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Macro and Micro-anatomy of Tokay Gecko's Reproductive Organs and Growth of External Body in Support on Reproduction Activities (Squamata: Gekkonidae: *Gekko gecko*)

[STRUKTUR MAKRO DAN MIKRO-ANATOMI ORGAN REPRODUKSI TOKEK RUMAH DAN PERKEMBANGAN EKSTERNAL TUBUH YANG MENYOKONG AKTIVITAS REPRODUKSI (SQUAMATA: GEKKONIDAE: *GEKKO GECKO*)]

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ABSTRACT

Tokay gecko (*Gekko gecko)* is an oviparous lizard that is distributed very widely in tropical Asia. The sexual maturity stage of the tokay gecko does not have seasonal reproductive activity; it reproduces throughout the year. The study on the reproduction organ and growth of external morphology of tokay gecko is the first contribution to further scientific information. Gonadal micro-anatomy that was shown by histology and macro-anatomy include the sex accessories that could be demonstrated in this lizard as a criterion for the reproductive activity of urogenital organs. Histological analyses were used the paraffin method, sectioned with 5 µm of thickness and stained by hematoxylin-eosin. The males and females of the tokay gecko have simple urogenital track systems, and also the males have simple hemipenis morphology. The testes' position inside the male body is not symmetric with the right testis is always in a higher position than the left testis. Based on analysis of the measurement of the posterior part of the body, the width of the cloaca, width of base tail, and length of hind limb showed asymptotic growth on the snout to vent length (SVL); SVL≥130 mm for females, and SVL≥150 mm for males. Asymptotic growth in the posterior part of the male and female body is a form of co-evolution, besides the male urogenital organs and the female reproductive system, which also support co-evolution in the reproductive organs.

Keywords: *Gekko gecko;* histology; tokay gecko; urogenital organs

ABSTRAK

Tokek rumah (*Gekko gecko)* adalah kadal ovipar yang distribusinya sangat luas di Asia tropis. Tahap kematangan seksual tokek rumah tidak memiliki aktivitas reproduksi musiman, tokek bereproduksi sepanjang tahun. Studi tentang organ reproduksi dan pertumbuhan morfologi eksternal tokek adalah kontribusi pertama untuk informasi ilmiah lebih lanjut. Mikro-anatomi gonad yang ditunjukkan oleh histologi dan makro-anatomi termasuk aksesori kelamin dapat ditunjukkan dalam kadal ini sebagai kriteria untuk aktivitas reproduksi organ urogenital. Analisis histologi dilakukan dengan metode parafin, tebal irisan 5 µm dan pewarnaan hematoksilin eosin. Tokek rumah jantan dan betina memiliki sistem jalur urogenital sederhana dan juga jantan memiliki morfologi hemipenis sederhana. Posisi testis di dalam tubuh jantan tidak simetris, testis kanan selalu dalam posisi testis kiri lebih tinggi. Berdasarkan analisis pada pengukuran bagian posterior tubuh, lebar kloaka, lebar pangkal ekor dan sistem tungkai belakang menunjukkan pertumbuhan asimptotik pada panjang moncong sampai anus atau *snout to vent length* (SVL); SVL≥130 mm untuk betina, dan SVL≥150 mm untuk jantan. Pertumbuhan asimptotik di bagian posterior tubuh jantan dan betina adalah bentuk ko-evolusi, selain organ urogenital jantan dan sistem reproduksi betina yang juga mendukung ko-evolusi dalam organ reproduksi.

Kata-kata kunci: *Gekko gecko;* histologi; tokek rumah; organ urogenitalia

INTRODUCTION

Tokay gecko (*Gekko gecko*) is an oviparous Squamata, which the fertilization of the egg occurs inside the female's body. Then a shelled egg is taken out from the body to go into a period of incubation which takes around 65 days (Das, 2016). According to Manthey and Grossmann (1997), adult females of tokay geckos lay eggs throughout the year with intervals of approximately 30 days before the next egg-laying time. Tokay geckos reproduce continuously without depending on the season. This pattern also occurs in house lizards, *Hemidactylus frenatus* (Goldberg and Kraus, 2016), both of which gekkonid species live sympatric in human habitation. Generally, Squamata reproduces offspring depending on the season, especially those of the species in the temperate regions, such as *Acanthodactylus erythrurus* (Castilla *et al*., 1992), *Anolis carolinensis* (Jenssen *et al*., 1995), *Takydromus woltery* (Luo *et al*., 2012), *Goniurosaurus kuroiwae* (Kurita and Toda, 2013), and *Anolis carolinensis* (Johnson *et al*., 2014). Most of the species in the tropics area were reproduce depending on the season (James and Shine, 1985).

Tokay gecko is a widespread reptile. They distributed in southern China, northeast Asia, and Southeast Asia included all islands in Indonesia (Caillabet, 2013; Reilly *et al*., 2019); tokay geckos are very adaptable in many different habitats. The gecko can live in the lowlands to altitude 1200 m above sea level (asl) and in primary forests, secondary forests, plantations, and human settlements (McKay, 2006; Bucol and Alcala, 2013; Singh and Choudhury, 2016). A large number of these species many used for consumption (Nijman *et al*., 2012; Caillabet, 2013). The current status of the tokay gecko is not comparable with the biological information in revealed the life of these reptiles, especially those living in Indonesia. The first information on the growth of tokay geckos in the juvenile stage was revealed by Nugrahani (2013). Then Kurniati and Phadmacanty (unpublished data) reported that the age and body growth of the sexual maturity stage. Morphological disclosure of internal reproductive organs, i.e. hemipenis morphology of tokay geckos were not yet available. The comparison of the hemipenis morphology of *G. smithii* and the others *Gekko* groups that live in southeastern mainland Asia and the Philippine Islands was revealed by Rösler *et al*. (2011). Tokay gecko's relatives, namely *Hemidactylus frenatus* and *H. platyurus* had been described by Das and Purkayastha (2012). In contrast, members of the Varanidae, *Varanus salvator* had been described by Kusuma *et al*. (2017) and Mafud *et al*. (2017).

The limbs, especially hind limbs, played a major role in the reproduction process. This phenomenon has been widely expressed in *Anolis* spp (Gredler *et al*., 2015; Tschopp *et al*., 2014; Klaczko *et al*., 2017), in which the lizard that changed the shape and size of the hind limb would affect the size and morphology of the hemipenis and the shape of the cloaca (Gredler *et al*., 2015; Nunes *et al*., 2014). Most of the function of the body morphology and the function of the hind limbs in the reproduction process has not been revealed in tokay geckos. This research was conducted to reveal the external morphological structure and histology of male and female reproductive organs of the tokay gecko. This research is the first contribution to the biology of the tokay gecko. Besides that, the study also revealed the function of external body parts that play a role in the reproduction process.

RESEARCH METHODS

Ethics Statement

Most of the samples were obtained from collectors in Central Jawa and East Java Provinces in freezing conditions, while some individuals were collected from residents' homes taken in living conditions. Euthanization was performed by following the rapid freezing method of the Underwood *et al*. (2013).

Measurement

A total of 95 specimens of tokay gecko consisted of 21 females (20 mature; 1 immature) and 74 males (69 mature; 5 immature) were used in morphometric measurements. The body measurement utilized a dial caliper with an accuracy approached 0.1 mm. Body length from the tip of the snout to the cloaca (SVL) was a determining factor in the analysis of correlations with other body parts measured. Another part measured was the length from the axilla to the groin (AG). Measurements of the hind limbs and rear body morphology were only for the right side of the specimen (Figure 1). The unit used in measuring was millimeters (mm). The posterior body parts measured were: femur length (FL), measured from the base of the upper femur to

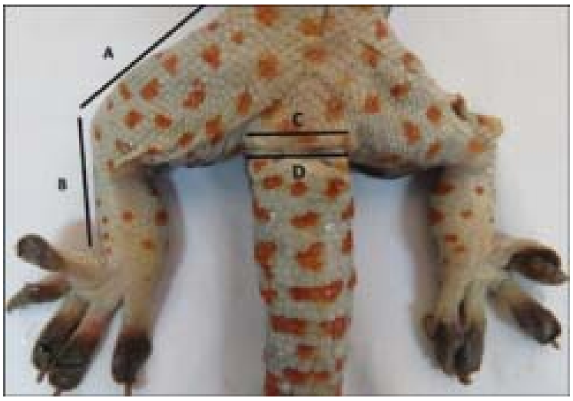

Figure 1. Measurement of posterior body parts. A. Femur length (FL); B. Tibia length (TL); C. Cloaca length (CL); D. Width of the tail base (BL); Hind limb length (HL) was FL+TL.

the knee joint; tibia length (TL), measured from the knee joint to the base of the outer finger joint; hind limb length (HL), was FL plus TL; cloaca length (CL), measured from the right corner to the left corner of the cloaca; width of the tail base (BL), measured at the base of the tail where located below the cloaca.

Measurement of the length and width of the immature and mature male testes used a digital caliper with 0.1 mm for accuracy. Testicular volume was calculated following Johnston (1999), i.e.:

V=4/3 <! ($L^2W/2$)

Where V was testis volume in mm^3; L was the length of testis and W was the width of the testis, L and W in mm.

Statistical Analysis

Linear regression between SVL and morphometry of other body parts was obtained from statistical analysis using Excel software version 2016. Minitab software version 17 was used to determine if the linear regression of the relationship between SVL and other body parts varied significantly or not. A value of $p<0.05$ indicated the two morphometric factors were significantly different.

Observation of Reproductive Organs

Reproductive tracts of ten mature females and males tokay gecko were observed after encryption. The reproductive organs were traced from the ovaries or testes to the cloaca by referring to *Anolis carolinensis* (Crews, 1978); after tracing, the reproductive organs and their ducts were photographed and then sketched to determine the position and parts that formed a single unit of the reproductive organ system.

Histology of parts of reproductive organs

Male reproductive organs were taken from specimens as a whole reproductive tract, included testes, ductus deferens, epididymis, and hemipenis. In contrast, the female reproductive tract was taken intact, included the ovary, infundibulum, uterus, and vagina based on the histological method by Kiernan (1990). Parts of the organs were fixed in a 10% NBF solution; after fixation, the organs were washed in a container with running tap water for 15 minutes, then dehydrated in multilevel ethanol concentrations, i.e. 70%, 80%, 90%, 90%, 95% and absolute ethanol, respectively. Then dehydration treatment was conducted for one hour for each concentration. Purification was achieved by soaking the organs in xylene (Merck, 1.08661.2500) for an hour, and then the organs were infiltrated in three levels of liquid paraffin, each of which took one hour; after that, the organs were embedded in a paraffin to make a block parrafin. Then the paraffin block was cooled until the paraffin froze. The organ was released from the mold and then cut with a rotary microtome (RX-860 Rotary type®, Yamato/PT Cahayatiara Mustika Sci Co, Cikarang, Indonesia) with a thickness of 5 ¼m. The histology tissue objects were stained by hematoxylin-eosin, and then the objects were observed under a microscope (Euromex-microscope®, Euromex Microscopen BV, Arnhem, Holland) that was connected to the camera. Finally, the object was photographed with magnification between 100-400 times.

RESULTS AND DISCUSSIONS

Some specimens that were used for microanatomy observation were specimens that have been preserved for a long time in the freezer at -20^0C. According to Baraibar (1984), histology of tissue from samples that have been in the freezer for such a long time would get osmotic disorder inside the cells. So, the cells were unable to absorb chemical dyes well; slightly coloured histology tissue was proven in this study.

Male Reproductive Tracy Macro-anatomy

The male urogenital organs of the Tokay gecko were similar to *A. carolinensis* (Crews, 1978). They consisted of a pair of testes, epididymis, and ductus deferens. A pair of kidneys were located in the posterior part of the epididymis and attached to the ductus deferens. The ductus deferens then continued to the hemipenis (Figure 2). The right testis was always higher than the left testis, and this position also belonged to *A. carolinensis* (Crews, 1978). The testes were connected to the epididymis duct and then proceeded to the ductus deferens duct. Finally, the ductus deferens ended up in the hemipenis, that located in the cloaca. On the posterior part of the ductus deferens, there was a pair of kidneys. On the anterior of the kidneys, there was a pair of the ureter. The urethra joined the ductus deferens before heading to the hemipenis.

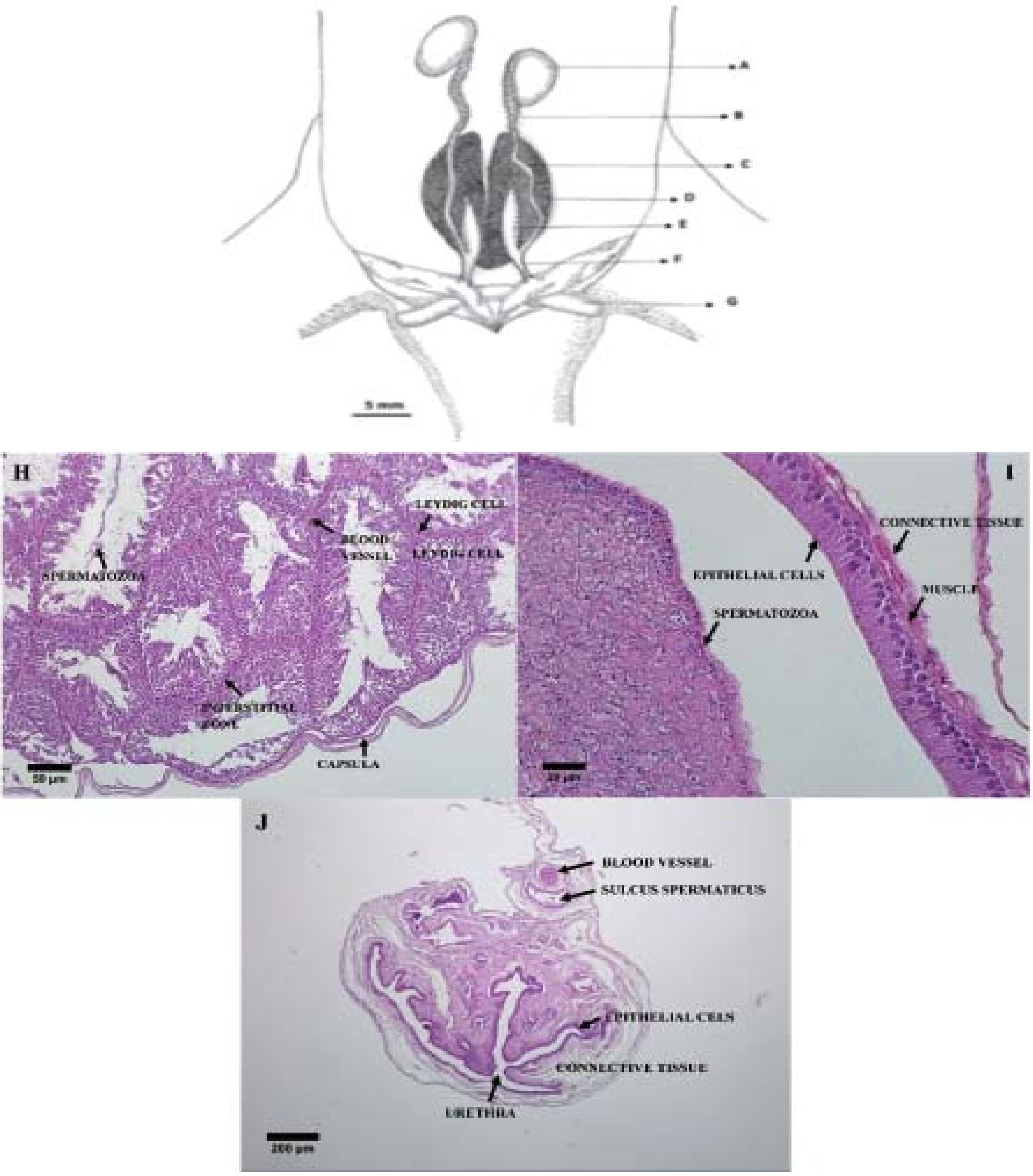

Figure 2. Reproduction organs and kidney of sexual maturity male of tokay gecko. A. Testis; B. Epididymis; C. Ductus deferens; D. Kidney; E. Ureter; F. Joining duct of ductus deferens and urethra; G. hemipenis. H. Histology of the testis (magnification 100 times). I. Histology of the epididymis (magnification 400 times). J. Histology of testis lobe (magnification 40 times).

The epididymal duct of the tokay gecko was squiggly at the top of the kidney. Then the ductus deferens duct straightened up and stuck to the kidney. The tokay gecko's ductus deferens were different from the ductus deferens of *A. carolinensis*, in which the ductus deferens kept twisting to the hemipenis (Crews, 1978). The hemipenis morphology of tokay geckos (Figure 3) was simple compared to other species in the *Gekko* spp. group (Rösler *et al*., 2014). The hemipenis of the Tokay gecko is smooth without any ornaments with a smooth surface of the lobe. The hemipenis of tokay geckos was similar to the hemipenis of *the Hemidactylus* spp group (Das and Purkayastha, 2012) that were members of the Gekkonidae. The outer morphology of the tokay gecko's hemipenis was smooth with no ornaments along the body to the top. In the top (head) part of the hemipenis, there were three bulges with smooth surfaces. Hemipenis of tokay geckos have no pair of pockets at the base of the tail; the right and the left hemipenis merged and lay horizontally parallel to the cloaca opening. Other than, that the hemipenis of the tokay gecko was unclear in the division of the hemipenis body part. The diameter of the hemipenis is not different between the truncus and the lobe. The right and left hemipenis are united to form the shape of a pipe.

Male Reproductive Microanatomy

Histological observations were conducted in the reproductive organs of mature males. The organ parts observed were testis, epididymis, and hemipenis. The histologies of the ductus deferens and urethra were not analyzed because their sizes were too small for histological processi. The parts of the male reproductive organs that successfully for histological analysis are described below.

Testes. The testes have thin capsules with seminiferous tubules where the spermatogenesis process was visible, and the tubules were surrounded by connective tissue (Figure 2H). The interstitial was tight and filled in with blood vessels and Leydig cells. Inside the tubules, some spermatogonia were close to the basal of the membrane. The stage of sperm development was visible, starting from spermatogonium, primary spermatocytes, spermatids, and spermatozoa. The cell structure that forms testis of tokay gecko was similar to the other species of Squamata included *Darevskia chlorogaster* (Choopani *et al*.,

Figure 3. Position, morphology and macro-anatomy of hemipenis on the sexual maturity stage of tokay gecko. Position of hemipenis at cloaca opening (above left); head of hemipenis which scrolls inward when inactive (above right); overall morphology of hemipenis (below).

2014), *Sceloporus* spp. (De la Cruz *et al*., 2015) and *L. strigata* (Hojati *et al*., 2016).

Epididymis. The outside layer of the epididymis was covered by connective tissue surrounded by the layers of muscle cells (Figure 2I). Inside epididymis, there was aligning of pseudostratified columnar epithelial cells with thin stereocilia on top, and in the lumen epididymis were spermatozoa shaped incomplete normal morphology. Histology of the epididymis of Tokay gecko was not much different with *Varanus salvator* (Mahfud *et al*., 2017).

Hemipenis. The histology of the hemipenis, the upper part where was the lobe in the truncus (Figure 2J). Sulcus spermatic was located close to large blood vessels. The urethra was a large lumen and surrounded by connective tissue, muscle cells, and glands. Inside the urethra, there was a part of the lobe that still folded. The tip of the hemipenis consisted of three bulks of the lobe. There were several tubercles on the surface of bulks. Referring to Karim (1998), it seemed that the tubercles were ornaments on the surface of the bulks that were not visible in macroscopic observations.

The male's urogenital organ of the tokay gecko looks simple (Figure 3), which is similar to the urogenital organ of *A. carolinensis* which member of Dactyloidae (Crews, 1978). However, the urogenital organ *Hemidactylus flaviviridis* (Mahendra, 1953), which is in the same family as the tokay gecko looks different from the urogenital organ of the tokay gecko. The position of the right testis being higher than the left testis on tokay gecko is similar with *A. carolinensis*, *H. flaviviridis,* and *Lacerta strigata* (Hojati *et al*., 2016). Information on the asymmetrical position of the testes in the male body also belongs to the blind snake's family of Leptotyphlopidae and Typhlopidae (Fox, 1965), *Phrynops geoffroanus* turtle of Chelidae (Cabral *et al*., 2011). Possibly other family groups besides those described above also have a testicular position that is not asymmetrical, but the available macro-anatomical visualization of male reptiles is still limited.

Male of tokay gecko enter sexual maturity at SVL 111.60 mm. The linear regression between SVL and testis volume for all males that entered adult sex was positively weak, but statistically significantly different (Table 1; Figure 5). Linear regression of males with SVL≥150 mm versus testis volume showed a weak positive correlation, but it still falls into a significantly different category (Table 1). The testis volume of the Tokay gecko was not influenced by the growth of SVL (Figure 5), but by hormones. This condition also occurred in the *Ctenophorus fionni* lizard (Johnston, 1999).

Hemipenis of the tokay gecko does not have sacs that are usually under the cloaca. These sacs are available on *A. carolinensis* (Ritzman *et al*., 2012) and *Hemidactylus* spp (Das and Purkayastha, 2012). The morphology of the hemipenis of gecko tokay looks like a fused organ. The right and left hemipenis merge in the middle, and the position is parallel to the cloaca (Figure 3). The ejection of all hemipenis bodies cannot be done by pressing the base of the tail.

The morphology of the tokay gecko's hemipenis is very different from the *G. smithii* hemipenis. The *G. smithii's* hemipenis has ornaments in the form of small protrusions at the top (Rösler *et al*., 2011), whereas the hemipenis of tokay gecko hemipenis is smooth on all parts of the body (Figure 3). Smooth bodies also belong to the hemipenis of *H. frenatus* (Das and Purkayastha, 2012); the difference in morphology between the two gekkonids is that the upper part of *H. frenatus's* hemipenis has two large swelling bumps when erecting, while the tokay gecko's hemipenis has three swelling bumps that swell when erection. According to Nunes *et al*. (2014). The presence of ornamentation on the hemipenis was used as the copulation anchor in the reproduction system of the female and could increase insemination's success. Thinking about the smoothness and no ornamentation of hemipenis morphology, it is likely that effectiveness in the copulation is not a priority in the reproduction process of the tokay gecko. Based on our observation, the adult male has a female as a fixed pair (monogamy); males do not need to fight with other males to get a female; because the male possesses a permanent couple. In the wild, the male controlled a certain place as their nest, where the area around the nest was his territorial area (Kurniati, 2020). In captivity, the couple of tokay gecko produced two eggs at an interval of one month (Manthey and Grossmann, 1997), this reproductive activity not being affected by the season.

Female Reproductive Macro-anatomy

The female reproductive tract of the tokay gecko consisted of a pair of ovaries that contained prospective eggs, a pair of the infundibulum which was connected to a uterine tube, a pair of the uterus, and a pair of the vagina which then continued to the cloaca (Figure 4). The ovaries,

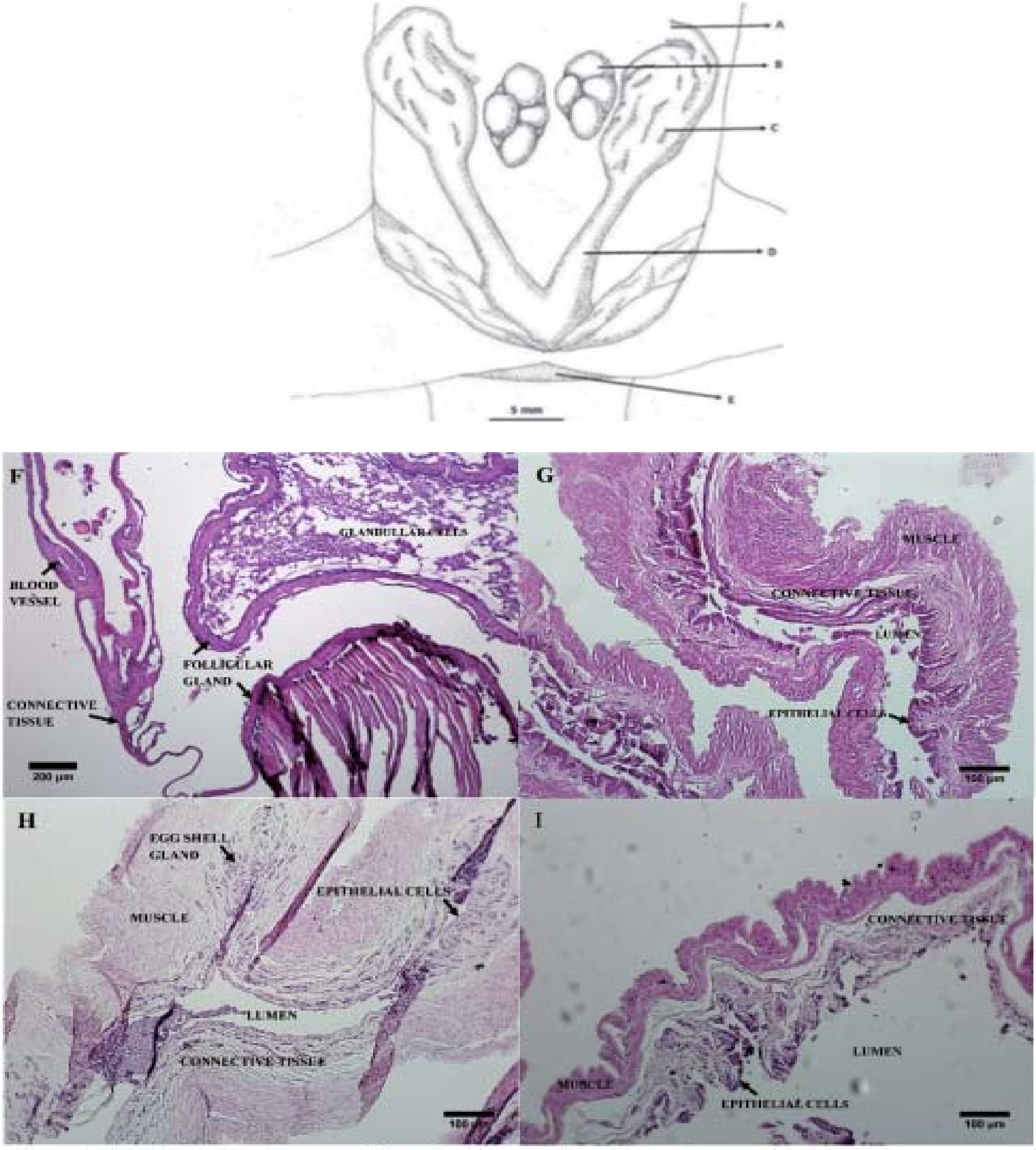

Figure 4. Reproduction system of female tokay gecko on sexual maturity stage. A. Infundibulum; B. Ovary with eggs; C. Uterus; D. Vagina; E. Cloaca. F. Histology of the ovary (magnification 100 times). G. Histology of infundibulum (magnification 100 times). H. Histology of the uterus (magnification 100 times). I. Histology of the vagina (magnification 100 times).

uterus, and vagina of the tokay gecko were thin and transparent. The uterus was the largest part of the reproductive tract of this species. The type of the tokay gecko reproductive system was bifid urodaeum; in reptiles, this form was the simplest type (Sanchez-Martinez *et al*., 2007).

Female Reproductive Microanatomy

For histological observations of the female reproductive organs, the observed organ parts were the ovary, infundibulum, uterus, and vagina. The parts of the female reproductive organs that had been successfully histologies are as follows:

Table 1. Statistical analysis of measurements. SVL versus measurements of the posterior body (CL, BL, FL, TL, HL), AG and testis volume for immature and sexual maturity group; adult male group (SVL≥150 mm), adult female group (SVL≥130 mm); linear regression, correlation (r2), and significant correlation on statistical analysis (*) in p<0.05.

Morphometric analysis	Sex and age stage	Range of SVL-mm	Number of samples	Average±SD (range)-mm	Linear regression	r^2	Statistical significance
SVL versus CL (Cloaca Length)	Male: Immature and mature	89.30-182.00	74	-SVL: 159.82±19.81 -CL: 8.70±1.98	y = 6.6304x + 101.19	0.3725	p=0.000*
	Male: mature	150.00-182.00	52	-SVL: 165.70±8.31 -CL: 9.14±1.59	y = 0.1367x + 164.45	0.0007	p=0.818
	Female: Immature and mature	90.10-144.35	21	-SVL: 118.04±16.49 -CL: 10.39±2.31	y = 0.067x - 1.8915	0.5635	p=0.000*
	Female: mature	130.00-144.35	5	-SVL: 139.14±5.69 -CL: 7.36±0.81	y = -0.0482x + 14.066	0.1153	p=0.576
SVL versus BL (Base tail Length)	Male: Immature and mature	89.30-182.00	74	BL: 14.50±2.82	y = 0.0897x + 0.2714	0.4707	p=0.000*
	Male: mature	150.00-182.00	52	BL: 15.24±1.97	y = -0.0037x + 15.863	0.0002	p=0.890
	Female: Immature and mature	90.10-144.35	21	BL: 10.39±2.31	y = 0.108x - 2.3552	0.5941	p=0.000*
	Female: mature	130.00-144.35	5	BL: 12.70±2.35	y = 0.0982x - 0.9569	0.0567	p=0.700
SVL versus FL (Femur Length)	Male: Immature and mature	89.30-182.00	74	FL: 23.62±2.85	y = 0.1065x + 6.6044	0.5471	p=0.000*
	Male: mature	150.00-182.00	52	FL: 24.34±1.85	y = 0.0201x + 20.998	0.0082	p=0.425
	Female: Immature and mature	90.10-144.35	21	FL: 18.37±2.12	y = 0.1068x + 5.771	0.6885	p=0.000*
	Female: mature	130.00-144.35	5	FL: 20.70±1.13	y = 0.0722x + 10.654	0.1315	P=0.549
SVL versus TL (Tibia Length)	Male: Immature and mature	89.30-182.00	74	TL: 21.77±2.66	y = 0.1041x + 5.1244	0.6018	p=0.000*
	Male: mature	150.00-182.00	52	T L 22.54±1.14	y = -0.028x + 27.192	0.0416	p=0.070
	Female: Immature and mature	90.10-144.35	21	TL: 16.40±2.22	y = 0.1286x + 1.223	0.9146	p=0.000*
	Female: mature	130.00-144.35	5	TL: 18.86±1.27	y = 0.143x - 1.0295	0.4107	p=0.244
SVL versus HL (Hind limb Length)	Male: Immature and mature	89.30-182.00	74	HL: 44.80±6.80	y = 0.2718x + 1.3602	0.6265	p=0.000*
	Male: mature	150.00-182.00	52	HL: 46.88±2.52	y = -0.0079x + 48.19	0.0007	p=0.819
	Female: Immature and mature	90.10-144.35	21	HL: 34.78±4.20	y = 0.2354x + 6.994	0.8555	p=0.000*
	Female: mature	130.00-144.35	5	HL: 39.56±2.34	y = 0.2151x + 9.625	0.2737	p=0.366
SVL versus AG (Length Axilla to Groin)	Male: Immature and mature	89.30-182.00	74	AG: 75.26±11.36	y = 0.4333x + 6.1606	0.859	p=0.000*
	Male: mature	150.00-182.00	52	AG: 79.10±4.89	y = 0.2888x + 30.542	0.2287	p=0.000*
	Female: Immature and mature	90.10-144.35	21	AG: 59.55±10.09	y = 0.5272x - 4.126	0.8597	p=0.000*
	Female: mature	130.00-144.35	5	AG: 69.24±1.88	y = -0.0379x + 74.58	0.0127	p=0.810
SVL versus testis volume	Male: all mature	111.60-82.00	69	Testis volume: 41.80±21.06	y = 0.2801x + 151.12	0.171	p=0.000*
	Male: mature	150.00-182.00	52	Testis volume: 43.47±20.70	y = 0.1207x + 160.61	0.093	p=0.04*

Ovary. The ovary of the tokay gecko was wrapped by solid connective tissue, where a bunch of eggs was inside (Figure 4F). In the connective tissue, blood vessels were scattered randomly. In the ovary, follicular cells lay at the edges of the eggs and were surrounded by granular cells.

Infundibulum. The lumen wall in the infundibulum is formed many folds (criptae), and there are columnar epithelial cells in the form of ciliated columns (Figure 4G). Beneath the epithelial cells appear lamina propria, tunica muscularis, and tunica adventitia.

Uterus. The walls of the lumen in the uterus is also formed many folds (criptae). The internal walls of the uterus are endometrium that formed by columnar epithelial cells with and without cilia. Epithelial cells are stand on the basals membrane with lamina propria below with many glands that function to form shells in the gecko eggs and blood vessels (Figure 4H). The gland produces a protein matrix for eggshells (Stewart *et al*., 2010). The thickness of the endometrium layer depends on the reproductive cycles of the animals. Below the endometrium, there is a myometrium layer with circular smooth muscles in the inner part and longitudinal muscles in the outer part. The muscle layers in the uterus appear thicker than in the infundibulum and vagina. Below the myometrium, there is a perimetrium with connectives tissue and blood vessels.

Vagina. The lumen wall of the vagina is also formed many folds (kriptae) (Figure 4I). There are the columnar epithelial cells with and without cilia that stand on basal membranes. The vagina of the tokay gecko has the thinnest layer of circular and longitudinal smooth muscle layers and has looser connective tissue than the infundibulum and uterus (Uribe *et al*., 1988). In reptiles, spermatozoa are usually stored in folds in the vagina, and some species of Gekkonidae store sperm in one part of the oviduct (Nogueira *et al*., 2011); but with the tokay gecko sample in this study is not yet known whether the vaginal folds can be used to store spermatozoa.

The female tokay gecko reproductive system also looks simple (Figure 4); the type of reproductive system resembled the shape of bifid urodaeum, as it belonged to the *Polychrus marmoratus* type of Polychrotidae (Sanchez-Martinez *et al*., 2007). The simple reproductive system also belonged to *H. flaviviridis* (Mahendra, 1953) which was in the same family as tokay gecko; the difference was only in the size of the uterus and infundibulum. The infundibulum of the tokay gecko was short, but the uterus was long and large, in contrast, the infundibulum of *H. flaviviridis* was long, but the uterus was short and small. The position of the right and left uterus in the tokay gecko was symmetric. However, the position of the *H. flaviviridis* uterus was asymmetric (Mahendra, 1953). This unequal position was also found in almost all species of snakes (Blackburn, 1998). The female reproductive system of the tokay gecko cannot be compared with the other members of Gekkonidae species, because visualization of this system is not available in the form of scientific publications; the few publications that revealed the comparative morphology of the female genitalia system were also stated by De-Lima *et al*. (2019).

The wall of the female reproductive tract of the tokay gecko has formed folds (kriptae), starting from the infundibulum, uterine, and then proceeding to the vagina. Several species of Gekkonidae can store sperm in the grooved wall of the infundibulum and vagina (Girling *et al*., 1997; Sever and Hamlett, 2002; Nogueira *et al*., 2011). However, based on our observation, sperm could be found in the folded wall of the infundibulum, uterus, and vagina. In this study, it was uncertain whether the sperm was found because the female has just copulated, or the sperm came from the results of copulation that occurred a long time ago.

Measurement of Body and Hind Limb

Recapitulation of the relationship between SVL and five measurements of the posterior body part (CL, BL, FL, TL, HL) and one measurement of the axilla to the groin (AG) are shown in Table 1. Five rear body measurements on the male and female groups have strong positive correlations and were statistically significantly different ($p < 0.05$) when the growth sequence starting from the immature to the adult stage; but for the adult male group with SVL≥150 mm and the adult female group with SVL≥130 mm, the rear body growth has entered asymptotic growth stage; except for SVL relationship with AG, the male group has a strong positive correlation. The correlation is very strong at the immature to the adult stage, and the correlation is strong enough in adult males with SVL≥150 mm. However, in the female group, the positive correlation is strong at the immature into the adult stage, whereas in adult females with

SVL≥130 mm, the AG growth is asymptotic.

The relationship between SVL and testis volume is shown in Table 1. The positive correlation is strong and statistically significantly different (r^2=0.171; p=0.000) when the growth sequence starting from the immature stage; but the positive correlation became very weak and statistically significantly different (r^2=0.093; p=0.04). In the relation between SVL and testis volume (Figure 5), it is shown that the testis volume of the adult male with SVL≥150 mm has a very wide range of the volume, which is between 22.77-64.17 mm^3 (43.47±20.70 mm).

Hind limb measurements versus SVL showed that growth of hind limb in males at SVL≥150 mm was asymptotic; whereas females were asymptotic at SVL≥130 mm (Table 1). The increase of SVL in males for individuals with SVL≥150 mm can be indicated by the fairly strong positive correlation between SVL versus AG (r^2=0.2287; p=0.000), this result proved that the increase of SVL caused by the rise of AG length; but the length of AG in females with SVL≥130 mm was asymptotic (r^2=0.0127; p=0.810), whit increasing of SVL caused by the head length.

The male's body size of the tokay gecko was 28% larger than the female's body size (Kurniati and Phadmacanty, unpublished data), but the male's posterior body parts played a role in the reproduction process. The posterior regions have not increased in length or width since SVL≥150 mm; this part was in synchronous with the growth of the posterior part of the female's body since SVL≥30 mm. Most likely asymptotic growth in the posterior regions of the male and female body as a form of co-evolution; moreover, the male's urogenital organs and the female's reproductive system also supported the occurrence of co-evolution in the reproductive organs. This phenomenon occurred in *Tropidurus torquatus* of the Tropiduridae family, which synchronous co-evolution of the reproductive organs has occurred since embryonic growth (De-Lima *et al*., 2019).

All specimens with SVL≥150 mm of 52 individuals were male in this study; however, there were no females with SVL≥150 mm. The longest SVL for females was 144.35 mm. Does the female tokay gecko never reach 150 mm in body size in the wild is it still a question mark? Based on Shahrudin (2013) experience with *G. smithii*, a female with SVL 176.21 mm died in a terrarium after one week of laying eggs. Was the cause of death of the female caused by the natural condition or stress inside a new environment, or was it stress due to male loss was not known with certainty; but the natural reproductive behaviour of *G. smithii* was not much different from natural reproductive

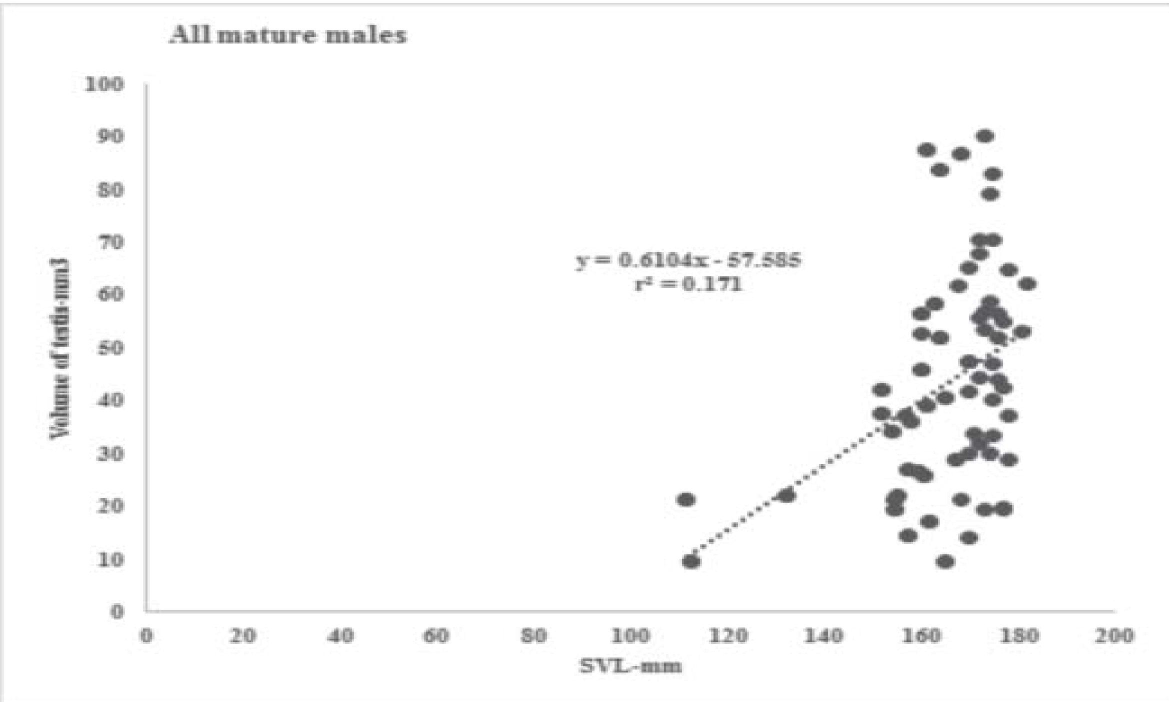

Figure 5. Snout to vent length (SVL) versus testis volume of tokay gecko.

behaviour of *G. gecko*, which can breed throughout the year with the number of eggs being two in every laying egg (Manthey and Grossmann, 1997; Shahrudin, 2013). No females of tokay gecko were found with SVL≥150 mm whether due to female body growth stopping to that size, or females susceptible to dying at SVL≥150 mm; but to answer all of those questions need further research.

CONCLUSION

Macro-anatomy of the male and female tokay gecko showed the gecko have simple reproductive organs; in males, it consists of a pair of testes, a pair of epididymis and ductus deferens and then continues to the hemipenis; whereas, in females, it consists of a pair of ovaries, a pair of the infundibulum, a pair of the uterus and a pair of the vagina which then continued to the cloaca. In micro-anatomy, the tissue cells that shaped male and female reproductive organs showed non-specific, not much different from other gekkonid members. The posterior part that supports reproductive activity in males grows asymptotically at SVL≥150 mm; while in females, the growth of this region is still linear.

SUGGESTION

In this study, there were no females with SVL≥150 mm in the samples. Histological studies on female individuals who died naturally in the wild or captivity are needed to answer the cause of the death.

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