

Change in diameters of the small intestine according to embryonic and early fetal growth

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Abstract

No previous studies have examined the diameter of the small intestine successively from the oral to the anal side of the small intestine. Therefore, the objectives of this study were to determine the successive intestinal diameters from the oral to the anal side (proximal to the distal) of the intestine, evaluate changes in diameter associated with growth, examine the effects of positional variation along the intestinal tract, investigate dynamic positional change from the extraembryonic coelom to the abdominal cavity, and assess the impact of complex tertiary intestinal loop formation. To this end, 14 human embryonic and fetal specimens with crown-rump lengths (CRLs) ranging from 25.6 to 69.0 mm were selected for high-resolution magnetic resonance imaging acquisition. The small intestines of the specimens were located in the extraembryonic coelom (herniation phase), transitioning phase, or abdominal cavity (return phase). The small intestine and mesentery were reconstructed in three dimensions, and the resulting morphological changes were observed and analyzed. Successive intestinal diameters from the oral to anal side of the small intestine were determined. Specifically, we observed the following: 1) gradual changes in the diameter of the position from the oral to the anal side in the jejunum-ileum, 2) the difference between the duodenum and jejunum-ileum, and 3) the difference between the superior part of the duodenum derived from the foregut and the remaining parts derived from the midgut. 4) Notably, the dynamic positional change from the extraembryonic coelom to the abdominal cavity, along with the rapid elongation and complex intestinal loop formation—a conspicuous phenomenon in the embryonic and early fetal periods—had little effect on the changes in diameter. This study indicates that increased diameter may serve as a useful indicator of intestinal development and differentiation, independent of tertiary intestinal loop formation and positional changes into and out of the abdominal cavity.

Keywords: Small intestine development; Embryonic growth; Intestinal diameter changes; Tertiary intestinal loops

Introduction

The intestinal tract has a general architectural framework comprising five concentric layers. From the innermost to the outermost, these layers include the mucosa, muscularis mucosae, submucosa, muscularis propria, and subserosa, which differentiate by the end of the embryonic period (Lewis 1912ab; O’Rahilly 1978; O’Rahilly & Muller 1987; Semba et al., 1984). These wall layers continue to develop throughout the fetal period.

The development of the mucous membrane in the intestinal tract is documented in classic anatomical and histological studies (Lewis 1912ab; Johnson 1910). The development of small intestinal mucosal villi, as described by Johnson (1910), is as follows. 1) Formation of vacuoles and intestinal pockets in the epithelium. Most of these structures are obliterated upon incorporation into the walls of the developing villi. 2) The villi originate from the formation of thickenings in the epithelium that are not preceded by longitudinal folds. The thickened epithelial plates undergo invagination and subsequently reduce cellularity, forming a single layer. 3) The presence of low longitudinal folds. The folds, which are between two and five, exhibit irregular heights and positions. Their presence likely reflects the slower expansion of the outermost gut layers compared with that of the epithelium. Irregularities observed along the top of the folds indicate villus development. Subsequently, the folds undergo expansion or absorption by rapidly growing villi, leading to their disappearance. 4) As the villi develop, they are arranged in longitudinal rows with varying regularities.

Based on elaborate classic observations (Lewis 1912ab; Johnson 1910), recent studies have argued the mechanism of villification. Shyer et al. (2015) proposed that the folding of growing chick epithelial tissue is driven by buckling caused by compressive forces from the underlying muscle layers. This mechanical deformation leads to the formation of ripples, within which the concentration of Sonic Hedgehog increases, guiding tissue patterning. On the other hand, Walton et al. (2016) demonstrated that villus formation is regulated by a complex cocktail of Bmps and Bmp signaling modulators secreted from mesenchymal clusters. This signaling activity creates patterns resembling those generated by a self-organizing Turing field.

Huycke et al. (2024) reported that changes in cell behavior within the mesenchymal layers initiate the folding in mice. Namely, the phase transition of the gut mesenchymal tissue from solid to fluid-like is the trigger, which aggregates mesenchymal cells into clusters, inducing folding. These studies indicate that the process of villus formation may differ in mammals and birds.

Few studies have provided quantitative measurements of intestinal diameter, especially at the embryonic and early fetal periods. Johnson (1910) measured the surface epithelium thickness, epithelial tube diameter, villi height and base diameter, and the length and diameter of intestinal and duodenal glands using elaborate three dimensional (3D) reconstructed wax models. The epithelial tube diameters at the duodenum (upper third) and midgut region in the specimens with crown-rump lengths (CRLs) of 30–55 mm were found to be 0.44–0.95 mm and 0.28 mm–0.75 mm, respectively. The exact sites and methods where the diameters were measured for the duodenum, jejunum, and ileum were not described in that study; therefore, the accuracy of the measured value was difficult to estimate. Malas et al. (2003) recently reported the morphometric value of the jejunum and ileum but not of the colon in a postmortem series of 131 fetuses with 10–40 weeks' gestation. Using autopsy and surgically resected specimens with 8–48 weeks' gestation, the thickness of the muscle layers of the small intestine and colon was measured, which showed a linear relationship between the thickness of circular and longitudinal muscles with gestational age (Lai et al., 2014). These studies indicate that measurements of the intestinal tract diameter are potentially helpful in evaluating intra-uterine normal growth.

The intestine undergoes rapid elongation during the embryonic and fetal periods. The intricate formation of the tertiary intestinal loops and the shift in position from the extraembryonic coelom to the abdominal cavity are notable for their dynamic nature. Several recent studies, including ours, have focused on these dynamic changes (Soffers et al., 2015; Ueda et al., 2018; Nagata et al., 2020; Ginzel et al., 2020; Kakeya et al., 2022). However, no studies measured the diameter of the small intestine except for the region at the umbilical ring (Soffers et al., 2015; Kakeya et al., 2022). It is unknown whether such dynamic changes affect the formation and growth of the layered structure and the increasing diameter of the small intestine.

Histogenesis proceeds from the rostral to the caudal end of the body (Huff, 2019). Therefore, different stages of differentiation may be observed along the rostral to caudal intestinal tract, with more differentiated morphology in the rostral than in the caudal tract. This feature may lead to discrepancies in the reported timelines of intestinal architecture differentiation in the literature. No previous studies have examined the diameter of the small intestine successively from the oral to the anal side of the small intestine. Therefore, the objectives of this study were to determine successive intestinal diameters from the oral to the anal sides (proximal to the distal), evaluate growth-related diameter changes, assess the positional effects along the intestine, examine the dynamic transition from the extraembryonic coelom to the abdominal cavity, and analyze the impact of complex intestinal loop formation in human embryonic and early fetal period.

Methods

Human Embryo Specimens

This study was approved by the ethics committees of Kyoto University Faculty and Graduate School of Medicine (approval numbers: E986, R0316, and R2224). Three human embryos (CRL: 25.6–32.3 mm, Carnegie stage [CS]: 23) and 11 human fetuses (CRL: 32.0–69.0 mm) were selected for this study (Table 1). Physiological herniation was observed in eight specimens. Five specimens were in the herniation phase (HN 1–5; CRL 25.6–32.3 mm), during which most of the intestine is in the extraembryonic coelom. Three specimens were in the transition phase (TS 1–3), during which the intestine is returning to the abdominal coelom (CRL 37.2, 41.0, and 43.0 mm). There was no physiological herniation in six specimens (return phase [RT1-6]; CRL 38.7–69.0 mm). The embryos were stored in 10% formalin solution at the Congenital Anomaly Research Center of Kyoto University (Yamaguchi & Yamada, 2018). According to the Maternity Protection Law of Japan, most specimens are obtained when the pregnancy is terminated for socioeconomic reasons. Specimens were collected from 1963 to 1995 according to relevant regulations. Written informed consent from the parents was not required. Instead,

parents provided verbal informed consent for specimen deposition, which was documented in the medical records. All specimens were anonymized and identified.

Image acquisition

Magnetic resonance imaging (MRI) was performed using a 7-T MRI system (BioSpec 70/20 USR; Bruker BioSpin MRI GmbH, Ettlingen, Germany) at the Medical Research Support Center, Graduate School of Medicine, Kyoto University. Various MRI coils and acquisition parameters were prepared for 7T-MRI. Appropriate settings were selected on the basis of the specimen size (Table 1, Supplementary Table 1).

3D reconstruction of the intestinal tract and mesentery

The MRI data were processed, and the intestinal tract (duodenum and small and large intestines) and associated mesentery were manually segmented using Amira software (version 5.50; Visage Imaging GmbH). Subsequently, the 3D images of these structures were reconstructed.

Definition of the four segments and tertiary intestinal loops in the small intestine

The oral end of the duodenum was defined on the basis of its 3D morphology, specifically the narrowing of the pyloric antrum at the anal end (O’Rahilly & Muller, 1987). The superior and proximal portions of the descending duodenum are derived from the foregut. The midgut inherently refers to the distal portion of the descending part of the duodenum and beyond; however, on the MR images, the pancreatic duct and ampulla of Vater, which mark the junction of the caudal foregut and midgut, could not be observed. On the basis of previous studies (Soffers et al. 2015; Ishida et al. 2025), we divided the small intestine into four segments (Segment-1, Segment-2, Segment-3, and Segment-4) and one colic segment. The boundaries of the small intestine segments were delineated by three short parts (Sps) of the mesentery (Sp1, Sp2, and Sp3), which corresponded to a thin dorsal mesentery as noted by Soffers et al., (2015), and the cecum (Figure 1a-i). The inflection point of the colon

was used as the caudal boundary of the midgut and hindgut, described previously as the future splenic flexure (Soffers et al., 2015). Approximately, Segment-1 corresponds to the duodenum, and Segments-2, -3, and -4 to the jejunal ileum in adults. Each segment was divided into tertiary intestinal loops. The folded structures of the associated mesentery proved to be useful indicators to discern each loop accurately (Ishida et al., 2025) (Figure 1a-ii).

Morphological observations and morphometry using magnetic resonance (MR) images

On MR images, mucosa and submucosa were recognizable in the same region at low intensity; the degree of the mucosal villi formation can be recognizable qualitatively but is difficult to estimate quantitatively. The muscular mucosa, the final layer to develop, is difficult to discern in the histology of the periods analyzed in this study (Huff, 2019). Proper muscular layers and serosa were recognizable as middle to high intensity. The border between the low-density inner layer and the high-intensity outer layer could be recognizable ambiguously to not be subject to further morphometry. The outer layer had high intensity, and the surface of the outer layer was clearly discernable from the mesentery and the adjacent intestine. Thus, it was suitable to segment the surface of the outer layer and subject it to the quantitative diameter measurements. The lumen cavity exhibited no or very low signal intensity, which was different from findings of the previous study (Hyde et al., 2020).

Amira software was used to measure the lengths of the midlines of the intestines for each segment and loop. For external diameter measurements, the outer boundary of the intestinal tract was segmented and successively visualized and measured using the Dragonfly software (version 2020.1.1; Object Research Systems, Montreal, Canada) (Figure 1b-i). The small intestine in Segment-1 was anatomically divided into four parts: the superior, descending, inferior, and ascending parts of the duodenum (Figure 1a-iii). The small intestine in Segments-2, -3, and -4 was divided by each tertiary intestinal loop. The software module was used to calculate the thickness as the diameter of a suppositional sphere fitted at each point. The distribution of hypothetical sphere diameters across the loops was displayed as a histogram. Since there were no significant changes in the diameter within the same

loop, except at the edge of the loop, i.e., the boundary with other loops, and the distribution of diameters was highly skewed around the mode, the mode on this histogram was used as the representative value of diameter for each tertiary intestinal loop (Figure 1b-ii).

Results

Gross view using 3D reconstructions

In the five specimens exhibiting the herniation phase, complex tertiary intestinal loop structures were observed in Segment-2, -3, and -4 of the extraembryonic cavity. However, these structures were not identified in Segment-1 of the abdominal cavity (Figure 2a, b; Supplementary Figure 1). The superior part of the duodenum was large, with a diameter of approximately 1.0 mm (indicated by red coloration on the surface map). In comparison, the diameters of Segments-2 to -4 were approximately half that size, with few changes from proximal to distal (ranging from 0.63–0.49 mm). The superior part of the duodenum consistently had the largest diameter, exceeding 1.0 mm in all specimens.

Among the three specimens in the transition phase, Segment-2 was located at the umbilical ring in one specimen with a CRL of 37.2 mm (Figure 2c), while Segment-4 was located at the umbilical ring in two specimens with CRLs of 41.0 and 43.0 mm (Figure 2d, e). In the two specimens with CRLs of 37.2 and 41.0 mm (Figure 2c, d), the diameter was found to be significantly larger in the upper part of Segment-1, followed by a gradual decrease from proximal Segment-2 to distal Segment-4. In the specimen with a CRL of 43.0 mm (Figure 2e), the diameters of the small intestine in Segments-2 and -3 were also greater than 1.0 mm. Notably, the loop was neither narrow at the umbilical ring, nor did its diameter change abruptly before or after passing through it.

In the six specimens in the return phase, all small intestines, including Segments-2, -3, and -4, were stored in the abdominal cavity (Figure 2-f, g). Segment-2 was situated on the left side of the abdominal cavity inferior to the splenic flexure of the colon. Segment-3 was situated at the midline of the abdominal cavity in the most caudal position. Segment-4 was situated on the right side of the abdominal cavity. At this point,

the diameters of the small intestine at Segments-1, -2, and -3 were greater than 1.0 mm, whereas the diameter of Segment-4 was smaller than that of the other proximal segments.

In all specimens, the diameters of the colonic part of the midgut (from the ileocecum to the inflection point of the colon) and appendix were smaller than that of any loop of the small intestine.

Length and number of the tertiary intestinal loops

The length of each segment and the total length increased linearly with the increasing CRL of the specimen, irrespective of the phase (herniation, transition, and return phases) (Figure 3a, Supplementary Tables 2 and 3). However, Segments-2, -3, and -4 lengths were almost similar, whereas Segment-1 was shorter than the other segments (Supplementary Table 3).

No tertiary intestinal loops were found in Segment-1 in all specimens. The number of tertiary intestinal loops in Segments-2, -3, and -4 increased with growth, and no apparent differences were observed in their numbers among the segments (Supplementary Table 4).

During the herniation and transition phases, the total number of tertiary intestinal loops from Segment-2 to Segment-4 exhibited a linear increase with increasing CRL, ranging from 11–22 (mean, 17.0) in the herniation phase and from 20–26 (mean, 22.7) in the transition phase. In the return phase, the total number of tertiary intestinal loops remained at 23–27 (mean: 24.3) (Figure 3b).

Cross-section of the intestinal tract

On the cross-sectional MR images of specimens with a CRL of 40 mm or less, the outer layer of the intestinal tract was discernible in high-intensity signals and distinctly delineated from the adjacent intestinal tract and mesentery tissue in all segments. Thus, the outer surface was segmented and subjected to morphometry. The mucosal tissue surrounding the lumen exhibited no uniform lower-intensity signal and no

discernible regions that could be identified as the apparent lumen in Segments-1 and -2, which may correspond to the villi and narrow lumen (TS1 in Figure 4). In contrast, short and rough villi and lumen (no signal intensity) were conspicuously observed in Segments-3 and -4.

On the MR images of the specimen with a CRL of approximately 40 mm, mucosa with well-developed villi were observed on the surface of the lumen in Segments-1, -2, and -3. The signal intensity reached a level comparable to that of the outer layer. In Segment-4, a layered structure was observed, although the luminal surface mucosal layer and outer muscular layer were thin, and no discernible villi were observed.

The outer layer was discernible as a high-intensity signal on the magnetic resonance images of the specimen with a CRL of approximately 50 mm. The mucosal layer with villi was detectable as a low-intensity signal in all segments (RT4 in Fig. 4). A layered structure was observed in the colon of all specimens, although the luminal surface mucosal layer and outer muscular layer were thin, and no discernible villi were observed. The diameter of the colon was small or of a similar size compared to Segment-4.

Diameter of the small intestine

In most of the specimens, the diameter of the superior part of Segment-1 (duodenum) was the largest of all loops, with a notable reduction in size in the remaining three parts of Segment-1 (Figure 5a, Supplementary Table 3). However, as the CRL was increased, the discrepancy in diameter between the superior part of Segment-1 (duodenum) and the remaining three parts of Segment-1 and Segments-2, -3, and -4 and (jejunum-ileum) diminished. In two specimens in the return phase (RT2 and RT5), tertiary intestinal loops with diameters larger than that of the superior part of Segment-1 (duodenum) were observed in the remaining parts.

The diameter of the small intestine from the oral end of Segment-2 to the anal end of Segment-4 (jejunum-ileum) exhibited a gradual and linear decrease, with occasional subtle increases and decreases (Figure 5a, Supplementary Table 5). The diameter from the oral end of Segment-2 to the anal end of Segment-4 (jejunum-ileum) was

approximated with a straight line, and the slopes of the lines were consistent across all specimens. Furthermore, the slope was unaltered throughout the positional transitions from the herniation and transition to the return phases (range ($\times 10^{-3}$), from -4.3 to -2.3; mean ($\times 10^{-3}$), -3.2) (Table 2). Present data indicate that the herniation, transition, and return phases do not differ in their effects on the diameter of the small intestine.

In the herniation phase, the diameter of the superior part of Segment-1 (duodenum) exceeded that of the remaining three parts of Segment-1. Nevertheless, it was nearly identical in the three distal parts of the specimens in the return phase (Figure 5b and Supplementary Table 3). The differences in diameter between the superior part of Segment-1 and the descending part ranged from 0.22 mm to 0.39 mm (mean 0.29 mm) in the herniation phase and from 0.18 mm to 0.40 mm (mean 0.32 mm) in the transition phase. In the return phase, the range of this difference was smaller than those in the herniation and transition phases (range: 0.05 mm to 0.17 mm, mean 0.10 mm), except for one specimen (RT3), in which the difference was 0.29 mm. A comparison of the remaining three parts revealed that the descending part was slightly larger than the inferior and ascending parts in specimens with small CRLs in the herniation phase (range of difference: 0.12–0.18 mm). In the other specimens, there was little difference among the three parts (Supplementary Table 3).

The diameter of the superior part of Segment-1 and the mean diameters of the remaining Segment-1 (duodenum), Segments-2, -3, and -4 (jejunum-ileum) exhibited a linear increase with increasing CRL, irrespective of the return condition (Figure 6). However, the superior part of Segment-1 exhibited a lower rate of increase than the other segments (slope=0.012). The average diameters of the remaining Segment-1 and Segments-2 and -3 also increased linearly at a rate that seemed almost identical (slopes for their remaining Segment-1, excluding the superior part, were 0.022, while that for Segment-2 was 0.024, and for Segment-3 was 0.023). The slope for Segment-4 was slightly smaller than those for Segments-2 and -3, at 0.019.

Discussion

Based on cross-sectional observations, the differentiation and increased height of

the inner layer (mucosa and submucosal layers), as described in previous studies (Johnson 1910; Lewis 1912ab), were detected with increasing CRL, suggesting that diameter growth is primarily driven by inner layer development. In contrast, the outer muscular layer remained thin, and further subdivision of the inner circular and outer longitudinal layers was undetectable. The thicknesses of the muscular layers may contribute minimally to diameter growth. The increase in diameter requires an increase in circumference; from this perspective, muscular layer growth may be one of the limiting factors in determining diameter.

Recent studies showed that the extrinsic and intrinsic innervation (derived from neural crest) of the intestinal wall preceded the concentric layers formation (Wallace & Burns, 2005; Sasselli et al., 2012). The delay between the passage of the migratory front of neural crest-derived neural cells and subsequent transformation of the architecture of the intestinal wall was 2–3 Carnegie stages in the human small intestine and approximately 4 Carnegie stages in the human colon. Kruepunga et al. (2020) noticed that the nerve extension along the branches of the superior mesenteric artery was blocked at the umbilical ring and did not reach the intestinal tract located in the extraembryonic coelom in the physiological umbilical hernia phase. The delay in innervation might result in the delayed formation of the layered structure of the tract and delayed increase in the diameter of the tract in the extraembryonic coelom. In our study, however, a linear reduction in the diameter from the oral to the anal region of the jejunum-ileum was observed in all specimens, suggesting that the growth and differentiation of the small intestine may proceed smoothly and continuously from the oral to anal tract in one dimension. Namely, the present results do not suggest that intestinal position (in the extraembryonic coelom or abdominal cavity) affects the diameter. Furthermore, other dynamic changes, such as rapid intestinal elongation and complicated tertiary intestinal loop formation, also appeared not to affect the diameter, suggesting that such dynamic, well-known morphological changes in the small intestine in the embryonic and early fetal period were independent of its diameter.

The diameter and its growth-related changes differed between the superior part, which belongs to the foregut, and the remaining three parts of Segment-1 (the duodenum), which belongs to the midgut. The latter exhibited similarities to

Segments-2, -3, and -4 (jejunum and ileum). Johnson (1910) described those vacuoles transitioned to villus formation earlier in the upper part of the duodenum (foregut) than in the lower part (midgut), and a structure called the “duodenal pocket” appeared only in the lower duodenum during the embryonic stage, similar to the intestinal pocket in the jejunum and ileum. Passage of the vagal neural crest-derived cells, appearing in the midgut 1–3 Carnegie stages after the appearance of the foregut, including Segment-1 as described previously (Kruepunga et al., 2020), might also result in the different features in the diameter and its growth-related changes between the upper part of Segment-1 and remaining three parts.

Recent clinical studies have measured the diameter of the intestine of the fetus at 20–41 weeks’ gestation *in utero* using sonography, indicating that the diameter increases with age (Lap et al., 2019). In all cases of progressive dilatation of the small intestine, pathology of the small intestine was confirmed postnatally. Furthermore, a significant correlation was identified between a substantial small intestinal diameter after 25 weeks’ gestation and the presence of small intestinal pathology (Lap et al., 2019). The application of diameter measurements may be useful as a discrimination marker of standard and abnormal intestine development. The lessons to be learned from our study are that the diameter gradually decreases from the oral to the anal end, suggesting that it is essential to set the anatomical site where the diameter will be measured when the diameter measurement is applied to clinical use. However, previous studies did not indicate the site of measurements accurately.

By applying *in utero* sonography at the human embryonic and early fetal period, the dynamic events of physiological umbilical herniation of the midgut in the extraembryonic coelom and its safe return in the abdominal cavity can be observed during normal development of the small intestine as well as during early diagnosis of omphalocele (Parulekar 1991; van Zalen-Sprock et al., 1997; Bogers et al., 2018). Although it remains difficult to examine the diameter of the small intestine as contents of the extraembryonic coelom in the embryonic and early fetal period, the data presented herein will serve as a valuable reference point for future studies, particularly because the resolution of sonography is expected to improve shortly.

Limitations

This study is limited in the following ways. First, three different conditions were used according to the sample size, which may have affected the significant digits of the morphometry for each specimen. Owing to the intrinsic resolution of MR images, only two layers of the intestinal tract were identified. Second, the anal end of the pyloric antrum was defined as the oral end of the duodenum. This definition does not refer to other anatomical landmarks, such as the pancreatic ducts and inferior vena cava, which could provide more accurate measurements and differentiation of the duodenum of the foregut and midgut. Third, the number of specimens included in the present study was small (N=14), which precluded a statistical examination of the segment-specific differences in the trend of increasing diameters with increasing CRLs. Finally, shrinkage for long-term preservation in a 10% formalin solution should be considered.

Conclusions

In this study, successive intestinal diameters from the oral to the anal sides of the intestinal tract were demonstrated using 3D reconstruction and morphometry. The dynamic positional change from the extraembryonic coelom to the abdominal cavity, along with rapid elongation and complex tertiary intestinal loop formation—a conspicuous phenomenon in the embryonic and early fetal periods—has little effect on the change in diameter.

Statement of Ethics

The ethics committee of the Kyoto University Faculty and Graduate School of Medicine approved this study, which used human embryos and fetal specimens (E986, R0316, and R2224).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

N.I., T.K., J. Matsubayashi: data analysis/interpretation

J. Männer. S.Y. H.I.: acquisition of data

T.T.: concept/design, critical revision of the manuscript

Data Availability Statement: Data are available on request.

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Supplementary Material

Supplementary Figure 1

Three-dimensional reconstructions of the intestinal tract

The intestinal tract from the duodenum to the midgut of the colon is shown in the right lateral (Hn1, Hn3, and Hn5) and frontal (Rt1, Rt2, Rt5, and Rt6) views. Scale bar indicates 1 mm.

Large reconstruction on the left: The diameter of the successive intestinal tract is color-mapped to the surface using rainbow colors. The diameters of the cecum, appendix, and colic part of the midgut are smaller than those of the small intestine in all the reconstructions.

Small reconstruction on the right: The location of four segments of the intestinal tract is indicated. The colors of the intestinal tube indicate Segment-1 (red), Segment-2 (yellow), Segment-3 (green), Segment-4 (blue) and colon (black). The numbers above the reconstruction indicate the total number of tertiary intestinal loops in the small intestine, with numbers per Segment-2, -3, and -4 in parentheses.

Note that the rainbow coloring ranges were changed according to increased CRL.

a, anal side; Ap, appendix; Ce, cecum; light blue line, umbilical ring; CRL, crown-rump length.

Figure Legends

Figure 1. Methods and definitions used in this study

a) Schematic diagram of four segments of the small intestine

i) The small intestine is divided into four segments: Segment-1 (red), Segment-2 (yellow), Segment-3 (green), and Segment-4 (blue) using the short parts of the mesentery (Sps. 1-3; purple lines) and ileocecum (purple dot).

a, anal side; o, oral side

+ The oral end of the duodenum in the present study corresponds to the end of the pyloric antrum (thick black). Note that this point was defined on the basis of morphological observations.

ii) Tertiary intestinal loops are defined by folded structures formed by the associated mesentery.

Black line: intestinal tract; pink and green membranes: mesentery; purple line: Sps.

iii) Segment-1 was anatomically divided into four parts: superior, descending, inferior, and ascending.

b) i) Visualization of the successive diameter using Dragonfly software

The diameter of the successive intestinal tract is color-mapped to the surface using rainbow colors. Rainbow color bars are indicated below.

ii) Methods showing the measurements of each tertiary loop using Dragonfly software.

The distribution of the diameter per loop is indicated on the histogram. The mode (arrowhead) represents the value of the diameter per loop. The lower limit of the histogram is standardized at 0.1 mm for all specimens and the upper limit set at three levels (1.0, 1.5, and 2.0 mm) for each specimen to ensure that all bins fall within the range.

Figure 2. Representative three-dimensional reconstructions of the intestinal tract

The intestinal tract from the duodenum to the midgut of the colon is shown in the right lateral (a-e) and frontal (f and g) views. Scale bar indicates 1 mm.

Large reconstruction on the left: The diameter of the successive intestinal tract is color-mapped to the surface using rainbow colors. The diameters of the cecum, appendix, and colic part of the midgut are smaller than those of the small intestine in all the reconstructions.

Small reconstruction on the right: Location of four segments of the intestinal tract is indicated. The numbers above the reconstruction indicate the total number of tertiary intestinal loops in the small intestine, with numbers per Segment-2, -3, and -4 in parentheses. S1 (red): Segment-1, S2 (yellow): Segment-2, S3 (green): Segment-3, S4 (blue): Segment-4

a, anal side; Ap, appendix; Ce, cecum; IP, inflection point; light blue line, umbilical ring.

Figure 3. Total length of the small intestine and number of tertiary intestinal loops

a) The total length of the small intestine

The distance from the oral end of Segment-1 to the anal end of Segment-4 (cecum) is calculated.

b) The number of tertiary intestinal loops in the small intestine with increasing CRL

white circle, herniation phase; cross, transition phase; black circle, return phase; CRL, crown-rump length.

Figure 4. Cross-sectional MR images of the intestinal tract per each segment

Representative MR images of three specimens with transition (TS1 and TS2) and return phases (RT4) for each segment. The CRLs of each specimen are indicated.

The degree of differentiation of the mucosa differs not only according to the CRL of the specimen but also according to the segments of the small intestine.

The scale bar indicates 0.5 mm.

The red, yellow, and green asterisks represent the intestine in Segment-1, Segment-2, Segment-3, and Segment-4, respectively.

The initials AP, IP and DP are all parts of the duodenum. AP; ascending part, IP; inferior part, DP; descending part; MR, magnetic resonance; CRL, crown-rump length.

Figure 5. The diameter of the small intestine

a) The diameter of the intestinal tract per each part (duodenum) and per each tertiary intestinal loop

The x-axis indicates the intestinal length from the oral end of the duodenum. The diameter of each part (superior, descending, inferior, and ascending) of the duodenum and that of each tertiary intestinal loop are plotted. Data from all specimens are indicated. Data from the tertiary intestinal loops of the umbilical ring are shown in red.

692 b) The diameter of each part [superior (orange), descending (blue), inferior (green),
693 and ascending (purple) parts]) in the duodenum per specimen was plotted.
694 Gray background indicates transition specimens.

695

696 Figure 6 Change in diameter of the small intestine per segment according to the
697 increase in CRL

698 a) Segment-1, b) Segment-2, c) Segment-3, d) Segment-4

699 For Segment-1, the data value at the superior part and the average of the remaining
700 three parts are indicated.

701 Approximate straight lines from each segment were imposed in d).

702 CRL, crown-rump length.

Table 1. Samples imaged using MRI systems

Phase	ID	CRL (mm)	MRI Condition*	Spatial resolution (isotropic, μm)
Herniation phase [n=5]	HN1	25.6	1	41.3
	HN2	29.1	1	46.9
	HN3	32.0	2	35.4
	HN4	32.3	2	54.7
	HN5	36.5	1	40.0
Transition phase [n=3]	TS1	37.2	2	54.7
	TS2	41.0	1	39.1
	TS3	43.0	2	60.5
Return phase [n=6]	RT1	38.7	2	40.0
	RT2	43.0	2	54.7
	RT3	43.5	2	54.7
	RT4	52.0	2	54.7
	RT5	56.5	3	70.3
	RT6	69.0	3	69.0

*The coil sizes used were 19 mm for condition 1, 35 mm for condition 2, and 72 mm for condition 3. Details of the MRI conditions are given in Supplementary Table 1.

CRL, crown-rump length

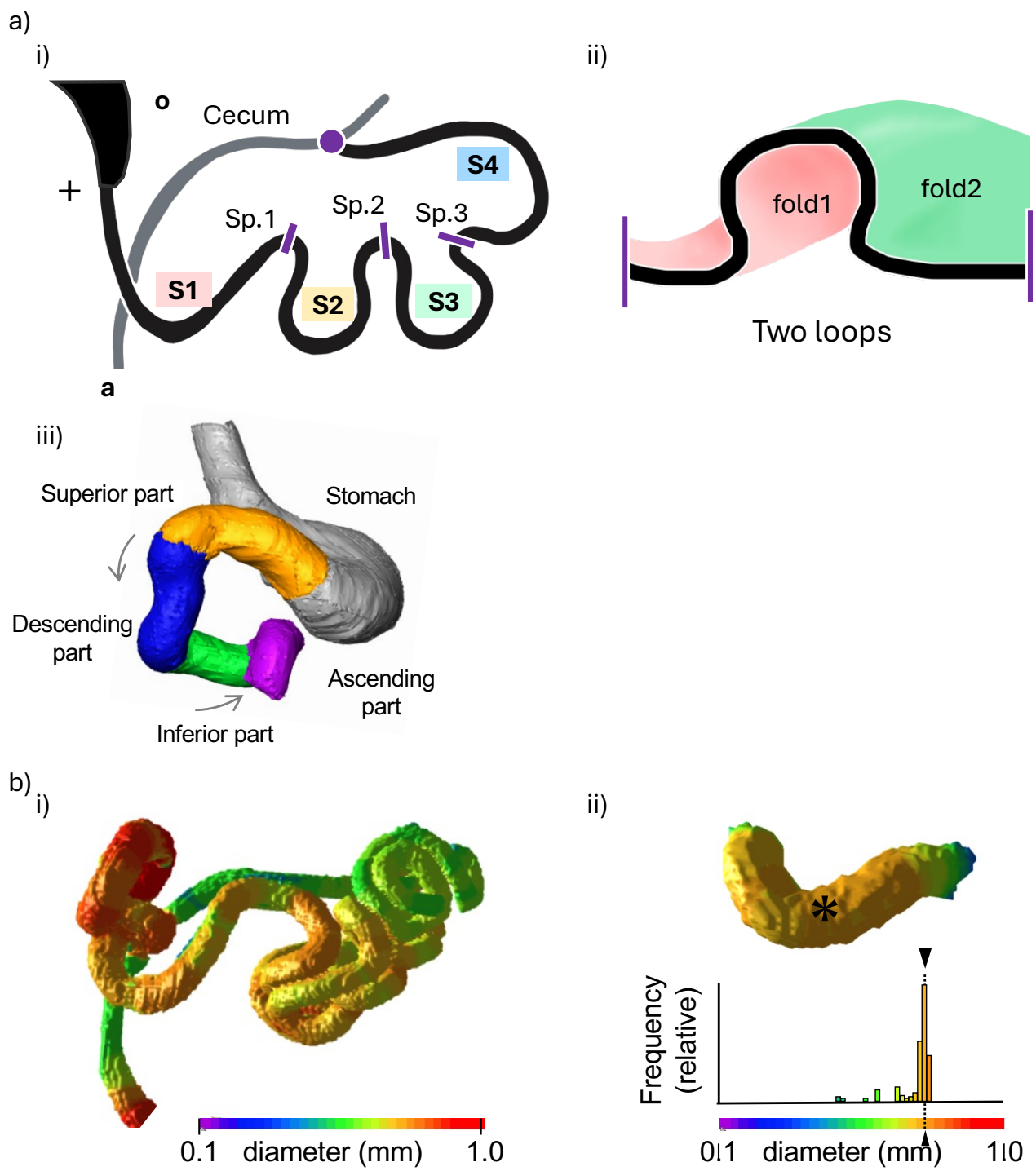


Figure 1

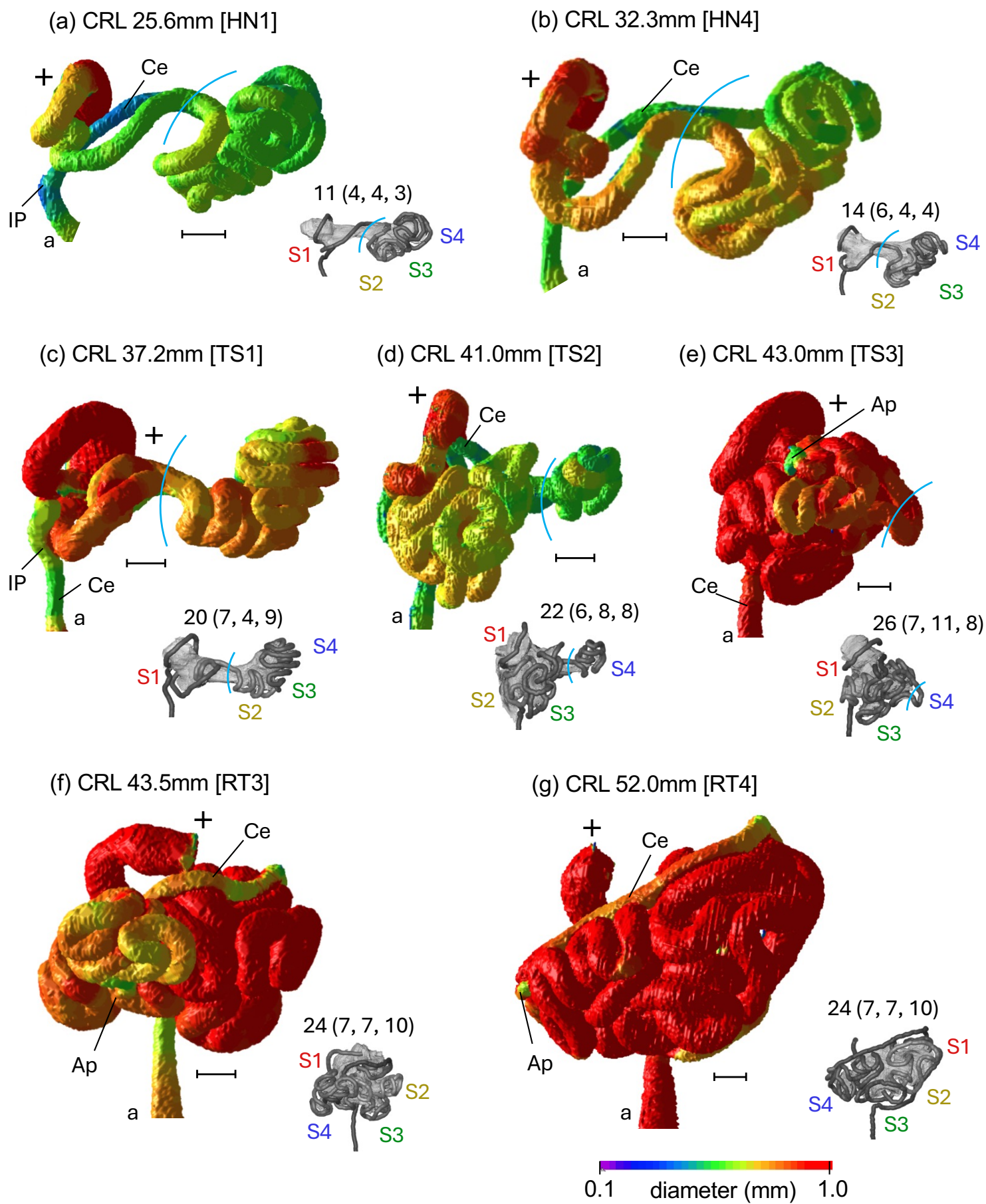


Figure 2

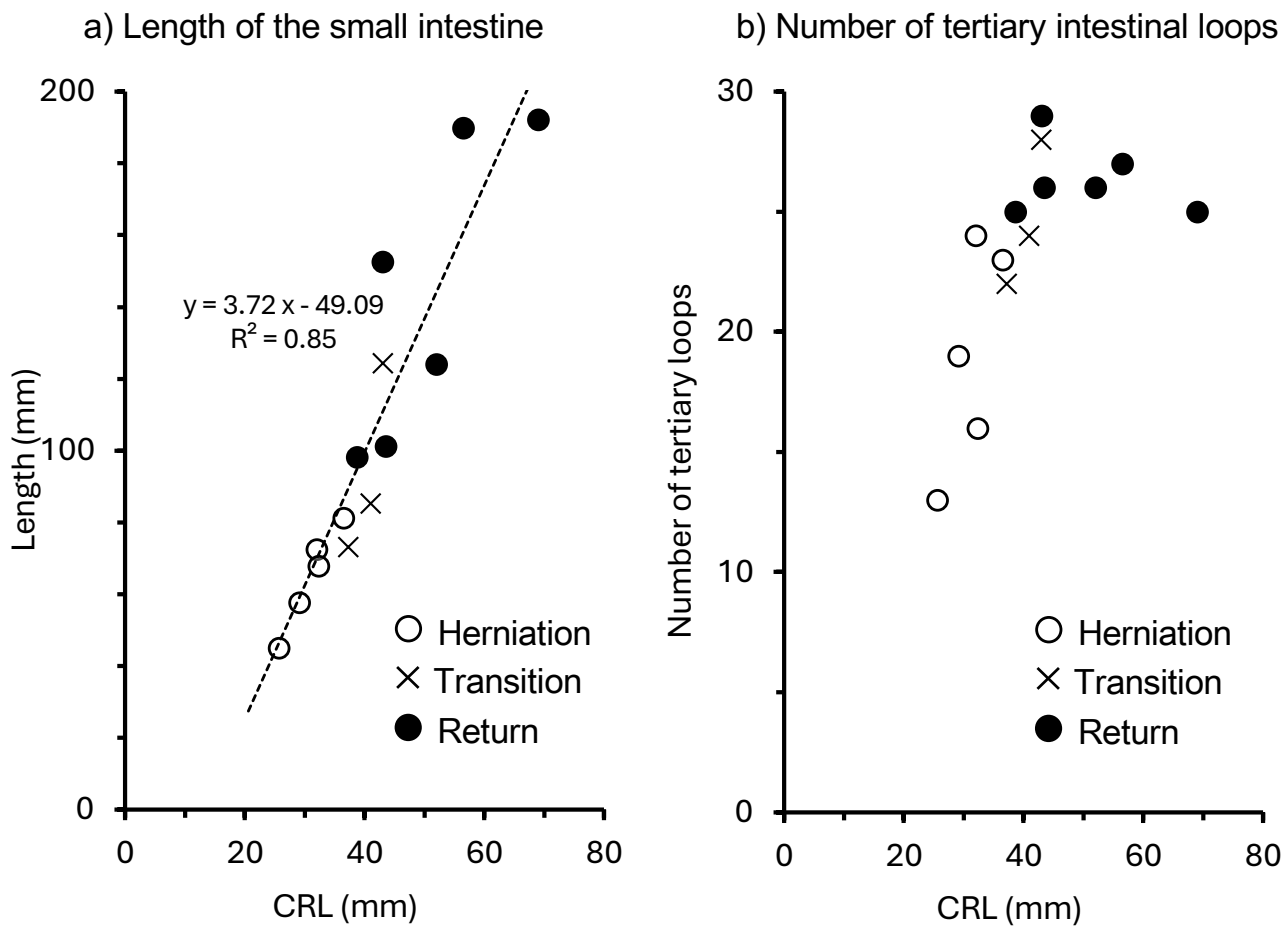


Figure 3

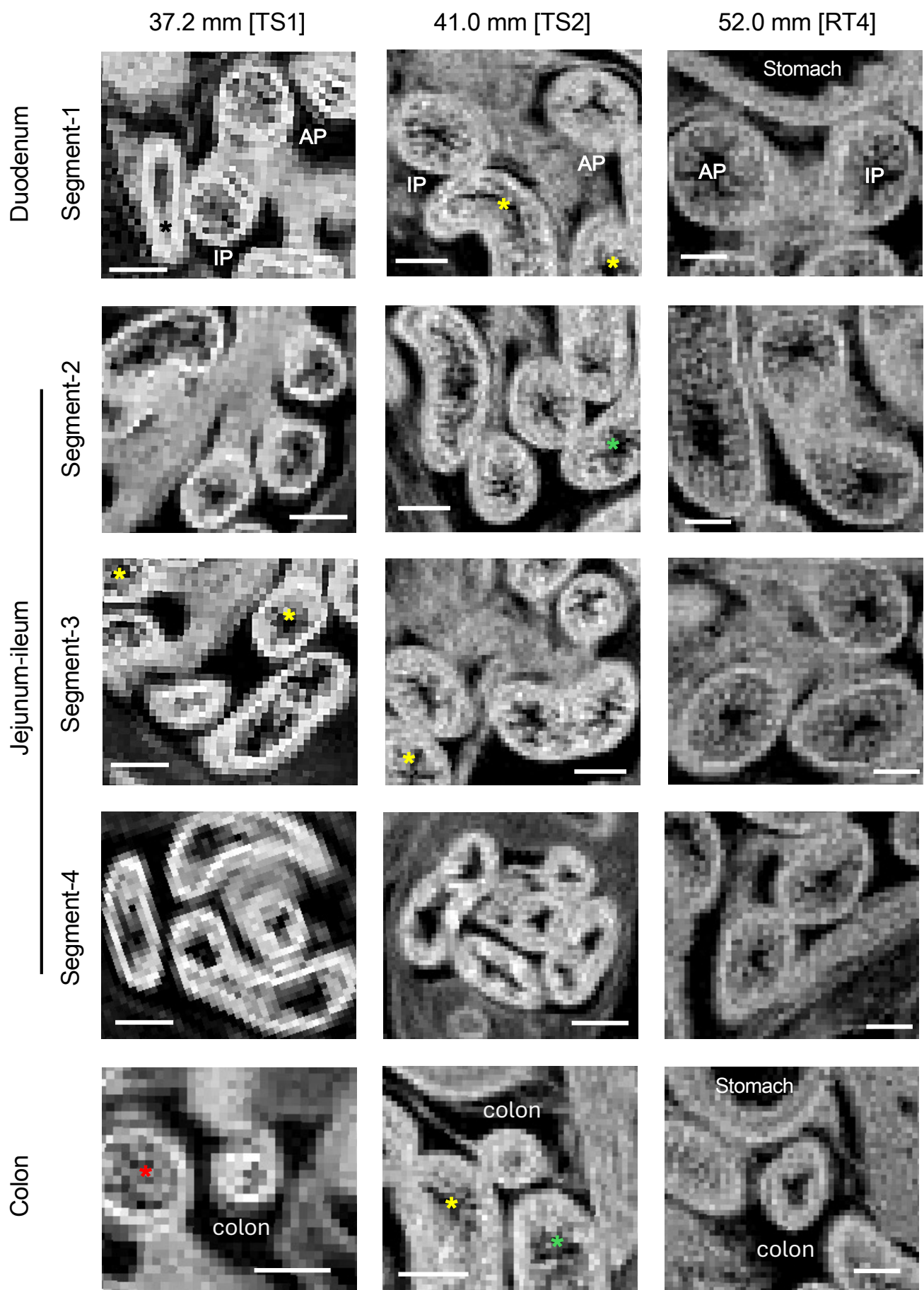


Figure 4

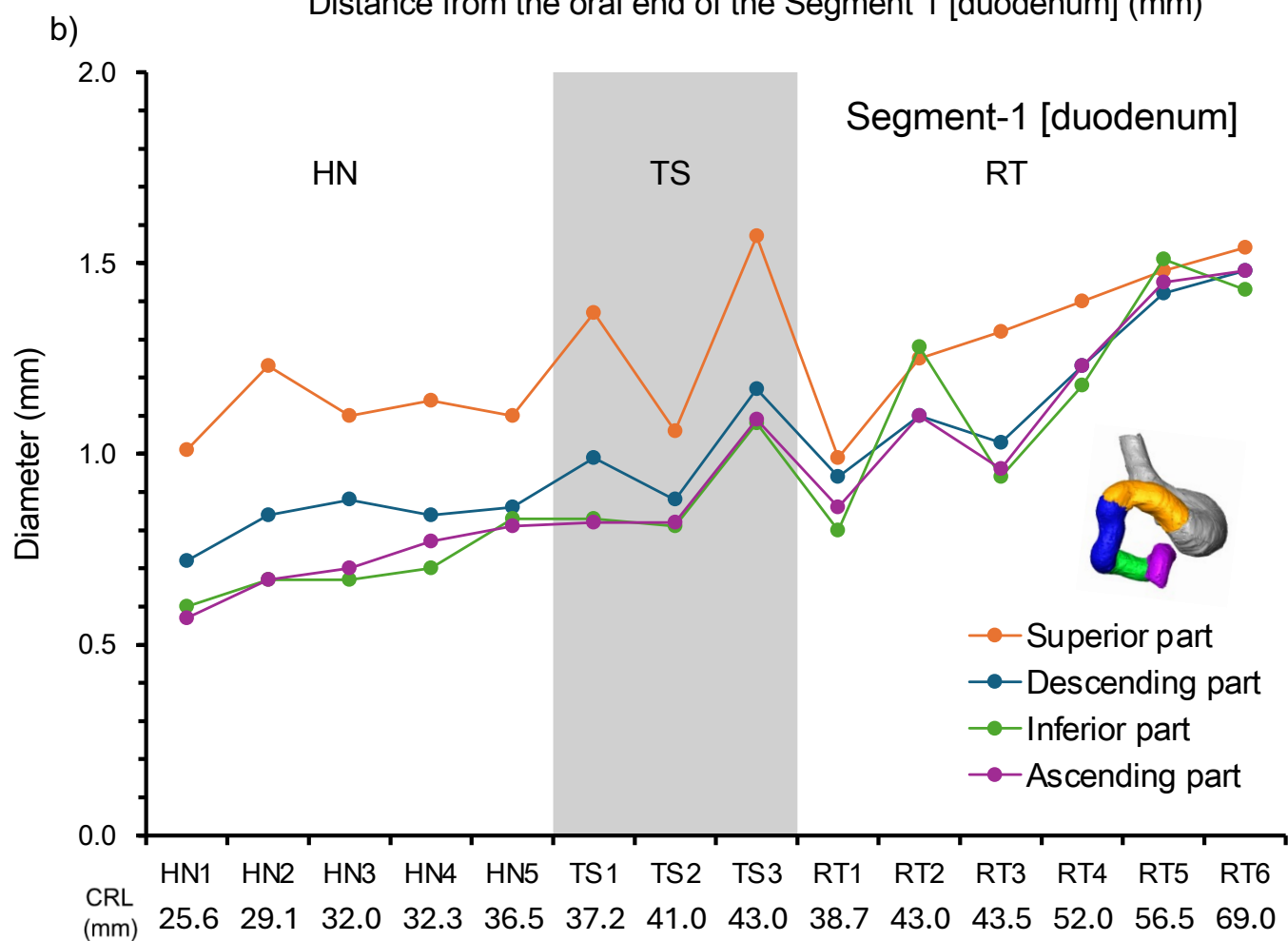
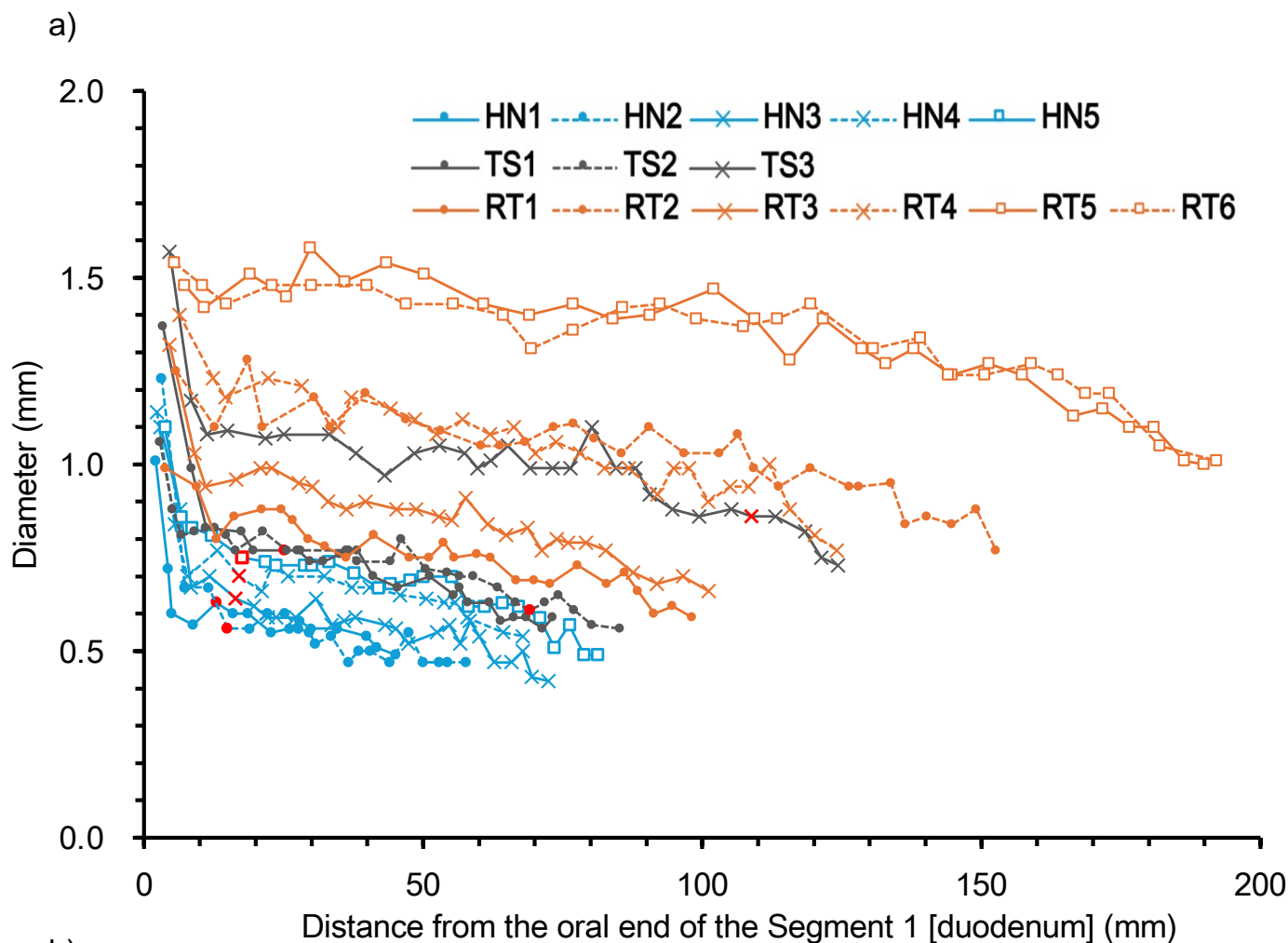


Figure 5

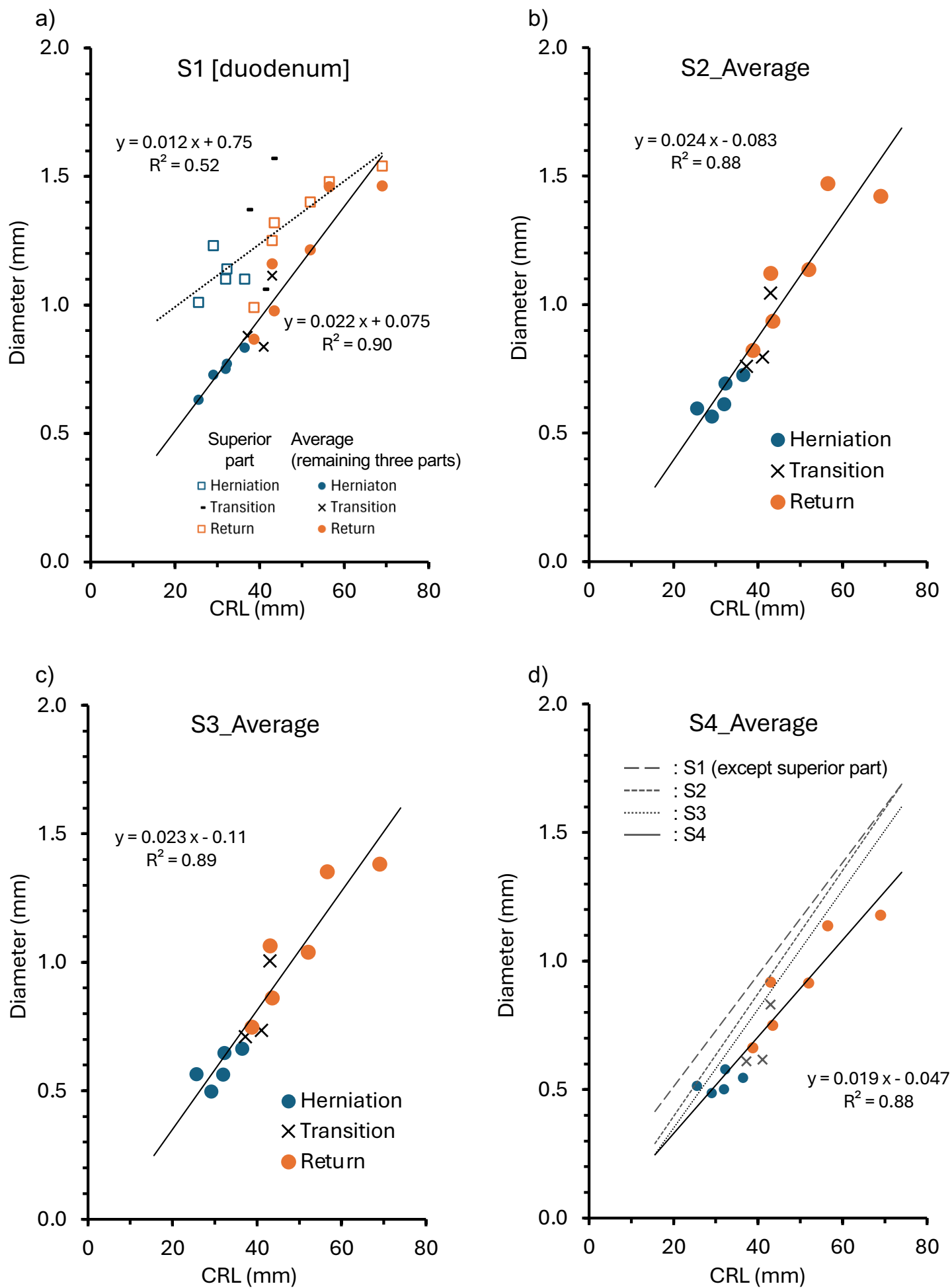


Figure 6