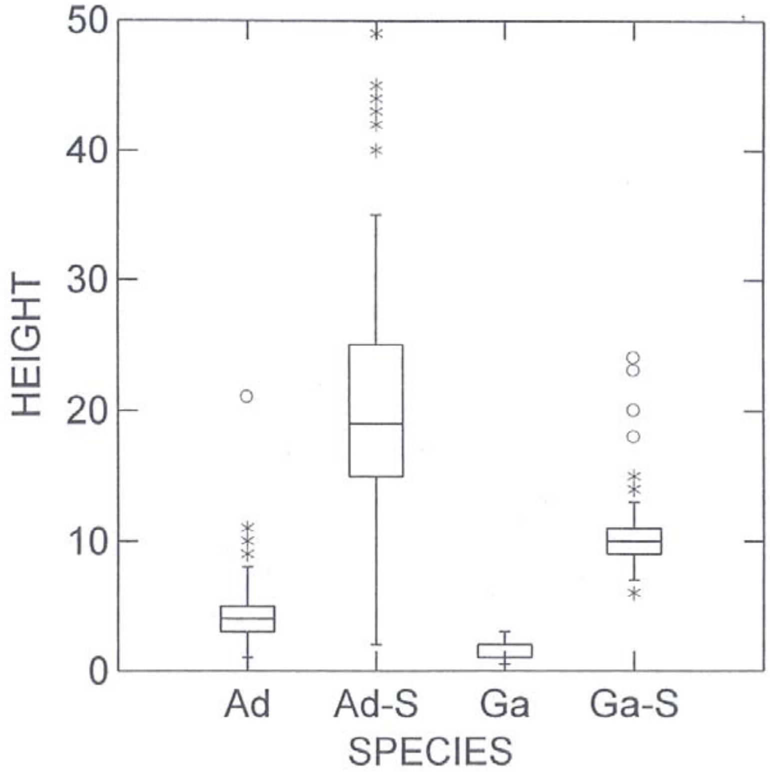

Figure 5. Box plots showing the fold length in *Aphanius dispar* and *Garra barriemiae* (Ad = *Aphanius dispar* intestine, Ad-S = *Aphanius dispar* stomach, Ga = *Garra barriemiae* intestine and Ga-S = *Garra barriemiae* stomach).

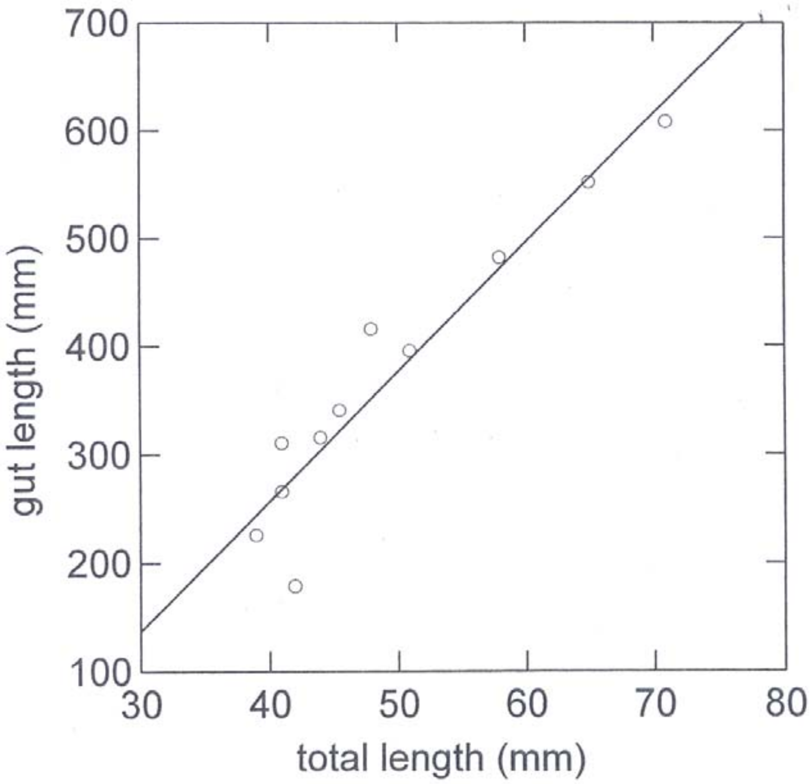

Figure 6. Relationship between total length and alimentary canal length in *Garra barriemiae.*

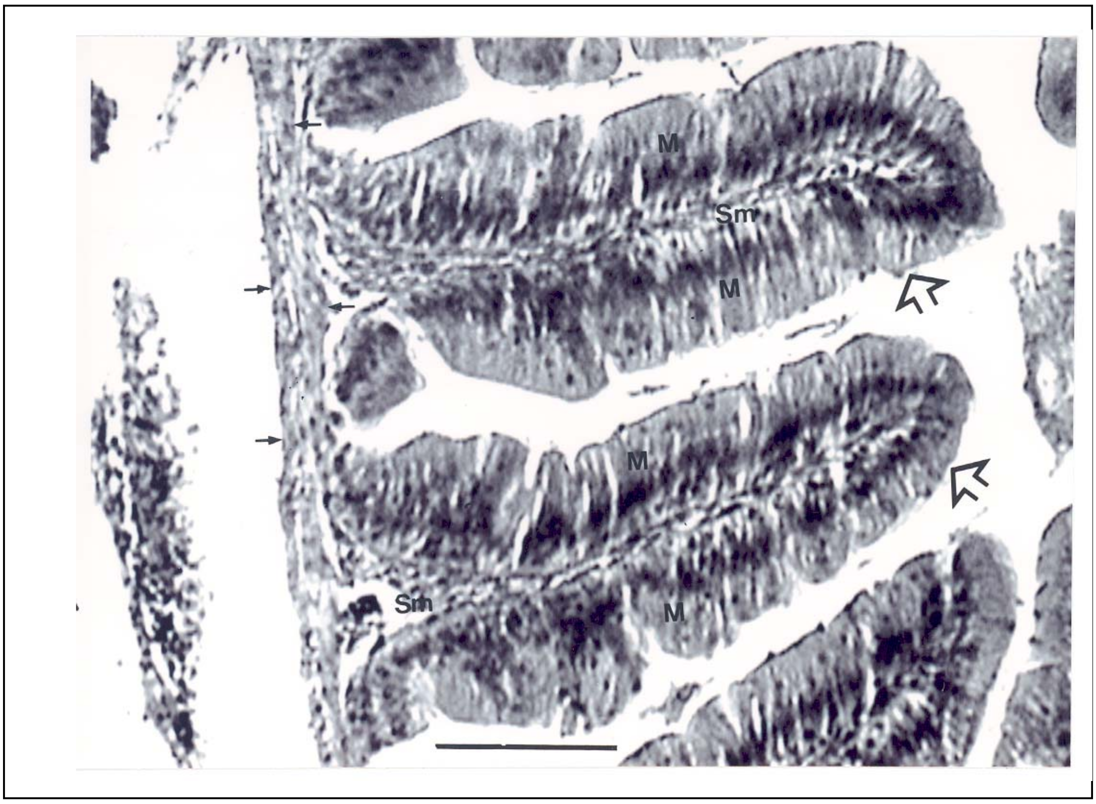

Figure 7. Light micrograph of a transverse section showing the histological organization of the 'stomach' of *Garra barriemiae* fish; rugae (folds) (large open arrowheads), mucosa (M), submucosa (Sm), muscularis (small arrows). Bar = 25 µm.

The histology of the 'stomach' is shown in Figure 7. It is composed of four different layers, mucosa, submucosa, muscularis and serosa all characterised by varying thickness. The mucosa is thrown into rugae with variable heights and widths. These are made of columnar epithelial cells with basal nuclei.

Figure 8 shows the histology of the 'intestine'. Its basic structure is the same as that of the 'stomach'. The rugae heights are variable and shorter than those of the 'stomach'. The structure of the columnar epithelium is the same as that in the 'stomach'.

Numbers of rugae in the 'stomach' and the 'intestine' as well as their heights in these two regions are given in Table 1 and Figure 5. Both the numbers (t-test, $t = 3.950$; $p < 0.001$) and rugae heights (paired t-test, $t = 32.726$; $p < 0.001$) are significantly different. There are no correlations between the numbers of rugae ($n = 25$; $r = 0.320$; $p > 0.05$) or between the heights of rugae ($n = 125$; $r = 0.055$; $p > 0.05$) in the 'stomach' and in the 'intestine'.

3.3 Comparison of *A. dispar* and *G. barreimiae*

A. dispar and *G. barreimiae* do not resemble each other in the gross anatomy of the alimentary canal. The histological organization of the 'stomach' and 'intestine' show very close resemblances. Table 1 shows the morphometric measurements of the 'stomach' and 'intestine' of both species. The ratio of the total length of fish to the length of alimentary canal in adult fish (total length / alimentary canal length) is different in both species. In *A. dispar* this ratio ranged from 0.40 to 0.66, while in *G. barreimiae* it ranged from 0.12 to 0.17. These ratios were significantly different (t-test on arcsin transformed data, $t = 17.346$; $df = 20$; $p < 0.001$). The mean numbers of rugae in both the 'stomach' and 'intestine' of *A. dispar* were significantly higher than those of *G. barreimiae* (Table 1).

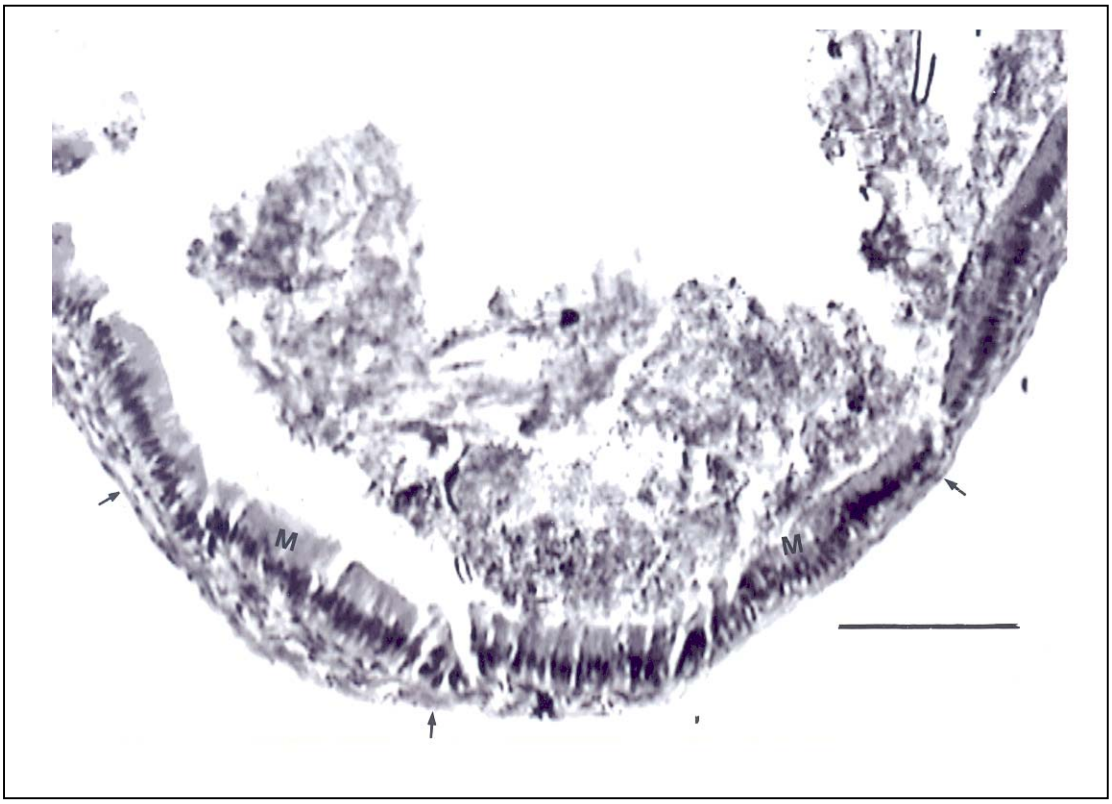

Figure 8. Light micrograph of a transverse section showing the histological organization of the intestine of the *Garra barriemiae* fish; mucosa (M) and muscularis (small arrows). Bar = 25 µm.

4. Discussion

The anatomical structure of the intestine, its relative length in particular, depends on the nature of food eaten by fishes (Kapoor *et al.* 1975; Anderson, 1986; Kuperman and Kuz'mina 1994). The length of the disgestive tracts in general not only increases from carnivorous to herbivorous diet, but their looping is more complex in herbivores than in carnivores and omnivores (Al- Hussainy 1949; Lagler *et al*. 1977). The gross anatomy of alimentary canals in *Aphanius dispar* and *Garra barreimiae* further confirms these findings. *A. dispar*, with a relatively short alimentary canal and a simpler loop is an omnivore which includes live animal food, while *G. barreimiae* with its extremely long alimentary canal and a complex loop is a herbivorous benthophage feeding on epilithic and epipsammic algae. The ratio of the total length of the fish to the total length of the alimentary canal in adult fish seems to be a good indicator of the feeding habits. The omnivorous *A. dispar* had a significantly higher ratio than the herbivorous *G. barreimiae*.

The histologies of the 'stomach' and 'intestine' of these two species are remarkably similar despite morphometric differences. It has been reported earlier that the height of microvilli decreased from the anterior to the posterior part of the fish intestine (Yamamoto 1966; Kayanja *et al.* 1975; Stroband 1977; Noaillac-Depeyre and Gas 1979; Anderson 1986). The number of rugae and the height of rugae significantly decreased from the anterior 'stomach' to the posterior 'intestine' in both species (Table 1). It appears that the columnar epithelial cells forming the brush-border along the margins of rugae are larger in the anterior 'stomach' region than in the posterior 'intestine' region.

Adequate discussion on the histology of the 'stomach' of *A.dispar* has been provided elsewhere (Ba-Omar *et al.* 1998; Ba-Omar and Victor, 2000). The histology of the alimentary canal of *G. barreimiae* has been provided for the first time in this study. Comparisons of the number and the height of rugae in both species are significantly different both in the 'stomach' and in the 'intestine' (Table 1). The total length (TL) ranges of *A. dispar* and *G. barreimiae* studied overlapped (Table 1). Therefore, the differences in histological measurements can not be explained by the differences in size range alone. The internal epithelia of the 'stomach' and the 'intestine' in both species have goblet cells and columnar cells with microvilli that appear as a brush border. These characters are similar to those reported for other cyprinids, but special cellular nests reported in the intestinal epithelia of some cyprinids were absent in these species (Al-Hussainy 1949). Whether the columnar cells with microvilli are equally absorptive in both 'stomach' and 'intestine' regions of both species requires histochemical verification.

The histological organization of the ‘stomachs’ described for both species here raises the question whether these are true ‘stomachs’. Several cyprinids have earlier been described as stomachless (Al-Hussainy 1949; Lagler *et al.* 1997). The 'stomach' in both species studied here is not as distinct as those described for freshwater fishes like pike, burbot and sunfish (Williams and Nickol 1989; Kuperman and Kuz'mina 1994), but nevertheless it is a morphological entity separated from the 'intestine' by a constriction.

Histological studies in *A. dispar* using light and electron microscopy did not show the presence of gastric glands ((Ba-Omar *et al.* 1998; Ba-Omar and Victor, 2000). Also gastric glands were not seen in the ‘stomach’ of *G. barreimiae*. It is often argued that the presence of gastric glands and their function are essential for defining a ‘true’ stomach. Stomachs store ingested food and by contraction facilitate the movement of food down the alimentary canal. Therefore, the absence of gastric glands alone need not qualify the absence of a ‘stomach’. Both *A. dispar* and *G. barreimiae* have ‘stomachs’ in the morphological sense and terms like ‘stomach-like pouch’ and ‘intestinal bulb’ are not necessary for their description. The histological organization of ‘stomach’ in both *A. dispar* and *G. barreimiae* indicates a greater surface area than that of ‘intestine’. It is quite likely that the ‘stomachs’ of these species play an important role in the absorption of food. Again the conventional thinking that the‘intestine’ is the only place where absorption of digested food takes place has to be readdressed. Based on the available evidence, this paper prefers to maintain that the cyprinodont *A. dispar* and the cyprinid *G. barreimiae* have demarcated ‘stomach’ and ‘intestine’.

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