

Comparative Gastrointestinal Morphology of Three Small Mammalian Insectivores: *Acomys spinosissimus* (Rodentia), *Crocidura cyanea* (Eulipotyphla), and *Amblysomus hottentotus* (Afrosoricida)

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Abstract

The gastrointestinal morphology was investigated in three mammalian insectivorous species, namely *Acomys spinosissimus*, *Crocidura cyanea*, and *Amblysomus hottentotus*. The aim of the study was to provide a comprehensive morphological comparison between the different species and to explore whether anatomical gastrointestinal adaptations are associated with the insectivorous diet of these species. The shape, proportional length, and proportional surface areas of the different gastrointestinal regions were recorded and compared in the three insectivores. Hematoxylin and Eosin (H&E) and Alcian Blue/Periodic Acid Schiff (AB/PAS) were used for morphological assessment. In all three species, the stomach was simple and uncompartimentalized. The internal aspect of the stomach in *A. spinosissimus* was hemi-glandular, containing stratified squamous epithelium in the fundus, with glandular epithelium in the body and pyloric region. However, *C. cyanea* and *A. hottentotus* had wholly glandular stomachs. Paneth cells were not observed in the intestinal tracts of *C. cyanea* and *A. hottentotus*. *Acomys spinosissimus* was the only species studied that had a cecum. The proximal colonic region of *A. spinosissimus* had V-shaped mucosal folds. Histologically, *C. cyanea* had villi throughout the entire gastrointestinal tract (GIT), whereas for *A. hottentotus* villi were not present in the most distal gastrointestinal regions. In both *C. cyanea* and *A. hottentotus*, longitudinal mucosal folds were present in the distal part of the colon. The GITs of *C. cyanea* and *A. hottentotus* showed little morphological differentiation namely, a simple, glandular stomach and the lack of a cecum.

Keywords:

morphology; gastrointestinal; insectivores

INTRODUCTION

Acomys spinosissimus (Southern African Spiny mouse), *Crocidura cyanea* (Reddish-grey Musk shrew) and *Amblysomus hottentotus* (Hottentot Golden mole) are insectivorous mammals that are widely distributed throughout Southern Africa (Mills and Hes, 1997). These three species belong to three different superorders, viz., Euarchontoglires, Laurasiatheria, and Afrotheria, respectively (Fig. 1; Springer et al., 2004, 2005; Wilson and Reeder, 2005; Beck et al., 2006; Meredith et al., 2011). *Acomys spinosissimus* belongs to the order Rodentia in the superorder Euarchontoglires, whereas *C. cyanea* and *A. hottentotus* were previously grouped in the order

Insectivora. The order Insectivora is currently replaced by three orders namely Afrosoricida (tenrecs and golden moles), Erinaceomorpha (hedgehogs), and Soricomorpha (shrews and moles; Wilson and Reeder, 2005). *Crociodura cyanea* and *A. hottentotus* belong to Soricomorpha and Afrosoricida, respectively.

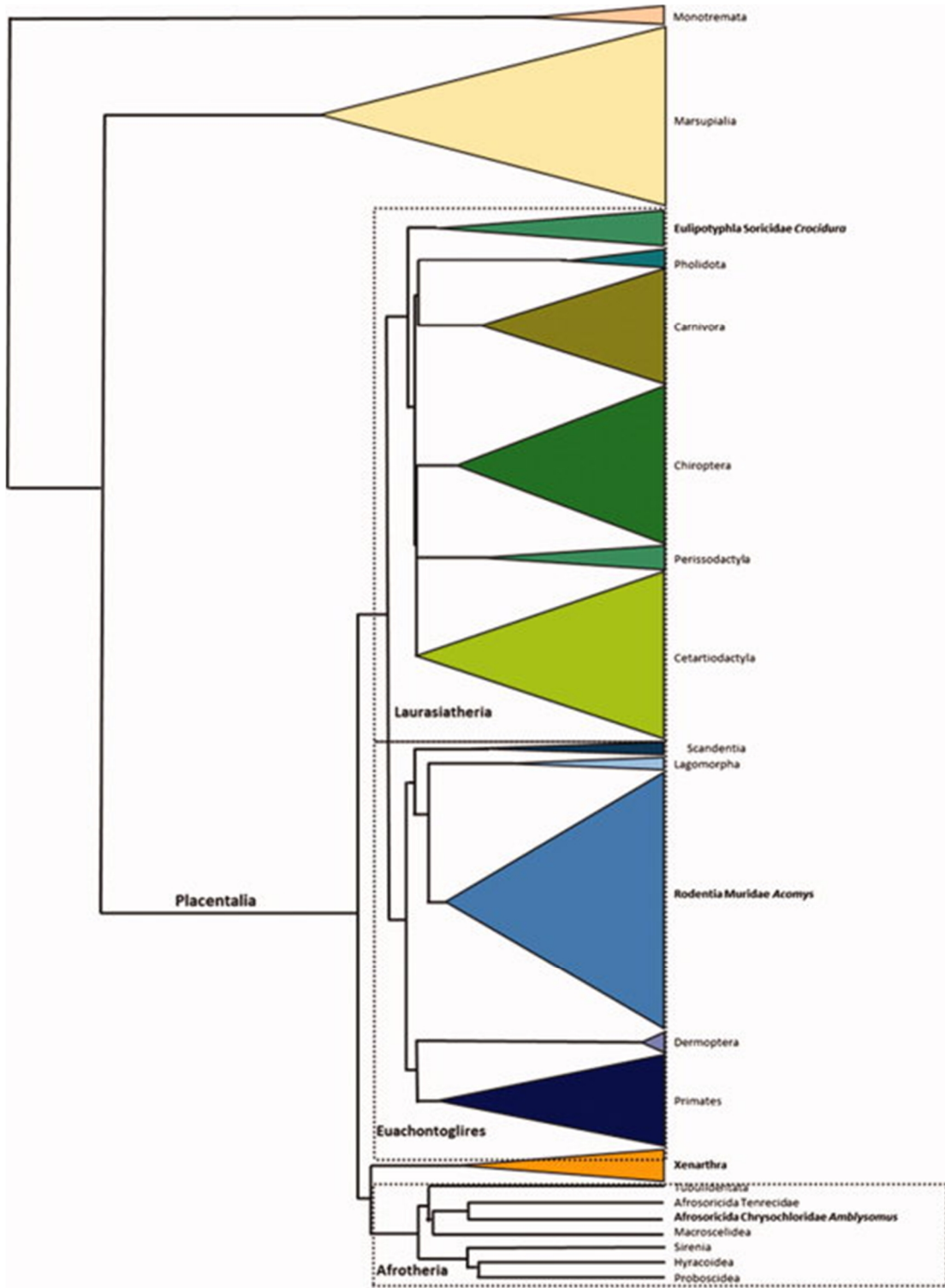


Figure 1. The phylogenetic relationships of *A. spinosissimus*, *C. cyanea*, and *A. hottentotus*, modified from Meredith et al. (2011).

The species examined in this study consume a wide variety of insects. *Acomys spinosissimus* consume mainly seeds and insects such as ants, millipedes, beetles, termites, spiders, and small snails (Vesey-FitzGerald,1966; Stuart and Stuart,2001). They sometimes opportunistically also ingest grass. Crocidurine species consume primarily invertebrates and only a small amount of leaf, seed or other plant material (Dickman,1995). Vertebrate remains such as the bones of small lizards, along with remnants of Isoptera (termites) and Araneida (spiders) have been reported in the faeces of *C. cyanea*. The insect taxa selected in the diet of the Reddish–grey Musk shrew have a relatively high ratio of body water to energy content (Churchfield,1990), which might be preferred in arid or water scarce environments. Heavily chitinized beetles are reportedly avoided by *C. cyanea*, but were prominent in the diets of *Crocidura fuscomurina* and *Crocidura hirta* (Dickman,1995). Hisaw (1923) reviewed the dietary preferences of different mole species and concluded that moles are carnivorous animals, rarely and unintentionally ingesting plant material. *Amblysomus hottentotus* consumes about 45 g of earthworms each day (excluding other insects) and is therefore classified as an insectivore/vermivore (Kingdon,1974a).

The morphology of the gastrointestinal tracts (GITs) of insectivores varies among different species and they often have a simple hindgut which lacks a cecum (Stevens and Hume,1995). Even though several morphological studies have been performed to relate the variations in gastrointestinal structures to different feeding habits (Chivers and Hladik,1980; Perrin and Curtis,1980; Langer,2002), relatively little is known about the intestinal morphology of insectivores (Kurohmaru et al.,1980) and particularly of the three species studied here. The aim of this study was therefore to do a morphological and morphometric analysis of the GITs of *A. spinosissimus*, *C. cyanea*, and *A. hottentotus*, thereby exploring whether anatomical GIT adaptations are possibly associated with their insectivorous diet.

MATERIALS AND METHODS

Intact fixed GIT specimens were obtained from the Department of Zoology and Entomology at the University of Pretoria: *Acomys spinosissimus* ($n = 10$), *Crocidura cyanea* ($n = 10$), and *Amblysomus hottentotus* ($n = 10$) specimens were used. Ethical approval for the use of these specimens was obtained from the animal ethics committees of the Universities of Pretoria and Stellenbosch. Nature conservation permits were also obtained prior to commencement of the study. Masses of the fixed intestinal tracts with intact mesenteries were recorded for all species. After removal of the mesenteries, the GITs were photographed and the total GIT lengths were measured on the antimesenteric border using a pliable, nonstretchable cord. The lengths of the different gastrointestinal regions were recorded and expressed as a percentage of the total GIT length.

The approximated macroscopic surface areas of the different intestinal regions were estimated by calculating the mean of two/three circumference measurements for each region, (recorded at corresponding positions in all three species) multiplied by the length of each region and expressed as a percentage of the total gastrointestinal surface area. The GITs were then opened using scissors along the antimesenteric border and the contents was gently rinsed out. The dissected GITs were pinned open in order to reveal the mucosal GIT surfaces and photographed using a digital camera (Sony DSC-H7) and a stereomicroscope (Leica MZ6) with a fixed camera.

For histological examination, tissue was harvested from *A. spinosissimus* ($n = 5$) from the following gastrointestinal regions namely the: corpus of the stomach, duodenum, middle of the small intestine, distal ileum, cecum, and proximal colon. Due to the lack of ceca in *C. cyanea* ($n = 5$) and *A. hottentotus* ($n = 4$), tissue for histology was harvested from the corpus of the stomach, duodenum, the middle, and distal intestinal regions and the colon (tissue for the distal intestinal region was harvested approximately 1 cm proximal to the GIT ending, whereas colonic tissue was harvested at the distal end of the GIT). The samples were routinely processed to wax and serial cross sections of each gastrointestinal region were cut at 4 μm , stained with Hematoxylin and Eosin (H&E) and Alcian Blue/Periodic Acid Schiff (AB/PAS). The tissue sections were examined and photographed with a Zeiss Axioskop2 light microscope. Hugin software (version 2011.0.0.0fd3e119979c, Pablo D'Angelo et al., Open Source, <http://hugin.sourceforge.net/download/>) was used to merge images together for the composite micrographs.

StatSoft STATISTICA software (StatSoft, version 10, 2011, Tulsa, Oklahoma, USA) was used to statistically analyze the measured macroscopic data. The F- (with Fisher LSD post hoc tests) and Kruskal–Wallis tests were used to compare the macroscopic measurements of the three species and the Spearman test was used to determine the allometric relationships for each species between the body mass (g) and GIT mass (g), and the body mass (g) and GIT surface area (mm^2).

RESULTS

The mean gastrointestinal and body masses, of animals used in this study are listed in Table 1. There were interspecies variations in the body masses because all animals were caught in the wild and no data on the physiological condition (juvenile, adult, and pregnant) of the species were available.

TABLE 1. List of the species used in the present study, including the origin of the preserved material, sample size, and mean gastrointestinal and body masses (\pm Std. Dev.)

Scientific name	Common name	Origin	n	GIT Mass (g)	Body Mass (g)	% GIT Mass to Body Mass
<i>Acomys spinosissimus</i>	Reddish-Grey Musk shrew	UP	10	4.37 (\pm 0.7)	21.11 (\pm 6)	18.05
<i>Crocidura cyanea</i>	Southern African Spiny mouse	UP	10	1.73 (\pm 0.4)	14.94 (\pm 4)	10.5
<i>Amblysomus hottentotus</i>	Hottentot Golden mole	UP	10	4.89 (\pm 1.4)	60.5 (\pm 10)	9.6

UP, Department of Zoology and Entomology, University of Pretoria.

Descriptive Gastrointestinal Morphology

The fixed intestinal tracts without mesenteries are shown in Figure 2, displaying the relative size and shape of the different compartments. The stomachs of all the species examined were unilocular (simple) and uncompartimentalized. The stomach of *A. spinosissimus* revealed a sharp angular incisure (Fig. 3), causing the cardia and pyloric regions to be positioned close together. The fornix of the stomach was elongated and extended above the cardiac region, giving the stomach a distinct U-shape.

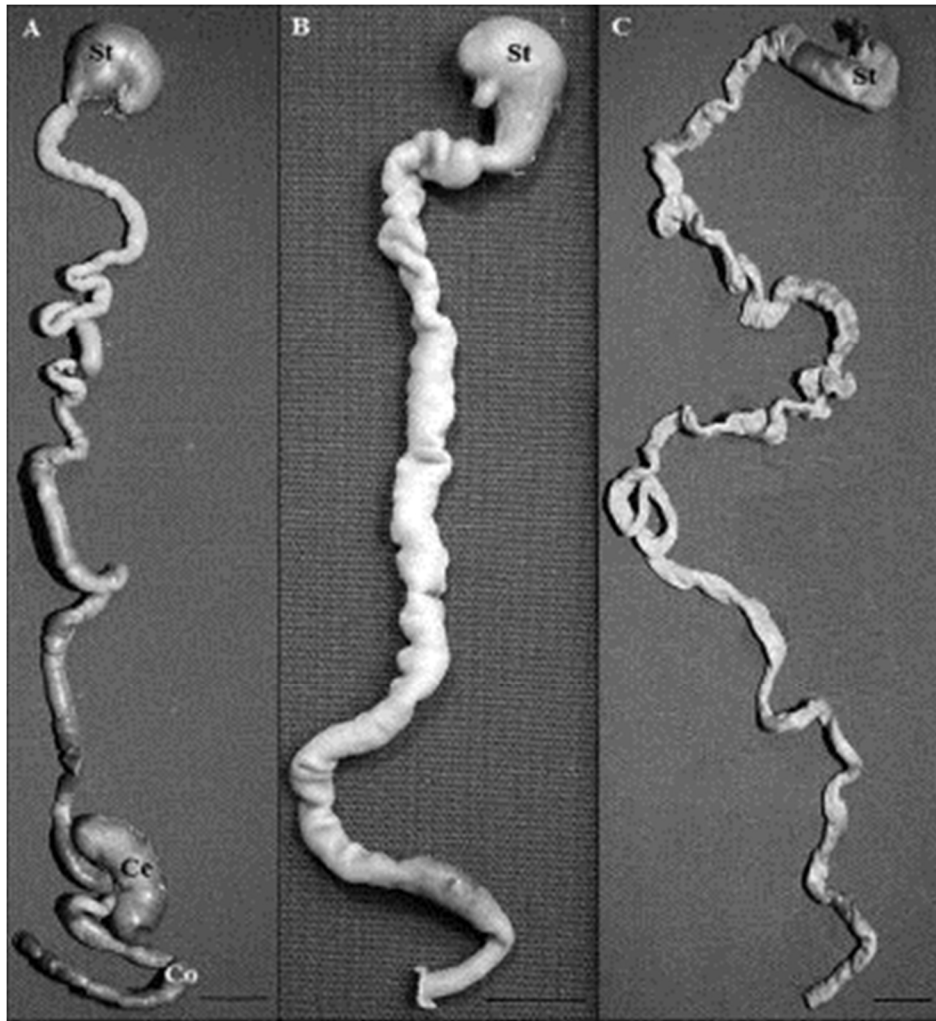


Figure 2. The GITs of the three insectivores without mesenteries. (A) *A. spinosissimus*, the only rodent species examined, had a cecum (Ce) which was absent in the GITs of *C. cyanea* (B) and *A. hottentotus* (C). St, stomach; Ce, cecum; Co, colon. Bar = 1 cm.

In both *C. cyanea* and *A. hottentotus* (Fig. 3), the stomach had an elongated pyloric region and a wide angular incisure with interspecies variations occurring in *C. cyanea* such as tubular-, J-shaped, or U-shaped stomachs.

The internal aspect of the stomach in *A. spinosissimus* was hemi-glandular (Fig. 3), with the stratified squamous epithelium covering the fundic region. The latter was demarcated from the glandular region by a macroscopically visible line or limiting ridge. The stomachs of *C. cyanea* and *A. hottentotus* were wholly glandular (Fig. 3) with a microscopically visible transition of stratified squamous to glandular epithelium at the cardio–esophageal junction.

Histological examination of the body (corpus) of the stomach revealed typical tubular gastric glands with numerous parietal cells and peptic cells in the gastric glands of *A. spinosissimus* and *A. hottentotus*. Peptic cells were present in the stomach of *C. cyanea*, but parietal cells were most abundant (Fig. 3). Brunner's glands were observed in the duodenal submucosa of all three

species. The villi and intestinal crypts were lined with tall columnar enterocytes, entero-endocrine- and goblet cells. Paneth cells were only observed in *A. spinosissimus*.

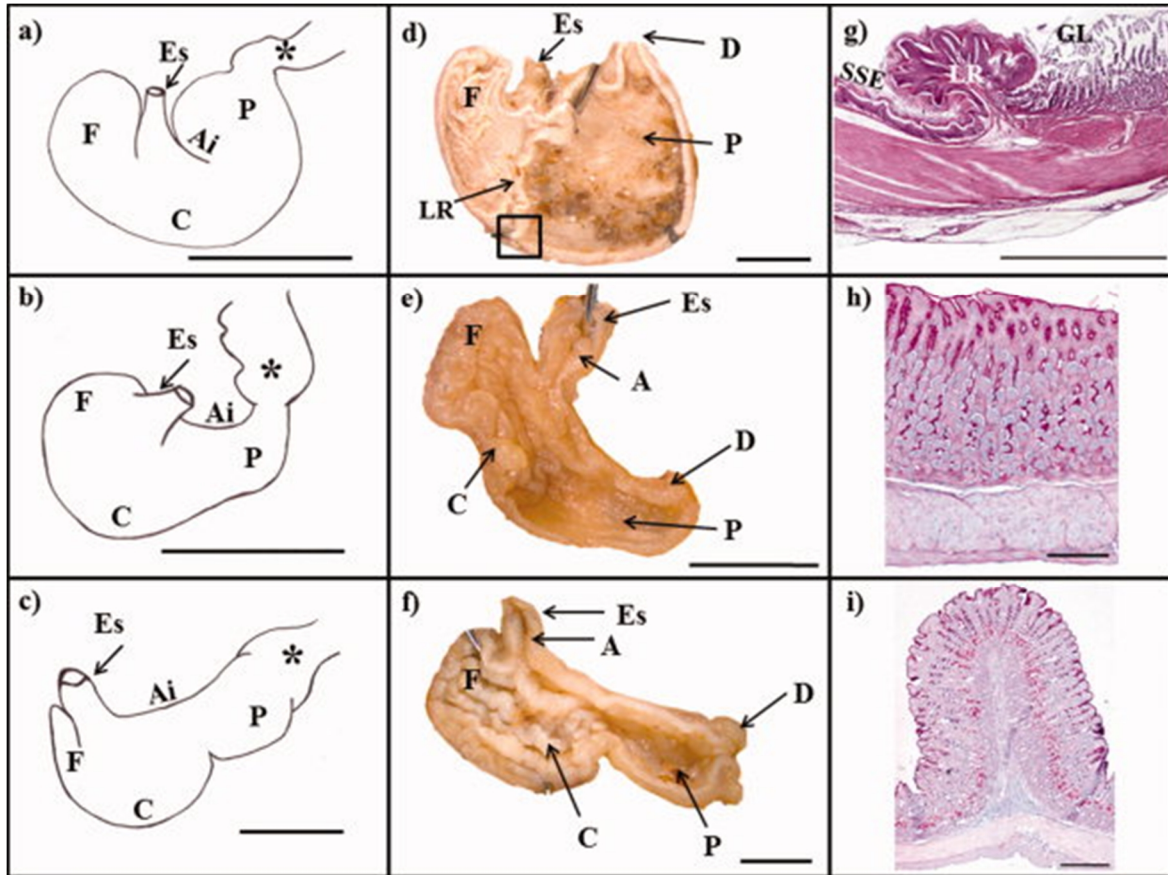


Figure 3. The shapes, macroscopic images, and the internal aspect of the stomachs of *A. spinosissimus*, *C. cyanea*, and *A. hottentotus*. Figures a, b, and c illustrate the unilocular stomachs of *A. spinosissimus*, *C. cyanea*, and *A. hottentotus*. The stomach of *A. spinosissimus* was U-shaped with a sharp angular incisure (Ai) whereas the stomachs of *C. cyanea* and *A. hottentotus* had a wide Ai. Bar = 1 cm. Macroscopic images of the internal aspect of the stomachs of *A. spinosissimus* (d), *C. cyanea* (e), and *A. hottentotus* (f). The internal aspect of the stomach of *A. spinosissimus* revealed a limiting ridge (LR) and rugae in the fundus (F). The boxed area on the *A. spinosissimus* stomach is histologically illustrated in image g. The stomachs of both *C. cyanea* and *A. hottentotus* had extensive rugae in the fundus (F) and corpus (C) regions. Bar = 1 cm. Microscopic images of the stomach of *A. spinosissimus* (g) revealed that the stomach is hemi-glandular and that the fundic/fundus region is covered with stratified squamous epithelium (SSE) which is separated from the glandular (GL) stomach by a limiting ridge (LR). Bar = 1000 μ m. In *C. cyanea* (h), the corpus stomach region has tubular gastric glands (Bar = 100 μ m) whereas the corpus region of *A. hottentotus* (i) demonstrates extensive rugae (Bar = 200 μ m). A, cardiac glands; Ai, angular incisure; C, corpus; D, position of the duodenum; Es, Esophagus; F, fundus/fornix; GL, glandular stomach; LR, limiting ridge; P, pylorus; SSE, stratified squamous epithelium; *, duodenum.

Of the three species studied, *A. spinosissimus* was the only species that had a cecum. The cecum was bean-shaped with the ileo-caecal and caeco-colic openings positioned close together. Villi were absent in the cecal mucosa which was typical of the mucosa of the large intestine. The external surface of the colon of *A. spinosissimus* was simple without taenia or haustra. Internally, V-shaped mucosal folds were observed in the proximal region of the colon (Fig. 4).

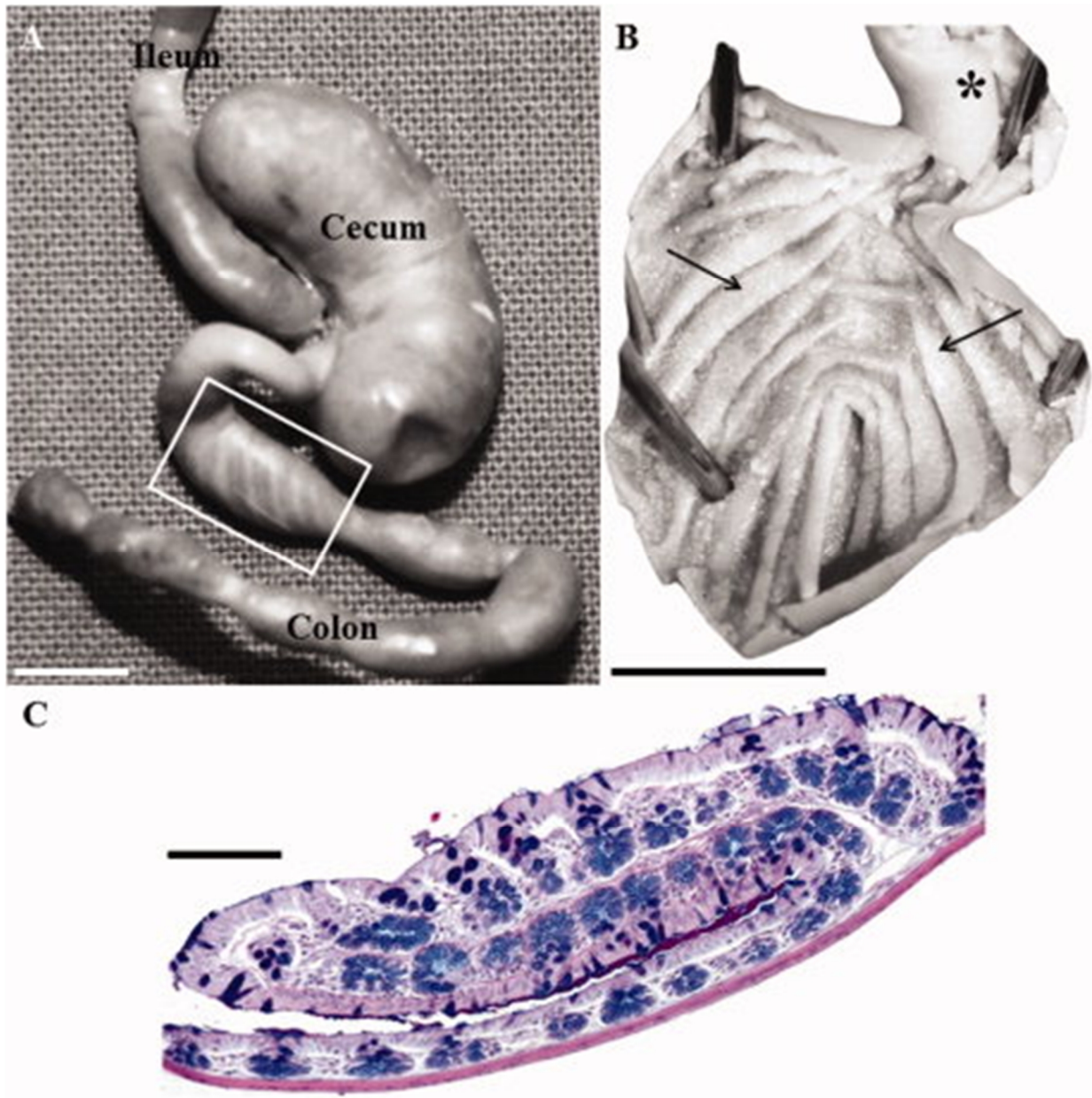


Figure 4. The macroscopic and microscopic images of the V-shaped mucosal folds in the colon of *A. spinosissimus*. (A) The boxed area illustrates macroscopically the spirally arranged mucosal folds in the proximal colon. Bar = 5 mm. (B) The proximal region of the colon was cut longitudinally on the antimesenteric border and pinned open to observe the V-shaped folds indicated with arrows. The asterisk indicates the proximal colon position. Bar = 5 mm. (C) A composite image of the transverse mucosal fold lined with crypts. Bar = 100 μ m. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

In *C. cyanea* and *A. hottentotus*, there was no clear anatomical division between the small- and large-intestines due to the lack of ceca in both species. Histologically, in *C. cyanea*, villi were observed throughout the entire GIT, whereas for *A. hottentotus* villi were not observed in the most distal part of the GIT. In both *C. cyanea* and *A. hottentotus*, macroscopic longitudinal folds (Fig. 5) were present in the distal colon for the last 2 and 6 cm, respectively. Histological sections revealed that these folds were typically mucosal folds.

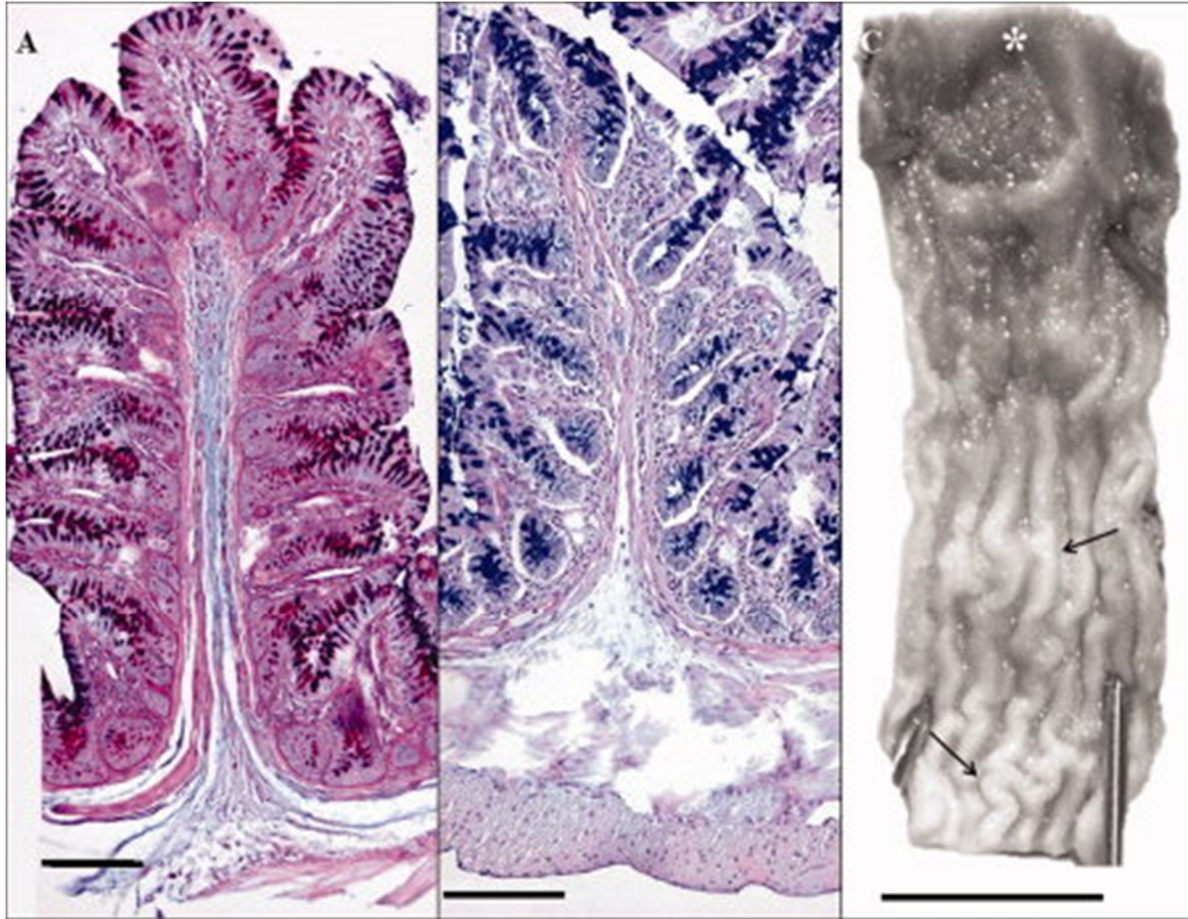


Figure 5. Microscopic and macroscopic images of the colons of *C. cyanea* and *A. hottentotus*. The microscopic images were stained with the AB/PAS technique. A composite image of the colon of *C. cyanea* (A) and *A. hottentotus* (B) shows the mucosa is thrown into a longitudinal fold. Bar = 100 μm . (C) The distal region of the colon of *A. hottentotus* was cut longitudinally on the antimesenteric border and pinned open to observe the longitudinal elevations/folds (arrows). Similar macroscopic folds were also observed in the distal colonic region of *C. cyanea*. The asterisk indicates the proximal position of the colon. Bar = 1 cm.

Statistical Analysis

As *C. cyanea* and *A. hottentotus* had no cecum and, therefore, no macroscopic demarcation between the small- and large-intestines, the entire length of the gut excluding the stomach was measured and referred to as the small intestine plus large intestine (from here on cited as SI + LI). This was also done to compare the gut lengths of all three species statistically. Thus, the SI + LI measurement of *A. spinosissimus* includes the lengths of the small intestine, cecum, and colon.

There were also statistically significant differences ($P = 0.03$) between the surface areas of the stomach and the combined surface area of the SI + LI of *A. spinosissimus* and *A. hottentotus* (Table 2, Fig. 6a,b). *Acomys spinosissimus* had the largest relative stomach surface area while *A. hottentotus* had the largest relative surface area of the SI + LI of all three species studied. *Crocidura cyanea* had an intermediate stomach and SI + LI surface area.

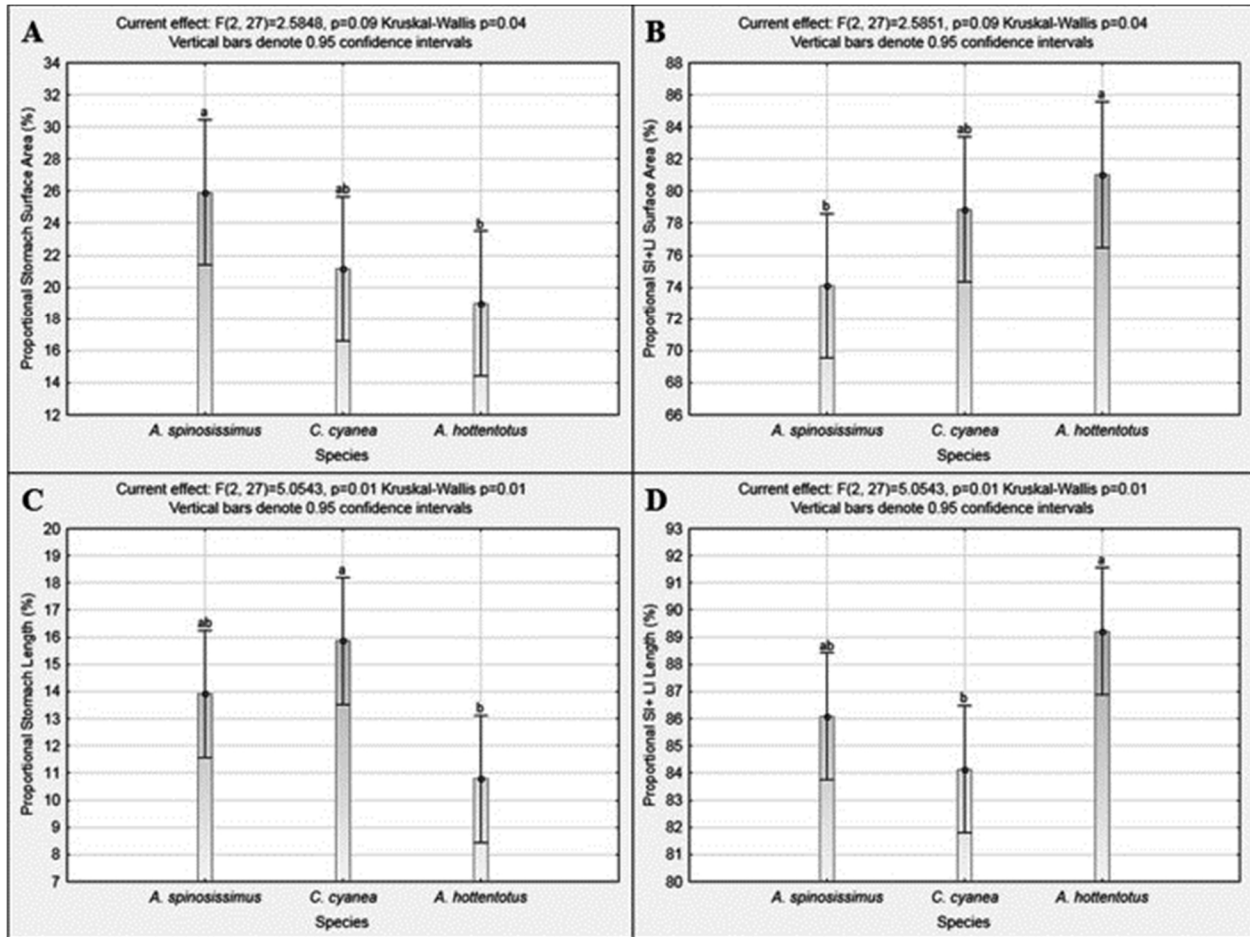


Figure 6. A graphical comparison of the mean surface area and length proportions of the stomach and SI + LI gastrointestinal regions of *A. spinosissimus*, *C. cyanea*, and *A. hottentotus*. Statistical significance ($P < 0.05$) between the insectivores is indicated by different letters of the alphabet. If the same letter/s is present above each of the species there are no significant differences between the animals. However, if the letter/s between the species is different from one another, statistical significance is indicated.

There were also statistically significant differences ($P = 0.05$) between the lengths of the stomach and the SI + LI of *C. cyanea* and *A. hottentotus* (Table 2, Fig. 6c,d). *Crocidura cyanea* had the longer stomach and *A. hottentotus* the longer SI + LI. *Acomys spinosissimus* did not differ significantly from *C. cyanea* and *A. hottentotus* with respect to the length of the stomach or the SI + LI length.

With reference to the allometric measurements of the insectivores, it appears as if the GIT mass increases with an increase in body mass. However, *C. cyanea* was the only species that indicated a significant allometric relationship between the body mass and the gastrointestinal mass ($r = 0.68, P = 0.03$; Table 3). None of the insectivores showed a significant allometric relationship between the body mass and the gastrointestinal surface area.

TABLE 2. The mean proportion (%) and Std. Dev (\pm) of the total GIT surface area and length of the anatomically distinct regions of the GITs of *A. spinosissimus*, *C. cyanea*, and *A. hottentotus*

	<i>A. spinosissimus</i>	<i>C. cyanea</i>	<i>A. hottentotus</i>
Proportional surface area (%)			
Stomach	25.91 (\pm 3.71) ^a	21.14 (\pm 10.16) ^{a,b}	18.97 (\pm 5.41) ^b
Small intestine	44.22 (\pm 3.99)	–	–
Small + Large intestine	74.09 (\pm 3.71) ^b	78.86 (\pm 10.16) ^{a,b}	81.03 (\pm 5.41) ^a
Caecum	16.17 (\pm 1.59)	–	–
Caecum + Colon	29.86 (\pm 1.26)	–	–
Colon	13.70 (\pm 1.99)	–	–
Ave total GIT surface area (mm ²)	5856.97 (\pm 532.97)	1778.64 (\pm 278.38)	8321.78 (\pm 2716.61)
Proportional length (%)			
Stomach	13.92 (\pm 2.01) ^{a,b}	15.86 (\pm 5.18) ^a	10.78 (\pm 2.85) ^b
Small intestine	56.64 (\pm 3.06)	–	–
Small + Large intestine	86.08 (\pm 2.01) ^{a,b}	84.14 (5.8) ^b	89.22 (2.85) ^a
Caecum	11.74 (\pm 1.26)	–	–
Caecum + Colon	29.45 (\pm 1.85)	–	–
Colon	17.70 (\pm 1.45)	–	–
Ave total GIT length (mm)	320.95 (\pm 21.98)	137.70 (\pm 17.05)	438.80 (\pm 100.32)

Different superscripts within a line indicate significant differences on a 5% level ($P < 0.05$) using the Fisher LSD post hoc test. The average total gut surface areas and lengths are given.

TABLE 3. Allometric relationships between the body mass (g), GIT mass (g), and GIT surface area (mm²), respectively

Specimens	<i>n</i>	Variable 1	Variable 2	Spearman <i>r</i> -value	Spearman <i>P</i> -value
<i>A. spinosissimus</i>	10	Body mass (g)	GIT mass (g)	0.34	0.34
<i>C. cyanea</i>	10	Body mass (g)	GIT mass (g)	0.68	0.03*
<i>A. hottentotus</i>	10	Body mass (g)	GIT mass (g)	0.19	0.60
<i>A. spinosissimus</i>	10	Body mass (g)	GIT surface area (mm ²)	–0.18	0.63
<i>C. cyanea</i>	10	Body mass (g)	GIT surface area (mm ²)	0.56	0.09
<i>A. hottentotus</i>	10	Body mass (g)	GIT surface area (mm ²)	0.14	0.70

*Statistically significant ($P < 0.05$) difference between species.

DISCUSSION

In a comparative gastrointestinal morphological study of 19 rodent species, Perrin and Curtis (1980) described the GIT of *A. spinosissimus* as ancestral due to its simple intestinal and cecal morphology; however, they noted that the ratio of stratified squamous to glandular epithelium in the stomach of *A. spinosissimus* reflected the advanced herbivorial condition. This relative simplicity of the GIT may be due to the fact that little cellulose fermentation is required as they ingest mainly insects and seeds and only rarely the foliage of grass (Vesey-FitzGerald, 1966; Stuart and Stuart, 2001). The GITs of the insectivores in this study shows similarities to the relatively short and simple digestive tracts of carnivores. The simple gastrointestinal morphology of carnivores generally correlates with the high digestibility of their food (Hume, 2002) and this may be the case in the insect-eating species studied here. The absence of a cecum in *C. cyanea* is similar to that observed in other shrews such as *Crocidura horsfieldi watasei* (Watase's shrew), *C. fuscomurina* (Bicolored musk shrew), *C. leucondon* (Bicolored shrew), *C. russula* (Greater White-toothed shrew), *Blarina brevicauda* (Northern Short-tailed shrew), *Neomys fodiens* (Eurasian water shrew), and *Suncus murinus* (Musk shrew; Kurohmaru et al., 1980, 1982; Hattori and Yamanouchi, 1984; Schieck and Millar, 1985; Langer, 2002). The absence of the cecum in *A.*

hottentotus is consistent with what has been observed in *Chrysochloris stuhlmanni* (Stuhlmann's golden mole) and in other mole species such as *Condylura cristata* (Star-nosed mole) and *Talpa europaea* (European mole; Schieck and Millar,1985; Stevens and Hume,1998; Langer,2002).

Perrin and Curtis (1980) suggested that it would be incorrect to conclude that the gastrointestinal morphology is directly correlated to the feeding habits of either the Cricetid or Murid rodents. Similar conclusions were also made by Gorgas (1967), who performed a large comparative study on various rodent species in attempting to relate GIT length to dietary preferences. Neither Perrin and Curtis (1980) nor Gorgas (1967) measured the relative surface areas of the different gastrointestinal regions. Perrin and Curtis (1980) realized the shortcomings of comparing gastrointestinal length only to diet and suggested that in future the gut surface area should be computed. The present study takes into account estimated surface areas which were expressed as a percentage of the total surface areas. This approach compensates for variation in intestinal and body masses between species and was successfully used to compare the GIT morphology of mole-rats (Kotzé et al.,2010). Various factors may influence exact measurements of GIT morphology, such as the quantity of ingesta in each gastrointestinal region at the time of death and tissue shrinkage and loss of pliability due to fixation. However, in the present study, all measurements were done on fixed intestinal tracts and all measurements were done in exactly the same manner.

By relating the gastrointestinal lengths to the diets of 35 species of small mammals, Schieck and Millar (1985) found that herbivores usually had longer large-intestines than omnivores and that the overall gut length in herbivores was longer than that of omnivores and carnivores. Since herbivores ingest plant material rich in fiber and low in calories, more time is needed for digestion to extract the necessary nutrients and this causes an increase in the volume and area of the large intestine (Wilczyńska,1998). In addition, Schieck and Millar (1985) concluded that the large intestine was a more accurate indicator of diet than the small intestine.

In *A. spinosissimus*, the relative surface area and length of the small intestine was greater than that of the combined cecum and large intestine. This indicates that fermentation may aid in digestion in this species but it is probably not of great importance. Of all three species, *A. hottentotus* has the largest and *C. cyanea* the smallest GIT with respect to relative surface area and length. *Acomys spinosissimus* has an intermediate GIT length and surface area between the latter two species. Although the comparison of body size and relative gastrointestinal length failed to show significant differences within a species, marked interspecies differences were observed between the three insectivores of the present study with the largest species (*A. hottentotus*) having the longest GIT.

In herbivorous mammals, gut capacity is linearly related to body mass (Clauss et al.,2007), therefore GIT mass might also be related to body mass. Allometric relationships of the insectivores of the present study revealed that the GIT mass increases with an increase in body mass, but it was only statistically significant in *C. cyanea*. Korn (1992) described that the lengths of the GITs can be influenced by food quality, season, and reproductive activity. For example, longer intestines are expected in the dry seasons (increased fiber in food) and in breeding females because of increased energy demands. However, this information was not available for the insectivores in this study. An increase of GIT mass along with body mass might compensate

for the energy needs of the animal. According to Langer (2002), small mammals need larger amounts of energy for survival than larger animals. The amount of energy needed, is related to the body mass of the animal. In addition, further allometric relationships of the insectivores in the present study revealed that the external gastrointestinal surface area does not significantly increase with an increase in body mass. Because these mammals are relatively small, an increase in gastrointestinal surface area could lead to an increase in energetic costs making these animals vulnerable to predation (Sibly, 1981). The insectivores in this study can possibly compensate for an increased body size through increasing the mucosal surfaces of the GIT with villi and plicae circulares, rather than increasing the external GIT surface.

The stomach in all three insectivorous species was unilocular (single compartment), similar to that observed in many rodents (Carleton, 1973; Perrin and Curtis, 1980), shrews and mole species (Myrcha, 1967). Similar to the findings of Perrin and Curtis (1980), the present study confirms the presence of stratified squamous epithelium in the proximal part of the stomach in *A. spinosissimus*. The hemi-glandular stomach was extensively investigated and described by Carleton (1973) in several species of New World cricetines (hamsters, voles, lemmings, new world rats, and mice), where he referred to the raised ridge, formed between the stratified squamous epithelium and glandular parts, as a bordering fold. Toepfer (1891) referred to this fold as the “limiting ridge” in the rat. Bensley (1902) has hypothesized that the increased cornification (keratinization) and relative reduction of glandular epithelium of the stomach mucosa might be due to the ingestion of abrasive foods such as the chitinous exoskeletons of insects and coarse grasses. However, this hypothesis is unlikely because several mammals consume coarse and abrasive foods and do not possess keratinized stomachs (Carleton, 1973). For example, the stomach of the beaver, *Castor canadensis* (Nasset, 1953), and Lagomorphs (Bensley, 1902; Todd, 1927; Dalke, 1942) are completely glandular despite the abrasive foods such as bark, leaves, grasses, and twigs which are ingested by the respective species. Vorontsov (1962) hypothesized that the increased keratinization of the stomach may be an adaptation to an herbivorous diet and that the keratinized region may function as a fermentation chamber where symbiotic bacteria can degrade cellulose. However, Carleton (1973) rejected Vorontsov's (1962) hypothesis and suggested that a reduction of glandular epithelium due to keratinization (nonsecretory region) in the rat stomach could cause salivary amylase to remain active for longer than in the human stomach, therefore allowing enhanced digestion to take place. As *A. spinosissimus* resides in arid regions (Kingdon, 1974b), Perrin and Curtis (1980) also suggested that the stratified squamous epithelium in the stomach may be an adaptation allowing for temporary food storage.

The presence of peptic cells in the stomachs of the three insectivores in this study correlated with the findings of Allison (1948) who observed peptic cells in the stomachs of *Eremitalpa granti* (Grant's Golden mole), *Elephantulus myurus* (Eastern Rock Elephant shrew), and *Suncus orangia* (shrew). Kanamori et al., (1989) detected peptic cells in *Suncus murinus* (House Musk shrew); however, Myrcha (1967) did not observe peptic cells in 27 species comprising of hedgehogs, moles and shrews.

The elongated pyloric regions observed in *C. cyanea* (Family Soricidae) and *A. hottentotus* (Family Chrysochloridae) were consistent with findings described by Myrcha (1967) in the Soricidae (shrews) and Talpidae (moles). He suggested that in these species, the pyloric region

undergoes elongation in order to compensate for an overload of the fundic region during large daytime food intakes, thereby increasing the volume of pyloric glands. However, in Talpidae the elongation of the pylorus did not result in an increased area of pyloric glands with relation to the total stomach length. The elongated pyloric region increases the pathway along which protein-rich food is subjected to gastric enzymes, allowing for prolonged digestion. Prolonged digestion in the stomach might result in faster nutrient uptake further in the digestive tract which could meet the increasing energy demands of the active *C. cyanea* and *A. hottentotus* species, the latter being most active at night.

Brunner's glands were present in the submucosa of all three species examined here. According to Takehana et al. (2000), they are present in all mammals and they secrete a slightly alkaline (pH 8.2–9.3) mucoid secretion, which protects the duodenal mucosa from auto-digestion by the acidic stomach contents (Young et al., 2006).

Similar to the findings of Kurohmaru et al. (1980, 1982) in the musk shrew and Watase's shrew, Paneth cells were not observed in *C. cyanea* and *A. hottentotus*. Paneth cells are typically located at the base of small intestinal crypts, and contain antibacterial enzymes and lysozymes in large secretory granules (Gartner and Hiatt, 2000). According to Satoh et al. (1986), Paneth cells are present in the intestinal crypts of many mammals except in carnivores. This was also observed by Sandow and Whitehead (1979), who reported that Paneth cells were absent in dogs and cats, suggesting that it is related to their carnivorous diet. Paneth cells were, however observed in *A. spinosissimus* in the present study as is the case in other rodents such as the rat, nude rat, mouse, guinea pig, and golden hamster (Satoh et al., 1990).

The structure of the cecum of 10 *A. spinosissimus* specimens examined in the present study had a broad sac-like appearance followed by a simple colon, consistent with the description of Perrin and Curtis (1980) who examined two *A. spinosissimus* specimens. Their study however failed to report on the V-shaped mucosal folds in the proximal part of the colon observed in all *A. spinosissimus* specimens in the present study. The arrangement of these folds in a V-shape, almost at right angles to the longitudinal axis of the colon, is suggestive of slowing down the passage rate of digesta and may therefore influence the retention time of food. These folds may also be part of a colonic separation mechanism (CSM) which separates more digestible food particles from the colonic content and returns these particles along with bacteria to the cecum while coarser food fragments are rapidly eliminated (Hume, 2002). The CSM has been observed in several mammalian and bird species (Sperber, 1985) and according to Hume (2002) nearly all myomorph rodents (mice, rats, gerbils, hamsters, lemmings, and voles) show morphological modifications of the proximal colon. Similar oblique folds have been observed in the rat (*Rattus norvegicus*) where their function is thought to facilitate the retrograde movement of a mixture of mucus and bacteria back into the cecum to form caecotrophes (Björnhag, 1994). The presence of the V-shaped folds in *A. spinosissimus* might therefore be suggestive of a CSM. Since the latter species inhabits arid regions (Kingdon, 1974b), a CSM could be beneficial during periods of limited protein-rich food (insects and seeds) and abundant coarse plant materials.

Compared to other rodents, the GIT of *A. spinosissimus* appears to have some similarities (gut length, ratio of small intestine:large intestine, cecum size) with omnivorous rodents such as *Tatera brantsii* (Highveld gerbil), *Tatera leucogaster* (Bushveld gerbil) and *Mus minutoides*

(African Pygmy mouse), when referring to the list of Perrin and Curtis (1980), which they ordered according to the degree of specialization toward herbivory.

As the GIT of *C. cyanea* and *A. hottentotus* both lacked a cecum, it was difficult to distinguish between the small- and large-intestines at the macroscopic level. The histological appearance of the GIT of *C. cyanea* was similar to that reported in the musk shrew and Watase's shrew (Kurohmaru et al., 1980, 1982) in that villi were observed throughout their entire GIT.

Villi in the large intestine are associated with a decreased gastrointestinal length (Kurohmaru et al., 1980) such as in birds. In both birds and bats (McNab, 1973), the decreased gastrointestinal length limits the capacity to store food because of flight constraints that do not allow sudden increases in the body mass. Sibly (1981) noted some disadvantages of a longer GIT, such as energetic costs of its maintenance and the additional mass thereof, might make the animal more vulnerable to predation. The relatively shorter GIT might be particularly important in Crocidurines as they are very active during the day and night (Meester, 1963; Pernetta, 1977; Genoud and Vogel, 1981; Baxter and Meester, 1982). Although some Crocidura species prefer a habitat at ground level, *C. cyanea* prefer a habitat in shrubs (35–95 cm above ground). Thus, the short GIT of *C. cyanea* may be an adaptation to decrease the storage capacity of food to accommodate its climbing habits. The passage of food through the GIT must, therefore, be fast with a high absorption rate (Keegan, 1977; Tedman and Hall, 1985). In the present study, villi were observed on the longitudinal colonic mucosal folds seen in *C. cyanea* only. Villi in the large intestine may also be an adaptation to increase the absorptive surface area of the GIT (Kurohmaru et al., 1980).

Unlike *C. cyanea*, the colonic mucosa of *A. hottentotus* lacked villi and its proportional SI + LI gastrointestinal length was longer than that of *C. cyanea*, possibly indicating that the passage rate of food is slower and more sufficient than in the latter species. Although the longitudinal colonic mucosal folds were observed in all specimens of *C. cyanea* and *A. hottentotus*, it is uncertain whether these folds are permanent structures or if they would disappear or flatten once the colonic regions are filled with digesta. It is plausible that the longitudinal mucosal folds may have been caused by postmortal muscle contractions of the underlying tunica muscularis. On the other hand, this is unlikely as these folds occurred only in a relatively circumscribed, small area of the GIT in all specimens examined and was not visible over the entire GIT length.

While the animals in the present study all consume insects, the relative proportions and types of insects in their diets differ. They also differ from one another with respect to phylogeny, habitat, and body size. The present study, therefore, highlights the gastrointestinal morphological differences in species representing Rodentia, Soricomorpha, and Afrosoricida.

Macroscopically, the intestinal tract of *A. spinosissimus* shows similarities with other herbivorous (*Aethomys shryophilus*, *Lemniscomys Griselda*) and omnivorous rodents (*Mus minutoides*, *Tatera leucogaster*) in that they possess a hemi-glandular simple stomach and a caecum (Perrin and Curtis, 1980). Similar to other crocidurine species and several other species belonging to the Soricidae and Talpidae families, *C. cyanea* lacks a caecum and has a relatively short intestinal tract. The GIT morphology of the order Afrosoricida, consisting of the families Chrysochloridae and Tenrecomorpha is not well described and this increases the need for further

morphological studies of these animals. *Amblysomus hottentotus*, similar to *Chrysochloris stuhlmanni* (Stuhlmann's golden mole) and some species of the family Tenrecidae (*Tenrec ecaudatus*, *Echinops telfairi*, and *Setifer setosus*) lacks a cecum (Langer, 2002). The present study confirmed that of the three insectivorous species examined, *C. cyanea* and *A. hottentotus* exhibited the most undifferentiated GITs, lacking both stomach differentiation and a cecum. This simple GIT is possibly reflective of their mainly protein rich insectivorous diet. We, therefore, hypothesize that the V-shaped and longitudinal folds in the colon may play a role in regulating the speed of digesta flow, thereby affecting the uptake of nutrients. This study will serve as a basis for further comparative morphological studies of the GITs of these and other insectivorous species.

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