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1 **Unique localization of disseminated pancreas in the esophagus of catfish (*Clarias gariepinus*)**
2 **with reference to sexual dimorphism**

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20 ***Running title***

21 Disseminated (esophageal) pancreas in African catfish

38 **Abstract**

39 Background: The fish pancreas composed of two portions: compact and disseminated. However, little
40 has been elucidated in catfish. The present study describes a unique localization of the disseminated
41 pancreas in African catfish. Methods: Paraffin sections were processed for either routine histological
42 examination following staining with hematoxylin and eosin (H & E), periodic acid Schiff's, or were
43 subjected to immunohistochemical staining for detection of both insulin producing β -cells and
44 glucagon producing alpha cells. Results: Our investigation showed that the pancreas of catfish
45 consisted of both compact and disseminated portions. The compact pancreas was embedded in the
46 mesenteric adipose tissue between the spleen, stomach, and liver. However, the disseminated one
47 showed unique localization in the tunica adventitia of the middle portion of the esophagus. The
48 pancreas consisted of two portions, exocrine and endocrine. Furthermore, in both types of pancreas,
49 the female showed a significantly higher ratio for the endocrine islet area/pancreatic tissue area than
50 that of the male. Also, a significantly higher ratio for both insulin and glucagon positive area/islet
51 area in the female pancreas (Compact and disseminated) than that of the male. In conclusion: The
52 present study provides evidence on a unique localization of the disseminated pancreas in the
53 esophagus of catfish. Furthermore, we revealed sex-related difference in the endocrine portion in both
54 pancreatic tissues with more development in the female. The study suggests that sex hormones could
55 be contributed to such sexual dimorphism. However, further investigation is required to compare the
56 degree of development during the spawning and resting seasons.

57

58 **Keywords:** Esophagus, Glucagon-secreting alpha cells, Insulin-secreting beta cells, Islets of
59 Langerhans, Pancreas.

60

61

62 **1. INTRODUCTION**

63 The Catfish is considered as one of the major species of the freshwater teleost fish and it represents
64 12% of all teleost (Wilson and Reeder, 2005). Additionally, the African Catfish (*Clarias gariepinus*),
65 despite their name, is widely distributed in Africa as well as in some areas of Asia and Europe (FAO;
66 Turan, 2016). Generally, the catfish in Africa, especially in Egypt showed high economic value as it
67 has been considered as an important source of food, and their significant contribution to income due
68 to their export to other countries all over the world (Zaghloul et al., 2017) . In the last three decades,
69 the catfish farming and production have been increased dramatically in Egypt due to their relatively
70 high growth rate and its ability to withstand hard environmental conditions (Shaalán et al., 2017) as
71 well as their high resistance to diseases (Shourbela et al., 2020) .

72 The fish species had more numbers of endocrine glands than that in mammals including
73 Corpuscles of Stannius, Ultimobranchial, Pituitary, pineal, chromaffin and interrenal tissue and islets
74 of Langerhans (Prasad et al., 2017). The pancreas is one of the most important glands associated with
75 the gastrointestinal tract and consists of both the exocrine (acini and pancreatic duct) and the
76 endocrine portion (islets of Langerhans) (Kaptaner, 2019). The exocrine part responsible for the
77 digestion of carbohydrates, lipids, and protein via secretion of enzymes (Senoo, 2000). The endocrine
78 portion releases hormones directly into the bloodstream that regulate the blood glucose level (Geyer
79 et al., 1996). On contrary to mammals, the pancreas in the majority of teleosts and carp fish is
80 composed of a compacted (main) portion (observed as a white mass associated with mesenteries
81 between spleen, gall bladder, and stomach), hepatopancreas (exocrine tissue scattered between
82 hepatocytes), and disseminated portion (Brinn, 1973; Naguib et al., 2009; Youson and Al-Mahrouki,
83 1999). These areas or islets are also known as Brockmann bodies consisting of both endocrine and
84 exocrine tissue, which can be found in variable percentage among the species often in conjunction
85 with smaller accessory islets (Agulleiro et al., 1993; Brinn, 1973; Epple, 1969; Mokhtar, 2015).

86 Even though investigations of the pancreas localization and structure in most teleost have already
87 been reported, little attention has been paid to the distribution and percentage of both endocrine and
88 exocrine part of the pancreas among both compact and disseminated types of the pancreas in African
89 catfish. Furthermore, there is a dearth of information about the sex-related differences in the
90 percentage of endocrine to exocrine portion as well as the ratio of both glucagon and insulin positive
91 cells in the islets among both types of the pancreas. Therefore, the aim of our research is to investigate
92 the distribution of pancreatic islets in different regions in catfish, with a comparison of the area ratio
93 of the islet and both α and β -cells among both sexes by both histological and immunohistochemical
94 means.

95

96 **2. MATERIAL AND METHODS**

97 **2.1 Experimental animals and ethics statement**

98 A total of twenty apparently healthy African catfish (*Clarias gariepinus*) of both sexes (10 males
99 and 10 females) with average one-year age were purchased from Behira commercial fish farms, and
100 They were transported in water tanks equipped with an air pump. The age was determined from the
101 recorded data by the commercial fish farms that was dealt with. The average length and weight of
102 male fishes were 46.0 ± 2.8 cm, and 600.0 ± 5.8 g, respectively, but that of females were 39.0 ± 3.5
103 cm, and 490.0 ± 5.8 g, respectively. The catfish sex was externally determined by examination of the
104 urogenital region (supplementary figure 1A and 1D). All experimental procedures were performed in
105 accordance with the ethics and regulations guide of the Animal Care and Use for laboratory animals,
106 including fish approved by the Institutional committee at Zagazig University, Faculty of Veterinary
107 Medicine with the approval number: ZU-IACUC/2/F/116/2018).

108 **2.2 Sample collection and fixation**

109 Following stunning of the catfish, a ventral midline incision was extended from the urogenital
110 opening toward the cranial region, and the esophagus (cranial, middle, and caudal portions), liver,
111 and adipose tissue within the mesentery in the triangular area between intestine, stomach, liver, and
112 gall bladder (supplementary figure 1C and 1F). Then, the specimens were immediately fixed in
113 Bouin's solution overnight at 4°C.

114 **2.3 Histological procedures and immunohistochemical staining**

115 After overnight fixation, the specimens were washed and were subsequently dehydrated in
116 ascending concentrations of ethyl alcohol, cleared in xylene (three changes/ thirty minutes each), then
117 embedded in melted paraffin (three changes/one hour each), then paraffin blocks were prepared.
118 Finally, 4 µm paraffin sections were obtained and used for either routine histological examination
119 following staining with hematoxylin and eosin (H & E), periodic acid Schiff's, or were subjected to
120 immunohistochemical staining for detection of both insulin producing β-cells and glucagon
121 producing alpha cells.

122 The immunohistochemical staining was performed according to our previous protocol (Elewa et
123 al., 2010). Briefly, the deparaffinized and rehydrated sections were subjected to antigen retrieval via
124 heating at 105 °C in 10 mM citrate buffer, pH 6.0 for 20 min. Then, the sections were kept at room
125 temperature for 30 min cooling. Then, peroxidase blocking was performed by incubating the sections
126 in a solution of 3% H₂O₂ in absolute methanol for 20 min at room temperature followed by washing
127 with distilled water and incubation with blocking serum (goat serum for insulin staining and mouse
128 kit for glucagon staining) for 1 hour at room temperature. For negative control, we incubated sections

129 with PBS instead of the primary antibody (the data not show). Then, the sections were overnight
130 incubated with the specific primary antibody at 4 °C (polyclonal rabbit anti-insulin “Cat. No.
131 Ab210560, Abcam”, and monoclonal mouse anti-glucagon “Cat. No. 14-9743-8, Invitrogen”) diluted
132 at 1:50. Such primary antibodies raised in mammalian species have been successfully used in several
133 previous studies to investigate the endocrine pancreas of various teleost species (Kaptaner, 2019).
134 Then, the sections were washed in Tris-Buffered Saline (TBS); followed by incubation with biotin-
135 conjugated secondary antibodies (goat anti-rabbit IgG antiserum for insulin stained sections and anti-
136 mouse IgG antiserum for glucagon stained sections) for 60 min, at room temperature. Then, the
137 sections were washed three times (5min/each) in TBS and followed by incubation with horseradish-
138 peroxidase conjugate for 30 min. Horseradish peroxidase activity was visualized by incubating the
139 sections for with a substrate-chromogen solution containing 3,3'-diaminobenzidine
140 tetrahydrochloride (DAB)- H₂O₂ solution for 3 min. Sections then were next washed in double-
141 distilled water and shortly stained with Mayer’s hematoxylin for counterstaining. For negative control
142 sections, TBS was added instead of the primary antibody.

143 Photographs from both immunohistochemical and H&E stained sections were captured using a
144 Sony super steady cyber shot digital camera (Dsc-W800, Sony, Japan) connected to an Olympus BX
145 21 light microscope at Histology and Cytology Department, Faculty of Veterinary Medicine, Zagazig
146 University, Egypt.

147 **2.4 Morphometrical examination**

148 Morphometrical measurements were performed using an Image J analysis free software (Fiji
149 image j; 1.51 n, NIH, USA) to measure the ratio of islet area/pancreatic area, the number of the islets/
150 total pancreatic area in both compact (in mesenteric adipose tissue) and disseminated (that was
151 observed in the wall of the middle portion of the esophagus) pancreas and comparing it among both
152 sexes using photomicrographs captured from H&E stained sections at 100x magnification.
153 Furthermore, we measured the glucagon and insulin positive areas in the compact and disseminated
154 pancreas in both sexes using photographs that have been captured from immune-stained sections of
155 both male and female fish at 400x magnification.

156 **2.5 Statistical analysis**

157 For comparison of the sex differences in the ratio of the islet area/pancreatic area, as well as the
158 positive area ratio of either insulin or glucagon/islet area. In the current investigation, we performed
159 the Mann-Whitney *U* test as a non-parametric test used to compare the non-parametrical
160 Morphometrical values of samples from both male and female. The data have been presented as mean
161 ± standard error (SE) and statistical significance was determined when *P* values ≤ 0.05.

162

163 **3. RESULTS**

164 **3.1 Anatomical features**

165 Morphologically, the adult African catfish has a long cylindrical shaped body, smooth skin
166 bearing no scales with blackish color on the dorsal and lateral surface as well as the grayish color
167 ventral surface. The male can be easily recognized externally from the female via the existence of the
168 sexual papilla in the urogenital region. Such sexual papilla was observed as a nipple-like projection
169 just behind the anal opening in males (supplementary Figure 1A) but was absent in females
170 (supplementary Figure 1D). Furthermore, internally the male testes appear creamy white color
171 (supplementary Figure 1B), and the female ovary had a brownish-red color (supplementary Figure
172 1E). The compact pancreas was observed to be concealed in mesenteric adipose tissue located in the
173 triangular area between the spleen, stomach, and liver (supplementary Figures 1E and 1F). The
174 esophagus of catfish is short, extending anteriorly from the pharynx and continued caudally in the
175 cardiac region of the stomach. The esophagus of catfish was divided into 3 portions (anterior, middle,
176 and posterior) according to shape and location. The anterior portion appeared as funnel-shaped, while
177 the other portions were tubular in shape. The posterior portion is covered by one of the liver lobes,
178 however the middle portion was not covered and began after the end of the funnel-shaped anterior
179 portion (supplementary Figures 1B and 1F).

180 **3.2 Histological observations**

181 **3.2.1 Histomorphometrical characterization of the compact pancreas**

182 The compact pancreas was observed to be embedded in the mesenteric adipose tissue. It consisted
183 of a poorly demarcated lobulated gland, with very delicate connective tissue (CT) septa extending
184 from a thin CT capsule. The parenchyma of the pancreas comprised of exocrine and endocrine parts.
185 The exocrine unit consists of tightly packed pancreatic acini and duct system. The endocrine portion
186 appeared as pale staining islets distributed among the exocrine tissue (Figures 1A to 1D). The
187 pancreatic islets in female were oval to ovoid-shape, but that of a male was of irregular shape and of
188 an apparently smaller size than that of females (Figures 1C and 1D). Interestingly, the average area
189 ratio of islet/pancreatic area was significantly higher in the female than that of males (Figure 1E). On
190 the contrary, the male showed higher numbers of islets/pancreatic areas but of non-significant
191 differences (Figure 1F).

192 **3.2.2 Histological characterization of various esophageal regions**

193 As shown in (supplementary Figure 2), the wall of the esophagus consisted of 4 tunics (mucosa,
194 submucosa, muscular, and adventitia). Histologically, the anterior, middle and posterior portions of

195 the esophagus could be distinguished by their mucosal folds, lining epithelium, luminal area. The
196 anterior portion of the catfish esophagus is characterized by the presence of highly folded mucosa
197 (elongated leaf-like folds that were consisted of primary, secondary, and tertiary folds) with a narrow
198 lumen ($251.67 \pm 14.84 \mu\text{m}$). The lining epithelia of such folds was characterized by the presence of
199 large polyhedral shaped club cells that showed a negative PAS reaction, as well as numerous PAS
200 positive goblet cells (supplementary Figure 2A and 2B). However, a moderate size lumen
201 ($573.82 \pm 43.76 \mu\text{m}$) and less folded mucosa was observed in the middle portion of the esophagus than
202 that of the anterior portion. Furthermore, the lining epithelia characterized by the absence of club
203 cells as well as less PAS positive goblet cells. Interestingly, a disseminated pancreatic tissue was
204 observed in their adventitial layer, in close contact with their muscular layer. Such pancreatic tissue
205 consisted of both endocrine and exocrine tissue similar to that of the compact pancreas
206 (supplementary Figure 2C and 2D). The posterior part of esophagus has the widest lumen
207 ($1235.61 \pm 74.76 \mu\text{m}$) and characterized by the absence of such disseminated pancreatic tissue
208 (supplementary Figure 2E and 2F).

209 **3.2.3 Histomorphometrical characterization of the disseminated (esophageal) pancreas**

210 The disseminated pancreas in catfish was noted to be in close contact with the tunica muscularis
211 of the middle portion of the esophagus in both sexes, where it was observed to be dispersed in their
212 adventitia (Figure 2A to 2D). Similar to the compact pancreas, the disseminated pancreas consisted
213 of exocrine and endocrine portions. The parenchyma of the former portion consisted of serous
214 secretory acini and excretory duct. The endocrine portion consisted of islets of Langerhans. The shape
215 of such islets was irregular to ovoid in males and oval to ovoid in females. Similar to the compact
216 pancreas, the morphometric measurements revealed a significantly higher ratio of the islet`s of
217 Langerhans area/ pancreatic area in the disseminated pancreas of the female than that of the male.
218 However, contrary to the compact pancreas, the female showed a higher percentage of islet`s number/
219 disseminated pancreatic area ratio than that of male but of non-significant difference (Figure 2E and
220 2F).

221 **3.2.4 Immunohistochemical examination of glucagon positive alpha cells and insulin positive 222 β -cells**

223 In the compact pancreas, the glucagon immune-positive alpha cells were spherical to spindle-
224 shaped and showed cytoplasmic processes in both sexes. In a male, such cells were located in the
225 peripheral area of the pancreatic islet and few cells were observed in the mantle region (Figure 3A
226 and 3C). However, in the female such cells were observed in both periphery, mantle, and in the center
227 and central region of the islet (Figure 3B and 3D). Statistical analysis showed a significant higher

228 glucagon immune-positive area ratio/total islet's area in the female than that of the male (Figure 3E).
229 In the disseminated (esophageal) pancreas, the glucagon immunoreactive cells were arranged in the
230 peripheral and the mantle region of the islet but were absent from the islet core (Figure 4A to 4D).
231 Similar to the compact pancreas, the female showed significantly higher glucagon positive area ratio/
232 total pancreatic islet's area in the esophageal adventitia than that of males (Figure 4E).
233 Figure 5A to 5D showed spindle-shaped insulin immunoreactive β cells occupying most of the islet
234 center. Furthermore, in males, a small cluster of beta cells was occasionally observed between the
235 exocrine acini (Figure 5C). Morphometrical analysis of the insulin positive area ratio/ islet's area
236 within the compact pancreas, revealed a significantly higher ratio of the females than that of males
237 (Figure 5E).
238 Examination of the islets within the esophageal pancreas denoted that the insulin immunoreactive
239 cells were located mainly in the islet's center as well as, occasional localized in the mantle region
240 (Figure 6A to 6D). Statistical analysis clarified that the insulin positive area ratio/ islet's area within
241 the esophageal pancreas was significantly higher in females than that of males (Figure 6E).

242

243 **4. DISCUSSION**

244 Catfish is one of the freshwater teleost fish that live near to the bottom. It is an omnivorous type
245 that has a convoluted tubular gastrointestinal tract (short esophagus, stomach, and followed by
246 intestine) and is known as stomach teleost (Lee et al., 2001; Mello et al., 2019; Moawad et al., 2017).
247 In fish, it has been reported that the alimentary pattern could affect hormonal secretion (Navarro et
248 al., 2002). Furthermore, two forms of the pancreas (compact and disseminated) were reported in
249 various fish species (Chen et al., 2006; Luchini et al., 2015; Mokhtar, 2015; Tocher et al., 2008;
250 Youson et al., 2006), however, reports explaining the localization and morphology of disseminated
251 portion in catfish remain scarce.

252 The present study provided a unique localization of the dispersed pancreas in the esophageal wall
253 of catfish (middle esophageal portion). Moreover, we revealed the sex-related difference in the
254 endocrine portion. Histologically, we could identify three portions of the esophagus in catfish
255 (anterior, middle, and posterior) according to lumen size, mucosal folds, and lining epithelium.
256 Conversely, only two esophageal portions (anterior and posterior) were reported in both catfish and
257 grass carp (Abd El Hafez et al., 2013). The anterior esophageal portion is characterized by highly
258 folded mucosa with a narrow lumen. The lining epithelia of the folds has large polyhedral club cells
259 and goblet cells. This was inconsistent with previous report by (Abd El Hafez et al., 2013). However,
260 in the anterior portion of sea bream esophagus, the club cells were absent and only two types of goblet
261 cells (mature and immature) was reported in (Abidi and Parwez, 2015) and (Abumandour and El-

262 Bakary, 2018). The middle and posterior portions showed moderate, and large size lumen, less folded
263 mucosa than anterior part, with the absence of club cells and a smaller number of PAS-positive goblet
264 cells. Interestingly, our results clarified a unique localization of the disseminated pancreas in the
265 adventitia of the middle portion of the esophagus in both sexes. However, in *Labeo calbasu*, Ghosh
266 and Chakrabarti, 2016 reported the presence of the exocrine pancreatic acini and ducts within the
267 hepatic parenchyma tissue as hepatopancreas and in spleen as spleenopancreas. In *Mystus gulio*, the
268 same authors stated that the disseminated pancreas was attached to the outer wall of the stomach.

269 Histologically, both compact and disseminated pancreas consisted of poorly lobulated glands with
270 very delicate connective tissue septa. Each lobule is composed of exocrine and endocrine portion.
271 The exocrine portion consisted of secretory pancreatic acini and pancreatic duct. Similar findings
272 were reported in the compact pancreas (Mokhtar, 2015; Naguib et al., 2009; Sheibani and Pahlavan
273 Yali, 2006). The endocrine portion appeared as pale staining islets distributed among the exocrine
274 tissue, similar to that recorded in other previous reports (Chakrabarti and Ghosh, 2015; Luchini et al.,
275 2015). Endocrine tissue consisted of Large principal islet and numerous smaller islets, as in most
276 previous studies (Groff and Youson, 1997; Stefan and Falkmer, 1980). The islets of Langerhans were
277 responsible for the secretion of insulin hormone by beta cells to maintain blood glucose level normal.
278 Alpha cells were responsible for the secretion of glucagon hormone that works to raise the
279 concentration of glucose and fatty acids in the bloodstream (Geser, 1976). On the other hand, it has
280 been reported that the islet organ in the hagfish consisted only of insulin and somatostatin cells with
281 absence of glucagon cells (Heller, 2015).

282 The present study revealed an oval to ovoid shaped islets in the female pancreas and of irregular
283 shaped in males, similar to that reported in grass carp (Mokhtar, 2015) and in lake van fish (Kaptaner,
284 2019), but showed large circular shaped in both sexes of the toadfish pancreas (Palazón-Fernández et
285 al., 2011). Islet cells were spherical and spindle in shape, as in red-eared slider (Ku et al., 2001). A
286 recent report from humans and animal models revealed sexual difference in the ratio of different
287 hormone-secreting cells in the pancreas of the same species (Parchami and Kusha, 2015).
288 Furthermore, it has been reported that prolactin secreted from the pituitary gland and placental
289 lactogen during pregnancy contributes to the expansion of β -cell mass (Huang et al., 2009).
290 Additionally, rapid enlargement of β -cell mass was observed during pregnancy (Gannon et al., 2018).
291 Similarly, in the current investigation, significant higher ratio of the area of pancreatic islet was
292 observed in female catfish than that of males. Additionally, the current investigation revealed
293 significant higher glucagon and insulin positive area in the islets of female catfish pancreas than that
294 of males. These results were inconsistent with other reports of an increase in glucagon content in

295 pancreases of female mice (Vagn Bonnevie-Nielsen, 1980, 1982). Therefore, we suggested that more
296 hormonal secretion from the female islets could be associated with the ovulation and spawning period.

297 In the male, glucagon positive cells were detected in peripheral and mantle regions of the islets
298 (Lee et al., 2003). On the contrary, in the female, the alpha cells were observed to be arranged mainly
299 in the peripheral, mantle. However, few cords were detected in the center of the islet. This was
300 consistent with other reports in *Lepisosteus osseus*, (Youson et al., 2001); in *Amia calva*, (Kong et al.,
301 2002) in *Cyprinus Carpio*, and in splenic pancreatic lobe of ddN mouse (Lee et al., 2010). Moreover,
302 similar to that reported in Bowfin (Scheuermann et al., 1991) and in *Silurus asotus* and *Siniperca*
303 *scherzeri* (Lee et al., 2001), the insulin-immunoreactive cells in both compact and disseminated
304 pancreas, were localized mainly in the center of the islet in both sexes. On the contrary, the beta cells
305 were reported to be localized in the periphery of pancreatic islets in angler fish (Johnson et al., 1976)
306 and in *Mugil auratus* and *Mugil saliens* (Lozano and Agulleiro, 1986).

307

308 **5. CONCLUSION**

309 In the catfish (*Clarias gariepinus*), two types of pancreas were observed; compact and disseminated
310 one. The former was detected pancreas in the triangular area between liver, stomach, spleen and
311 gallbladder. The later type was embedded in the adventitia of the middle portion of the esophagus.
312 Interestingly, we clarified some regional distribution of both insulin and glucagon positive cells in
313 the pancreatic islets of both types. Additionally, we reported sex-related differences in the size of the
314 islet's as well as the positive area ratio of both insulin and glucagon cells with more significant values
315 in the females than that of males.

316

317 **Data availability statement**

318 on what request the data that is presented in this manuscript will be available to the readers. It can
319 be on "requesting the corresponding author request".

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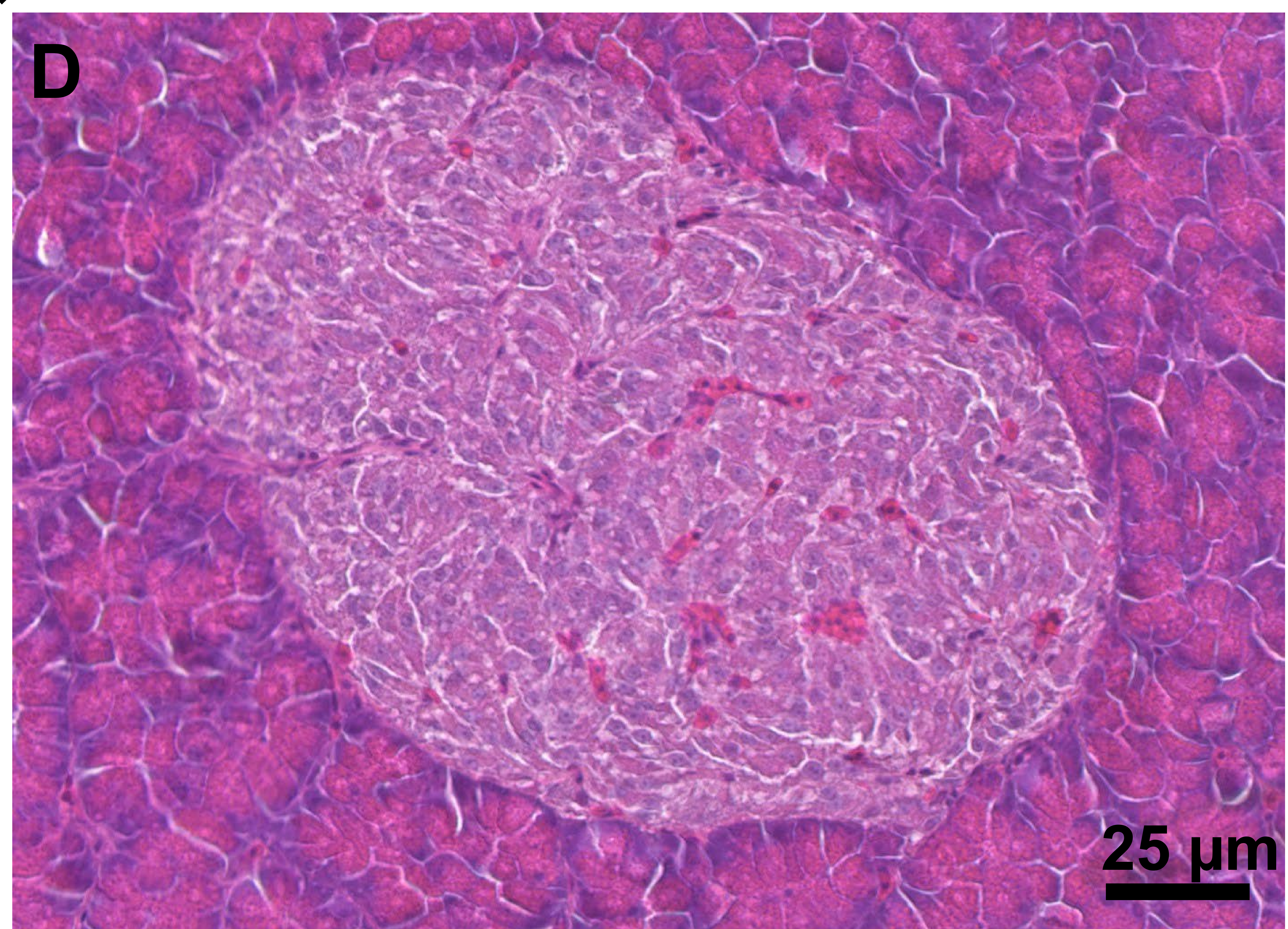
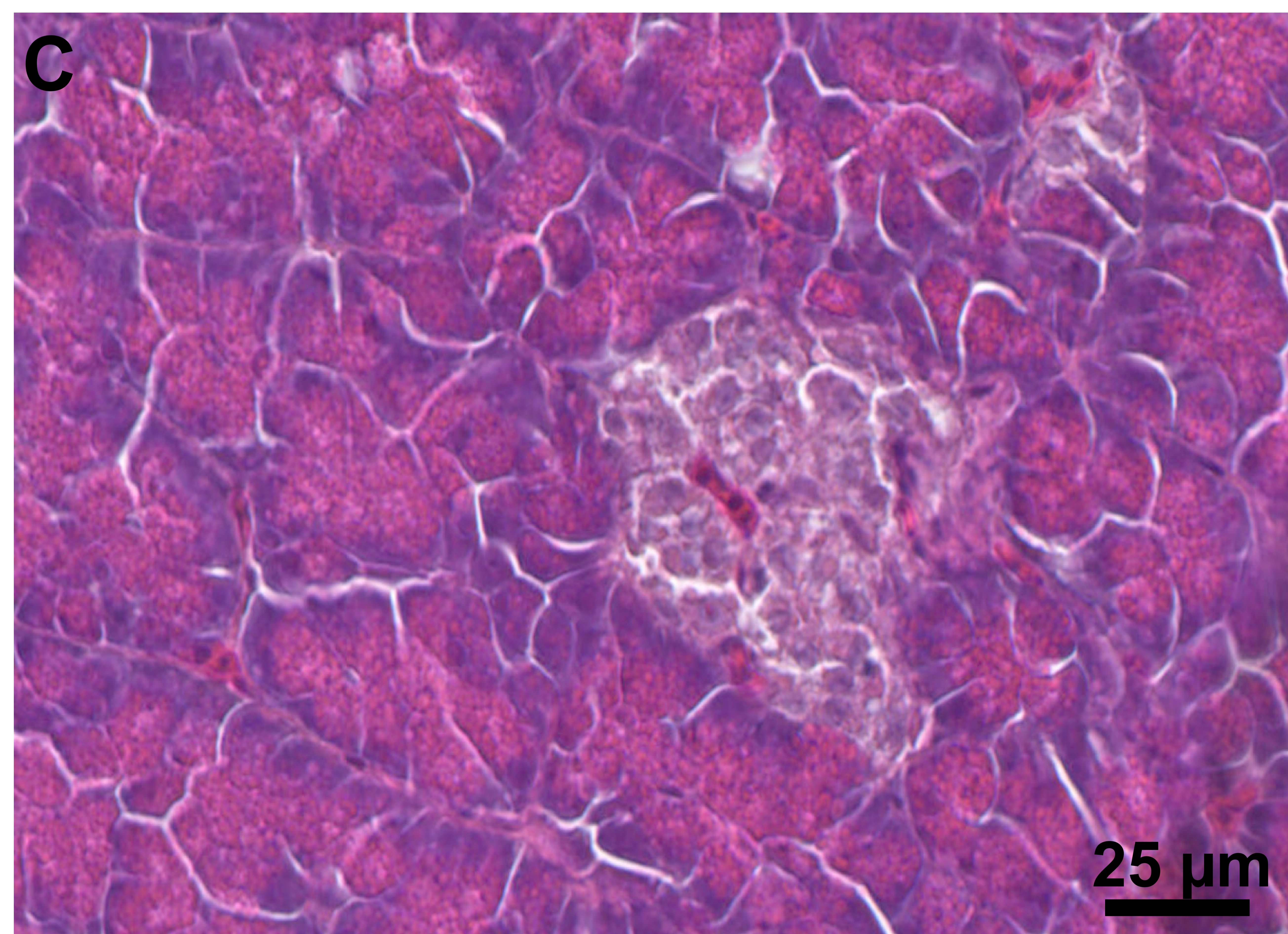
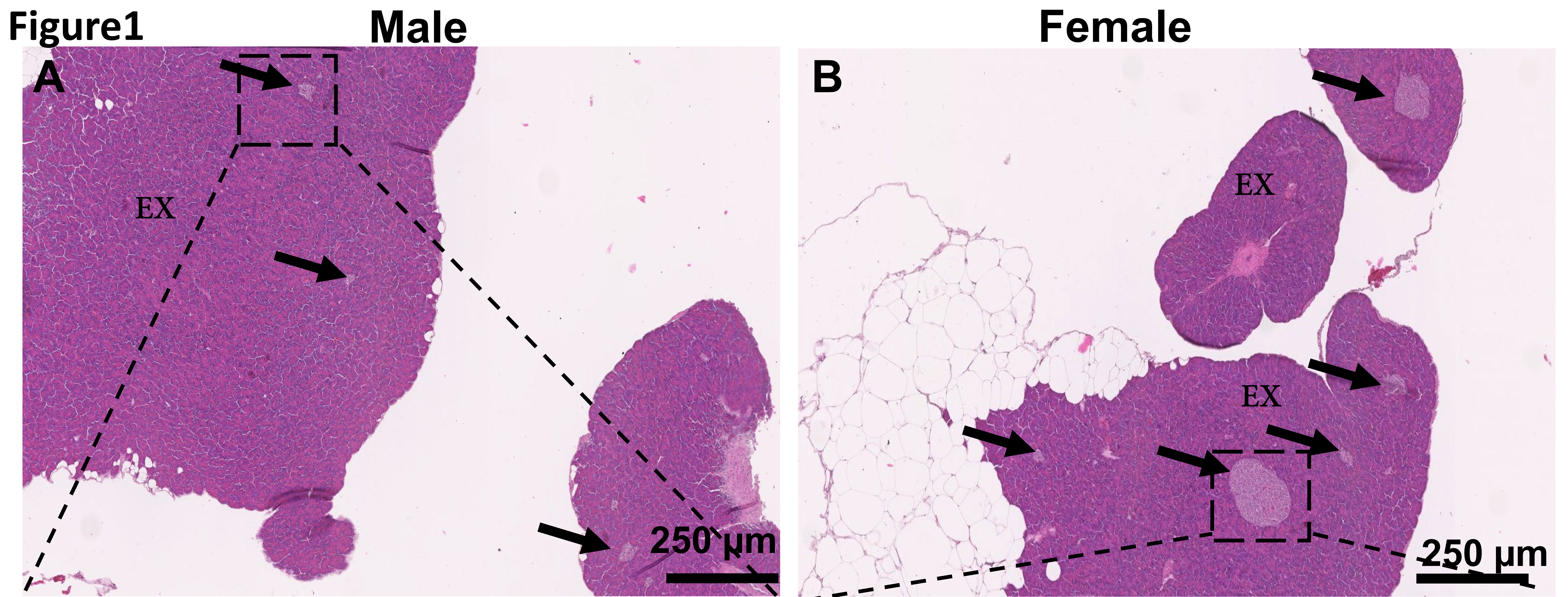
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E Ratio of islet area/ total pancreatic area

F Islet number/ total pancreatic area

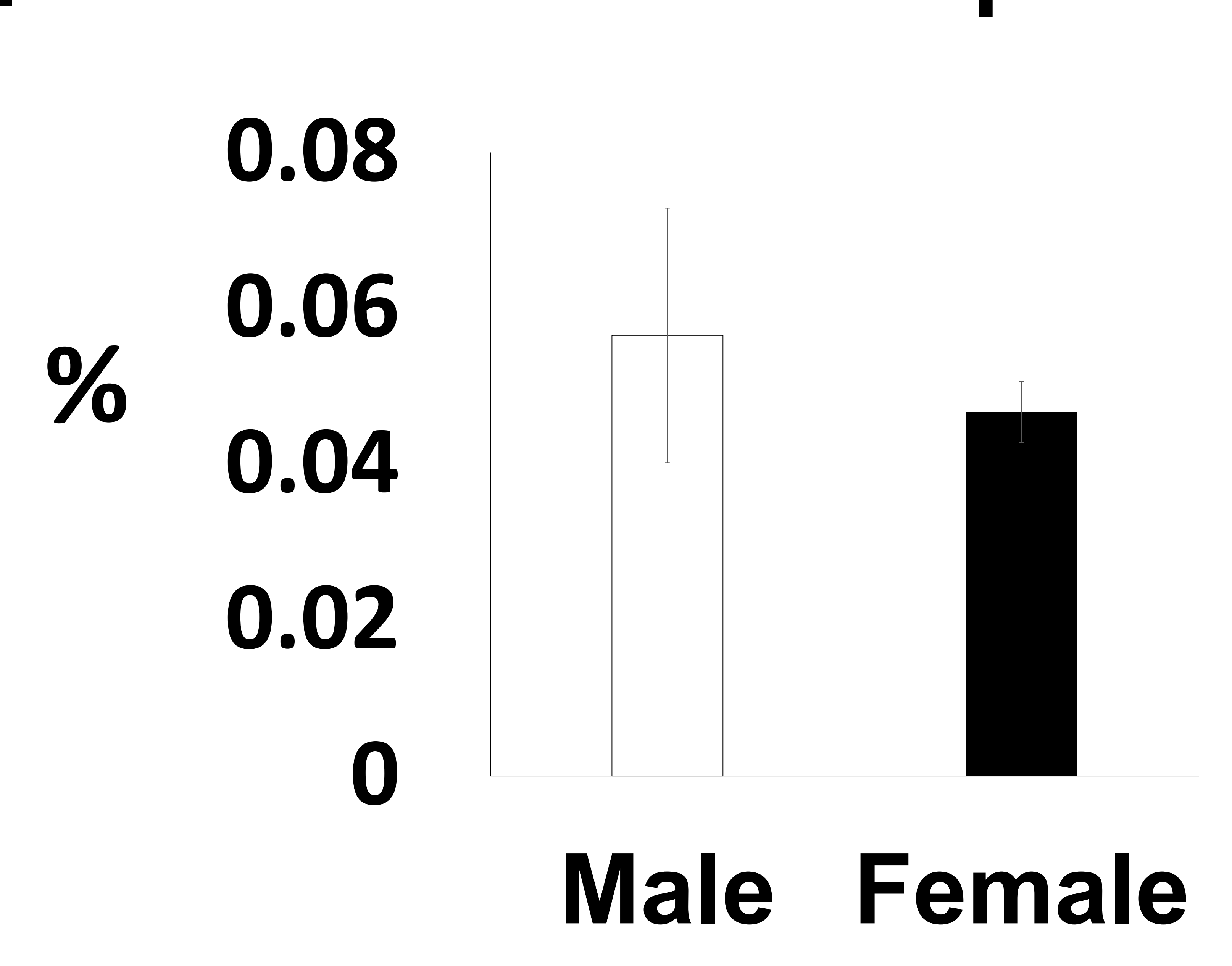
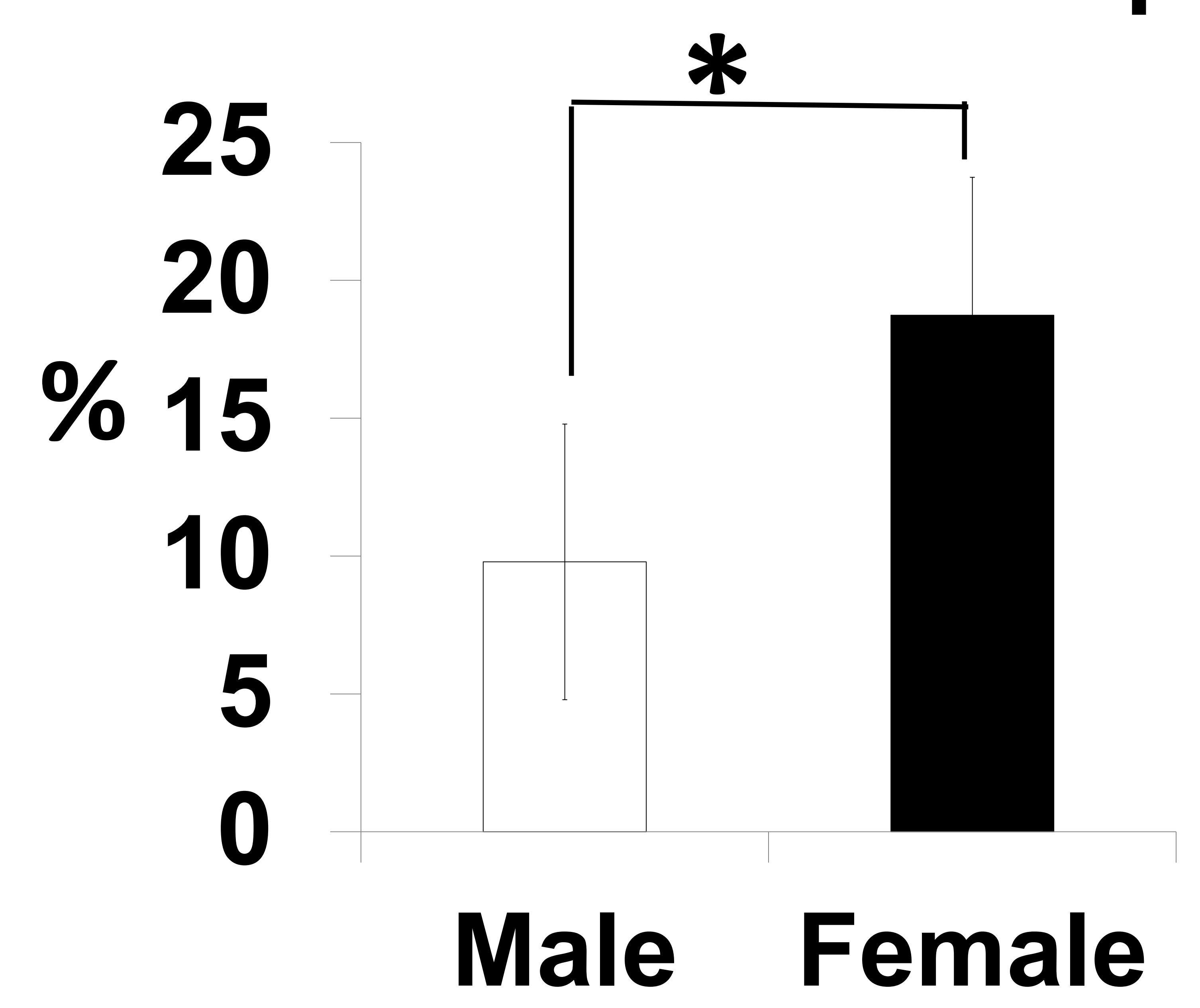


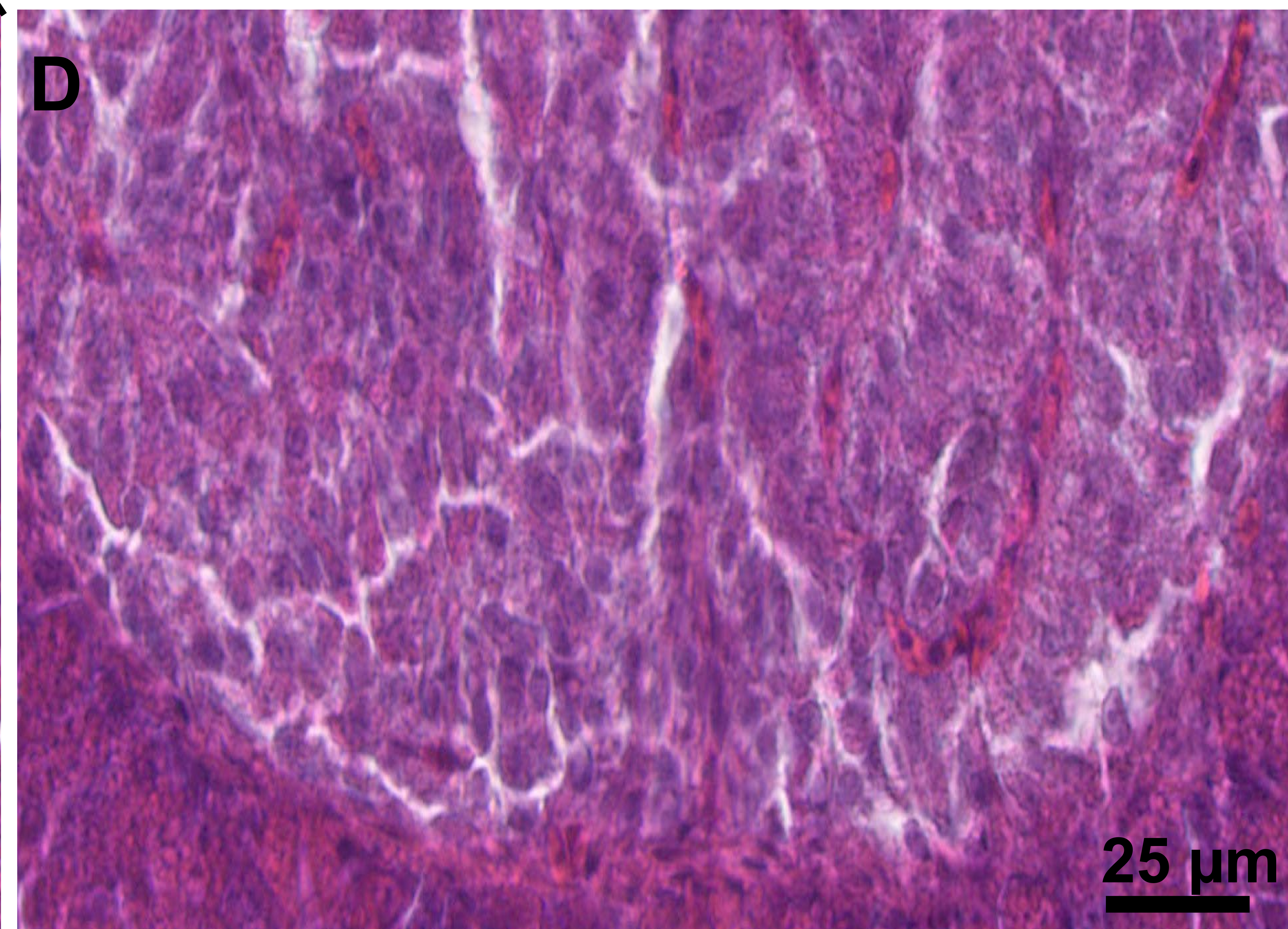
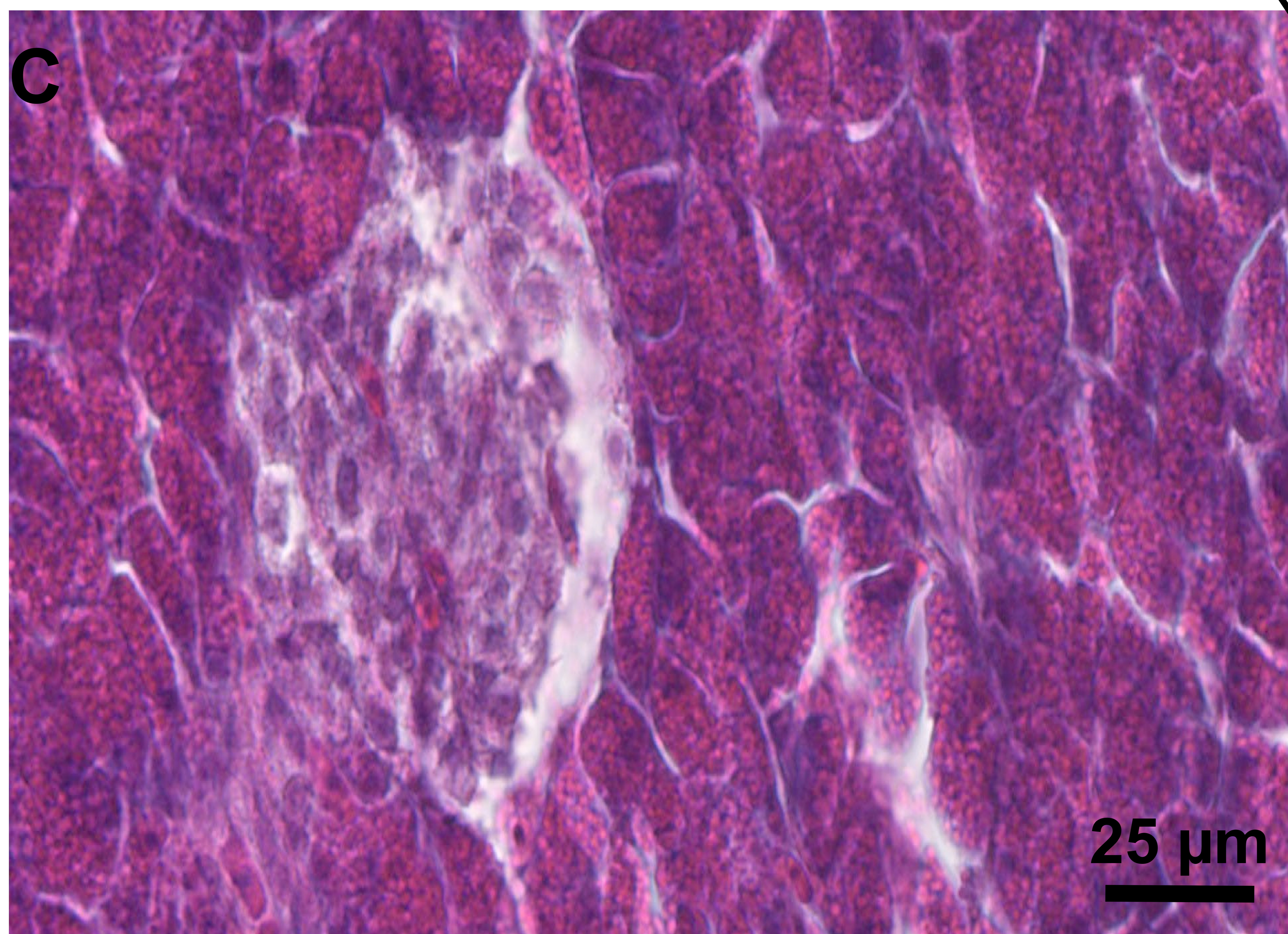
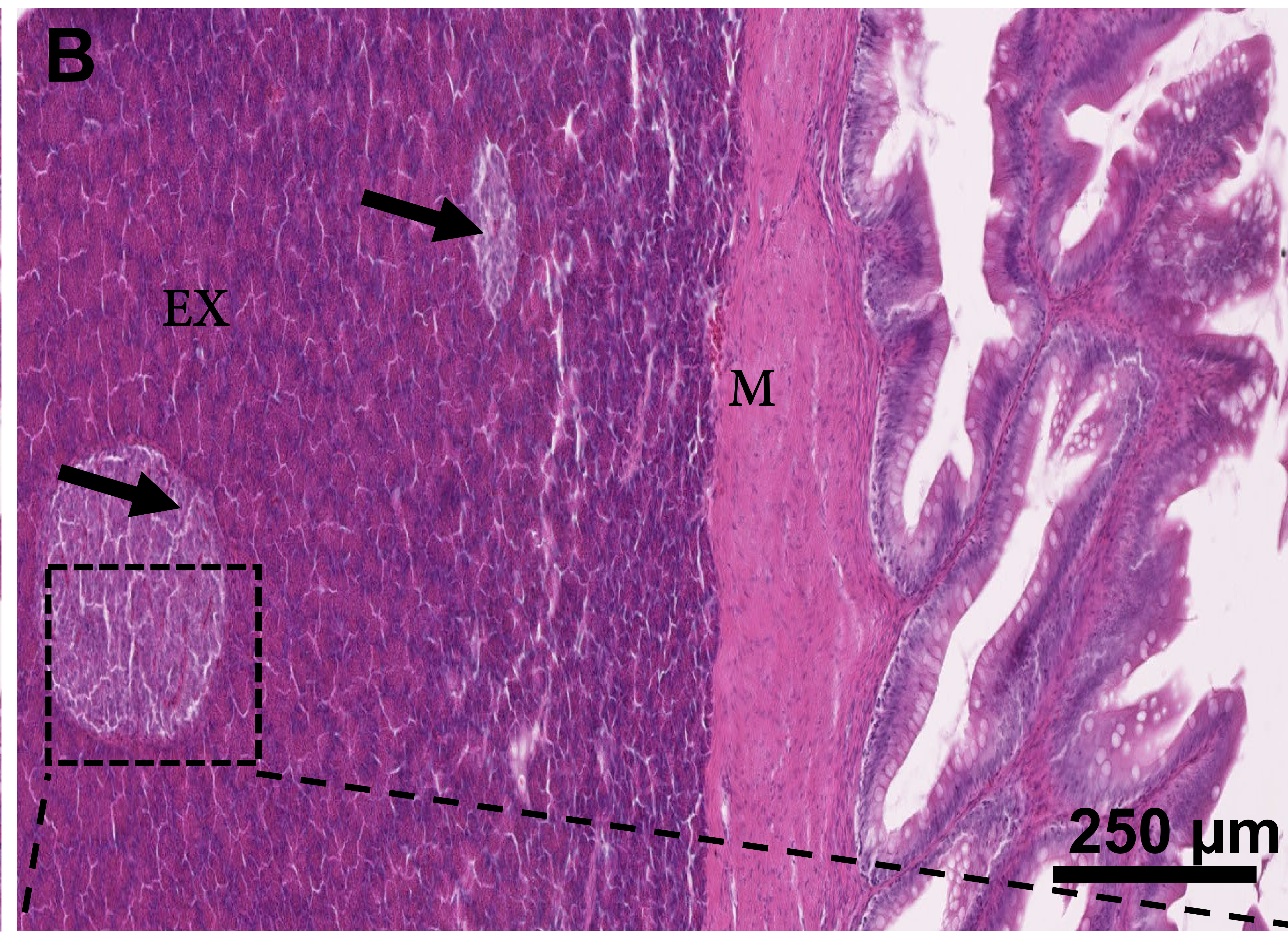
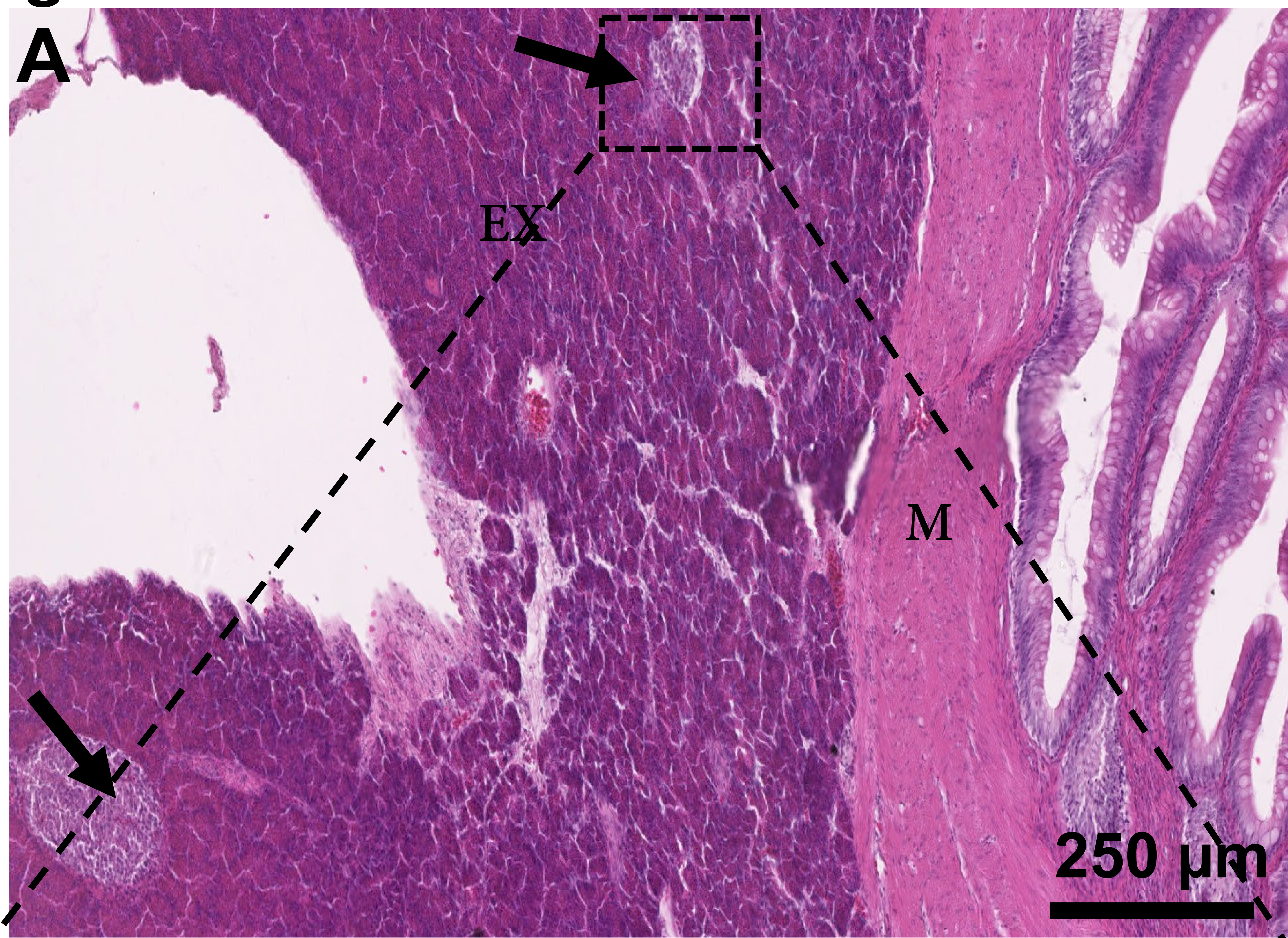
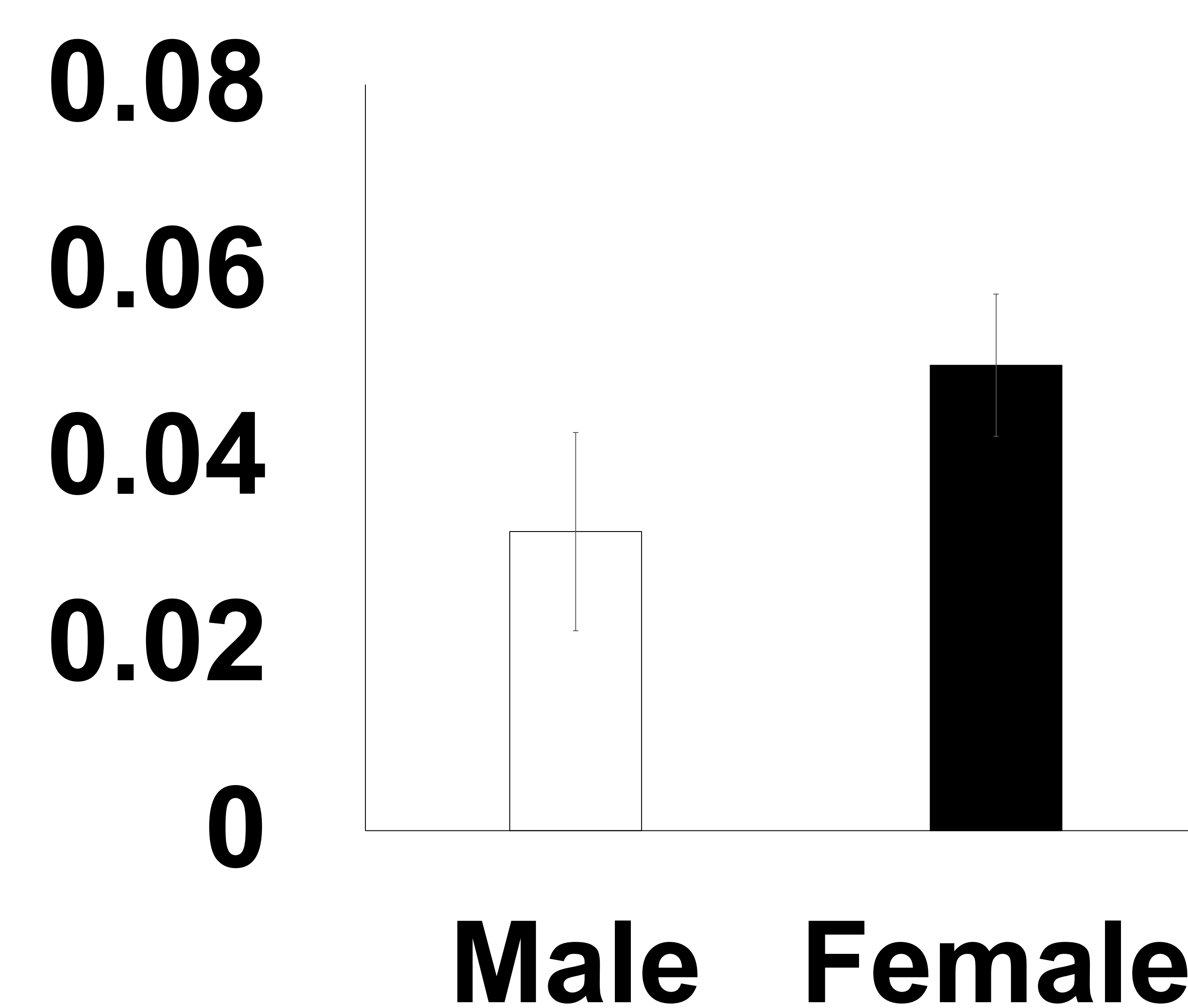
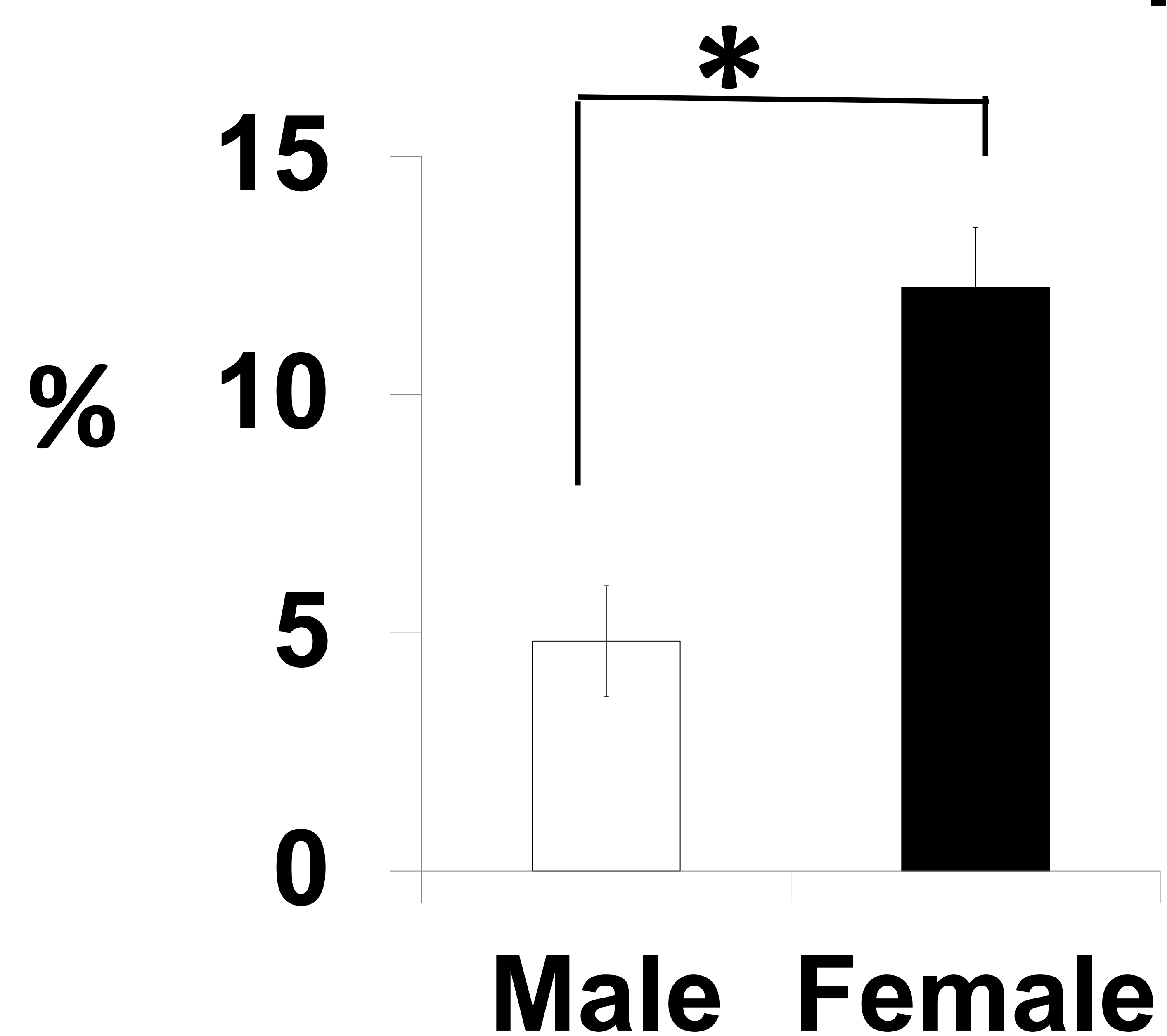
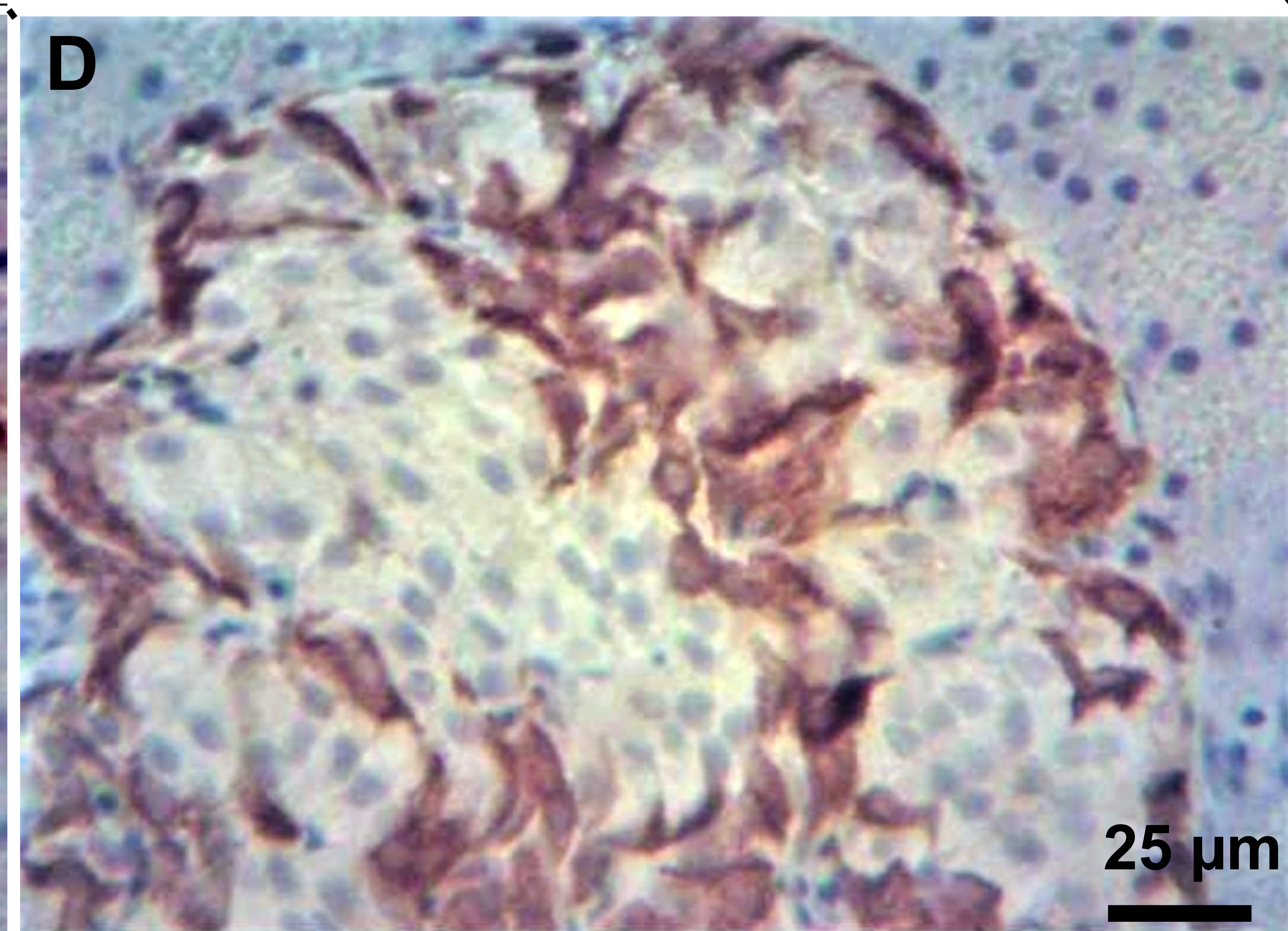
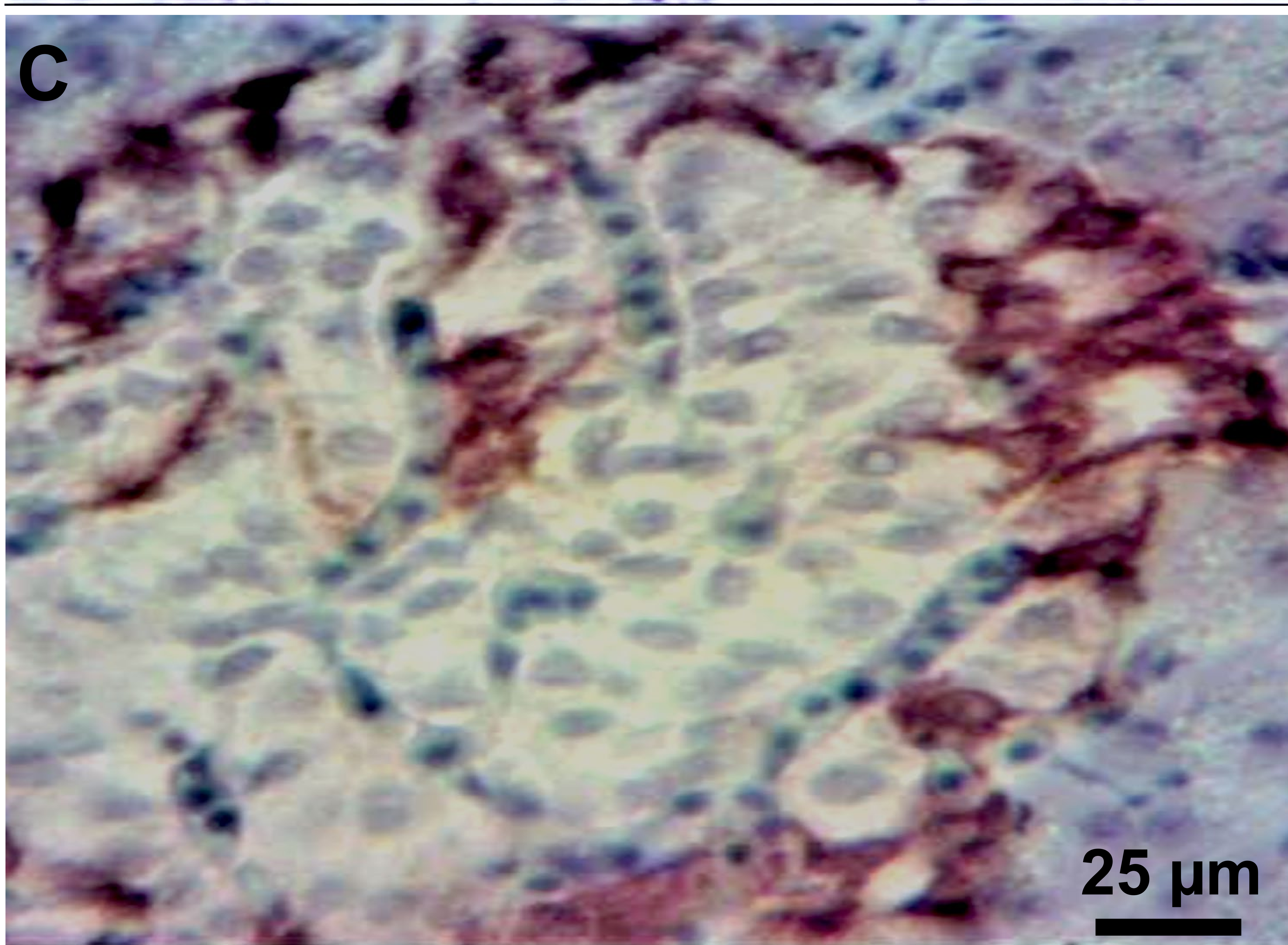
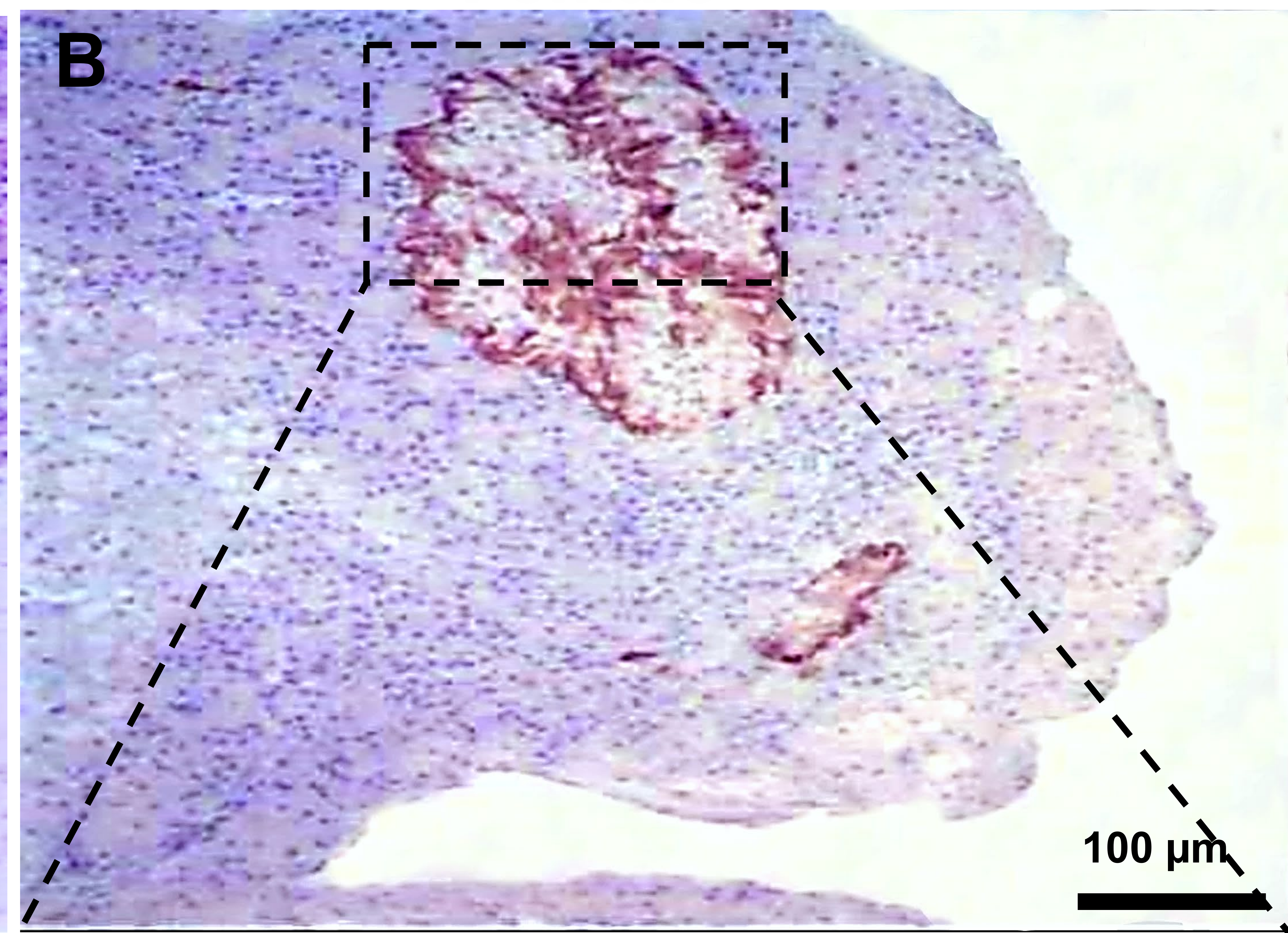
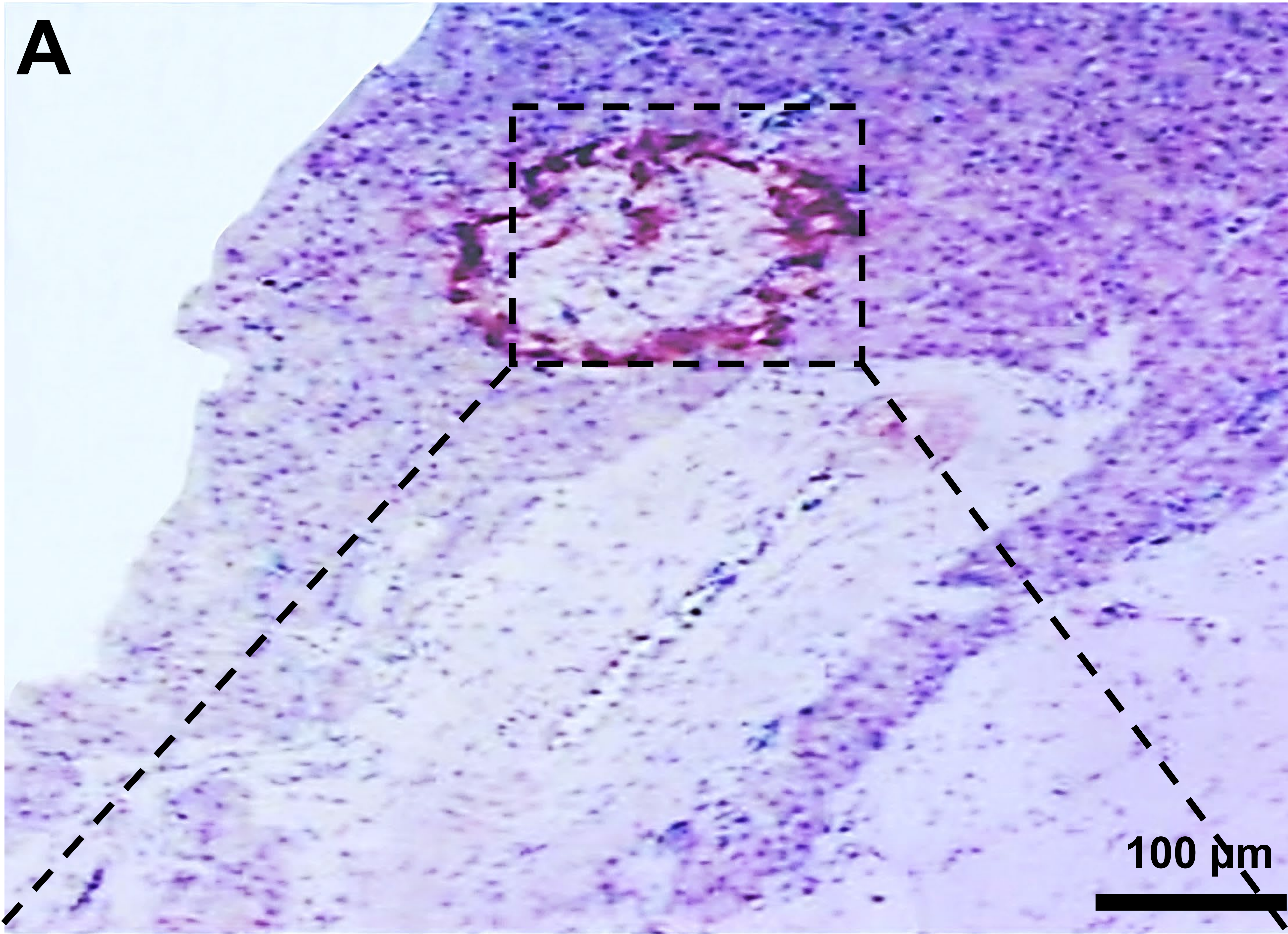
Figure 2**Male****Female****E Ratio of Islet area/ total pancreatic area****F Islet number/ total pancreatic area**

Figure 3

Male

Female



E Ratio of glucagon positive area/ islet area in compact pancreas

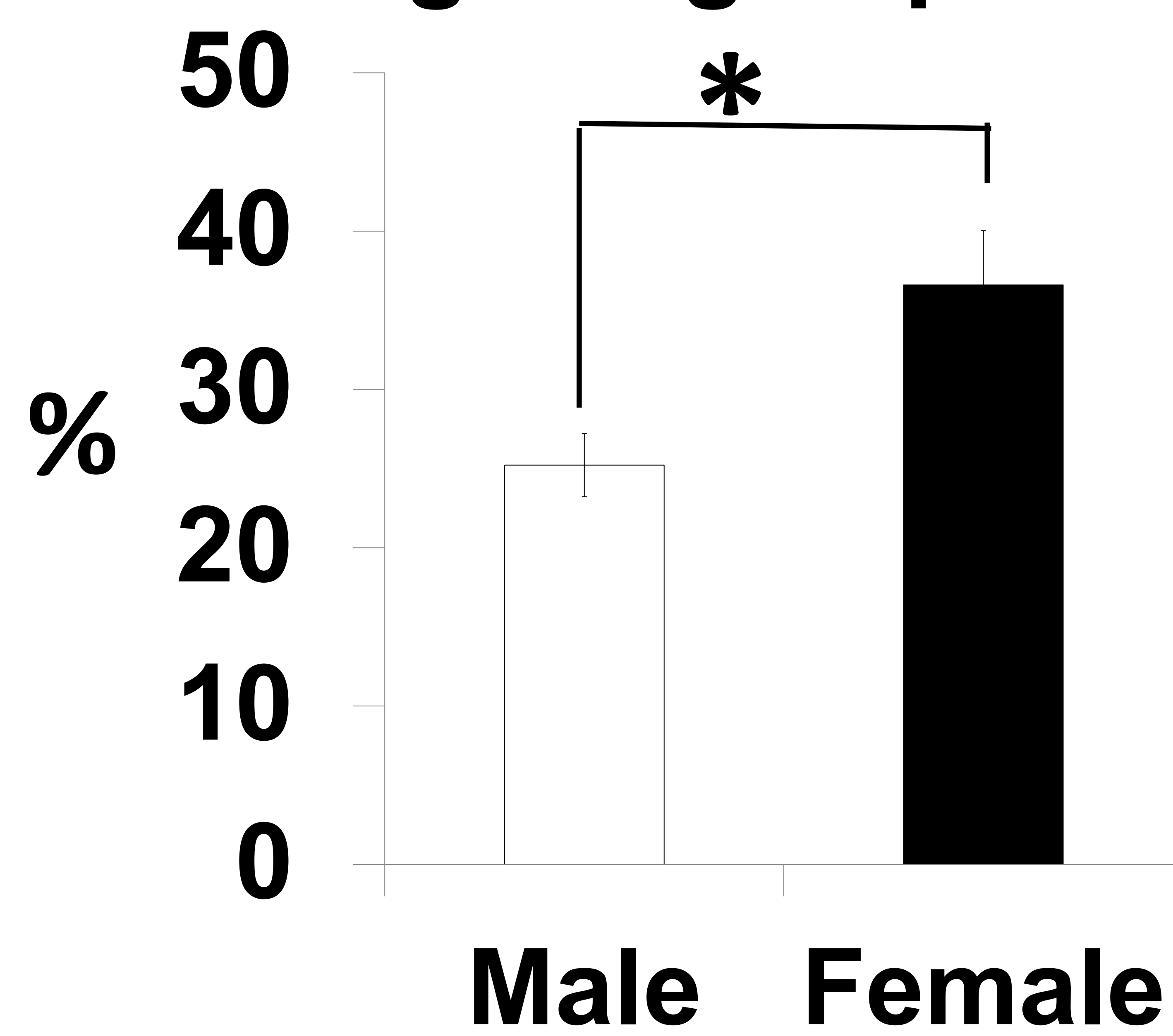
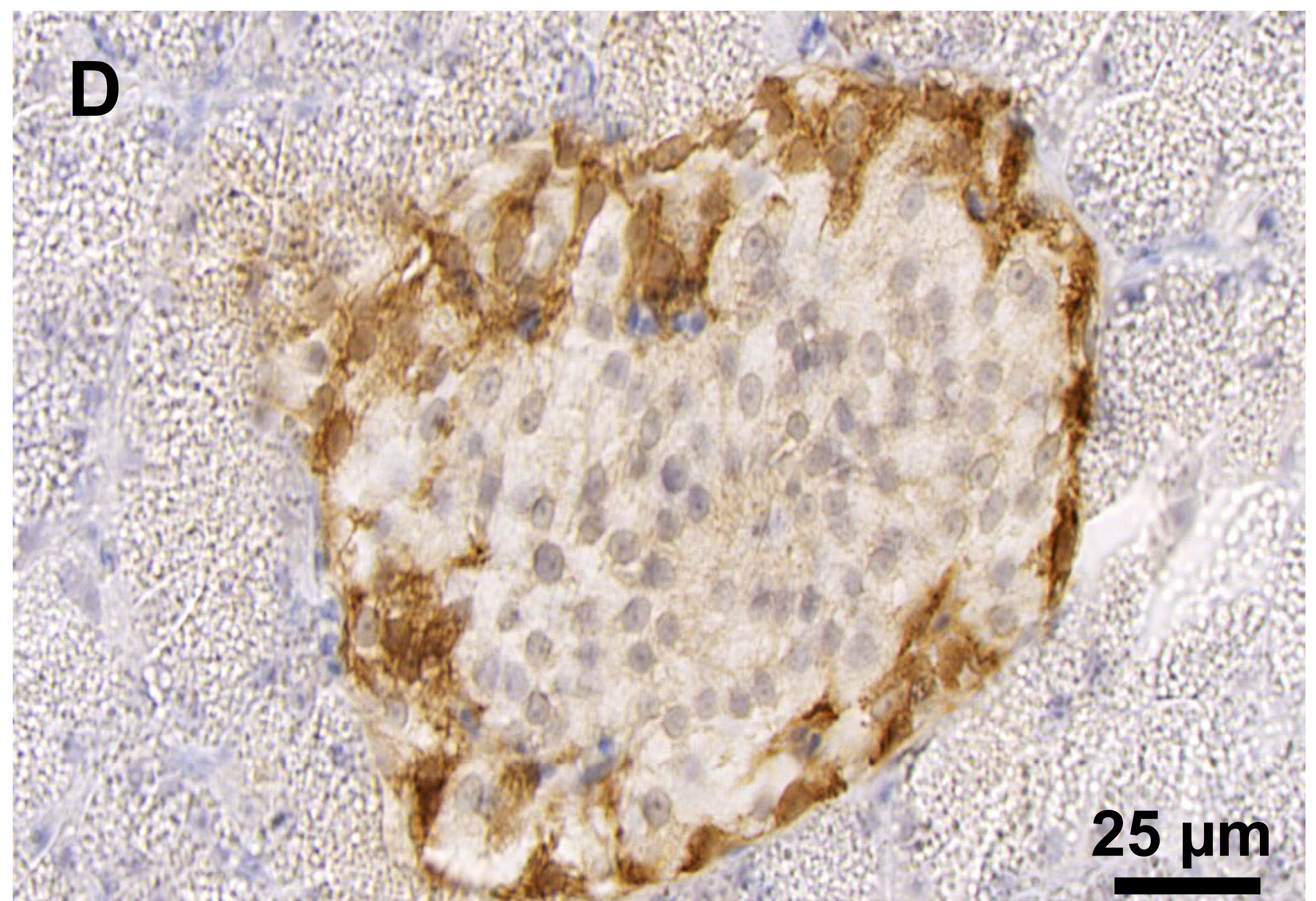
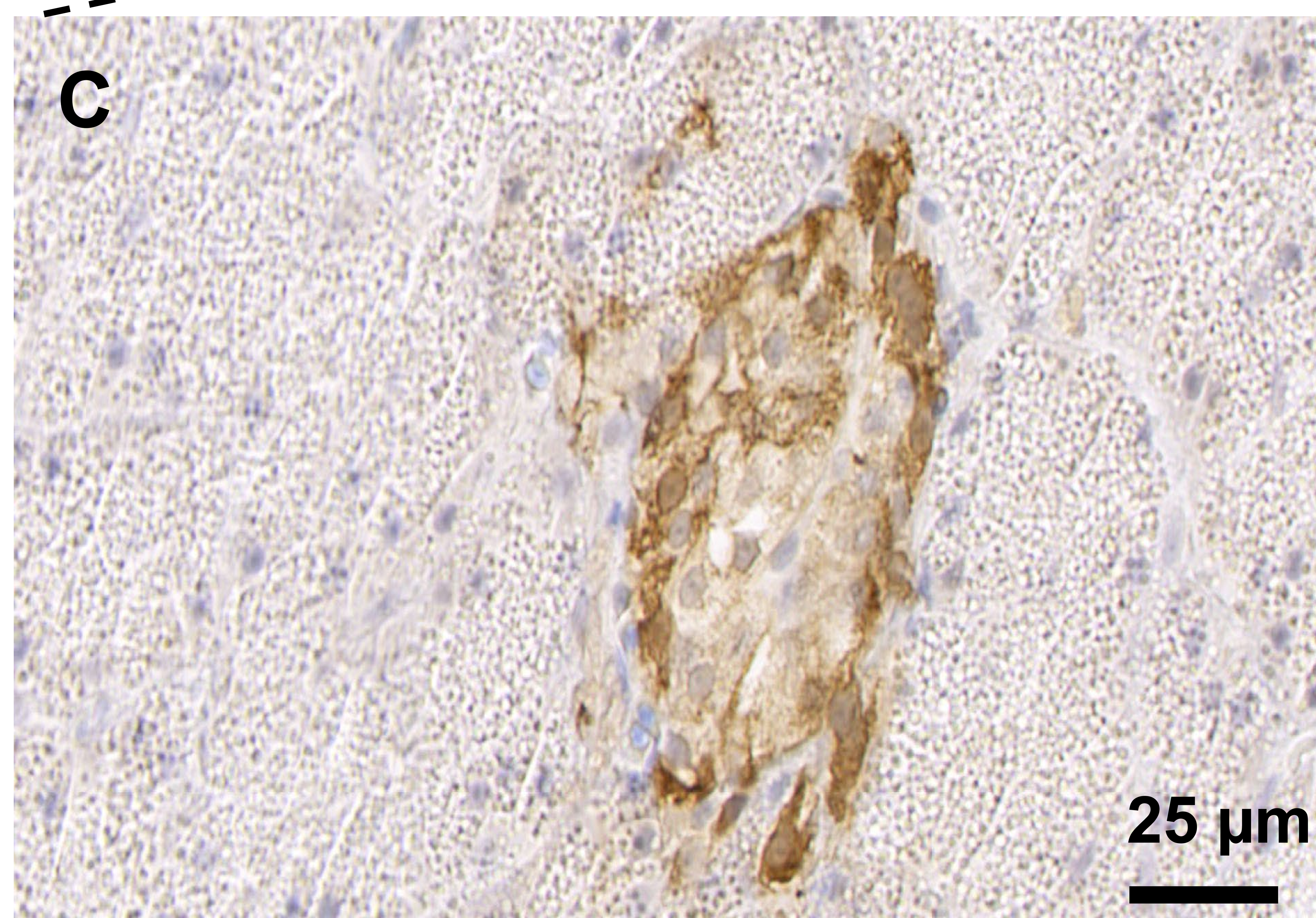
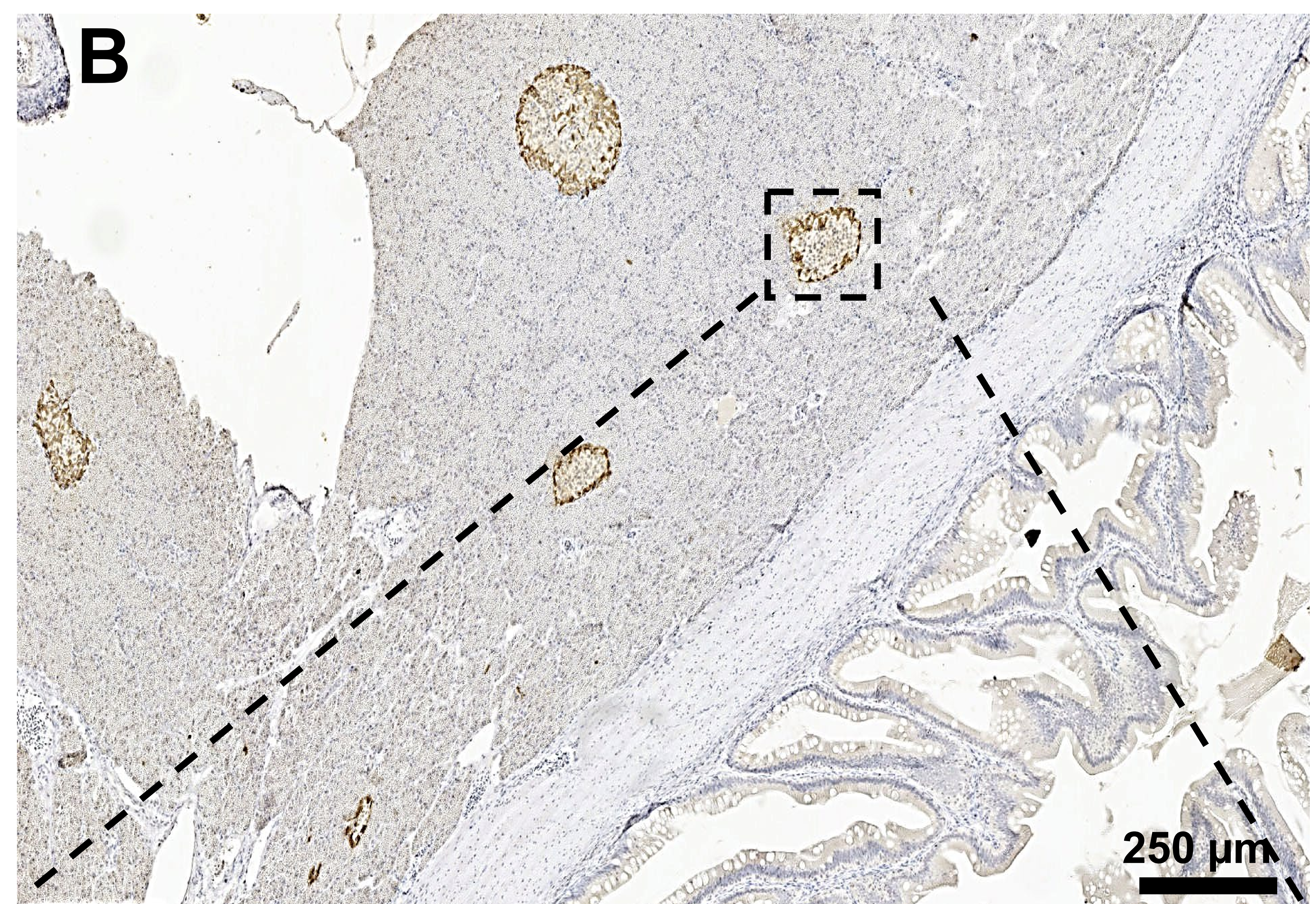
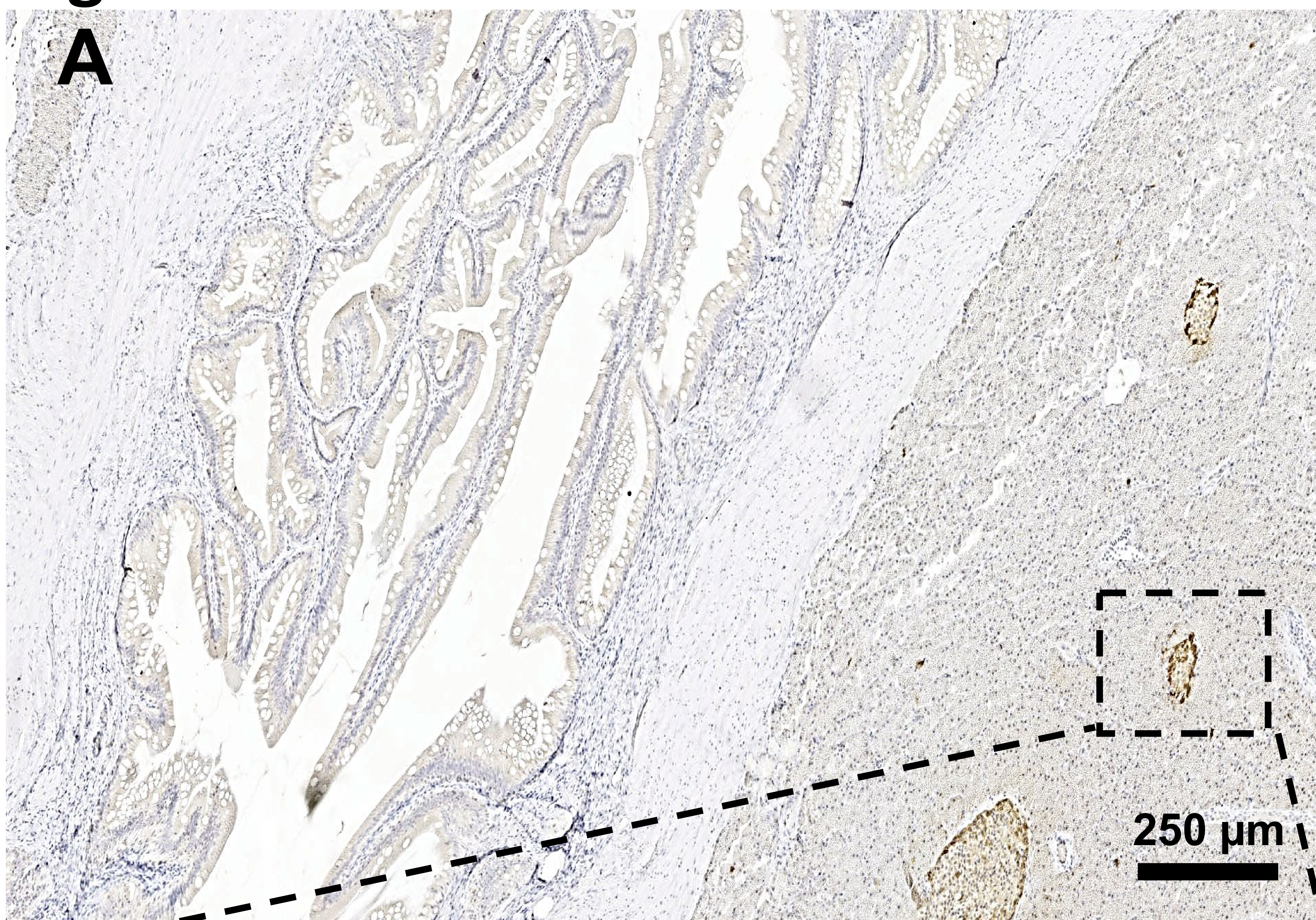


Figure 4

Male

Female



E Ratio of glucagon positive area/ islet area in esophageal disseminated pancreas

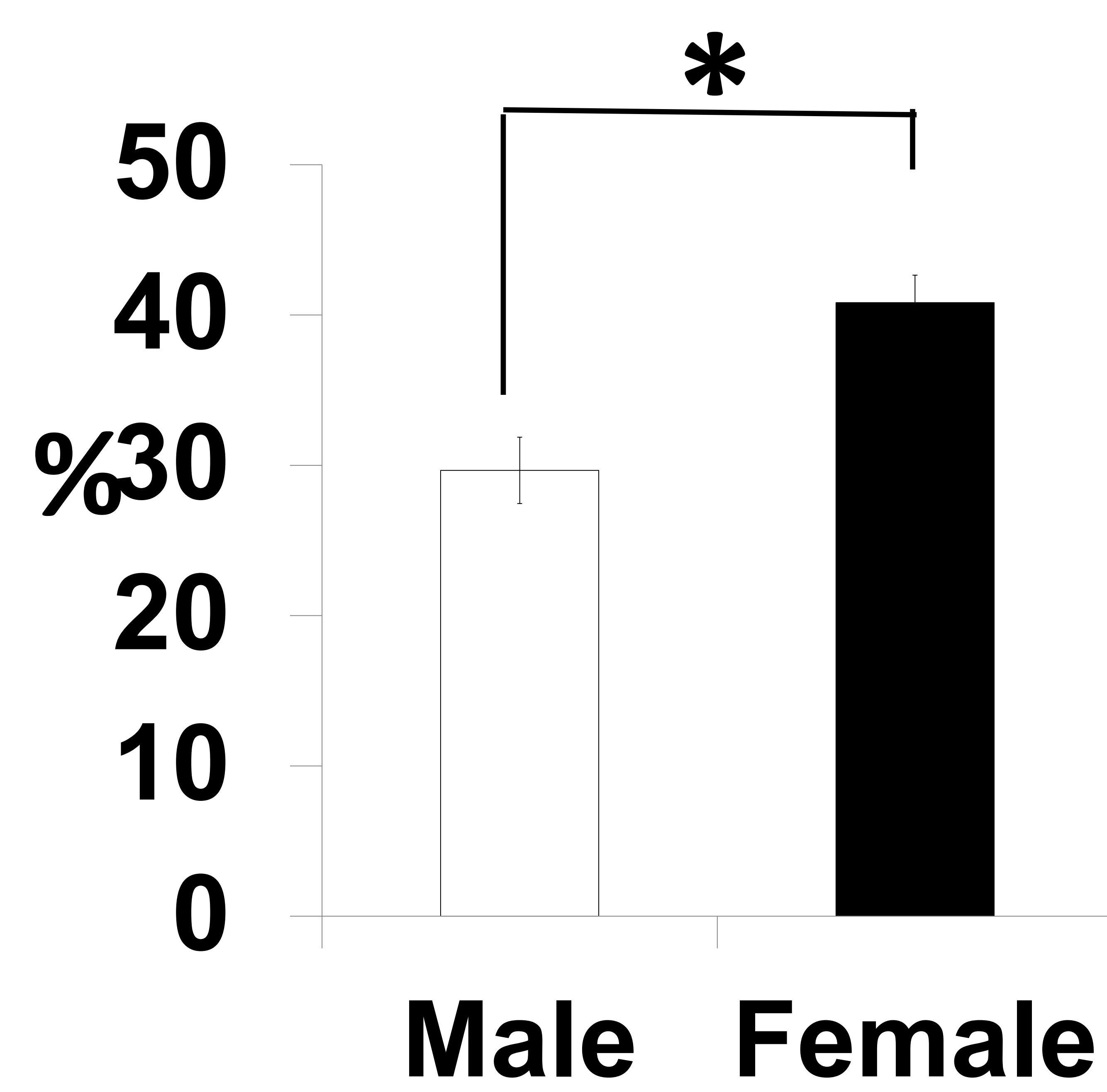
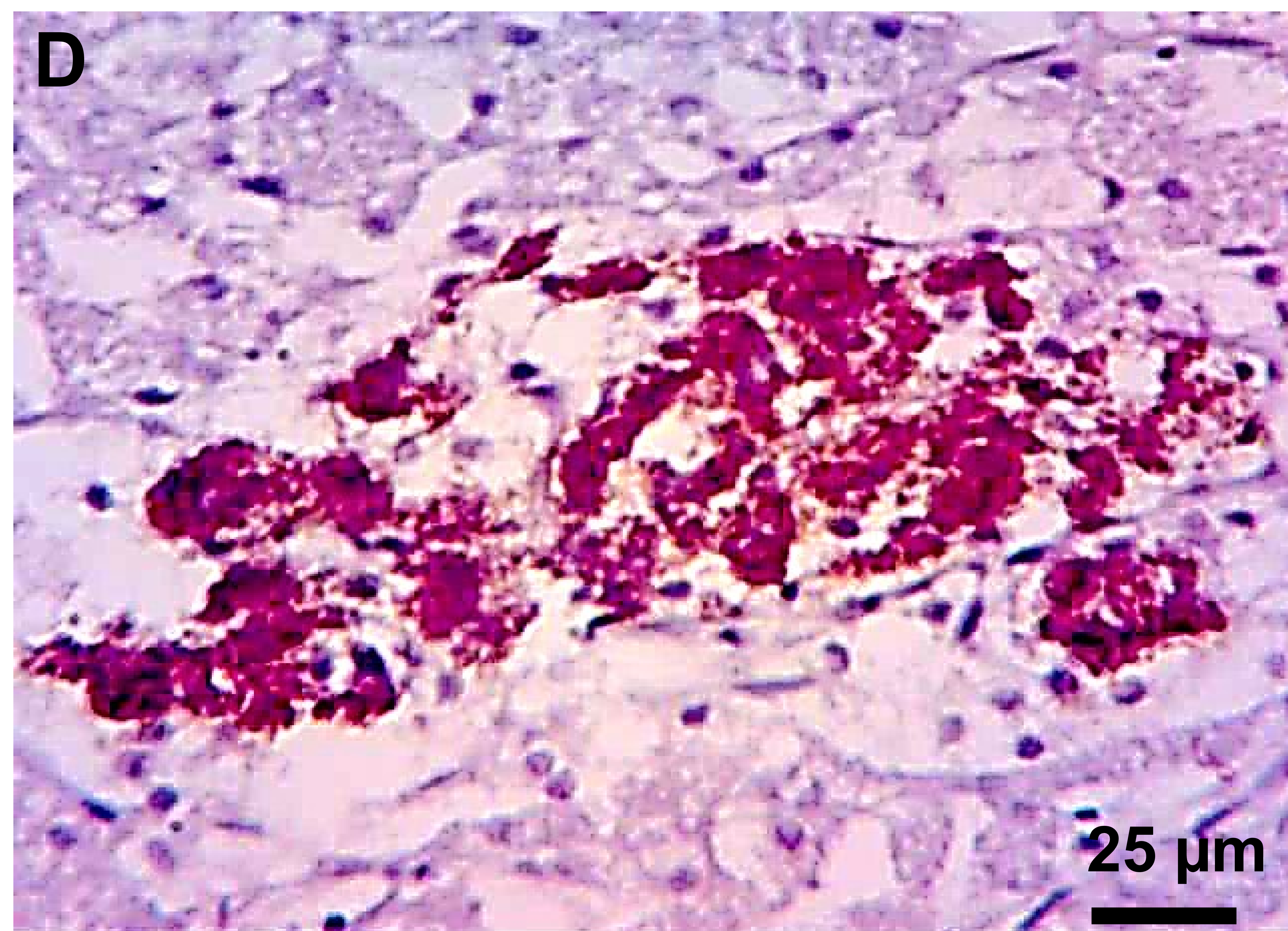
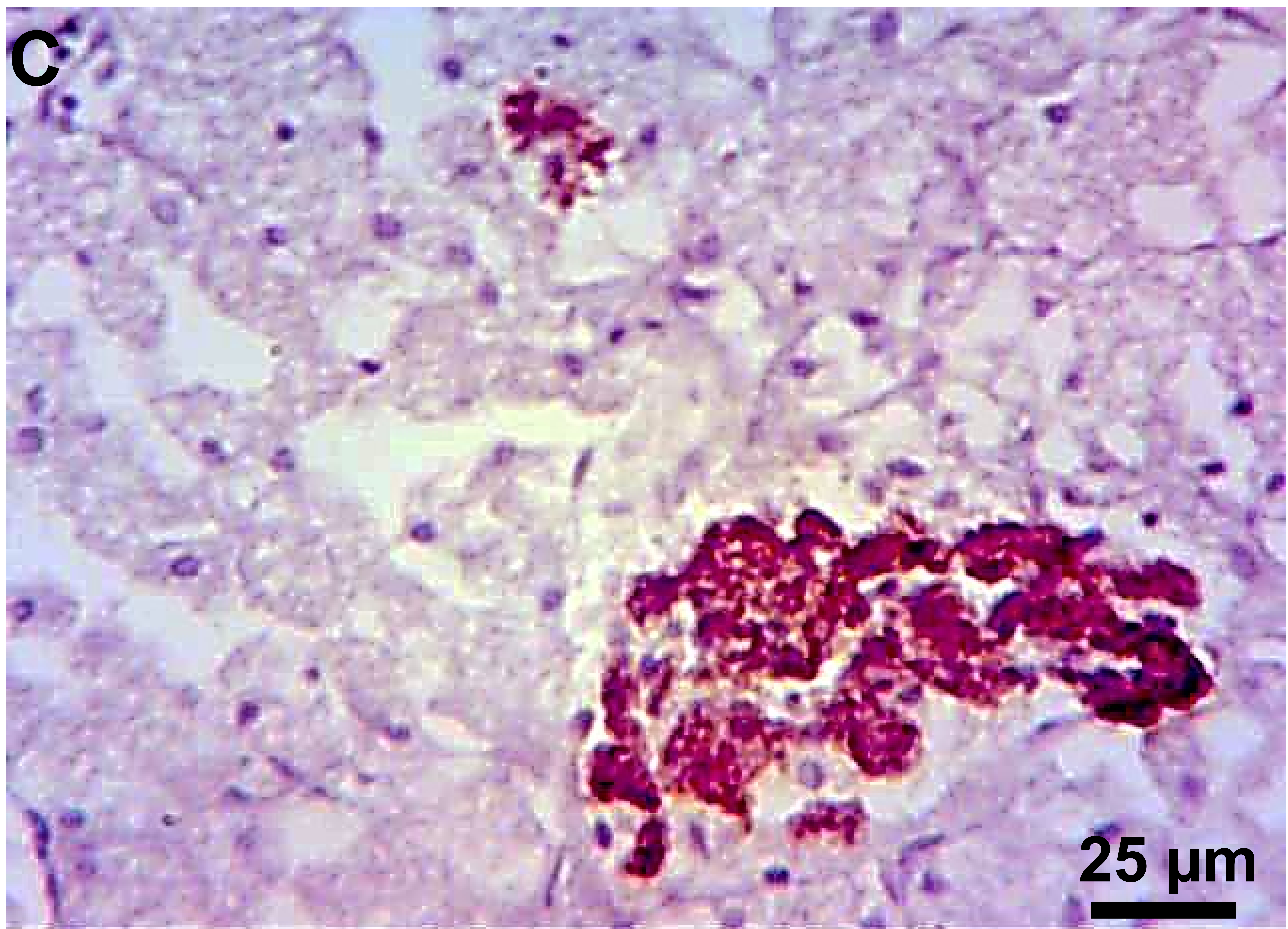
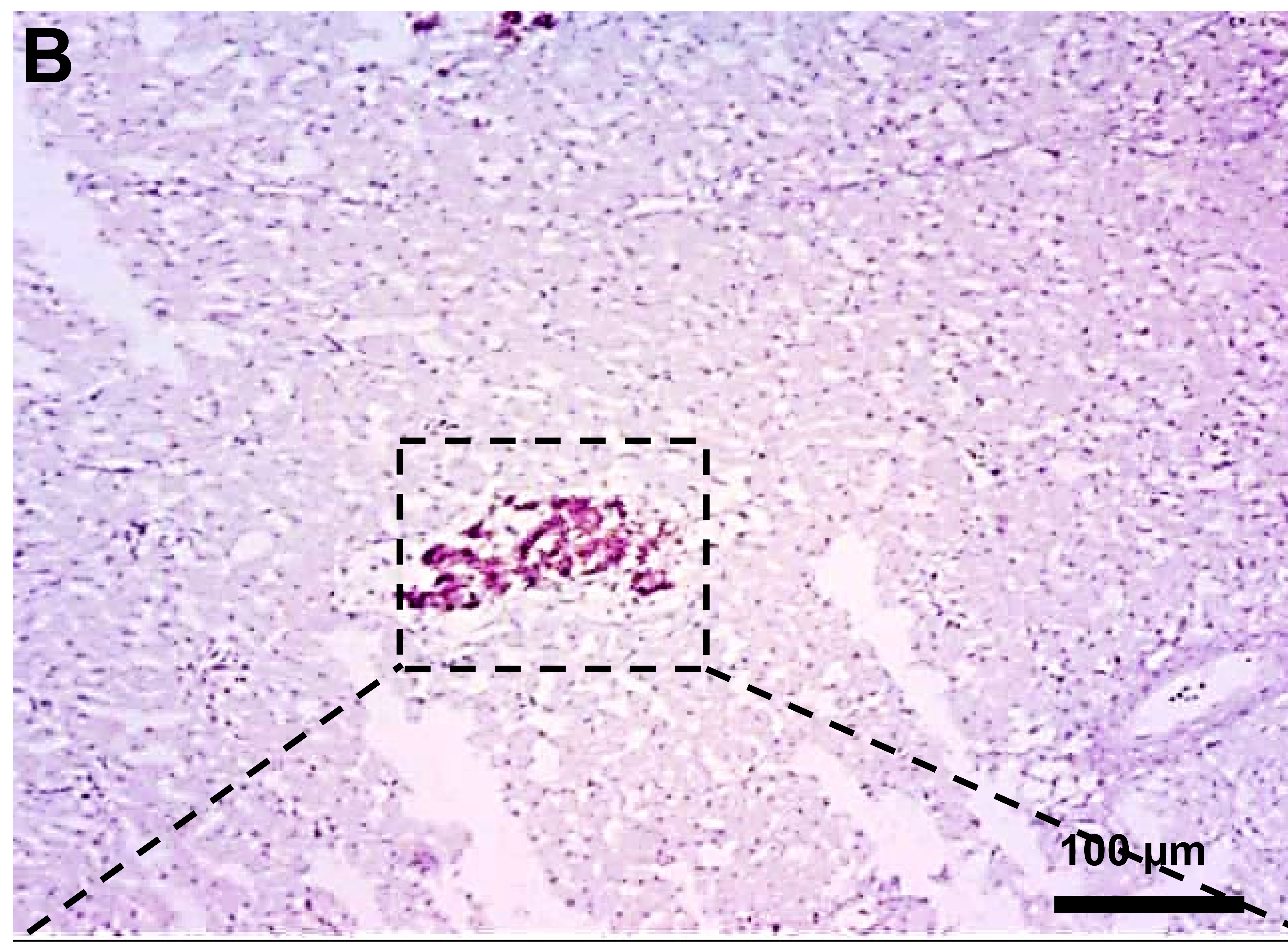
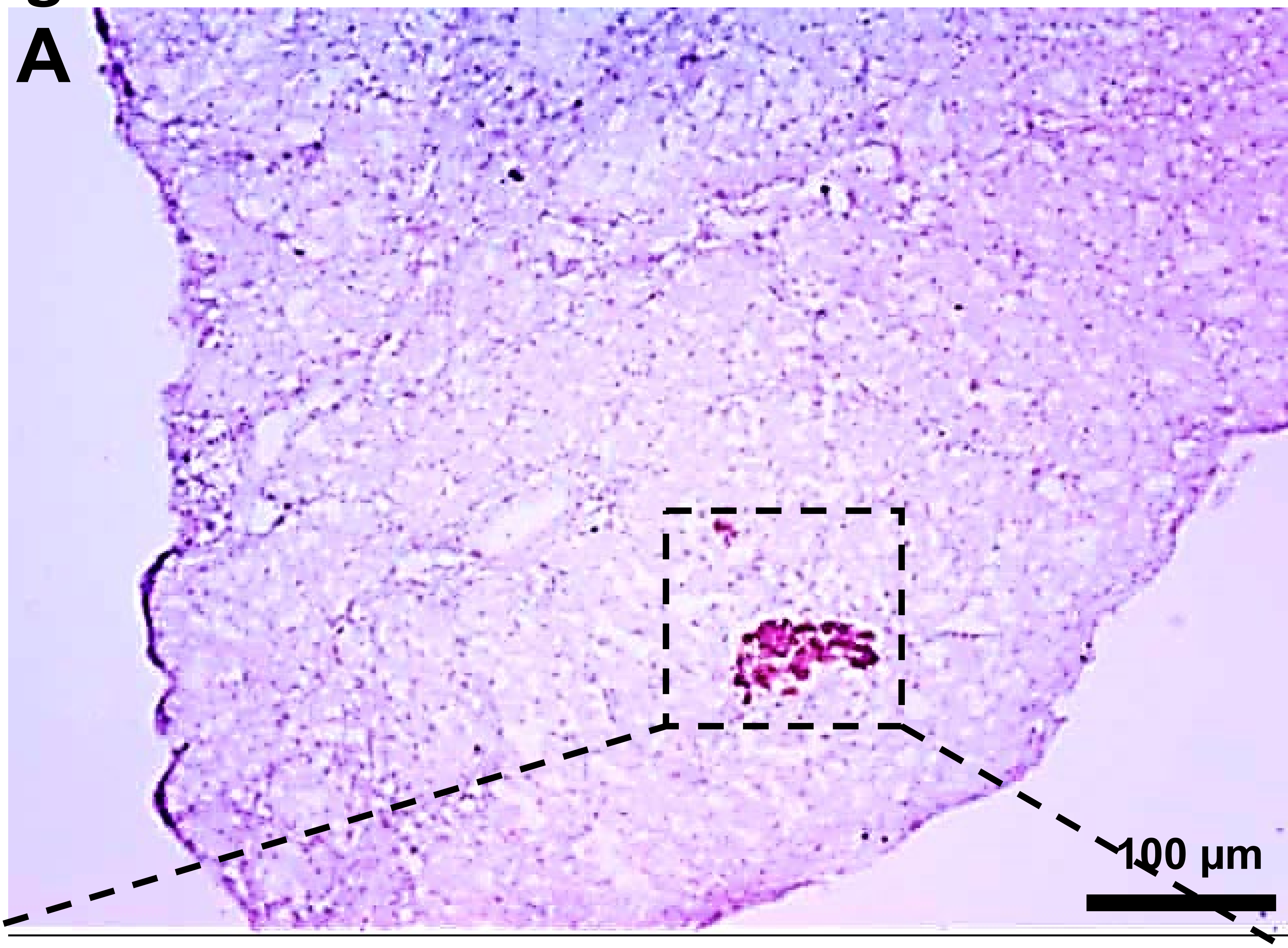


Figure 5

Male

Female



E Ratio of insulin positive area/ islet area in compact pancreas

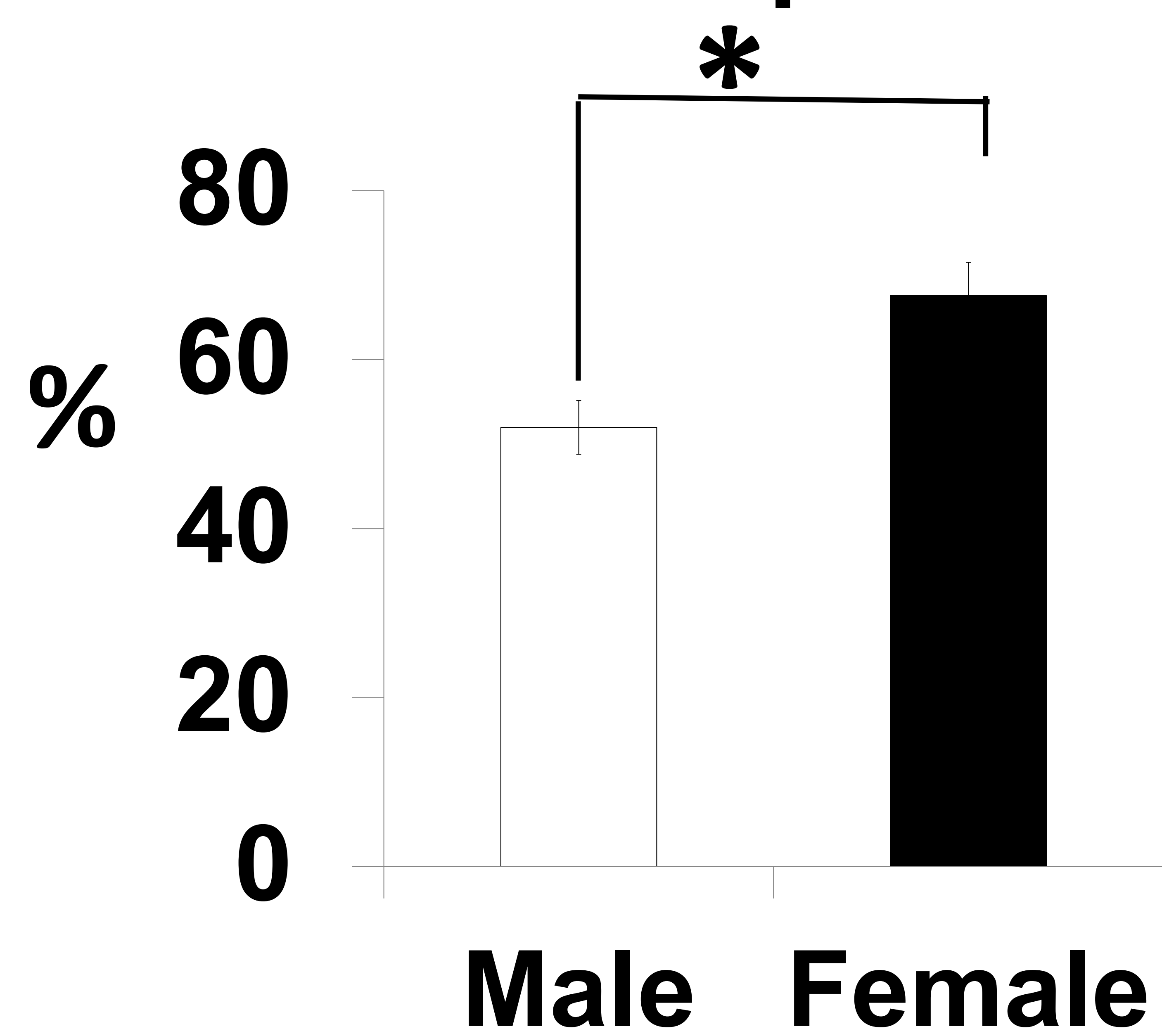
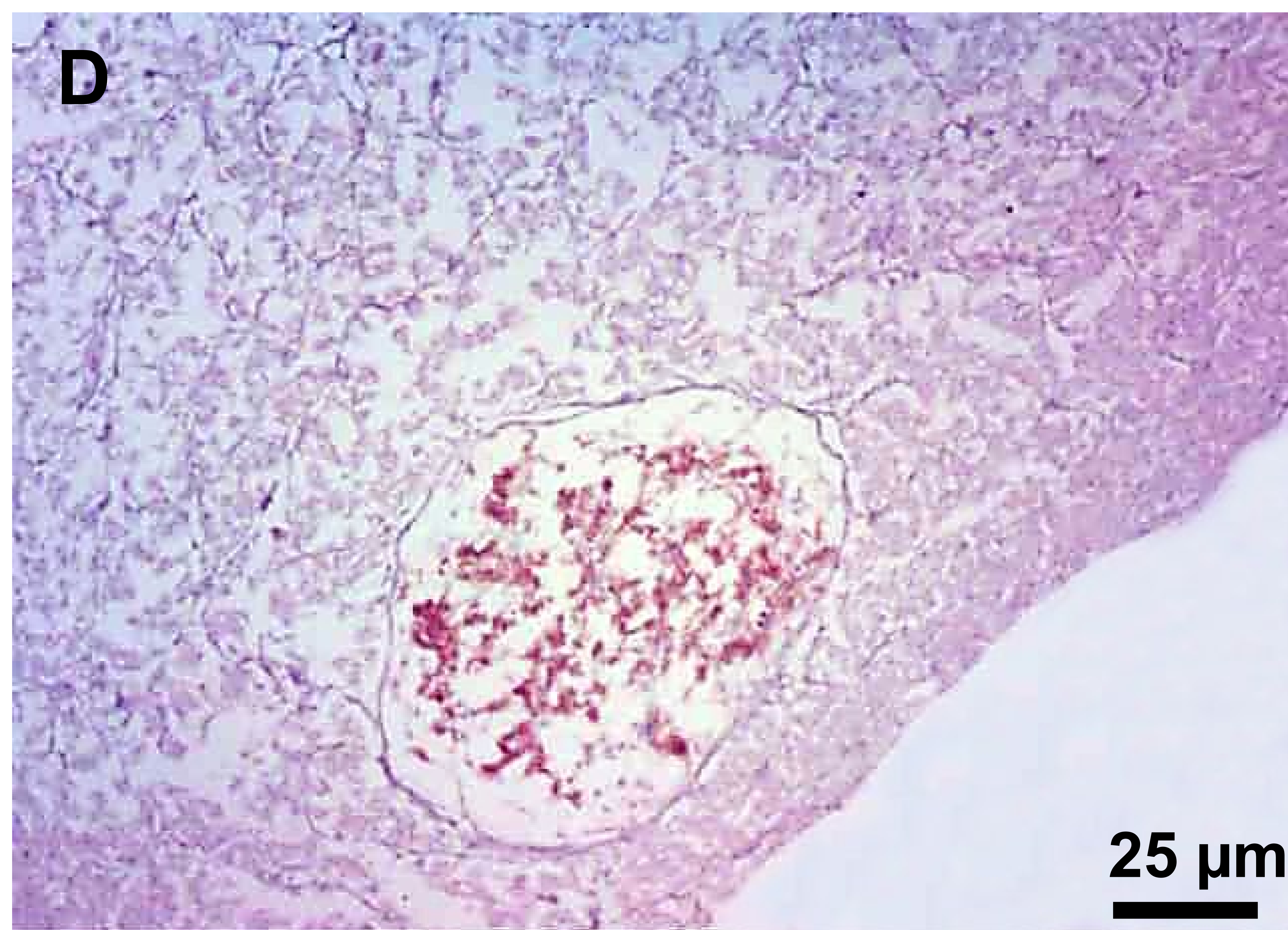
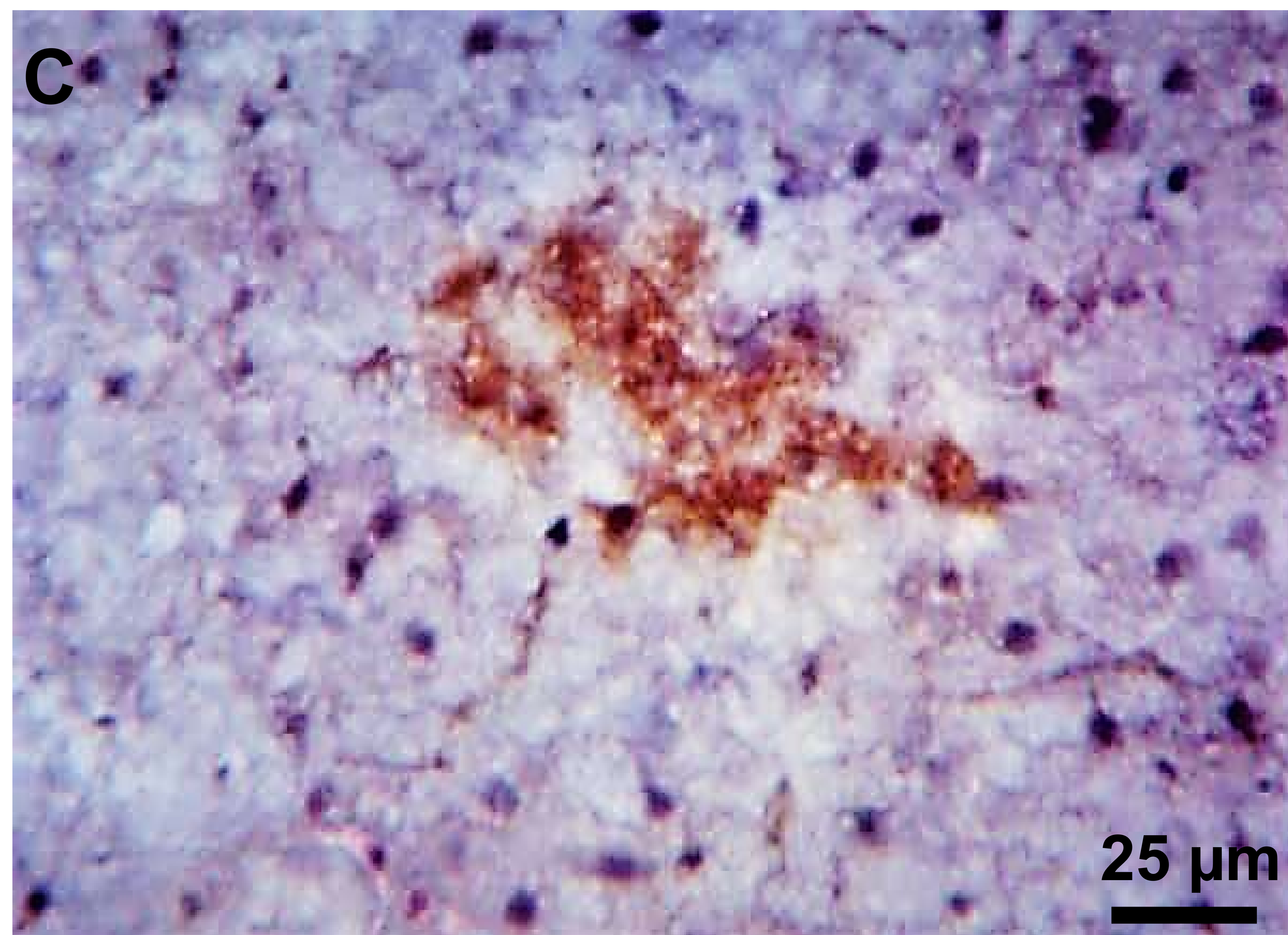
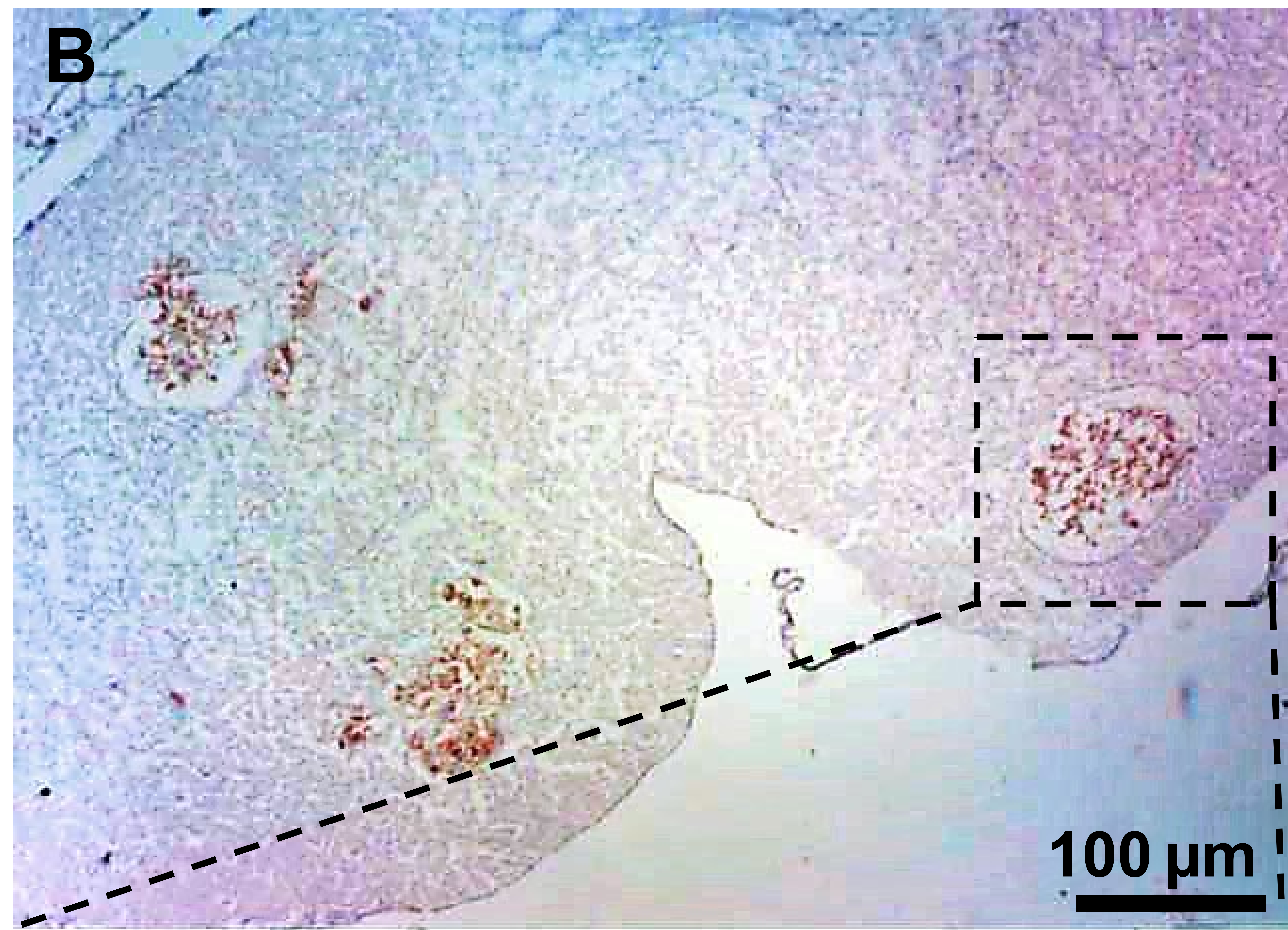
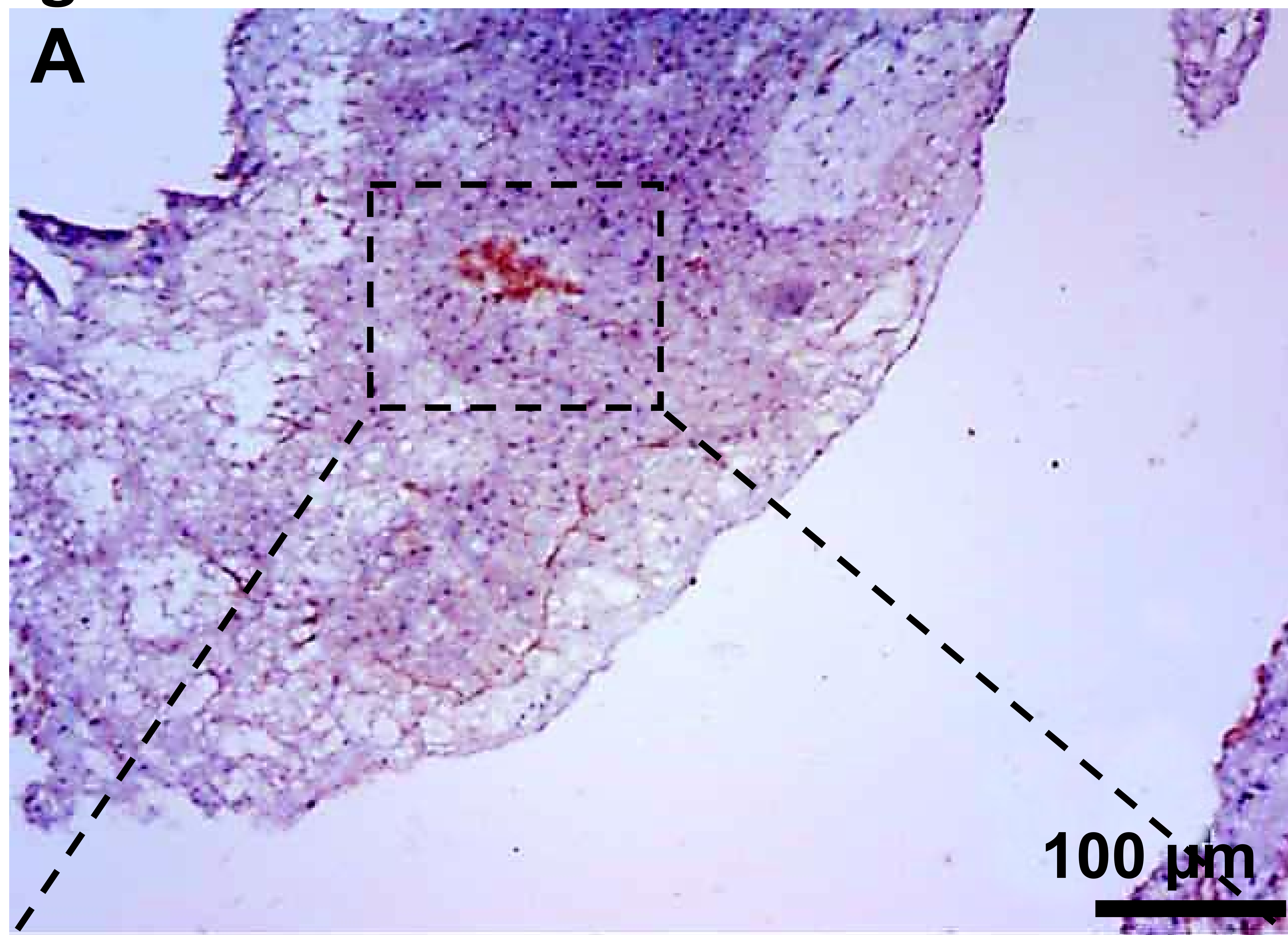


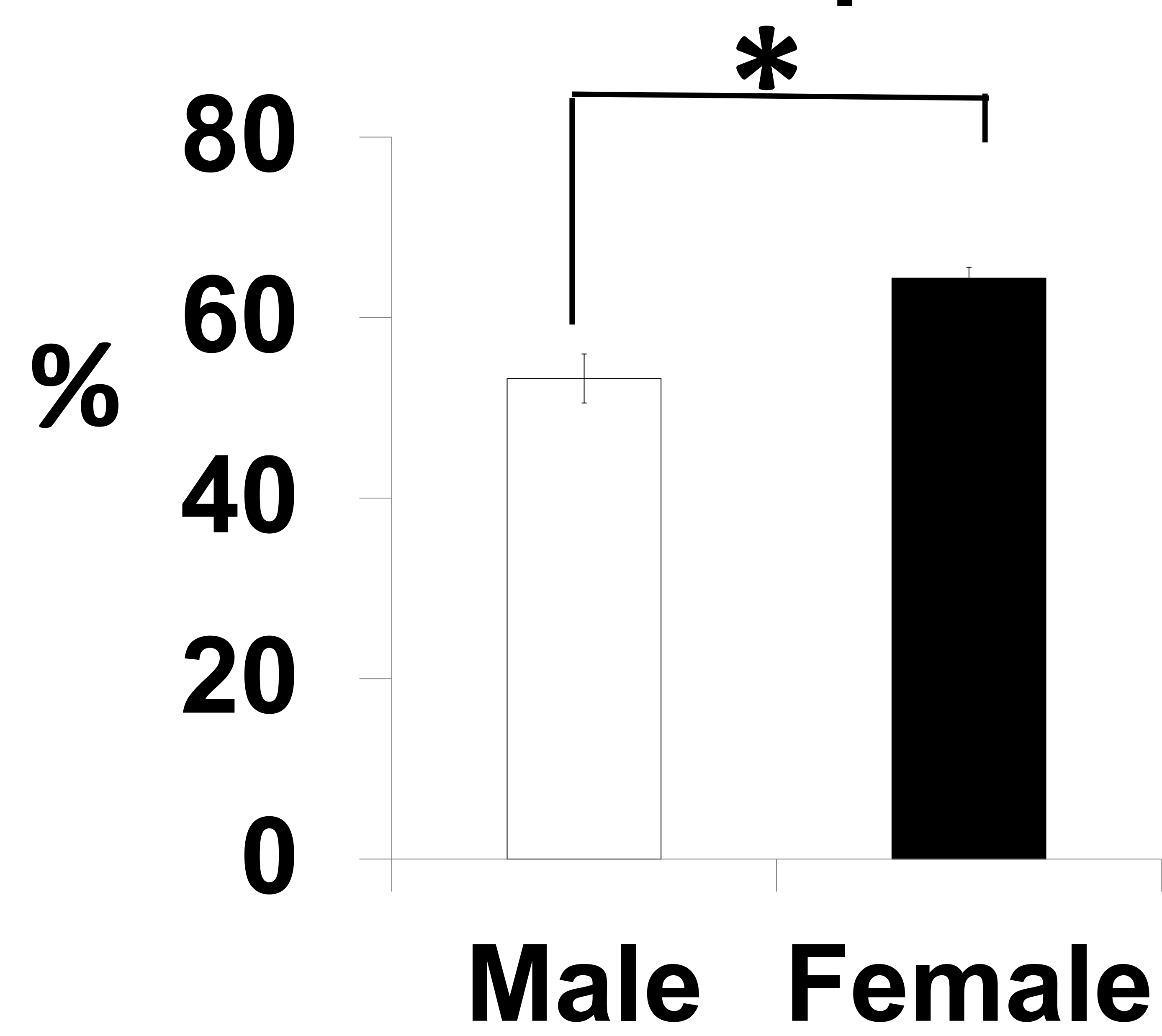
Figure 6

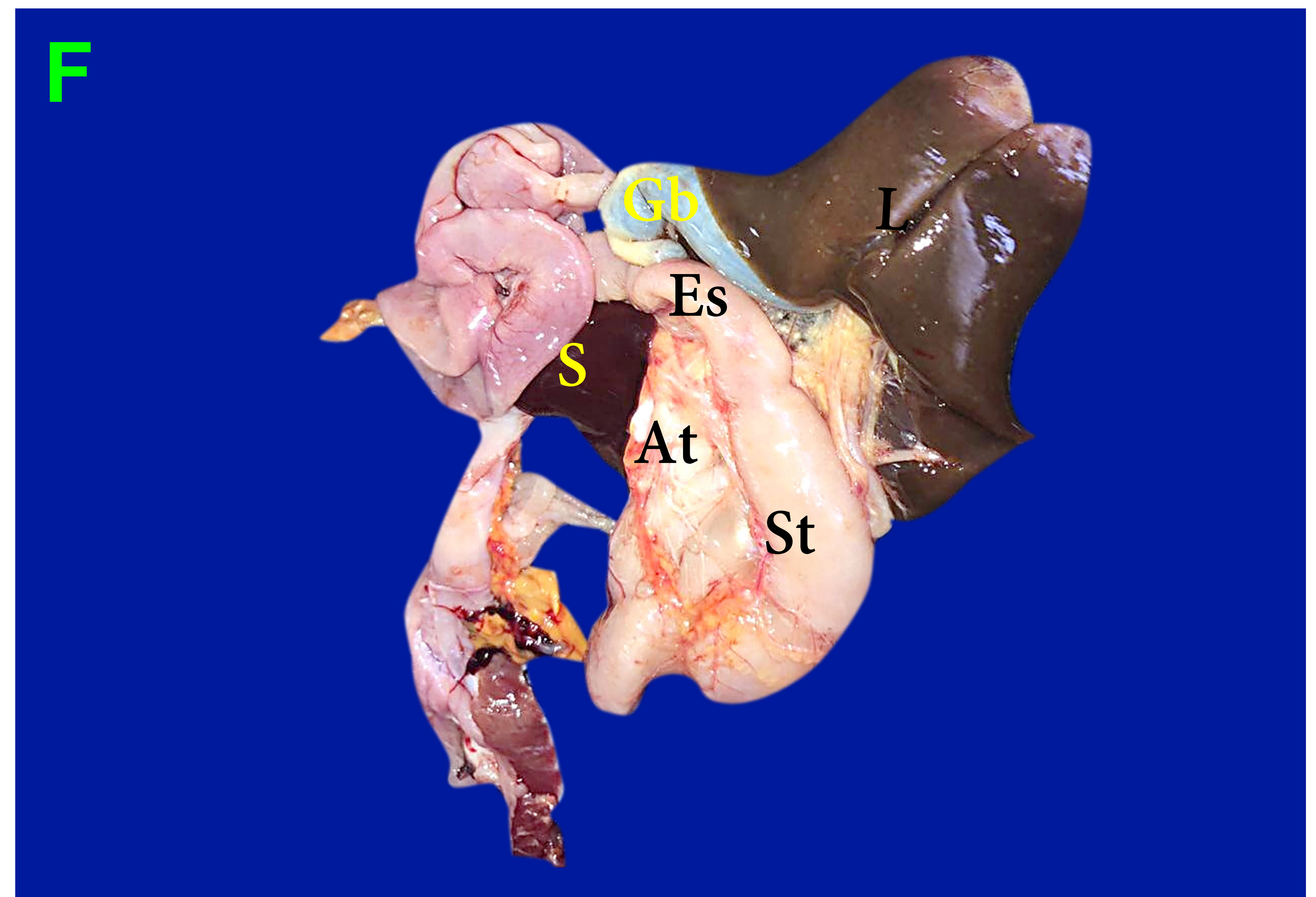
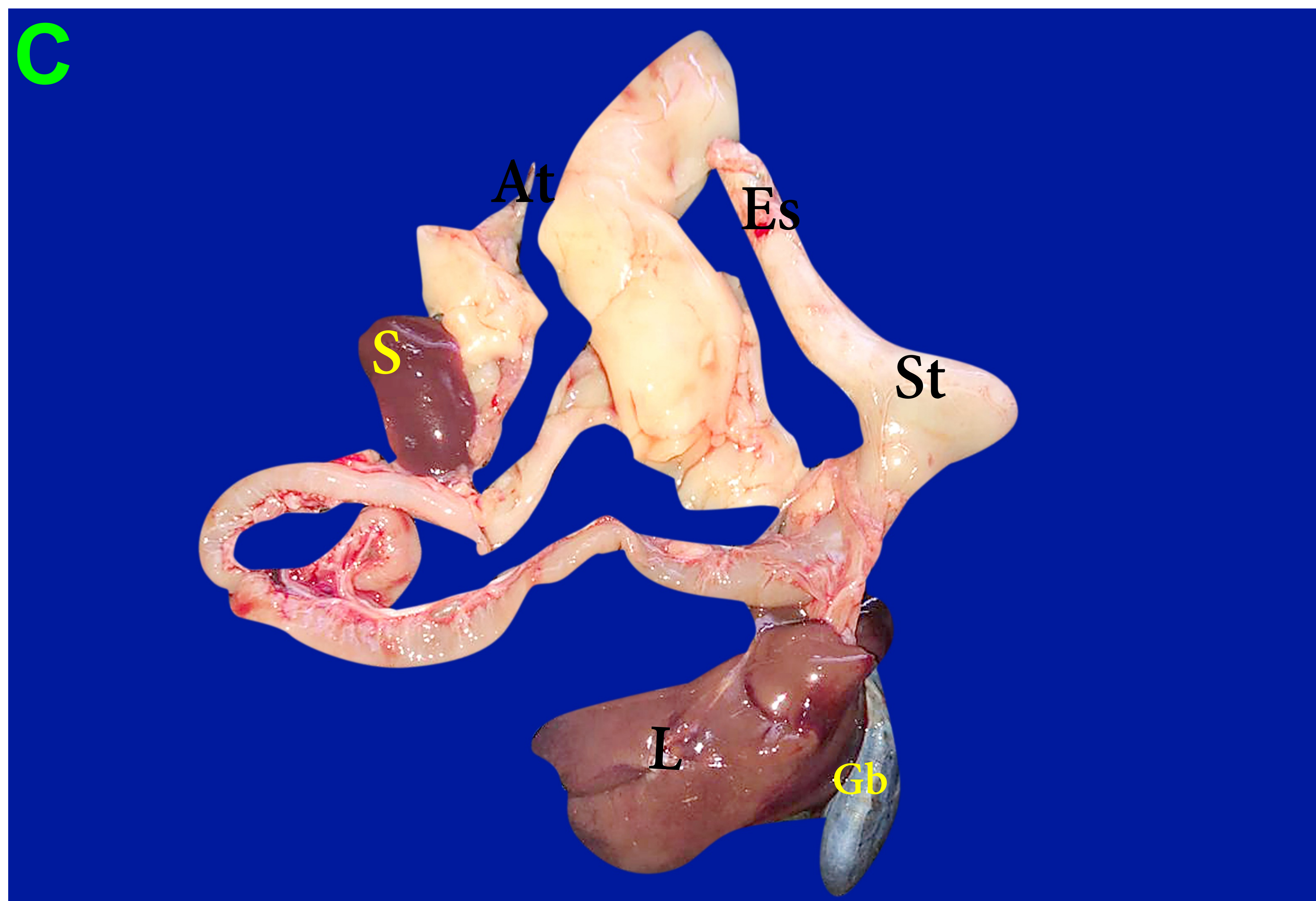
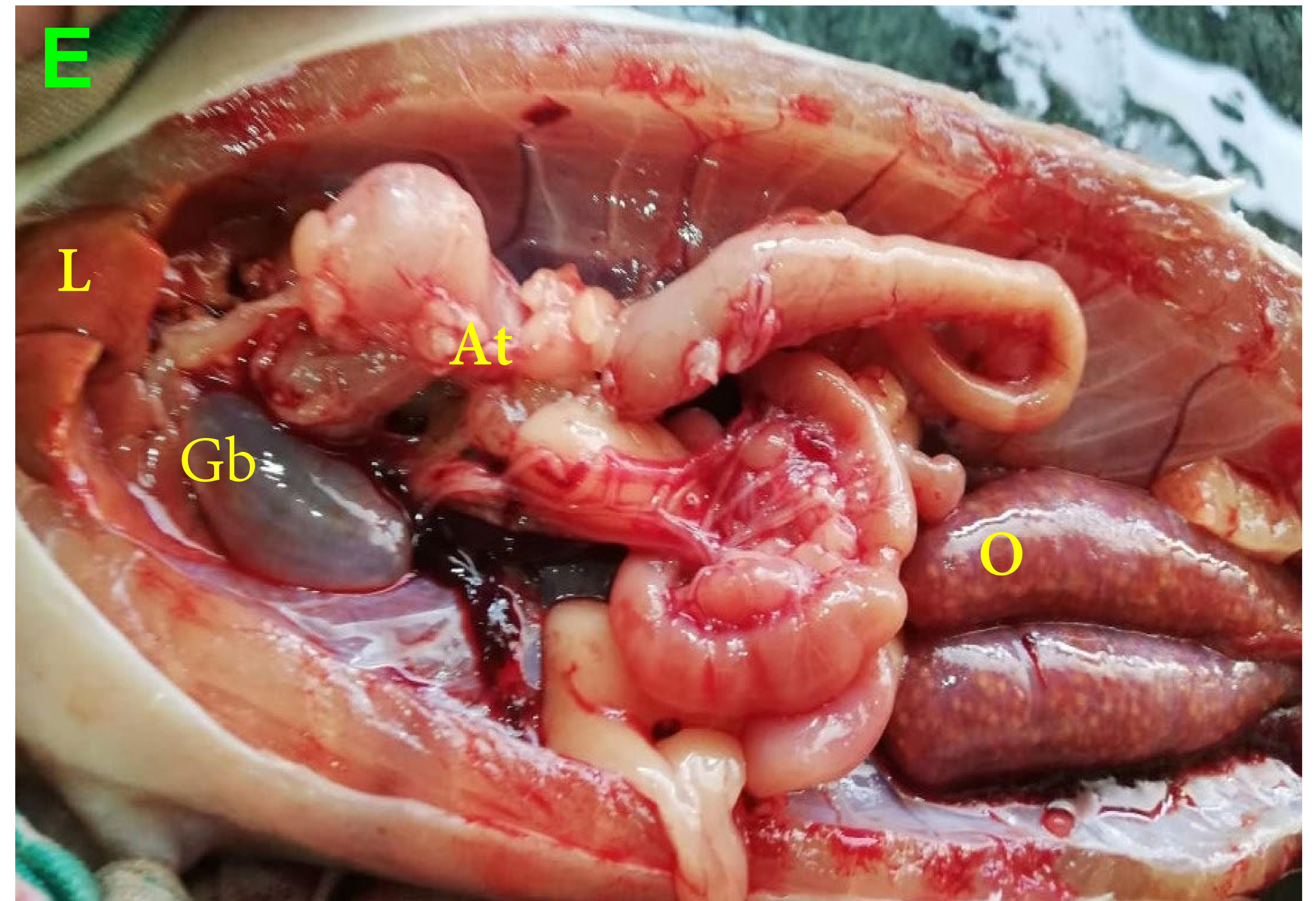
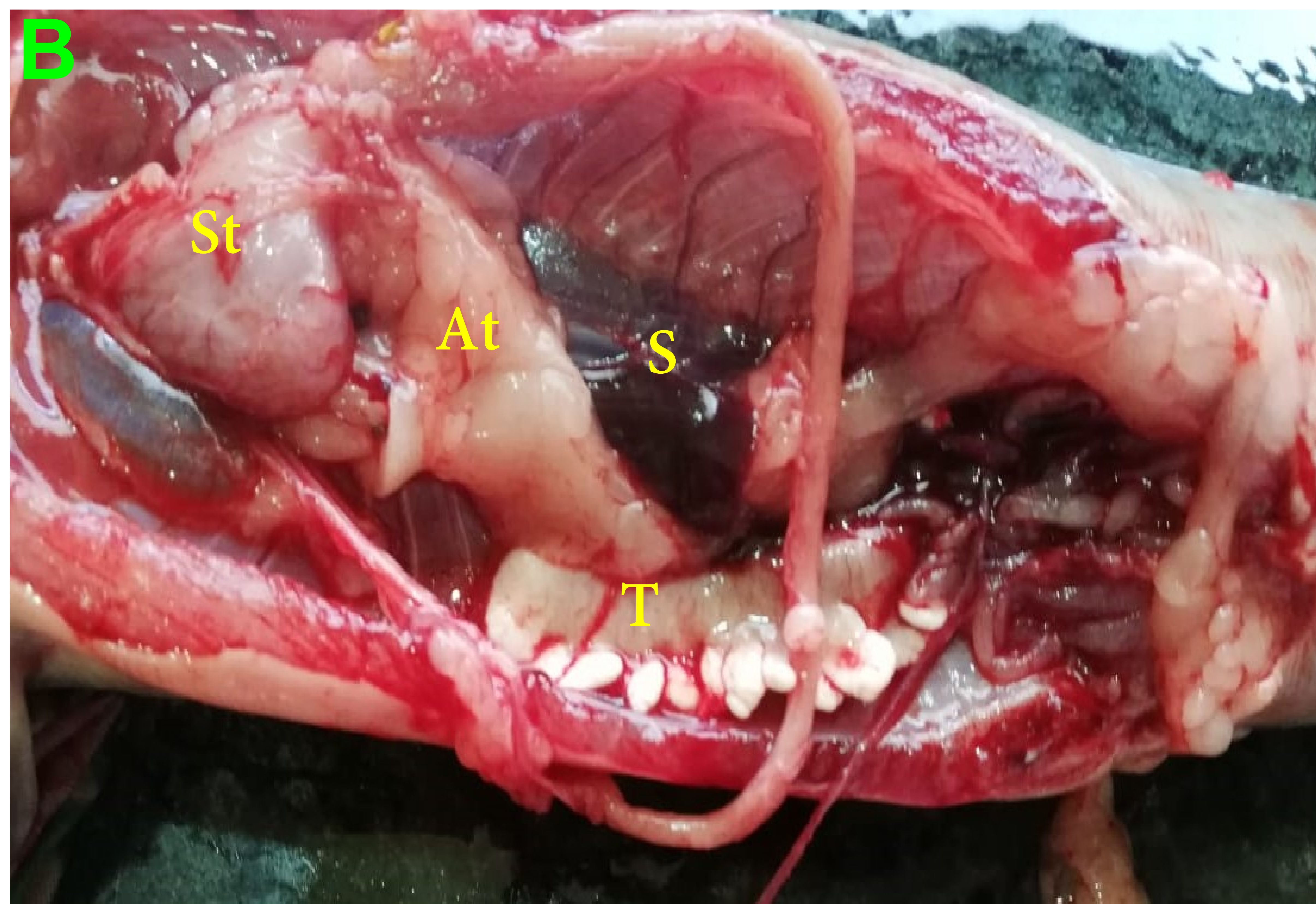
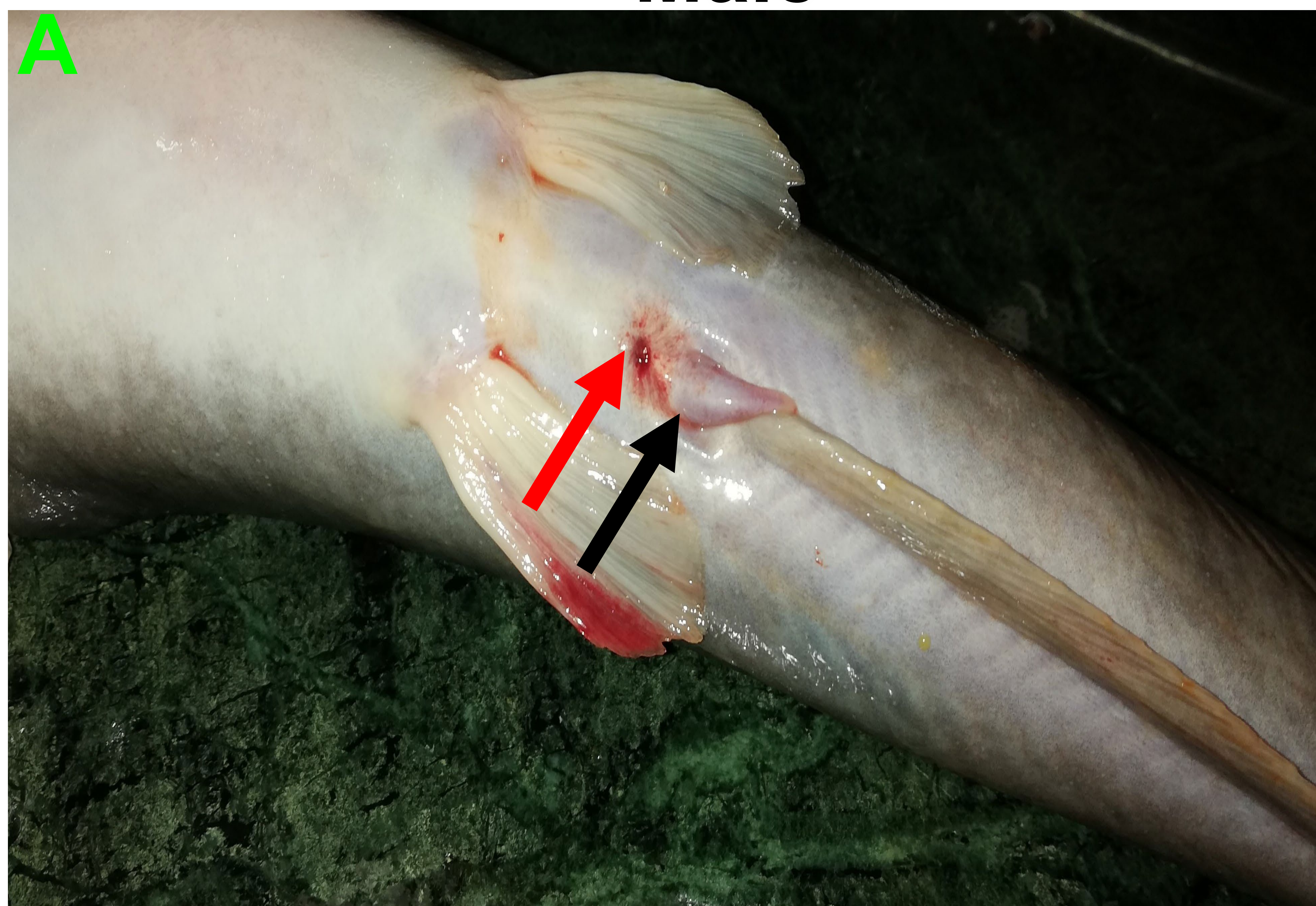
Male

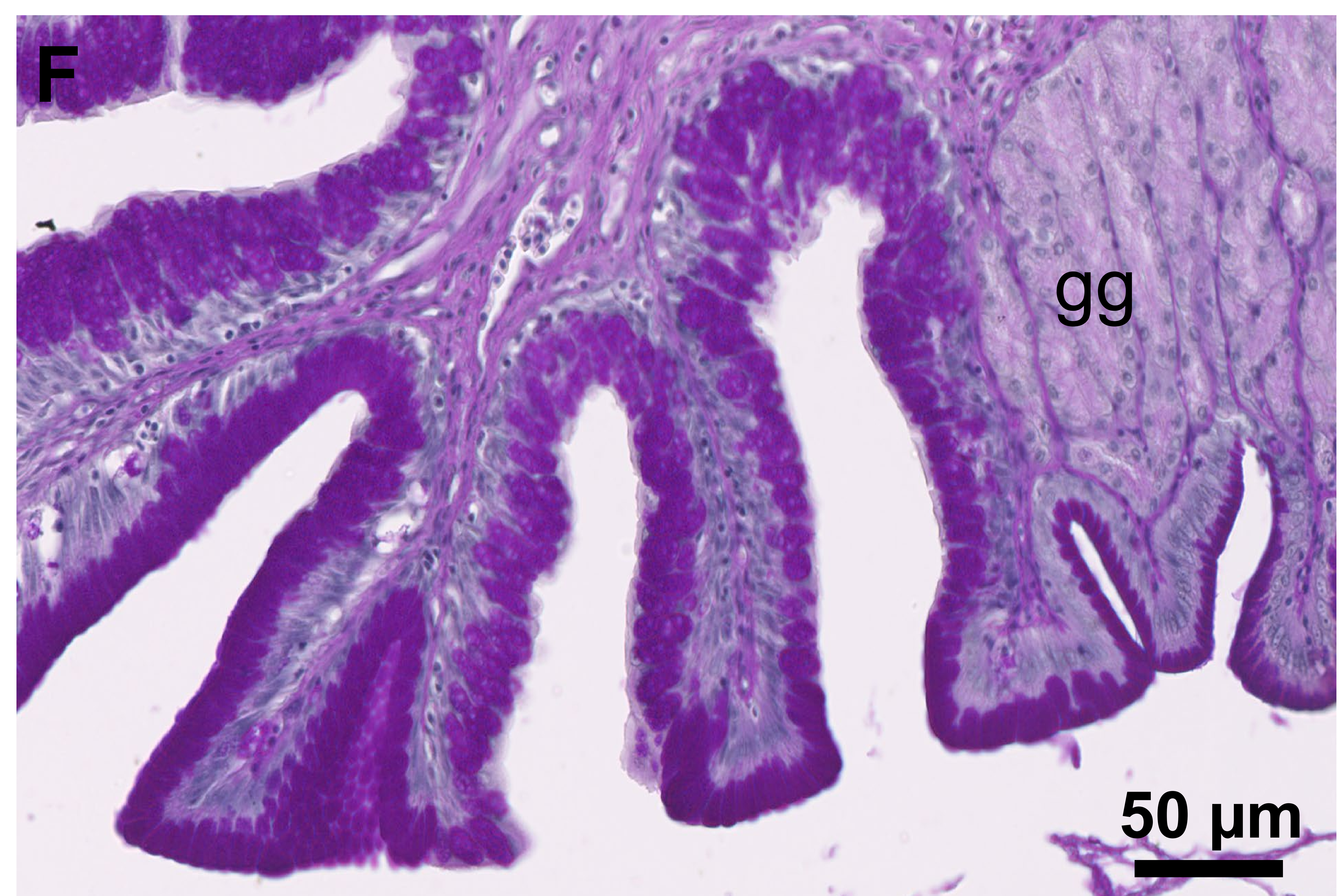
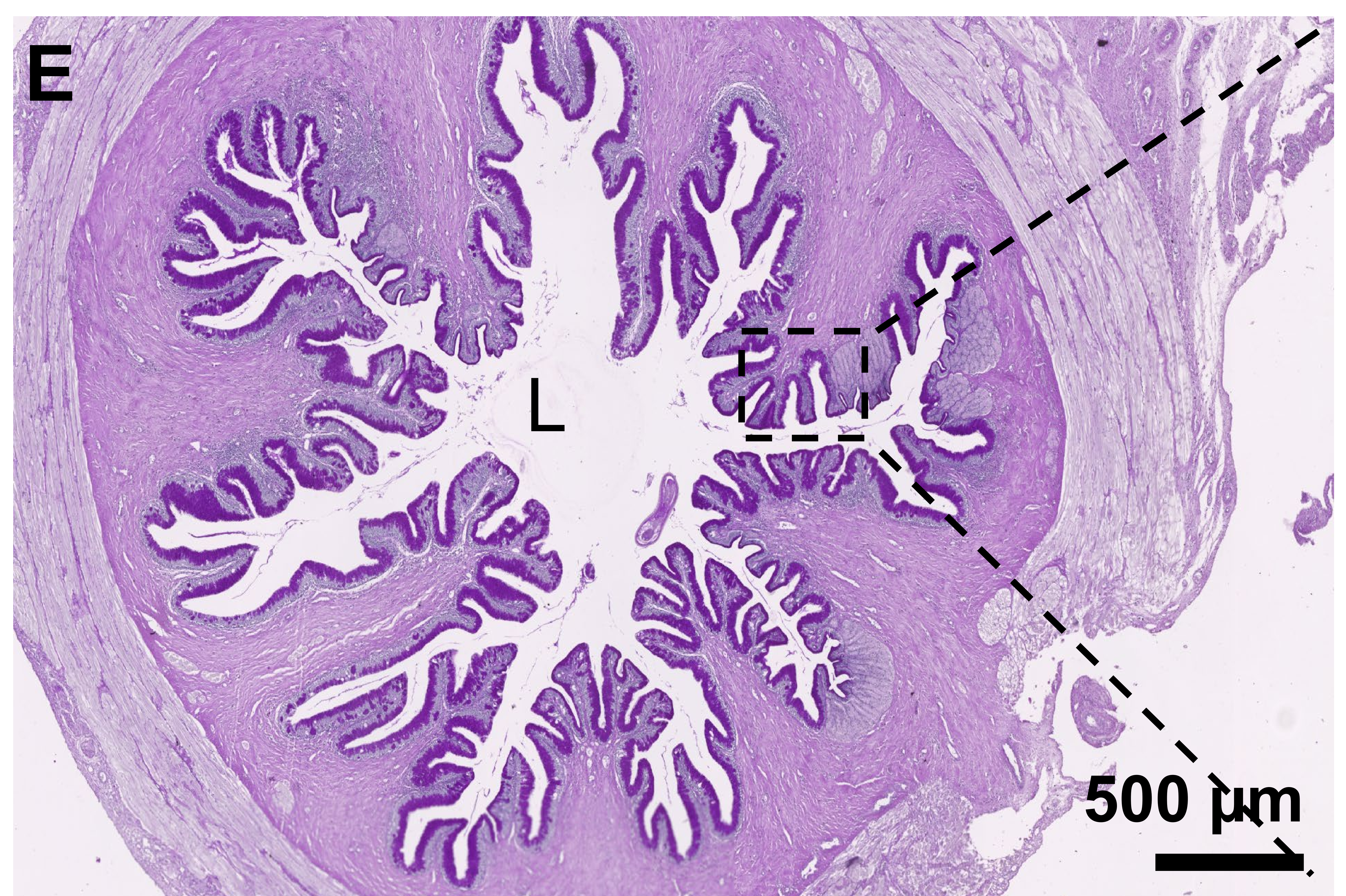
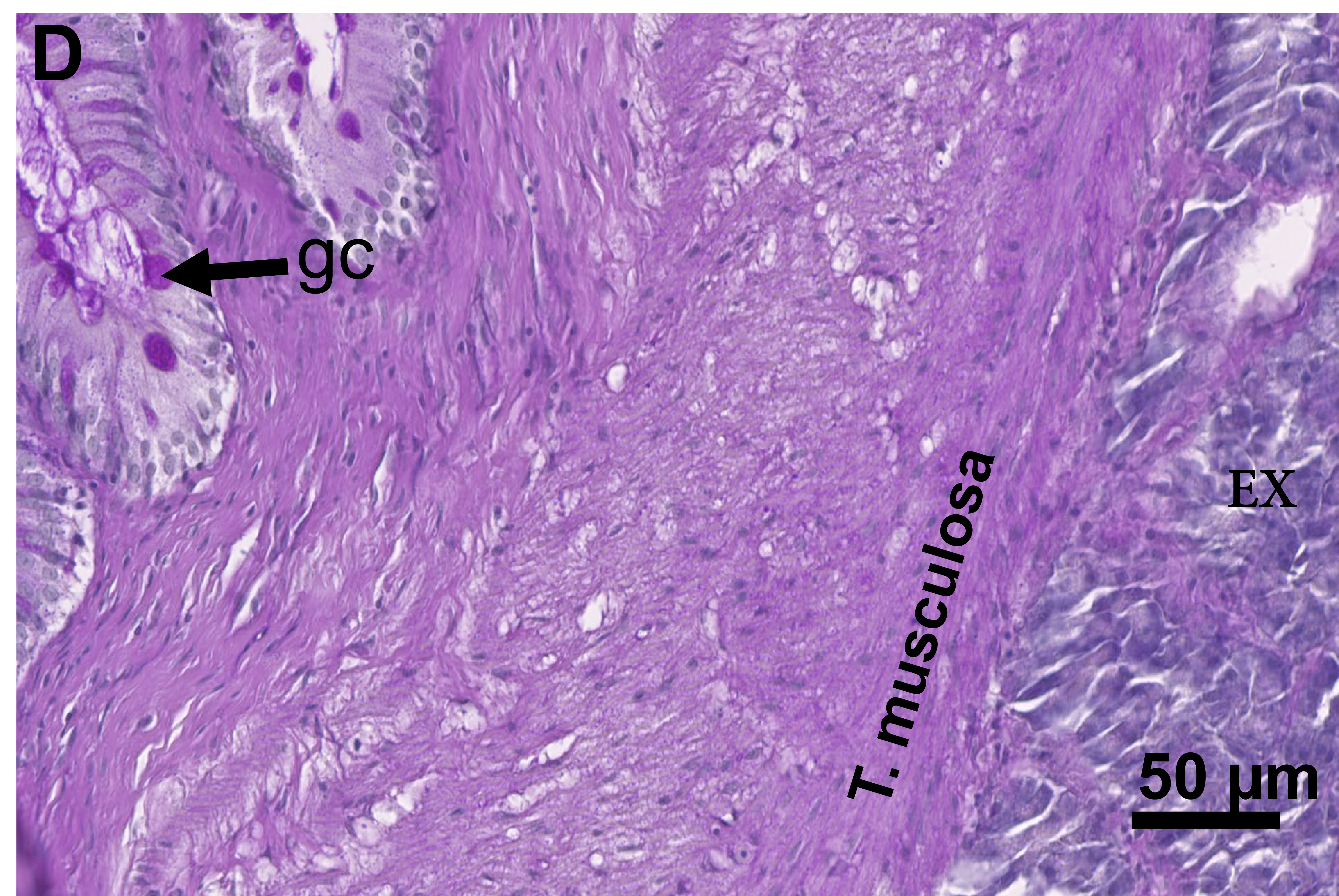
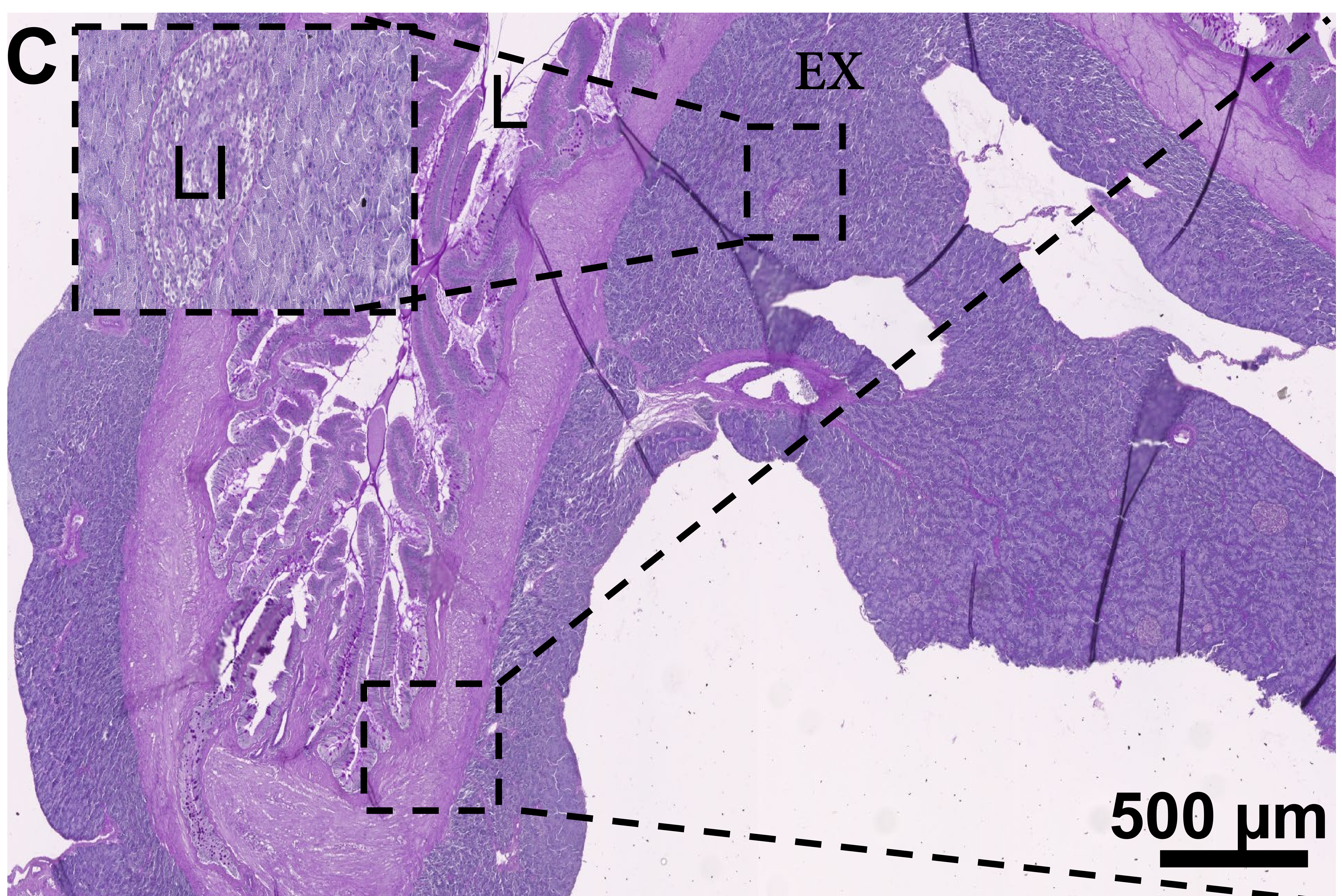
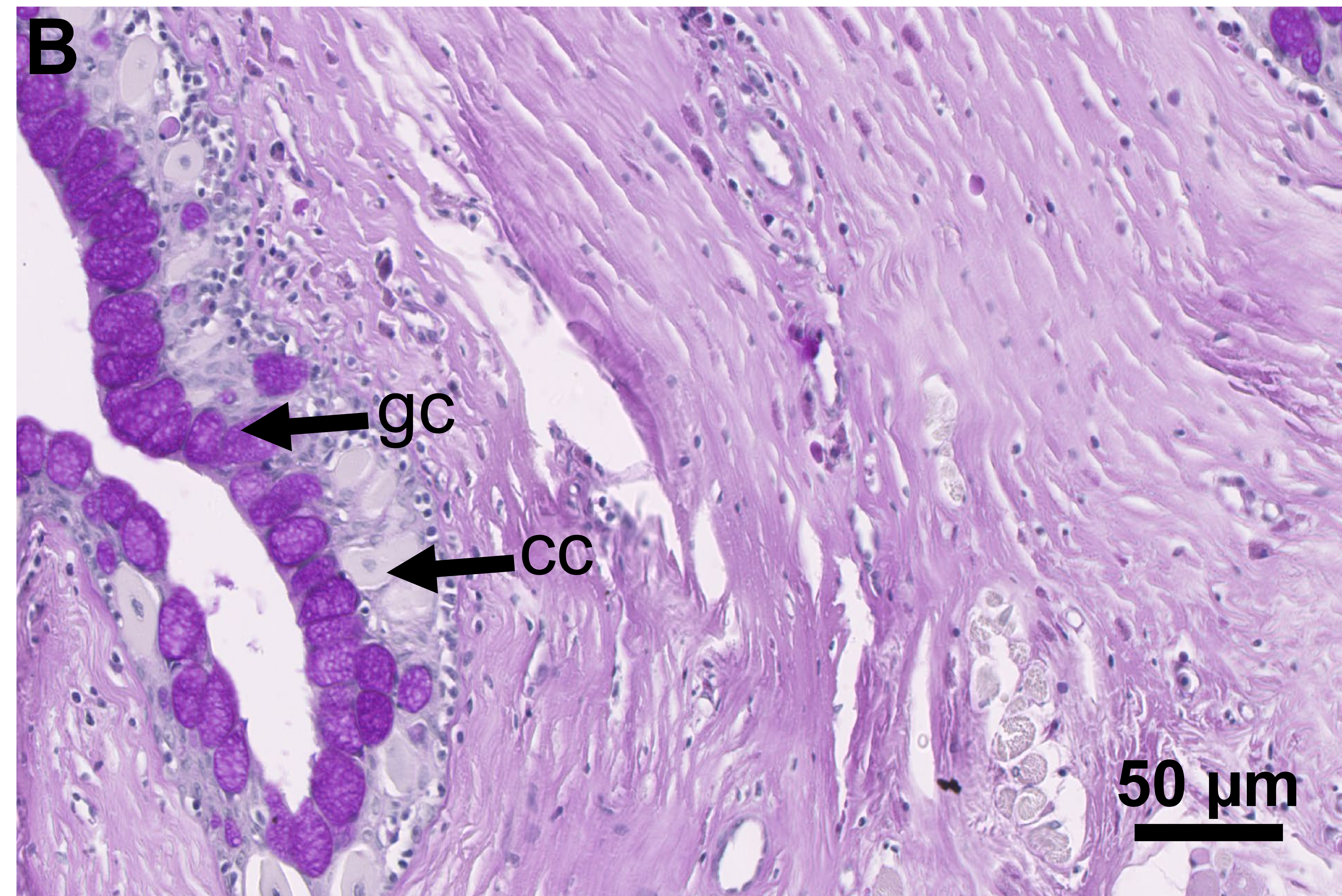
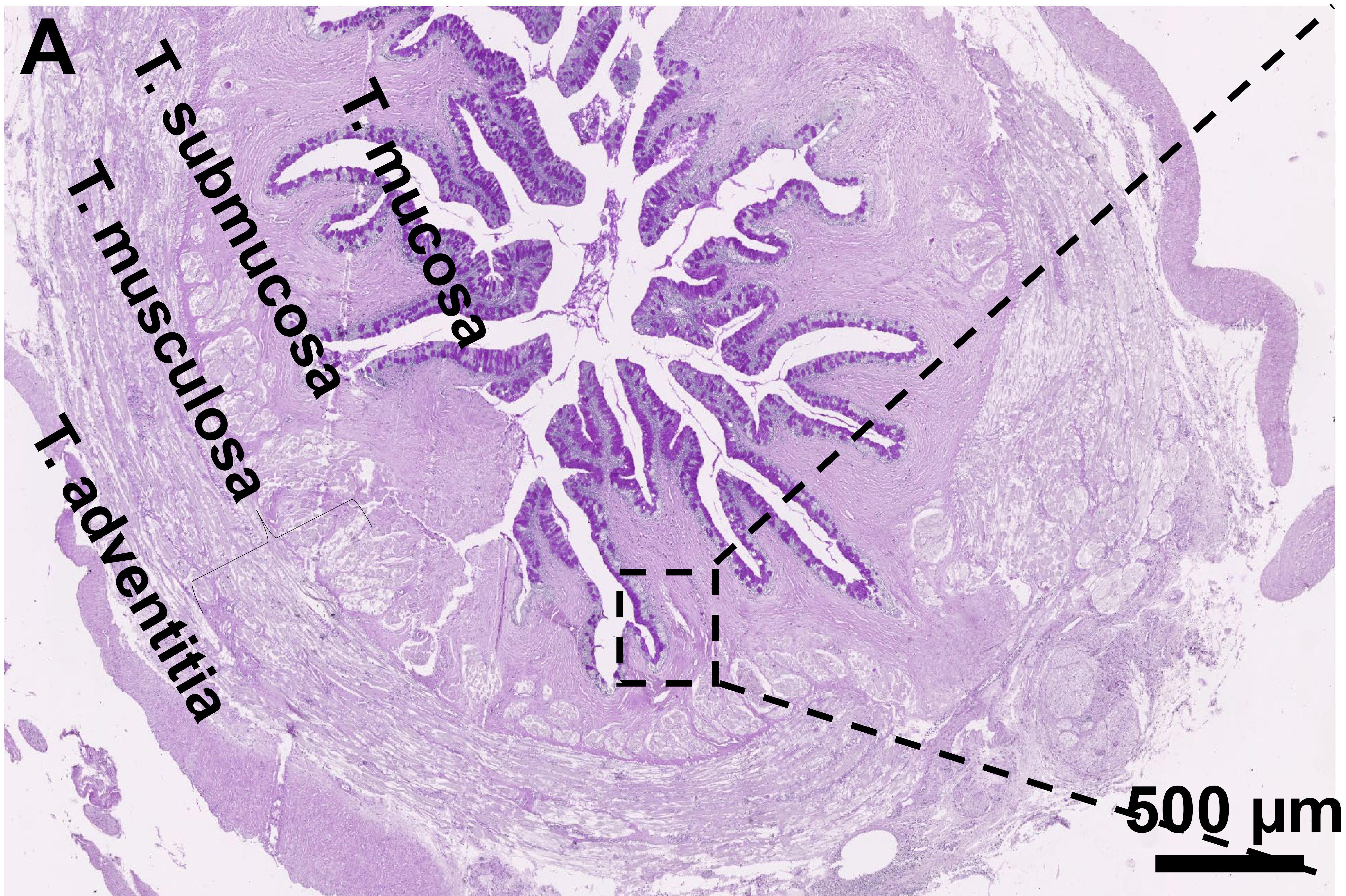
Female



E Ratio of insulin positive area/ islet area in esophageal disseminated pancreas







1 **Figures legend**

2 **Figure 1:** Photomicrographs of H&E stained sections for the catfish compact pancreas within the
3 mesenteric adipose tissue in both sexes: (A, B) showed the pancreas lobules with endocrine
4 pancreatic islets (arrows) between exocrine tissues (EX). (C, D) showed higher magnification for
5 rectangle areas in (A, B). (E) Graph showing (*) significant differences in the ratio of islet
6 area/total pancreatic area in the mesenteric adipose tissue in both sexes. (F) Graph showing no
7 significant difference in the ratio of islet number/ total pancreatic area in both sexes.

8 **Figure 2:** Photomicrographs of H&E stained sections for the dissiminated pancreatic tissue within
9 the esophagal adventitia tissue. (A, B) showed the pancreatic exocrine tissues (EX) with the
10 endocrine pancreatic islets (arrows) located within esophagal adventitia layer and inclose contact
11 with the tunica musculosa of the esophagus (M). (C, D) showed higher magnification for rectangle
12 areas in (A, B). (E) Graph showing (*) significant differences in the ratio of islet area/ total
13 pancreatic area within the esophagal adventitia tissue in both sexes. (F) Graph showing no
14 significant difference in the ratio of islet number according to total pancreatic area in both sexes.

15 **Figure 3:** Immunohistochemical staining of the glucagon- positive cells in the compact pancreas
16 within the mesenteric adipose tissue. (A) Glucagon-immunopositive cells in male catfish are
17 arranged mainly in the periphery with few cells in the mantle regions. (B) Glucagon-
18 immunopositive cells in female are detected in the periphery, the mantle and in the central region.
19 (C, D) showed higher magnification for rectangle areas in (A, B). (E) Graph showing (*)
20 significant differences in the ratio of glucagon positive area/ islet's area.

21 **Figure 4:** Immunohistochemical staining of the glucagon- positive cells in the disseminated
22 pancreas. (A, B) showed the exocrine and endocrine pancreatic islets within the esophageal
23 adventitia layer in both male (A) and female (B) catfish. (C, D) showed higher magnification for

24 square areas in (A, B), glucagon positive cells are detected in the periphery and few in the mantle
25 regions. (E) Graph showing (*) significant differences in the positive area ratio of glucagon/
26 pancreatic islets within the esophageal adventitia tissue.

27 **Figure 5:** Immunohistochemical staining of the insulin- positive cells in the compact pancreas
28 within the mesenteric adipose tissue. (A, B) showed the pancreatic islets in male and female catfish
29 with insulin positive cells in the center of islet. The female islet is apparently larger than in the
30 male. (C, D) showed higher magnification of square and rectangle areas. (E) Graph showing (*)
31 significant differences in the positive area ratio of insulin/islets area.

32 **Figure 6:** Immunohistochemical staining of the insulin- positive cells in the disseminated
33 pancreas. (A, B) showed the exocrine and endocrine pancreatic islets within the esophageal
34 adventitia layer in both male (A) and female (B) catfish. The pancreatic islet is apparently larger
35 in female than that in male. (C, D) showed higher magnification of square areas. (E) Graph
36 showing (*) significant differences in the positive area ratio of insulin/ total islets area within the
37 esophageal adventitia tissue.

38 **Supplementary figure 1:** Gross morphology of catfish (*Clarias gariepinus*) and pattern of
39 gastrointestinal tract and distribution of pancreatic tissues. (A) adult male catfish with genital
40 papillae (black arrow), caudal to the anal opening (red arrow). (D) adult female catfish. (B, E)
41 Internally, the gastrointestinal tract in male (B) & female (E) include stomach (St), liver (L), spleen
42 (S), gall bladder (Gb), pancreatic tissues within the mesenteric adipose tissue (At), testes (T) and
43 ovary (O) in male and female, respectively. (C, F) showed esophagus (Es) and pancreatic tissues
44 localized in the triangular area between stomach (St), liver (L), spleen (S) and gall bladder (Gb).

45 **Supplementary Figure 2.** Photomicrographs of periodic acid Schiff's stained sections for the
46 catfish esophagus: (A, B) Anterior portion of the esophagus. Notice the layers of esophagus (tunica

47 mucosa, submucosa, muscularis, adventitia), highly folded mucosa, numerous goblet cells (gc),
48 clear cells (cc). (C, D) Middle portion of the esophagus. Notice moderate sized lumen (L),
49 moderately folded mucosa with less goblet cells (gc), with the presence of disseminated pancreas
50 including exocrine (EX) and endocrine portions represented by islets of Langerhans (IL) in the
51 tunica adventitia and in close contact to the tunica muscularis. (E, F) Posterior portion of the
52 esophagus. Notice large sized lumen (L), less folded mucosa, with the presence of gastric glands
53 (gg) at the junction with the cardiac region of the stomach.