

Morphometric Study of *Cuora amboinensis* in the Ex-Situ Conservation Area of Bengkulu University

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ABSTRACT

The Southeast Asian box turtle, Cuora amboinensis, known locally as the "Batok Turtle" is one of the world's most endangered turtle species. Conservation is needed to protect this species from the threat of extinction. Conservation efforts are complex and involve multiple scientific disciplines. This research was conducted in the Ex-situ Conservation area of Bengkulu University, to determine the morphometric variations of adult C. amboinensis and determine the differences between males and females based on body size as a conservation effort. The research subjects were 20 adults (8 men and 12 women) of C. amboinensis. Several morphometric measurements that have been carried out include head width, carapace length, carapace width, curved carapace length, curved carapace width, carapace height, plastron length, plastron width, length of the midline on all plastron scales (sugar, humerus, pectoral, abdominal, femoral and anal), tail length, tail thickness, and body weight. Next, a t test was carried out to determine the morphometric characteristics of male and female C. amboinensis. The results of the analysis show that there are significant differences. C. amboinensis males have greater carapace length, tail length and tail thickness than females. C. amboinensis females have greater carapace height and humeral scale diameter than males.

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1. INTRODUCTION

The Southeast Asian box turtle or broken-breasted turtle or ambon turtle (turpepel) and in scientific language *Cuora amboinensis* were described by Daundin in 1802. *C. amboinensis* is a species of freshwater turtle that tends to spend most of its time in water or semi-aquatic (Das & Gupta, 2017). Rice fields, rivers, swamps, and lakes are the natural habitat of *C. amboinensis*. This species is widespread in Southeast Asia and Wallacea, inhabiting an area of 3,136,203 km2. The range of *C. amboinensis* is wider than that of other turtle species throughout the world, with a range of around 1,076,798 km². Unlike all other species in the genus Cuora which are usually found in East Asia and Indochina, *C. amboinensis* is found on mainland Southeast Asia as well as on all large islands in the Sundaland Region (Southeast Asia and East Asia) (Ernst et al., 2016). The islands of Sulawesi, Kalimantan, Java, and Sumatra including Bengkulu province are the distribution areas of *C. amboinensis*.

C. amboinensis has several identified subspecies, namely *Cuora amboinensis* amboinensis which is distributed in eastern Indonesia (Ambon and Sulawesi), *Cuora amboinensis couro* in western Indonesia (Sumatra, Java, Sumbawa, and small islands), *Cuora amboinensis Kamaroma* from the southern mainland Asia which is distributed in Singapore, Malaysia, the Philippines and Kalimantan (Indonesia), and *Cuora amboinensis lineata* in Myanmar (Ernst et al., 2016; Diba et al., 2022). Ernst et al., (2016) explained that only two subspecies are recognized, namely *C.a. amboinensis* and *C.a. Kamaroma*. Neither *couro* nor *lineata* is validated so *C.a. couro* is synonymized with the subspecies *C.a. amboinensis* and *C.a. lineata* with subspecies *C.a. Kamaroma*.

Massive environmental changes, habitat fragmentation, and illegal trade have resulted in this species' numbers continuing to decline (Das & Gupta, 2017). Illegal trade in protected animals occurs in almost all parts of the world. Illegal wildlife trade is an environmental problem, the most important extinction crisis in general

caused by human activities (anthropocene) (Gray et al., 2017). As a result of continuous over-exploitation in its distribution area in Indonesia, the average number of adult *C. amboinensis* is decreasing (Silahooy & Huwae, 2020). In the last 20 years, this species has continued to experience a population decline of up to 50-80% due to over-exploitation, thus placing this species in a higher conservation category, namely Endangered (EN), which previously in 2000 was still in the Vulnerable (VU) category (Cota et al., 2020).



Figure 1. C. amboinensis in the Ex-situ Conservation area of Bengkulu University

Bengkulu University's ex-situ conservation is a place for biodiversity in Bengkulu province, one of which is C. amboinensis (Figure 1). Ex-situ conservation is an area for protecting animals outside their natural habitat. This conservation is the result of collaboration between the University of Bengkulu, Indonesia and the University of Carolina Greensboro, United States. The role of conservation is to protect and save Sumatran turtle species obtained from findings from the community and animal lover groups in Bengkulu province and its surroundings (Sari et al., 2019). Conservation activities also carry out breeding efforts to maintain the C. amboinensis population in the Bengkulu province area. Breeding results can be returned to their natural habitat (Ruyani et al., 2022).

Knowing the sex of turtles has a role in conservation efforts. The sex of reptiles can be identified and determined during the egg incubation process by looking at the temperature indicators in the nest. Low temperatures will produce male turtles and high temperatures will produce female turtles (Ario et al., 2016). Exsitu conservation areas are natural biodiversity conservation areas. The egg-laying and hatching processes were not specifically monitored, so sex determination based on the temperature in the nest during incubation could not be identified. Sex determination in the ex-situ conservation area of Bengkulu University can be done by observing secondary sexual characteristics and morphometric measurements on the body morphology of C. amboinensis.

Conservation is an activity that is full of challenges from the study of scientific and socio-cultural aspects so the implementation process involves various scientific disciplines (Silahooy & Huwae, 2020). The field related to variations and changes in the size and shape of organisms or objects is morphometrics (Septyaningsih et al., 2014). An in-depth study of the morphometric characteristics of male and female C. amboinensis is very important for conservation efforts. Understanding the morphometric characteristics, apart from being a reference in identifying and describing morphology, are also part of what can support the successful conservation, cultivation, and breeding efforts of *C. amboinensis* in the ex-situ conservation area of Bengkulu University.

2. RESEARCH METHODS

a. Research Design, Time and Place

This research uses a quantitative approach with morphometric methods. The study consisted of 20 samples (8 males and 12 females) of adult *C. amboinensis* from the ex-situ conservation area of Bengkulu University which had previously been inventoried and identified to obtain a uniform sample group, to have better comparability. Adult *C. amboinensis* is characterized by relatively similar reproductive behavior and size including carapace length and width (Ernst et al. 2016). Gender is determined by examining physical characteristics or secondary sexual characteristics. The research was carried out from September 2023 to October 2023 in the ex-situ conservation area of Bengkulu University.

b. Morphometric Measurements

C. amboinensis was measured using traditional morphometrics using scales, calipers, and measuring tape with three repetitions. The measurement object consists of 17 morphometric patterns which are divided into 5 aspects, namely head, carapace, plastron, tail, and body weight (Ernst et al., 2016). There are 2 ways to measure the length and width of a carapace, namely straight line and curved measurements. Straight-line measurements were carried out with 0.1 mm dial calipers (Figure 2) (A.B. Somers et al., 2017; Ann Berry Somers & E. Matthews, 2006). Variables measured: Head Width (HW); the longest carapace length (Carapace Length/CL) does not have to be in the middle of the line; carapace width (Carapace Width/CW); the height of the carapace (Carapace Height/CH) at the suture that separates the second and third vertebral scales; the longest plastron length (Plastron Length/PL) does not have to be in the middle of the line; plastron width (Plastron Width/PW) at the chest fracture between the pectoral and abdominal scales; and the length of the medial suture of all plastron scales (Figure 2). The arrangement of all plastron scales according to Duro et al. (2021) starts from Gular (Gul), Humeral (Hum),

Pectoral (Pect), Abdominal (Abd), Femoral (Fem), and Anal (An); tail thickness (Tail Thickness/TT) is measured at the bottom of the cloaca and the top of the tail; and tail length (Tail Length/TL) from the cloaca hole to the tip of the tail. Other measurements using a measuring tape include the length of the curved carapace (Curved Carapace Length/CCL) from the tip of the scale anterior to the posterior; the width of the curved carapace (Curved Carapace Width/CCW) from the tip of the rightmost marginal scale to the left and body weight (Weight/W), the turtle was weighed by placing it on a digital scale (maximum capacity 10 kg).

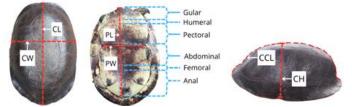


Figure 2. Morphometric measurements

CL: Carapace Length. CW: Carapace Width, PL: Plastron Length, PW: Plastron Width, CCL: Curved Carapace Length. CH: Carapace Height

$$\mathbf{A} = \frac{\mathbf{A}}{\mathbf{A} + \mathbf{B}}$$

Note

A= Plastron Scales

B= Plastron Length

The calculating results are displayed in table form for descriptive analysis and images as support

3. RESULTS AND DISCUSSION

General morphometric data on *C. amboinensis* in the Bengkulu University ex-situ conservation area can be seen in Table 1. Morphological characteristics of turtles can be identified based on the head, body, and tail shape. The head has a width of 28.8 mm with a range of 25.4-33.2 mm. The body consists of the carapace and plastron. The carapace has a dome-like shape with an average arch length of 223.3 mm and an arch width of 225.2 mm. The plastron part has 6 scales which have different sizes, the average plastron length is 174.4 mm with a range of 157-194 mm and the average plastron width is 83.4 mm with a range of 76.7-90.7 mm. *C. amboinensis* has a cone-shaped tail with the base of the tail being larger than the tip of the tail with an average tail length of 21.8 mm with a range of 16.5-29.5 mm. The average body weight of adult *C. amboinensis* was 996.9 mm with a range of 745.7-1256.3 mm. In detail, the sizes of the morphometric parts can be seen in Table 1.

Table 1. Morphometric data of adult *C. amboinensis* living in the Bengkulu University ex-situ conservation area (n=20)

Dout	Morphometrics (mm)	Marris (CAL Destation	Range	
Part		Mean, ±Std. Deviation	Minimum	Maksimum
Head	Head Width (HW)	28,8 ±2,0	25,4	33,2
Carapace	Carapace Length (CL)	188,1 ±7,6	172,3	201,7
	Carapace Width (CW)	139,8 ±6,7	133,0	159,0
	Curved Carapace Length (CCL)	222,3 ±10,9	203,7	249,7
	Curved Carapace Width (CCW)	225,2 ±9,3	211,0	244,0
	Carapace Height (CH)	80,9 ±6,0	69,0	95,0
Plastron	Plastron Leight (PL)	174,4 ±8,9	157,0	194,0
	Plastron Width (PW)	83,4 ±4,0	76,7	90,7
	Gular (GUL)	31,4 ±4,8	12,8	36,0
	Humeral (HUM)	$5,8 \pm 1,6$	2,5	8,0
	Pectoral (PECT)	36,1 ±2,7	32,6	42,6
	Abdominal (ABD)	45,0 ±3,7	37,5	51,5
	Femoral (FEM)	9,3 ±1,9	6,6	13,2
	Anal (AN)	47,6 ±3,1	42,3	53,3
Tail	Tail Length (TL)	21,8 ±4,7	16,5	29,5

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Part	Morphometrics (mm)	Mean, ±Std. Deviation	Range	
rari			Minimum	Maksimum
	Tail Thickness (TT)	6,8 ±1,4	5,2	8,9
Weight (W)=	(g)	996,9 ±121,4	745,7	1256,3

Based on the morphometric table above, some parts can be used as determinants to differentiate the secondary sexual characteristics of the sexes of *C. amboinensis*. Male and female turtles tend to be different sizes. As shown in Figure 3, male and female turtles have different sizes in each morphometric section. The male gender has a larger size in morphometrics: head width (HW), carapace length (CL), carapace curve length (CCL), center line length of Gular scales (Gul), femur (Fem), tail length (TL) and thickness. tail (TT). The female gender is larger in morphometrics, carapace width (CW), carapace arch width (CCW), carapace height (CH), plastron length (PL), plastron width (PW), humeral scale midline length (Hum), pectoral (Pect), Abdominal (Abd), Anal (An) and Body weight (W). In terms of body size, female turtles are dominantly larger than male turtles. The larger size of some morphometric parts of female *C. amboinensis* is directly proportional to the measurement of the turtle's body weight. The body weight of female turtles is greater than that of males. The results of a more detailed morphometric data analysis can be seen in Table 2.

 Table 2. Morphometric data of adult male and female *C. amboinensis* living in the ex-situ conservation area of Bengkulu University (n males= 8, n females= 12)

Dout	Morphometrics (mm)	Mean, ±Std. Deviation		Range (Minimum-Maksimum)	
Part		Male	Female	Male	Female
Head	Head Width (HW)	$30,3 \pm 2,0$	$27,6 \pm 1,2$	28,1-33,2	25,4-28,9
Carapace	Carapace Length (CL)	191,7 ±4,8	$185,7 \pm 8,3$	185,0-201,7	172,3-200,0
	Carapace Width (CW)	137,7 ±3,3	$141,2\pm\!\!8,1$	134,0-145,0	133,0-159,0
	Curved Carapace Length (CCL)	$229,3 \pm 10,3$	217,7 ±8,9	215,3-249,7	203,7-233,0
	Curved Carapace Width (CCW)	222,1 ±7,8	$227,3 \pm 10,0$	211,0-231,3	214,0-244,0
	Carapace Height (CH)	$75{,}9{\ \pm}5{,}0$	84,2 ±4,2	69,0-85,0	79,0-95,0
Plastron	Plastron Length (PL)	172,1 ±4,0	$176,0 \pm 11,0$	166,3-177,7	157,0-194,0
	Plastron Width (PW)	81,1 ±3,8	84,9 ±3,6	76,7-89,2	79,7-90,7
	Gular (GUL)	$33,2\pm 2,3$	$30,1 \pm 5,7$	29,0-36,0	12,8-34,6
	Humeral (HUM)	$5,0 \pm 1,8$	$6,5 \pm 1,1$	2,5-7,7	4,4-8,0
	Pectoral (PECT)	$35,0\pm 2,1$	$36,8\pm 2,9$	32,6-39,3	33,1-42,6
	Abdominal (ABD)	$41,0 \pm 1,7$	$47,6 \pm 1,9$	37,5-42,8	45,3-51,5
	Femoral (FEM)	$10,2 \pm 2,0$	$8,8 \pm 1,6$	6,8-13,2	6,6-10,8
	Anal (AN)	$46,5 \pm 1,7$	48,4 ±3,7	43,8-48,8	42,3-53,3
Tail	Tail Length (TL)	$26,0\pm 2,2$	$17,7 \pm 1,2$	23,4-29,5	16,5-19,7
	Tail Thickness (TT)	8,1 ±0,5	5,5 \pm 0,4	7,4-8,9	5,2-6,3
Weight (W	/)=(g)	992,8 ±94,0	999,6 ±140,7	897,3-1130,3	745,7-1256,3

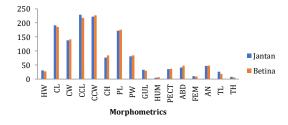


Figure 3. Morphometric values of adult males and females

Table 3 shows that male *C. amboinensis* has a longer carapace arch length than females. The difference in the curved length of the male and female carapaces shows a significant difference, with a significant *p*-value of 0.015. Based on the t-test results, the carapace height of female *C. amboinensis* was significantly higher than that

of male *C. amboinensis*, with a significance *p*-value of 0.001. The results of the t-test on the length of the midline of the humeral scales also showed a significant difference with a significant *p*-value of 0.036, female *C. amboinensis* had a longer humeral scale midline length than male *C. amboinensis*. Very significant differences were also shown in tail length and tail thickness with the same significant *p*-value of 0.000, male *C. amboinensis* had greater tail length and tail thickness than female *C. amboinensis*.

 Table 3. Results of morphometric t-test analysis of adult C. amboinensis in the ex-situ conservation area of Bengkulu University

Morphometric	Significance <i>p</i> -value
Curved carapace length (CCL)	0,015
Carapace height (CH)	0,001
Gular (GUL)	0,036
Tail length (TL)	0,000
Tail thickness (TT)	0,000

The discussion is divided based on five measurement aspects, namely: head, carapace, plastron, tail, and body weight.

a. Head

Based on the results of measurements on 20 adult *C. amboinensis* individuals (8 males and 12 females). Head morphometrics of *C. amboinensis* show differences between males and females. Moldowan et al., (2016) explained that the size and head structure of several types of turtles from the Emydidae and Geoemydidae families showed sexual dimorphism. The range and average head width (HW) show that the smallest head width is 25.4 mm while the largest head width is 33.2 mm. The head width of males is greater than that of females (Table 2). Similar to the analysis by Yuliana et al., (2023), the heads of males are larger than females. The larger head of a male turtle is a sexual characteristic because it can provide an advantage in fights over food and mates with other males.

b. Carapace

Independent sample t-test results on the carapace of adult *C. amboinensis* from the four variables tested, namely carapace length, carapace curve length, carapace curve width, and carapace height. Two of them showed significant differences between male and female sex in *C. amboinensis*, while the other variable, namely carapace width, was only analyzed using range and average because it did not meet the t-test requirements (Table 2). The morphometrics of the male and female sexs that show significant differences are in the curved length of the carapace and the height of the carapace.



Figure 4. Differences in carapace arch length and carapace height of male and female *C. amboinensis :* A: Male B: Female

The independent sample T-test on the size of the carapace curved length (CCL) between male and female turtles showed a significant *p*-value of 0.015 (table 3), which was smaller than 0.05 so it was declared statistically significant. The curved carapace length of male turtles is on average larger than that of female turtles. The average length of the curved carapace of male turtles is 229.3 ± 10.3 mm with the smallest size being 215.3 mm to the largest being 249.7 mm, while females have a smaller average of 217.7 ± 8.9 mm, the smallest size. 203.7 mm and the largest 233.0 mm, with a mean difference value of 11.6 mm. The difference in the size of males and females in the carapace curve length variable is directly proportional to the carapace length variable, which states that the carapace length value for males is longer than for females. The shape of the carapace affects the curved length of the carapace, in female turtles the carapace is circular while in males it is more oval or oblong (Setiadi, 2015). The length of the marginal scales, especially the 8-12th marginal scales on the posterior or supracaudal side, also influences the morphometric length of the carapace arch. Supracaudal scales in male turtles are longer and repeating compared to females which have straight and short scales.

C. amboinensis shows significant differences in morphometric carapace height (CH) between males and females with a significance *p*-value of 0.001 < 0.05. Female turtles were taller than males (Table 2). The average carapace height of female turtles is 84.2 ± 4.2 mm higher than that of male turtles which only has an average of

 75.9 ± 5.0 mm. The dome-like shape of the carapace in female turtles is higher, providing more space inside the carapace when containing eggs (Figure 4), similar to the explanation by Ernst et al., (2016) that female turtles have The carapace is wider and taller (especially in the posterior part of the carapace) to accommodate a larger number of eggs, whereas according to Campos-such, (2016) males have a smaller and aerodynamic carapace to make it easier when they want to return to their position when upside down. The difference in height between male and female turtles can also be related to the thickness of the carapace. Nurazizah et al., (2022) explained that in general, the carapace thickness of female turtles tends to be thicker than that of male turtles.

c. Plastron

In plastron morphometrics, there are eight measuring variables (Table 1). Plastrons were analyzed based on the ratio index of plastron scales to plastron length in male and female C. amboinensis. The comparison index data consists of 6 morphometric characters on the plastron (Table 4). Male C. amboinensis has a higher index in the midline length of the gular and femoral scales, while the humeral, pectoral, and abdominal scales of male C. amboinensis have a lower index than females. There are similar proportions of anal index between male and female C. amboinensis.

Indeks	Male	Female
Gular (GUL)	0,16	0,14
Humeral (HUM)	0,02	0,03
Pectoral (PECT)	0,16	0,17
Abdominal (ABD)	0,19	0,21
Femoral (FEM)	0,05	0,04
Anal (AN)	0,21	0,21

The results of the index analysis found that the proportion of plastron scale sizes of male and female C. amboinensis tended to be unequal except for the anal scales. The plastron scale comparison index value is in line with morphometric calculations. The difference in the proportion of plastron scale size is influenced by the turtle's biological factors, male C. amboinenis has a concave plastron in contrast to females which have a flatter plastron, this is a secondary sexual characteristic for turtles. The concave plastron will make it easier for C. amboinensis males to mount females during the mating process. The flat plastron in female C. amboinensis functions to provide more space for the reproductive organs when storing eggs. All plastron morphometrics were also analyzed using an independent sample t-test, humeral scales showed significant differences between male and female sex in C. amboinensis in the Ex-situ Conservation of Bengkulu University.



Figure 5. Differences in the length of the midline of the humeral plastron scales in male and female C. amboinensis A: Male B: Female

The humeral scales (Hum) show a "p-value" sig value. (2-tailed) of 0.036 < 0.05 (Table 3), then as a basis for decision-making in the t-test, it can be concluded that there is a significant (real) difference between the average length of the medial suture in the humeral scales of male turtles and female turtles. Male turtles have a shorter humeral suture (midline on the plastron) than females by 1.5 mm. This value shows the difference between the morphometric average length of the medial suture of the humeral scales in male and female C. amboinensis. The diameter of the humeral scales in male C. amboinensis is 5.0 ± 1.8 mm, while females have an average length of 6.5 ± 1.1 mm (Table 1). The average size of the medial suture of the humeral plastron scales in females is longer than in males, directly proportional to the average length of the plastron. Females have a longer mean plastron size than males (Table 2). Data in research by Nurazizah et al., (2022) also shows that the difference in average plastron size in C. amboinensis is longer in females than in males. d. Tail

Independent sample t-test on 2 tail variables, namely tail length (TL) and tail thickness (TT), the results were statistically significant. The tail of male C. amboinensis is longer and thicker than that of females which is shorter and slender (Table 2). Similar to research data conducted by Dayeni et al., (2020) shows that the tail of male C. amboinensis is longer than that of females. Male turtles have longer tails and have cloacas located further apart than females. The location of the cloaca is far away on the tail of male turtles because the function of the cloaca, apart from being a digestive organ, is also a reproductive organ (Figure 6A). The function of the tail, which

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is also a reproductive tool, provides differences in the length and thickness of the tail in turtles. The longer and thicker tail of a male turtle will make it easier to accelerate during the mounting process. Baizurah & Das, (2021) identification of male turtles can be seen through a longer tail and the location of the cloaca which is far from the base of the tail.

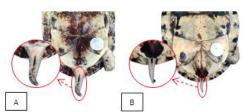


Figure 6. Differences in tail length in C. amboinensis A: Male B: Female

e. Weight

Analysis of the body weight variable (W) found that the average body weight of female turtles was greater than that of males (Table 2). It is directly proportional to the measurement of carapace width, plastron width, and plastron length which affect body size and shape. Female turtles have a heavier weight because their mobility behavior is not like males which are relatively active. Karyadi et al., (2023) male turtles will move to attract the female's attention and chase the female when they are about to mate. The male's light body weight will benefit mobility during mating. Females do not actively seek out males for mating purposes.

4. CONCLUSION

There are significant morphometric differences in *C. amboinensis* in terms of carapace arch length, carapace height, humeral scale midline length, tail length and tail thickness between males and females. *C. amboinensis* males have a larger size in terms of carapace curve length, tail length and tail thickness, while females have a larger size in the length of the midline of the humerus scales and carapace height. Identification of male and female sex in grouping and conservation breeding efforts for *C. amboinensis* can be seen in this aspect. It is hoped that future research will measure several other variables such as foot length and sole width.

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