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RESEARCH ARTICLE

HISTOLOGICAL STUDY OF STOMACH OF *LYCIASALAMANDRA FAZILAE* AND *LYCIASALAMANDRA FLAVIMEMBRIS*

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ABSTRACT

Histological examinations were conducted in order to describe the tissues on the stomach of the critically endangered *Lyciasalamandra fazilae* (Başoğlu and Atatür, 1974) and *L. flavimembris* (Mutz & Steinfartz, 1995). In this study two adult females, two adult males and one juvenile from Dalyan, Turkey (*L. fazilae*) and one adult male and one juvenile from Marmaris, Turkey (*L. flavimembris*) were examined. Tissue samples were taken from the freshly dead animals found on the road during the night field work. They were taken into alcohol for the histological studies. Tissue samples were fixed in Bouin's solution and routine histological techniques were applied. Hematoxylin and Eosin stains were used to identify cells and structures of the tissues. Stomach is covered with gastric columnar epithelial cells and glandular epithelium which are contained with gastric glands. Tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa were observed as mucosa layers. There was no significant difference between males and females in mucosa layers where gastric glands are situated. The histological structure of stomach of both species between males and females and between adults and juveniles were found to be mostly similar. Results from the literature review has indicated that the stomach histology show a similarity with the other Urodela species.

Keywords: *Lyciasalamandra fazilae*, *Lyciasalamandra flavimembris*, Stomach, Histology

1. INTRODUCTION

The digestive system of amphibians has two major part; a digestive track and digestive glands. The anatomy and histology of the digestive system is flexible and may give different responses to variation in environmental conditions [1]. Digestive track of amphibians begins with the buccal cavity, the pharynx, oesophagus, stomach and small and large intestines. Mostly the general morphology of the digestive track is similar within the amphibians, show some differences in dimensions [2]. Digestion begins in the stomach for the amphibians. Most amphibians are carnivores but in some species their diet varies according to age.

The distribution of *Lyciasalamandra* [3] is restricted to a small, ~350 X 50 km region of southwestern Turkey between Muğla and Antalya, plus three offshore Greek islands. The Turkish Lycian salamanders are currently classified as seven allopatric species *L. antalyana, L. atifi*, *L. billae*, *L. fazilae, L. flavimembris* and, *L. luschani*. The seventh described species, *L. helverseni* inhabits the

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Greek islands of Karpathos, Kasos and Saria opposite the westernmost Anatolian species. Ecologically, Lycian salamanders are all terrestrial, ovoviviparous and aestivating species [4]. They are most active during the cooler winter months from December to March. According to Veith et al. (2001) generalized ecological information on all Turkish *Lyciasalamandra* species can be summarized by as follows; they are restricted to karstic limestone with precipitation exceeding 800 mm annual rainfall, most localities are below 500 m elevation, and typical habitat is pine forest on northerly exposed slopes. However, little is known about the specific ecological microhabitat requirements for each *Lyciasalamandra* species.

Lyciasalamandra flavimembris is an endemic species found around Marmaris and Ula. It spreads at altitudes up to 600 m above sea level. They are found in stony areas (with limestone rocks) within the pine forests or maquis that are not destroyed or modified [5]. The species previously thought to be subspecies of *Mertensiella luschani* were reclassified under the genus Lyciasalamandra. While making this classification, apomorphic base changes that are different from all other Salamandridae in the mitochondrial genome of Lyciasalamandra genus were noticed [3].

L. fazilae classified as Endangered by the IUCN is restricted to the southern Anatolian coast, Turkey Muğla to Fethiye and ranges from sea level up to 1,000 m asl [5]. The species can be distinguished from other Lyciasalamandra species by the fact that the head and body are orange reddish in color. The dorsal bump in the tail base of male individuals can be used for gender identification.

There are several studies on the histology of Urodela species [6]–[11]. Recent studies were conducted on the histological studies on stomach of *Triturus karelinii*, *Mertensiella luschani* and *Salamandra infraimmaculata* [8], [10]. According to Koca and Karakahya (2014) the stomach tissues of *T. karelinii* and *M. luschani* show similar characteristics. To date from Lyciasalamandra genus only *Lyciasalamandra luschani* (former name: *Mertensiella luschani*) stomach histology was examined [8]. More studies using histochemical, morphological and immunohistochemical have been used to identify the differences of gastrointestinal tube between the anuran species [12]–[16].

In recent years, decreases in amphibian species and populations have been accepted as a problem with the global dimension. The main reasons for this situation are the change, fragmentation or disappearance of habitats. Increasing UV-B radiation in recent years can directly affect amphibians or slow down growth, causing sublethal effects such as making the immune system inoperable. In addition, pollution caused by chemical stressors (pesticides, heavy metals, nitrogen fertilizers, acidification) has also been shown to cause deaths and other sublethal effects (slow growth, developmental or behavioral abnormalities, decreased reproductive success, weakened immune system, hermaphroditism). The objective of this study is to describe the histological characteristics of the stomach of *L. fazilae* and *L. flavimembris* and compare with the previous studies and contribute to the literature in this area.

2. MATERIAL AND METHOD

A total of five specimens of *L. fazilae*, two adult females, two adult males and one juvenile from Dalyan, Turkey and one adult male and one juvenile of *L. flavimembris* from Marmaris, Turkey were collected from the freshly dead animals found on the road during the night field work in 2014-2016. SVL (Snout-vent length) measured from the tip of the snout to the posterior edge of the cloaca, is the standard measure of body size in caudate amphibians [17]. After measurement process, they were

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taken into alcohol for the histological studies. The stomach tissues of seven species were collected and fixed in Bouin's solution for 48 hours. Stomach tissues were dehydrated in ethanol series and embedded in paraffin. Using a microtome (RM 2145; Leica), histological sections of 5 µm were obtained and stained with Hematoxylin and Eosin (HE) (Humason 1962). Stained sections were photographed with a digital camera (Kameram 5) connected to an Olympus light microscope (Olympus CX31).

3. RESULTS

The average SVL and total length of adult *L. fazilae* sampled from Dalyan and *L. flavimembris* sampled from Marmaris were 64.13 and 120.88 respectively (Table 1). The juvenile *L. flavimembris* found from Marmaris was smaller than the *L. fazilae* Dalyan specimen.

Table 1. Snout-vent length (SVL), Total length and sex categories of *L. flavimembris* and *L. fazilae*.

Species	Sex	SVL (cm)	Total length (cm)
L. fazilae	Adult Female	65.69	117.63
L. fazilae	Adult Female	58.5	112.62
L. fazilae	Adult Male	62.35	118.65
L. fazilae	Adult Male	62.79	113.91
L. fazilae	Juvenile	41.09	77.32
L. flavimembris	Adult Female	71.34	141.57
L. flavimembris	Juvenile	35.21	64.72

The stomach of both *L. fazilae* and *L. flavimembris* divided into two distinct parts; the fundus and the pylorus histologically. As for almost most amphibians and reptiles the stomach was histologically divided into two parts; fundic and pylorus regions, we also examined these two parts in both species examined stomach. [18], [19]. Stomach is lined with columnar epithelial cells and glandular epithelium contained with gastric glands. The presence of the simple columnar epithelium in this study also show similarity with the other vertebrates. [20]. Mucosa layers were observed as tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa in both two species (Fig. 1a,b; Fig. 2a,b,c).

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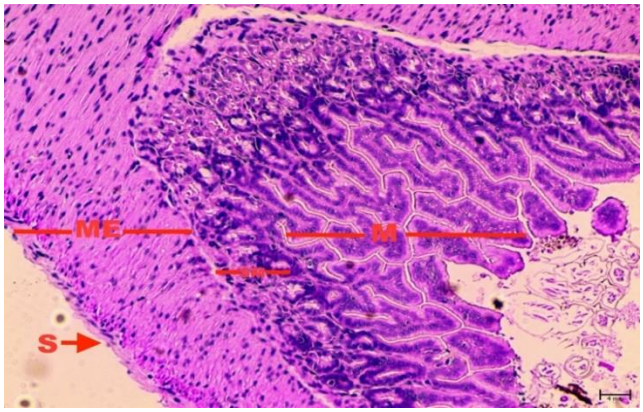

Figure 1a. Histological sections of the mucosa *L flavimembris* (Juvenile). Muscularis externa (ME), submucosa (SM), mucosa (M), serosa (S).

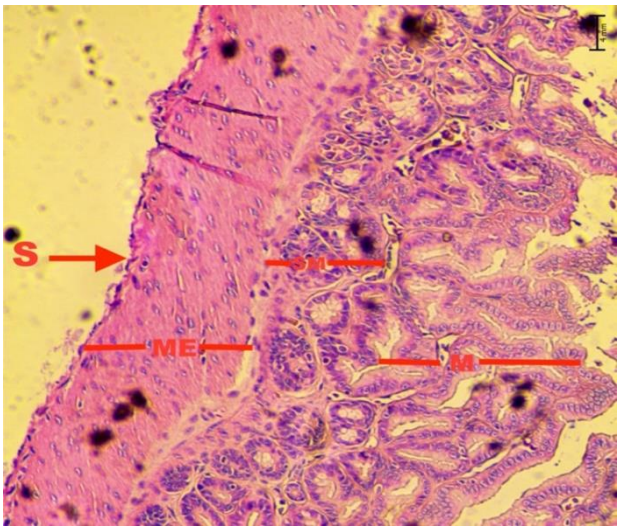

Figure 1b. Histological sections of the mucosa *L. flavimembris* (Female). Muscularis externa (ME), submucosa (SM), mucosa (M), serosa (S).

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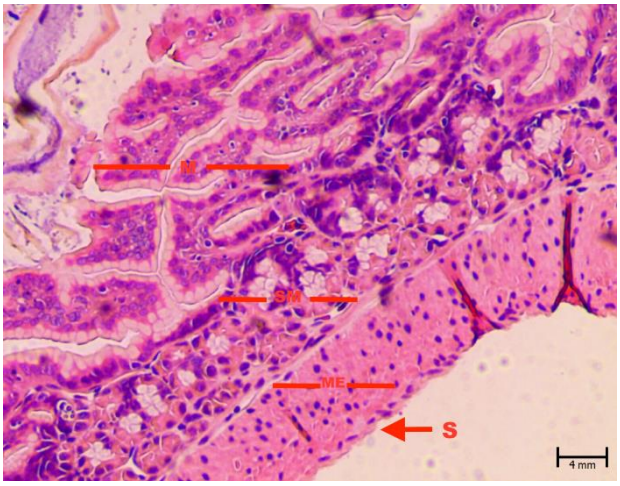

Figure 2a. Histological sections of the mucosa *L. fazilae* (Juvenile). Muscularis externa (ME), submucosa (SM), mucosa (M), serosa (S).

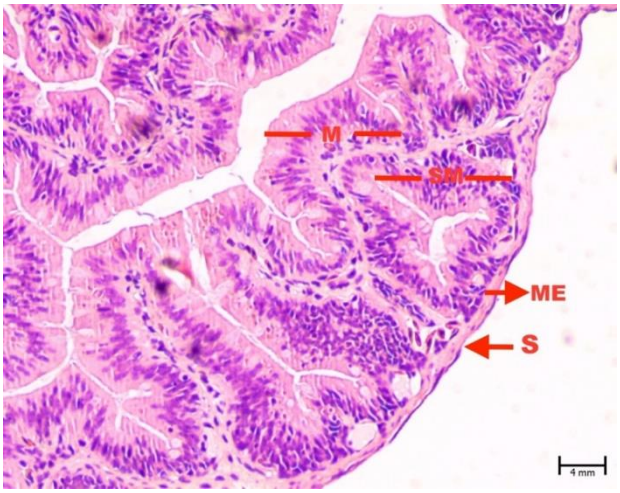

Figure 2b. Histological sections of the mucosa *L. fazilae* (Male). Muscularis externa (ME), submucosa (SM), mucosa (M), serosa (S).

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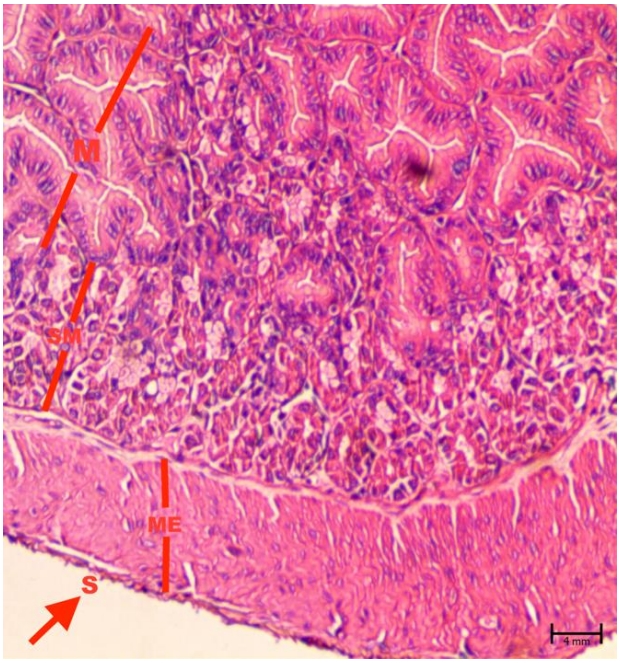

Figure 2c. Histological sections of the mucosa *L. fazilae* (Female). Muscularis externa (ME), submucosa (SM), mucosa (M), serosa (S).

Mucous secreting columnar epithelium covers the mucosa membrane. Fundic glands are mostly simple tubular type, besides pyloric glands are usually branched tubular type (Fig.3). Internal and external muscle layers form the muscular membrane of the stomach.

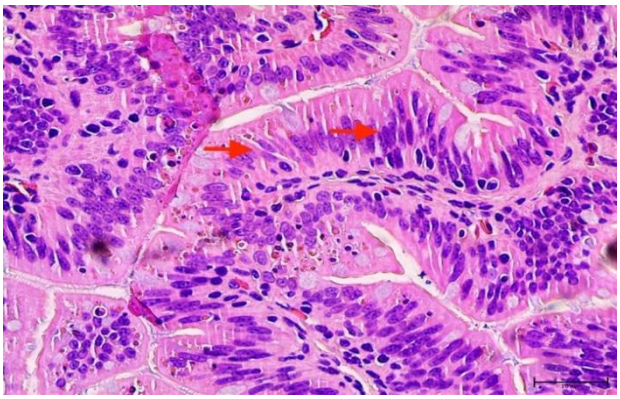

Figure 3. Gastric mucosa of *L. fazilae*. Parietal cells (arrow).

4. DISCUSSION AND CONCLUSION

There was no significant difference between males and females in mucosa layers where gastric glands are situated. The histological structure of stomach of *L. fazilae* between males and females and between adults and juveniles were found to be mostly similar.
It was reported that there is no fundus in amphibians [21] but in further studies based on the morphological and histological features, the region around the entrance of the oesophagus called as cardiac area and the anterior slight dilation is called as fundus, the major part called as corpus and the posterior part called as pylorus.
To date this is the first study describing the histological characteristics of the stomach of *L. fazilae* and *L. flavimembris.* The present description of the stomach revealed similar histological characteristics between *L. fazilae*, *L. flavimembris* and *Mertesnsiella luschani* [8]. And also, the histological characteristics of stomach for *L. fazilae and L. flavimembris* showed a similarity compared to other amphibians [8], [10], [15], [18], [20].

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