Maturity and Reproductive Characteristics on Tokay Gecko (*Gekko Gecko* Linnaeus, 1758) From Java
[Kematangan dan Karakteristik Reproduksi pada Tokek Rumah (*Gekko Gecko* Linnaeus, 1758) di Pulau Jawa]

Tizar Gusli[1], Muhammad Alif Fauzi[2], Quraisy Zakky[3], Amir Hamidy[4], & Bambang Irawan[1]

[1)]Faculty of Sciences and Technology, Department of Biology, Airlangga University, Surabaya, Indonesia, 60115. [2)] Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia [3)] Indonesia Herpetological Society (Penggalang Herpetologi Indonesia/PHI), Bogor, Indonesia [4)] Laboratory of Herpetology, Museum Zoologicum Bogoriense, Research Center for Biosystematics and Evolution, National Research and Innovation Agency, Widyasatwaloka Building, KST Soekarno, Jl. Raya Jakarta Bogor Km 46, Cibinong, Bogor 16911, West Java, Indonesia
Email:Tizar.gusli-2021@fst.unair.ac.id;alifauzi313@gmail.com; zakkyquraisy@gmail.com; hamidyamir@gmail.com; bambangirawan@unair.ac.id.

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ABSTRACT

One of the reptiles that massively exploited and traded for export commodities in Indonesia is the tokay gecko (*Gekko gecko*). Uncontrolled exploitation possibly threatened its wild population. Currently, there needs to be more biological information regarding this species. Thus, a biological approach is required to support a sustainable harvesting program, primarily through reproductive biology approaches. This study provided information about the minimum size for mature individuals of both sexes. We measured morphological characters and examined reproductive system of 136 males and 324 females from the legal processing house *Gekko gecko*. Our results show that males reproduce at a minimum SVL of 134,09 ± 22,89 mm, while females are 121.21 ± 8.85 mm. There is a correlation between SVL and TL in male and female geckos. Significant differences in the length and width of testes were observed between mature and immature geckos ($p<0.05$). A relationship was found between SVL and the volume of the right and left testes in mature male geckos, but it was not observed in immature geckos. Significant differences in the volume of the right and left testes were also found between mature and immature male geckos ($p<0.05$). In female geckos, significant differences in follicle length and width were observed between mature and immature individuals ($p<0.05$). Additionally, differences in oviduct width were found between mature and immature female geckos ($p<0.05$). There is a correlation between SVL and follicle length in the virgin and Type 1 categories, while a correlation between SVL and follicle width was only found in Type 1 geckos. An asymmetry pattern in follicles was also discovered in female geckos.This finding can contribute to formulate recommendation for sustain harvest-size of *Gekko gecko* in Indonesia.

Keywords: Reproduction, Reptile, Sustainable Harvesting, Tokay Gecko

Abstrak

Salah satu reptil yang dieksploitasi secara besar-besaran dan diperdagangkan sebagai komoditas ekspor di Indonesia adalah tokek rumah (*Gekko gecko*). Eksploitasi yang tidak terkendali mungkin mengancam populasi liar mereka. Saat ini, diperlukan lebih banyak informasi biologis tentang spesies ini. Oleh karena itu, pendekatan biologis diperlukan untuk mendukung program pemanenan yang berkelanjutan, terutama melalui pendekatan biologi reproduksi. Studi ini menyediakan informasi tentang ukuran minimum individu dewasa dari kedua jenis kelamin. Kami mengukur karakter morfologis dan memeriksa sistem reproduksi dari 136 jantan dan 324 betina dari *Gekko gecko* rumah pemrosesan yang sah. Hasil kami menunjukkan bahwa jantan bereproduksi pada SVL minimum 134,09 ± 22,89 mm, sementara betina adalah 121,21 ± 8,85 mm. Terdapat korelasi SVL dan TL pada tokek jantan dan betina. Terdapat perbedaan panjang dan lebar testis yang signifikan antara tokek mature dan immature ($p<0.05$). Terdapat hubungan antara SVL dan volume testis kanan dan kiri pada tokek jantan mature, namun tidak ditemukan pada tokek immature. Terdapat perbedaan signifikan antara volume testis kanan dan kiri pada tokek jantan mature dan immature ($p<0.05$). Pada tokek betina ditemukan perbedaan signifikan panjang dan lebar folikel pada tokek mature dan immature ($p<0.05$). Selain itu terdapat pula perbedaan lebar oviduk pada tokek mature dan immature($p<0.05$). Terdapat korelasi antara SVL

dan Panjang folikel pada kategori tokek virgin dan type 1, sedangkan korelasi SVL dan lebar folikel hanya ditemukan pada type 1. Ditemukan pula pola asymmetry follicle pada tokek betina. Temuan ini dapat memberikan kontribusi untuk menformulasikan rekomendasi ukuran panen yang lestari dari *Gekko gecko* di Indonesia.

Kata Kunci: Reproduksi, Reptil, Pemanenan Berkelanjutan, Tokek rumah

INTRODUCTION

The Tokay gecko (*Gekko gecko*) is one of the harvested reptiles from the wild for its meat and skin (Lwin *et al*. 2017) and Chinese medicine (Li *et al*. 1998) in large quantities. This species is highly distributed in Indo-Malayan regions (Bauer *et al*. 2009), from Bangladesh, northeastern India, Nepal, Bhutan, Southern China, Taiwan throughout Southeast Asia, from Myanmar, Laos, Thailand, Cambodia, Vietnam, Peninsular Malaysia, Philippines, Singapore, and Indonesia (excluding Papua) (Das 2015; Uetz & Hallerman 2022).

This commercially important species is an export commodity from Indonesia. 4.297.706 individuals and 258.157 kilograms of meat have been exported from Indonesia (2019 and 2020) (CITES Trade Database). Up to now, this species is listed in the Least Concern of IUCN Redlist database. The wild population still might be threatened due to immense harvesting in large numbers (Lwin et al., 2017). Consequently, this species' trade status has been elevated to Appendix II since 2019 (CITES 2019).

Biological information is fundamental in sustainable harvesting management (Sinclair *et al*. 2016). Few biological approaches have been conducted previously to support the sustainable harvesting program for Tokay geckos by estimating the wild population regionally (Kurniati 2019; Fauzan *et al*. 2022). Formerly, a reproduction approach is mentioned in Kurniati & Phadmancanty (2022). Nevertheless, the reproduction approach in more significant samples has not been conducted for Tokay geckos, but has been done several times in snakes (Natusch & Lyons 2012; Natusch *et al*. 2016; Natusch *et al*. 2019; Zakky *et al*. 2022). Male and female geckos enter reproductive age in less than one year, where female geckos can produce eggs, and in male geckos, sperm is found in perfect form (Kurniati & Phadmacanty 2022). Female geckos can lay eggs throughout the year, and in one clutch, they can produce two eggs (Manthey & Grossman, 1997) with an incubation period of 65 days (Das 2016). Female geckos will lay eggs again within 30 days, whereas within a year, female geckos can lay up to 12 eggs (Manthey & Grossman 1997).

Our study aims to determine the reproductive characteristics of *the Gekko gecko* based on the reproductive conditions and body size of the harvested individuals in existing industries. It would also support the sustainable harvesting program by determining the minimum size for harvesting based on the reproductive characteristics of the *Gekko gecko* in Indonesia from hunters and collectors to processing houses and exporters level as harvest size recommendation.

MATERIALS AND METHODS

We examined the carcasses of *Gekko gecko* from processing houses in East Java, collected from three districts: Banyuwangi (East Java), Madura (Madura Island), and Klaten (Central Java). These specimens are individuals with intact tails, as legal processing houses only process specimens with intact tails in accordance with the standard quality of dried gecko products. Biological attribute data of the specimens were collected following the method of Natusch *et al*. (2019): snout-vent length (measured from the tip of the snout to the cloaca), tail length (TL, measured from the base of the tail to the tip of the tail), testis length (measured from the anterior to the posterior testis), testis width (measured from the outer side to the inner side), oviduct width (from the outer side to the inner side), and body mass. Both testis length and width were measured on both the right and left sides.Maturity was determined by checking the condition of the vas deferens (convoluted or non convoluted) and oviduct (transparent, thick, or scarred), as well as counting and measuring the diameters of primary and secondary follicles (vitellogenic follicles) and the corpus luteum and corpus albicans in the

ovary. Specimens were considered mature if the vas deferens was convoluted in males and the oviduct was thickened in females. We also conducted a regression analysis using the tail length (TL) and snout-vent length (SVL) to observe sexual dimorphism between male and female geckos. All statistical analysis were conducted by R studio (4.1.2) (R Core Team, 2021

Here, we state that the samples are from existing industries; fortunately, no living tokay geckos were harmed for the purpose of this research.

RESULTS

A total of 504 carcasses tokay gecko (136 males and 324 females) were examined All.

Sexual Dimorphism

We found the presence of sexual dimorphism between male and female geckos. Our data showed that male shorter than female (SVL 134.09 ± 22.89 vs 128 ± 7,90) (Table 1).

Male Reproduction Characteristics

We found significant differences in the measurements of several reproductive characteristics in male geckos. The measured characteristics included the length of the right and left testes, the width of the right and left testes, as well as the volume of the right and left testes. All measured variables indicated differences in the size of reproductive organs between mature and immature male geckos. Mature male geckos exhibited larger length, width, and volume ratios compared to those that had not entered the reproductive maturity phase (Table 2).

Female Reproduction Characteristics

We have found a significant difference in the size of reproductive organs between mature and immature female geckos. Female geckos that have entered the sexual maturity phase are characterized by a thickened oviduct. Significant differences in the size of reproductive organs were observed in terms of follicle width, follicle length, and oviduct width. In mature female geckos, the reproductive organs are larger compared to immature ones.

Asymmetry Follicles Allocation in the ovaries and embryos in oviducts

We have discovered an asymmetric ratio pattern in the follicles of female geckos. The cause of this asymmetry has not been identified so far, but similar patterns have been observed in other reptile species, especially snakes. We also found a ratio of one for embryos, indicating that the embryos in the right and left oviducts are equal in number in six female geckos.

Reproductive organs of male and female gecko

We have found differences in morphology between immature and mature male gecko organs. Mature male geckos are characterized by convoluted vas deferens, while in immature geckos, clear vas deferens with no convolutions are observed. In mature female geckos, there is thickening of the oviduct, while in immature geckos, the oviduct remains transparent. We also found the presence of corpus luteum and corpus

Table 1. Body and testes measurements in male individuals (mean ± standard deviation)

	Immature	**Mature**
n	43	136
Mass (g)	27.01 ± 5.49	43.40 ± 13.41
SVL (mm)	119.88 ± 20.87	134.09 ± 22.89
TL (mm)	103.32 ± 0.92	108.65 ± 1.14
Right testis length (mm)	5.20 ± 0.52	6.76 ± 0.50
Right testis width (mm)	2.58 ± 13.62	2.99 ± 20.35
Right testis volume (mm^3)	29.06 ± 1.11	48.97 ± 0.79
Left testis length (mm)	4.94 ± 0.54	6.41 ± 0.53
Left testis width (mm)	2.54 ± 13.62	3.06 ± 21.92
Left testis volume (mm^3)	26.61 ± 4.28	49.61 ± 19.38

albicans in the female geckos we observed (Figure 10 c).

DISCUSSION

Sexual Dimorphism

We found morphological differences between male and female individuals where female geckos are longer than male geckos, where the average male ratio ± SD is 0.886034 ± 0.06786 with a correlation value of 0.63 ($p<0.05$), while in female geckos it was 0.915792 ± 0.052945 with a correlation value of 0.72 ($p<0.05$) (Figure 1). Similar to Kurniati & Phadmacanty (2022), where the average SVL of male geckos is significantly longest than the females.

Male Reproduction Characteristics

Mature male geckos have a body mass of 43.40 ± 13.41 g (n = 136). SVL 134.09 ± 22.89 mm, right testis length 6.76 ± 0.50 mm, right testis width 2.99 ± 20.35 mm, right testis volume 48.97 ± 0.79 mm^3.In the same group have a left testis length 6.41 ± 0.53 mm, left testis width 3.06 ± 21.92, and left testis volume 49.61 ± 19.38 mm^3. Immature male geckos had a body mass of 27.01 ± 5.49 g (n = 43). SVL 119.88 ± 20.87 mm, right testis length 5.20 ± 0.52 mm, right testis width 2.58 ± 13.62 mm, and right testis volume 29.06 ± 1.11 mm^3. In the same group have a left testis length 4.94 ± 0.54 mm, left testis width 2 .54 ± 13.62 mm, and left testis volume 26.61 ± 4.28 mm^3 .

There is a difference in the average testicular length found in the right and left testis. In mature male geckos, testicles were found to be longer compared to immature geckos. The result of the one-way ANOVA test showed *p*-value 7,93x10-24, where there is a significant difference between each category of male gecko in terms of testicular length ($p<0.05$) (Figure 2).

It was also found in the width of the testicles, where there were differences in the average thickness of the testicles in the two categories of

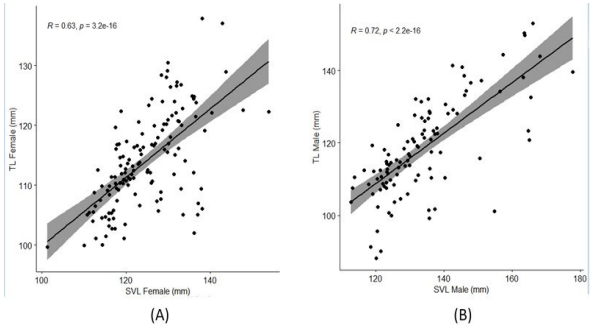

Figure 1. Correlation between SVL and Tail Length (TL) in female geckos (A) and male geckos (B).

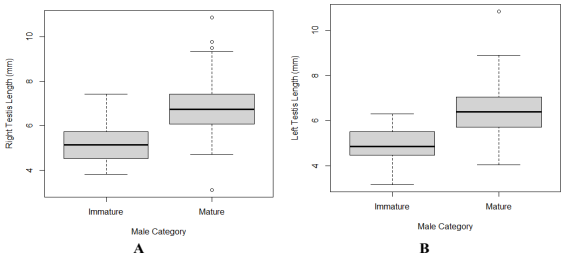

Figure 2. (A) Boxplot of male gecko categories towards right testicular length; **(B)** Boxplot of male gecko categories towards left testicular length .

Table 2. Body and Reproductive organ measurements in female *G. gekko* (mean ± standard deviation)

	Virgin	Mature	Mature Type 1	Mature Type 2	Mature Type 3
Samples (N)	63	149	21	17	74
Mass (g)	26,41 ± 4,25	34,69 ± 7,22	30,58 ± 7,15	29,77 ± 4,65	35,44 ± 7,32
SVL (mm)	118,17 ± 4,42	128 ± 7,90	121,21 ± 8,85	121,50 ± 6,42	130,91 ± 7,44
Oviduct Width (mm)	3,42 ± 0,85	6,75 ± 1,23	5,66 ± 1,44	6,42 ± 1,43	6,88 ± 1,31
Primary follicle length (mm)	2,59 ± 0,44	3,07 ± 0,57	2,82 ± 0,76	3,22 ± 0,68	3,03 ± 0,57
Primary follicle width (mm)	2,24 ± 0,37	2,79 ± 1,70	2,46 ± 0,62	2,77 ± 0,58	2,90 ± 2,36
Secondary follicle length (mm)	-	7,23 ± 2,24	-	7,44 ± 1,55	-
Secondary follicle width (mm)	-	12,75 ± 2,24	-	7,01 ± 1,83	-
Egg/embryo length (mm)	-	16,39 ± 2	-	-	16,91 ± 1,99
Egg/embryo width (mm)	-	11,37 ± 1,40	-	-	11,83 ± 1,99

male geckos. Mature male geckos have wider testicles compared to immature geckos. The results of the one-way ANOVA test show *p*-value 0.007, where there is a significant difference between each oviduct condition on follicle length (p<0, 05) (Figure 3).

There is a difference in the average testicular volume in immature and mature male geckos (Figure 4). This difference is found in both testes, the right testis and the left testis. The results of the ANOVA analysis show a significant difference between the volume of the left and right testicles in the immature and mature gecko categories where the p-value < 0.02. Differences are also evident in the relationship between SVL length and the increase in the volume of the left and right testes in immature and mature tokay geckos. When tokay geckos enter the mature phase, the testes' volume increases in accordance with the increase in SVL length. However, this does not occur in immature tokay geckos (Figure 5). The significant differences between the three previous variables, including length, width, and testicular volume in male geckos, indicate that enlarged testicular size is one of the characteristics of male geckos that have entered the sexual maturity phase (Kurniati & Phadmacanty 2022)

It is clear that mature males have larger testes in size. Like in other reptiles, an increase in testes volume occurs in the period approaching the breeding season, as shown by the increase in length and width of both testicles regarding the pattern of the breeding season (Natusch et al., 2019), due to an increase in spermatozoa production in both testes (Jadhav & Padgaonkar, 2011).

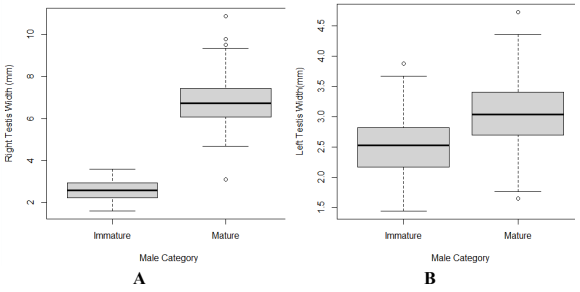

Figure 3. (A) Boxplot of male gecko categories towards right testicular width; **(B)** Boxplot of male gecko categories towards left testicular width.

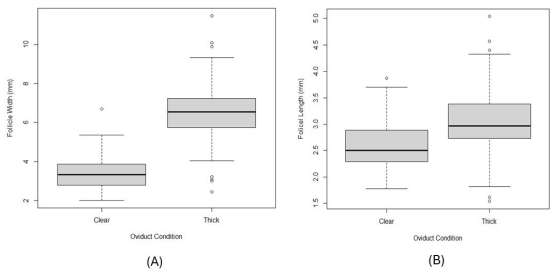

Figure 4. Boxplot of left testicular volume (A) and right testicular volume (B).

Female Reproduction Characteristics

The total number of female geckos observed was 324 individuals. We put these individuals into five groups based on the possessed reproductive characteristics of each individual.

Virgin:

As discussed earlier, we also found reproductively immature geckos in the industry, which had a mass range of 26.41 ± 4.25 gr (n =

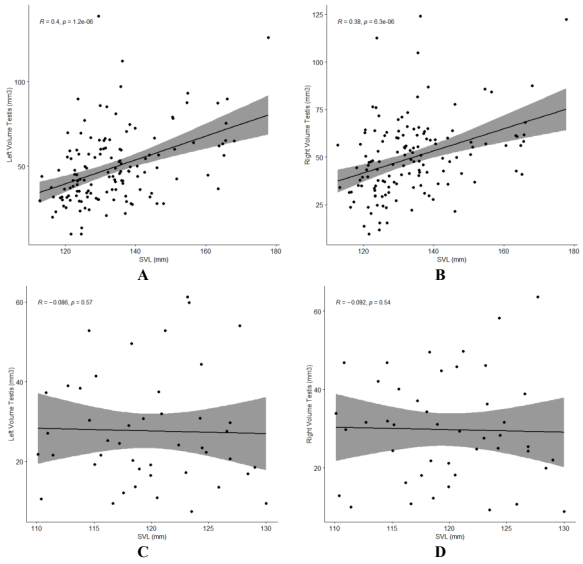

Figure 5. Corelation between SVL and the left testis volume (A) and right testis volume (B) in mature tokay geckos, and the relationships between SVL and the left testis volume (C) and right testis volume (D) in immature tokay geckos.

63), SVL 118.17 ± 4.42 mm, oviduct width 3.42 ± 0.85 mm, primary follicle length 2.59 ± 0.44 mm, secondary follicle width 2.24 ± 0.37 mm, number of right follicles 5.90 ± 0.85 and left follicles 5.90 ± 0.85. In geckos in this category, the oviduct has not thickened and looks transparent (clear) (Figure 6).

Mature - Type 1:

Mature geckos that have never reproduced have a mass range of 30.58 ± 7.15 g (n = 21), SVL 121 ± 8.85 mm, oviduct width 5.66 ± 1.44 mm, primary follicle length 3.22 ± 0 .68, and primary follicle width 2.64 ± 0.62 mm, number of right follicles 6.05 ± 0.69, number of left follicles 5.65 ± 0.67. In this group of geckos, one individual was found who had an embryo with a length of 15.03 mm and a width of 10.83 mm with a total of two embryos, one on the left and one on the right, with the condition of the oviduct, which was found to have thickened. (Figure 7)

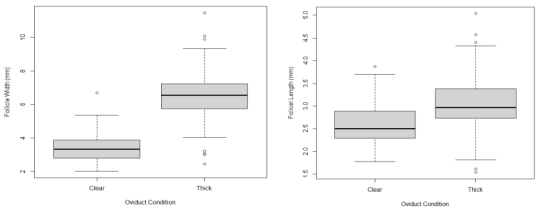

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Figure 6. (A) Boxplot of Oviduct Condition towards primary follicle width; **(B)** Boxplot of Oviduct Condition towards primary follicle length.

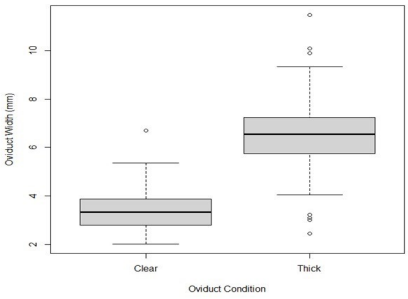


Figure 7. Boxplot of Oviduct Condition against Oviduct Width.

Mature - Type 2:

In the group of adult geckos that were about to reproduce for the first time, the mass range was 29.77 ± 4.65 g (n = 17), SVL 121.50 ± 6.42 mm, oviduct width 6.42 ± 1.43 mm, length primary follicle 3.22 ± 0.68 mm, primary follicle width 2.77 ± 0.58 mm, secondary follicle length 7.44 ± 1.55 mm, secondary follicle width 7.01 ± 1.83.

Mature - Type 3:

In this group, mature female geckos had a mass range of 34.69 ± 7.22 g (n=149). SVL 128 ± 7.90 mm, oviduct width 6.75 ± 1.23 mm, primary follicle length 3.07 ± 0.57 mm, primary follicle width 2.79 ± 1.70 mm, secondary follicle length 7.23 ± 2.24 mm, secondary follicle width 12.75 ± 2.24 mm, embryo length 16.39 ± 2 mm, embryo width 11.37 ± 1.40 mm, right follicle 6.07 ± 0.79 and left follicle 5 .91 ± .88. In this group of geckos, there were ten individuals with the number of embryos varying from one to two embryos per individual.

The adults (type 3) have thickened oviducts, the mass range was 35.44 ± 7.32 g (n = 74), SVL 130.91 ± 7.44 mm, oviduct width 6.88 ± 1.31 mm, primary follicle length 3.03 ± 0.57 mm, primary follicle width 2.90 ± 2.36 mm. In this group of geckos, seven individuals had embryos with a length of 16.91 ± 1.99 mm and a width of 11.83 ± 1.99 mm.

The female group approaches the sexual maturity phase at an SVL size of 112 mm. This value was also the same for the male geckos that we observed. This size is close to Kurniati & Phadmacanty (2021), who stated that the size of females entering the adult phase at SVL is 98 mm and males are 110 mm.

The females with thickened oviducts have longer (mm) average widths than those with clear oviducts. The one-way ANOVA test results showed p-value 0,74x10-3 where there was a significant difference between each oviduct condition and follicle width ($p<0.05$). There was also a difference in the average length of primary follicles in the two oviduct conditions. In thickened oviducts, the average length of the follicles is longer than the length of the follicles in the clear oviduct condition. The one-way ANOVA test results showed p-value 2,94x10-26, where there was a significant difference between each

oviduct condition and follicle length ($p<0.05$).

We found that there were also differences in the average oviduct width in the two oviduct conditions. In thickened oviducts, the average width of the oviducts is longer than that of the oviducts in the clear oviduct condition. The one-way ANOVA test results showed p-value 2,94x10 -26,, where there was a significant difference between each oviduct condition and follicle length ($p<0.05$).

We also found differences in the relationship between SVL and the length and width of left and right primary follicles in each category. A linear increase in SVL length with an increase in follicle length was only observed in virgin tokay and Type 1 ($p<0.05$), while in the other categories, there was no significant relationship between SVL length and primary follicle length (Figure 8). We also observed a linear relationship between SVL length and the width of primary follicles, where a linear relationship was only found in female Type 1 tokay in all categories ($p<0.05$), while in the other categories, it was not observed.

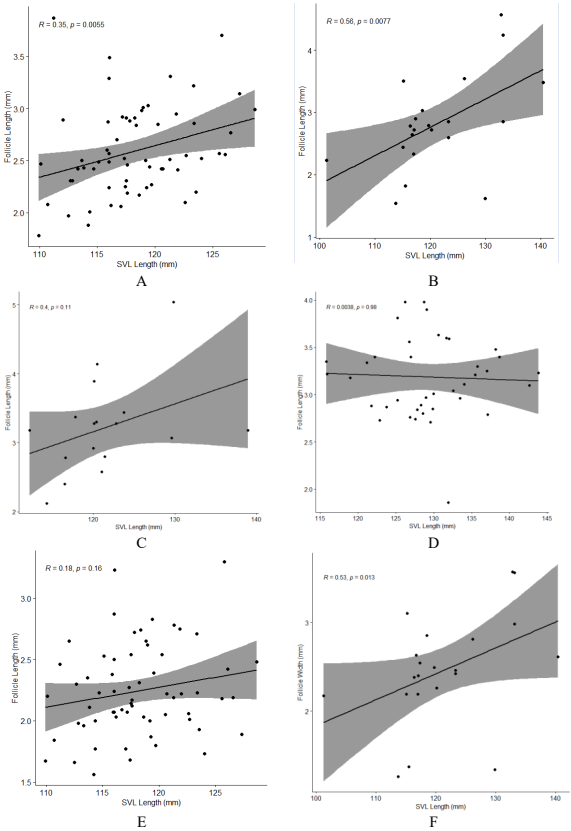


Figure 8. The correlation between SVL and Follicle Length in virgin tokay geckos (A), Type 1 (B), Type 2 (C), and Type 3 (D), and the regression graphs of SVL and Follicle Width in virgin tokay geckos (E), Type 1 (F), Type 2 (G), and Type 3 (H).

Asymmetry Follicles Allocation in the ovaries and embryos in oviducts

The zero value in Figure 9A represents a 1:1 ratio, where the value on the right side of zero represents a ratio skewed to the right, while the one on the left represents a skewed ratio to the left.

The number of individuals who had a follicle ratio on the right was 25 individuals, with an average ratio of 1.19. In comparison, individuals who had a follicle ratio on the left were 49 individuals with an average follicle ratio of 0.83. The reason behind the asymmetry in geckos remains unclear. However, this asymmetry is also found in other species of reptiles (Zakky 2022, Perez *et al.* 2019, Aldridge 1979). Figure 9B shows the ratio of embryos in the right and left oviducts (n=6) with a value of 1, which means the number of embryos found in both oviducts is the same.

Reproductive organs of male and female gecko

Figures 10A, B show the reproductive organs of a female gecko with an embryo. Female geckos have matured reproductive organs and ovaries containing potential egg cells (Kurniati & Phadmacanty 2021). The condition of the reproductive organs (Figure 10 C) occurs in sexually immature geckos possessing a clear

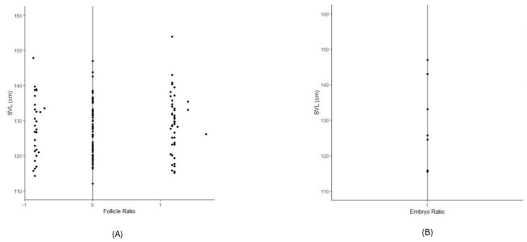
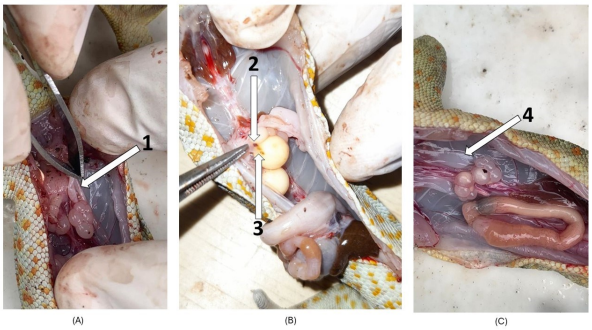


Figure 9 Note: Ratio values are in the form of positive and negative only for illustrative purposes.

oviduct (not yet thickened). Geckos laid their eggs in sufficient space. Naturally, they lay eggs on tree bark; commonly, in urban habitats, they are in electricity poles (Kurniati 2019). This activity is also found in different species in the Gekkonidae family (Shahrudin, 2016; Krysko et al., 2003). After that, the gecko will hatch after an incubation period of 65 days (Das 2016).

Macroanatomically, male geckos consisted of several parts: testes, epididymis, ductus deferens, and hemipenis. In this specimen, the right testis has a higher position than the left testis. In the testes, the process of spermatogenesis and sperm development occurs. The testes of *G. gecko* are linked to the epididymis, which is then connected to the ductus deferens and ends in the hemipenis (Figure 11). The epididymis of *G. gecko* is grooved, and inside it is a lumen containing spermatozoa and a smooth texture (Kurniati & Phadmacanty, 2021). Furthermore, it has similarities with other members of the Gekkonidae family (Das & Purkayastha 2012). The right and left hemipenes in *G. gecko* are fused and shaped like a tube, cannot be distinguished between the hemipenis stem and the lobe, and have a position parallel to the cloacal opening (Kurniati & Phadmacanty, 2021).



Figures 10. Comparison of the reproductive organs condition in mature (A & B) and immature (C) female geckos (1. Thick Oviduct, 2. Corpus albicans, 3. Corpus Luteum, 4. Transparent Oviduct) .

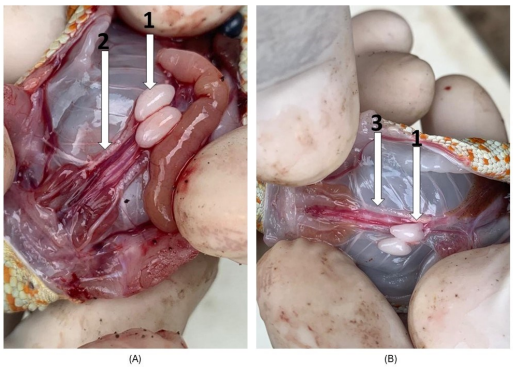


Figure 11. Reproductive organ conditions on male gecko (1. Testes, 2. Convoluted Ductus deferens/vas deferens, 3. Clear Ductus deferens/ vas Deferens)

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