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Reproductive parameters of the Sunda pangolin, *Manis javanica*

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Abstract. The observations of Sunda pangolin reproductive parameters in this paper were based on the wild-caught animals and those that had spent time in captive environments, however, when analyzing the results, we did not consider differences in terms of breeding habits between the two. Still, this research has led to an increase in knowledge of the breeding habits of the Sunda pangolin. Our results suggest that there is no breeding season or season of parturition for the Sunda pangolin, which breeds all year round. We estimated the gestation period in this species to be around six months. Sexual maturity occurred at one year old or as early as six-seven months old in some individuals, and requires further investigation. Each Sunda pangolin in this study gave birth to one offspring at a time. The sex ratio at birth was 0.875:1 (♀:♂) (n = 15); and the weaning age was estimated at four months with a weight of 1.19 ± 0.50 kg (n = 3), which concurs with recent research. Findings in this study will contribute to future analyses of population dynamics, species conservation, and both *in situ* and *ex situ* management of the Sunda pangolin. Despite this contribution, further studies are needed on the reproductive parameters of Sunda pangolin.

Key words: age, sexual maturity, mating season, gestation period, weaning age, litter size

Introduction

Sunda pangolins (*Manis javanica*) belong to the mammalian order Pholidota and are one of eight extant species of pangolin (Nowak 1991, Corbet & Hill 1992, Gaubert & Antunes 2005). They are distributed in Southeast Asia, including Thailand, Myanmar, Indonesia, Vietnam, Lao PDR, Cambodia, Malaysia, and Singapore (Payne et al. 1985, Gaubert & Antunes 2005, Francis 2008). In the last few decades this species has experienced precipitous population declines due to over-exploitation, and destruction of its natural habitat. Based on these trends Sunda pangolins were assessed as critically endangered on the IUCN Red List of Threatened Species in 2014 (Challender et al. 2014). The Sunda pangolin is also listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2014), with zero quotas for all wild-caught specimens traded for primarily commercial purposes, which were established at CoP11 in 2000.

Reproductive parameters can influence studies of populations, analyses of population dynamics, and predict future population trends, and they are useful for *in situ* and *ex situ* management of wildlife populations. However, owing to the elusive nature and increasing rarity of the Sunda pangolin it is extremely hard to sample data in the wild. Therefore, data on the biology and ecology of the Sunda pangolin, especially its reproduction are limited (Wu et al. 2005, Challender 2008). There has also been little research in this area. Lim & Ng (2008) studied the home range, and natal den use of a female Sunda pangolin using radio tracking technology in Singapore. Yang et al. (2010) divided 51 confiscated Sunda pangolins into four age groups according to indicators such as scales, hair, degree of claw wear, relationship between mother/infant, and body weight, and analyzed their population structure. With respect to reproductive data, Lim & Ng (2008) estimated the period of maternal care to be approximately three to four months. "Wrestling" behaviour between a female Sunda pangolin and its 4-month-old offspring was also observed by Challender et al. (2012) – observations that support the assumption of Lim & Ng (2008).

Moreover, research estimating parameters including gestation period, number of offspring, weaning age, and weight can provide useful information for the management of captive pangolins. Estimates of age at sexual maturity and understanding potential preferences for mating at a particular time of year

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could also benefit captive management (Challender 2008). In this study, reproductive parameters of Sunda pangolins rescued from illegal trade and maintained in captivity were analysed to provide an evidence base pertaining to mating season, gestation period, sex ratio at birth, litter size, breeding age, and weaning age. These findings should improve understanding of the breeding habits of these species and may inform future conservation efforts, both *ex situ* and *in situ* (e.g. population modelling).

Material and Methods

Subjects

The subjects of this study were 31 pregnant female Sunda pangolins (*Manis javanica*). Data for seven subjects was obtained from the literature (Lim & Ng 2008, Challender et al. 2012) and provided by Singapore Zoo and the Wildlife Rescue Center of Guangdong Province (J.J. Zou, pers. comm.). The other 24 animals were confiscated from illegal trade and sent by the Forest Police to the Pangolin Research Base for Artificial Rescue and Conservation Breeding of South China Normal University (PRB-SCNU) between June 2010 and August 2013. Fourteen of the 24 females were already pregnant before they arrived at the PRB-SCNU. The remaining ten pangolins conceived in captivity. Of the 24 trade-confiscated subjects, nine died during pregnancy, one gave birth to a dead premature foetus and 14 successfully gave birth to 15 offspring. One subject, MJ75, was pregnant twice, the first occurring in the wild and the second in captivity, and which resulted in the successful birth of one offspring on each occasion.

Housing

At PRB-SCNU each rescued pangolin was housed individually in a separate enclosure within a large room. The room was 45 m in length, 12 m in width, and 3 m in height and was equipped with thermal insulation, ventilation, and lighting, and was designed to effectively prevent animals from escaping. A total of 30 enclosures were arranged down two sides of the room with 15 enclosures on each side. A keeper corridor with 1.8 m wide ran between the two rows of enclosures. Each enclosure was 3.0 m in length, 5.0 m in width, and 1.5 m in height. A nest box approximately 0.8 m in length, 0.8 m in width, and 0.6 m in height with an entrance 0.25 m in length by 0.25 m in width was provided in one corner each enclosure for each pangolin to sleep in. Sunda pangolins are known to defecate in water (Challender et al. 2012), and a water pool was provided (0.6 m in length × 0.5 m in width × 0.3 m in depth) in each enclosure. Fallen trees were also provided for environmental enrichment. The nest boxes and enclosures had a concrete floor, covered with 0.1 m thick layer of sandy soil (sand: clay = 1:1). Cotton rags were also added to the nest boxes to provide bedding in winter. The room was also equipped with under-floor heating and an air humidifier to maintain the temperature and humidity of the enclosures at 18-30 °C and 60-90 %, respectively throughout the year.

Husbandry

On arrival at the PRB-SCNU, most of the Sunda pangolins were observed to be hungry, carried traumatic injuries, were experiencing stress, exhibiting a strong desire to escape, and refused to eat an artificial diet. To improve the animal's welfare, the Research Center provided individual housing, treated any diseases which were apparent, and avoided unnecessary disturbance until animals were settled in their captive environment. We defined this as the disappearance of stress behaviour, the recovery of health, and the unforced consumption of an artificial diet. When this was achieved, at irregular intervals, one female and one male Sunda pangolin were selected and housed together

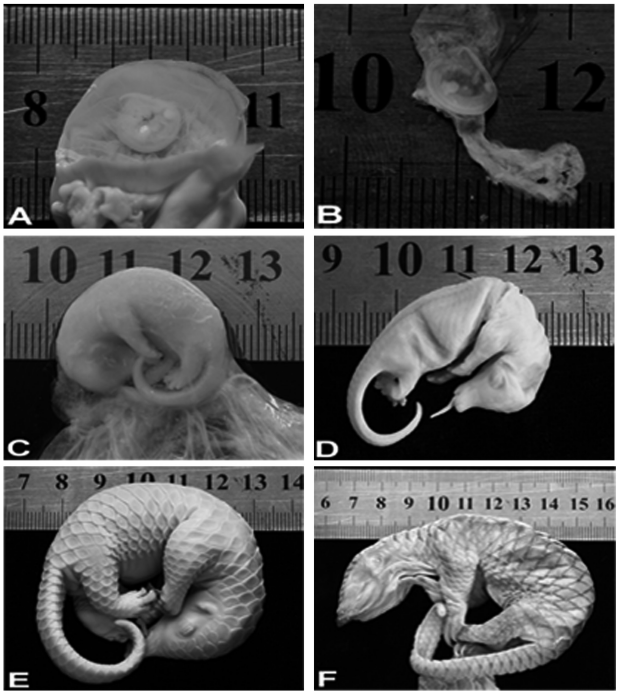

Fig. 1. Six foetuses in different development stages found in their mothers' uterus between June 13 and 23, 2011. Note: the development level of each foetus was more mature than the previous one from A to F. A, B and C are separate foetuses MJ-Rb, MJ-Hb and MJ-Gb; they were each found on June 15, 2011; D and F are separate foetuses MJ-Kb and MJ-Jb, both of them were found on June 23, 2011; E is foetus MJ-Fb, it was found on June 13, 2011.

Fig. 2. This picture shows two young riding on the tail of a female Sunda pangolin – suggesting it gave birth to twins (by Shibao Wu in 2009).

for mating. At irregular intervals, their sexual responses were observed and when it was suspected mating had occurred the female was housed individually.

Results and Discussion

Parturition date and mating season

A total of 22 Sunda pangolin births were recorded (Table 1). We observed that Sunda pangolins were born throughout the year with the exception of May, August, and December (Table 1), suggesting that breeding in this species is aseasonal. Within the 10 day period between 13 to 23 June 2011, six dead female Sunda pangolins in captivity were found to have been pregnant before they arrived at PRB-SCNU (10 June, 2011), with foetuses at different stages of development (Fig. 1). The foetus MJ-Rb had unformed limbs (only white but could be seen); its head, eyes, nose, external ears, and other organs could not be distinguished (A in Fig. 1). The foetus MJ-Jb was close to full development, it weighed 130 g, thereby reaching an estimated birth-weight of Sunda pangolin, 141.43 ± 15.47 g (n = 7, see Zhang et al. 2013), the body surface was covered with overlapping scales, with hair between the scales, and eyes, external ears, and other organs developed (F in Fig. 1), so we believe it could have been viably born.

Table 1. Parturition date of Sunda pangolins included in this study.

No.	Mother ID	Date arrived in captivity	Mating site	Parturition date	Young ID	Source
1	NITA	Unknown	Singapore Zoo	January 14, 2011	WANITA	Singapore Zoo
2	MJ-X1	Before May 1, 2012	PRB-SCNU	January 23, 2013	MJ-X1b	PRB-SCNU
3	MJ65	June 10, 2011	PRB-SCNU	February 14 2012	MJ132	PRB-SCNU
4	MJ60	May 12, 2011	PRB-SCNU	March 10, 2012	MJ133	PRB-SCNU
5	QFY-17	November 9-22, 2012	PRB-SCNU	March 22, 2013	QFY-34	PRB-SCNU
6	MJ-X2	Before May 1, 2012	PRB-SCNU	March 22, 2013	MJ-X2b	PRB-SCNU
7	MJ-X3	Before May 1, 2012	PRB-SCNU	April 12, 2013	MJ-X3b	PRB-SCNU
8	ANGGUN	Unknown	Singapore Zoo	April 25, 2011	JELITA	Singapore Zoo
9	MJ69	June 10, 2011	Wild	June 11, 2011	MJ83	PRB-SCNU
10	MJ-B	June 10, 2011	Wild	June 11, 2011	MJ-Bb1	PRB-SCNU
11	MJ81	June 10, 2011	Wild	June 11, 2011	MJ-Cb1	PRB-SCNU
12	MJ-D	June 10, 2011	Wild	June 16, 2011	MJ-Db1	PRB-SCNU
13	MJ72	June 10, 2011	Wild	June 21, 2011	MJ-Eb1	PRB-SCNU
14	GDWR1	July 23, 2002	Wild	July 24, 2002	GDWRb1	WRC-GD[λ]
15	GDWR2	July 23, 2002	Wild	July 25, 2002	GDWRb2	WRC-GD[λ]
16	GDWR3	July 23, 2002	Wild	July 25, 2002	GDWRb3	WRC-GD[λ]
17	MJ6	Unknown	Wild	September 8-17, 2005	MJ6-Y	Lim et al. 2008
18	MJ75	June 10, 2011	PRB-SCNU	September 20, 2012	MJ188	PRB-SCNU
19	MJ90	December 14, 2011	PRB-SCNU	October 2, 2012	MJ189	PRB-SCNU
20	MJ75	June 10, 2011	Wild	October 8, 2011	MJ85	PRB-SCNU
21	MJ70	June 10, 2011	Wild	October 22, 2011	MJ88	PRB-SCNU
22	P11	Unknown	Wild	November 22, 2007	P19	Challender et al. (2012)

[λ] Wildlife Rescue Center of Guangdong Province.

Table 2. Estimated gestation period of the nine Sunda pangolins with successful pregnancies and parturition in captivity included in this study.

No.	Mother ID	Date arrived in captivity	Mating date	Parturition date	Estimated gestation period (day)	Young ID	Father ID	Source
1	MJ65	June 10, 2011	August 15-October 2, 2011 (48 d)[λ]	February 14, 2012	135-183	MJ132[a]	MJ66	PRB-SCNU
2	MJ60	May 12, 2011	August 19-November 10, 2011 (52 d)[λ]	March 10, 2012	120-203	MJ133[a]	MJ71	PRB-SCNU
3	MJ75	June 10, 2011	March 28-April 15, 2012 (18 d)[λ]	September 20, 2012	158-176	MJ188[a]	MJ71	PRB-SCNU
4	MJ90	December 14, 2011	March 28 and April 4, 2012	October 2, 2012	181 or 188	MJ189[a]	MJ66	PRB-SCNU
5	MJ-X1	Before May 1, 2012	July 16-September 8, 2012 (54 d)[λ]	January 23, 2013	137-191	MJ-X1b1	Unknown	PRB-SCNU
6	MJ-X2	Before May 1, 2012	September 10-November 15, 2012 (66 d)[λ]	March 27, 2013	134-200	MJ-X2b1	MJ120	PRB-SCNU
7	MJ-X3	Before May 1, 2012	September 10-November 15, 2012 (66 d)[λ]	April 12, 2013	150-216	MJ-X3b1	MJ120	PRB-SCNU
8	NITA	Unknown	July 1-September 30, 2010 (92 d)[λ]	January 14, 2011	106-198	WANITA	Panjang	Singapore Zoo
9	Anggun	Unknown	October 1, 2010-January 1, 2011 (93 d)[λ]	April 25, 2011	115-207	JELITA	Panjang	Singapore Zoo

[a] Stillbirth, well developed, excluding premature possibility. [λ] Numbers in brackets show the mating duration.

These findings support the notion that breeding is aseasonal. In a survey of hunters, it was often reported that they could see young pangolins riding on the back of their mothers' tails in the wild throughout the year. Some respondents also remarked that they had seen mount behaviours (a male pangolin attempting to copulate with a female pangolin) throughout the year (Y.L. Zhou, pers. comm.). Where pangolins have been confiscated from illegal trade, the authors have been able to examine them and have found young pangolins suckling from dead females that also had foetuses in different stages of development in their uteri. All of the above suggests that there is no specific mating season for the Sunda pangolin and that it may breed throughout the year. However, mating seasons have been reported for the Chinese pangolin (*Manis pentadactyla*), Indian pangolin (*M. crassicaudata*), and Cape pangolin (*Smutsia temminckii*). Regarding the Chinese pangolin most births occurred from late autumn to early spring (in the northern hemisphere), and its mating behaviour was mainly observed to occur in late spring, summer, and early autumn (see Heath & Vanderlip 1988, Heath 1992a, Wu 1998, Cheng et al. 2000, Yang et al. 2007); for the Cape pangolin it is thought that the mating season is late summer to early autumn and birthing season in the southern-hemisphere winter (Heath 1992b). This suggests that the mating season of the Chinese pangolin and Cape pangolin are different, and differ from the Sunda pangolin. However, births of the Indian pangolin have also been observed throughout the year (Heath 1995, Mohapatra & Panda 2014), and may in fact be similar to those of the Sunda pangolin, though further research is needed to confirm this. Nonetheless, it does suggest the existence of interspecies differences in the breeding habits of pangolins.

Gestation period

In this study, a total of nine cases of successful conception and parturition in captive female Sunda pangolins were recorded, seven from the PRB-SCNU and two from Singapore Zoo (Table 2). With the exception of the female MJ90, all pangolins were housed for mating for at least 18 days (Table 2). Only the dates of parturition were recorded, but exact conception dates are unknown, making it difficult to determine the specific length of gestation. When we infer the gestation period of Sunda pangolin, calculated from the start and end date of housing a female together with a male, to the date of parturition separately, we can calculate a "likely" minimum and maximum gestation period. As such, we estimated the

Table 3. Weight and data of confirmed pregnancy among the 24 female Sunda pangolins at the PRB-SCNU included in this study.

No.	Mother ID	Pregnancy confirmed time	Mother weight (kg)	Father ID	Mating site
1	MJ148	August 24, 2012	1.75	Unknown	PRB-SCNU
2	MJ-R	June 15, 2011	1.95	Unknown	Wild
3	MJ90	April 4, 2012	2.16	MJ66	PRB-SCNU
4	MJ116	August 24, 2012	2.68	Unknown	PRB-SCNU
5	MJ-L	July 2, 2011	2.78	Unknown	Wild
6	MJ60	November 29, 2011	3.06	MJ71	PRB-SCNU
7	MJ-D	June 16, 2011	3.10	Unknown	Wild
8	MJ81	June 11, 2011	3.18	Unknown	Wild
9	MJ-H	June 15, 2011	3.20	Unknown	Wild
10	QFY-17	December 13, 2012	3.22	Unknown	Wild
11	MJ-B	June 11, 2011	3.23	Unknown	Wild
12	MJ69	June 11, 2011	3.30	Unknown	Wild
13	MJ-G	June 15, 2011	3.40	Unknown	Wild
14	MJ-X2	January 15, 2013	3.55	MJ120	PRB-SCNU
15	MJ70	July 19, 2011	3.73	Unknown	Wild
16	MJ65	October 2, 2011	3.76	MJ66	PRB-SCNU
17	MJ-J	June 23, 2011	4.00	Unknown	Wild
18	MJ-X1	January 15, 2013	4.02	Unknown	PRB-SCNU
19	MJ72	June 21, 2011	4.14	Unknown	Wild
20	MJ12	July 5, 2011	4.40	MJ26	PRB-SCNU
21	MJ-X3	January 15, 2013	4.30	MJ120	PRB-SCNU
22-1	MJ75	July 19, 2011	4.58	Unknown	Wild
22-2	MJ75	April 5, 2012	5.54	MJ71	PRB-SCNU
23	MJ-F	June 13, 2011	4.70	Unknown	Wild
24	MJ-K	June 23, 2011	-	Unknown	Wild

gestation period of the Sunda pangolin to be between 106-207 days (Table 2). In this study, there were three females (MJ75, MJ65 and MJ90), and the exact dates they mated with the males were recorded. The female MJ75 mated on the evening of 28th March 2012 and gave birth on 20th September 2012, giving a gestation period of 176 day (Table 2). Female MJ65 mated on four consecutive days (August 16-19, 2012), giving an estimated gestation period of 178-181 days if the pregnancy was caused by one of several mating events (Table 2). The female MJ90 mated with a male (MJ66), twice – on the 28th March and 4th April 2012, and gave birth to one young on 2nd October 2012, indicating that at least one of the two mating events was successful and suggests a gestation period of 181 or 188 day (Table 2). At Singapore Zoo, the female Sunda pangolin "Nita" was observed to mate with the male animal "Panjang" on 11th, 13th, and 16th July 2010 suggesting a gestation period of between 182 to 187 day (Table 2). Based on these data it is likely that the Sunda pangolin has a gestation period of 176-188 day (around six months). Lekagul & McNeely (1988) thought that the Sunda pangolin had a pregnancy of two to three months, but this is inconsistent with our observations. In the present study, data from direct observations on multiple subjects suggests the gestation period of the Sunda pangolin is no less than three months and is more likely to be around six months.

Age at sexual maturity and weight of pregnant pangolins

In the PRB-SCNU, a total of 24 female Sunda pangolins were confirmed to be pregnant and had an average weight of 3.49 ± 0.90 kg (1.75-5.54 kg, n = 24). Most weighed three to five kg (n = 19, Table 3). It was also observed that Sunda pangolin young could reach a weight of three to five kg one year after they were born, with the mean growth rate 10.44 g/day (n = 3). Based on this we infer that Sunda pangolins likely reach sexual maturity and reproductive age when they are one year old. MJ148, which became pregnant in

captivity and was the pregnant female that weighed the least was confirmed to weigh only 1.75 kg while pregnant. Similarly, MJ-R died on the fourth day after her arrival, and during dissection it was confirmed the animal was pregnant (weight 1.95 kg). Based on the growth rate of captive Sunda pangolins observed at PRB-SCNU, we estimated the ages of MJ148 and MJ-R to be 180-200 days when they were confirmed to be pregnant, indicating that the females MJ148 and MJ-R could start to breed at six to seven months old, but other subjects were typically one year old approximately.

There have been suggestions that pangolins reach sexual maturity at two years old (MacDonald 2006). However, it is thought that the Chinese pangolin may begin to breed at around 1-1.5 years old or even earlier (see Liu & Xu 1981, Heath 1992a, Chin et al. 2011), and which is closer to the age of sexual maturity in Sunda pangolin that we present here.

Sex ratio at birth and litter size

There were seven females and eight males among the 15 newborn pangolins born at the PRB-SCNU (Table 1), suggesting a sex ratio in this species of 0.875:1 (♀:♂, n = 15). Based on the 22 cases of births of Sunda pangolins, females gave birth to one offspring at a time (Table 1). Dissection of the nine dead pangolin females also revealed that each was pregnant with only one young (Table 1). One other pangolin miscarried, and this resulted in a stillbirth (Table 1).

These results suggest that the Sunda pangolin typically gives birth to one offspring at a time and though births involving two or more offspring are reported in the literature (e.g. MacDonald 2006), births of more than one offspring were not recorded in this study. However, the corresponding author of this paper also saw two similar-sized Sunda pangolin young simultaneously riding the mother's tail (Fig. 2), suggesting that Sunda pangolins may also have two young at a time on same occasions, and which warrants further research. The Chinese pangolin, Indian pangolin, Cape pangolin, and tree pangolin (*Phataginus tricuspis*) are reported to give birth to one young each time (van Ee 1966, Masui 1967, Menzies 1967, Ogilvie & Bridgwater 1967, Shi & Wang 1985, Heath & Vanderlip 1988, Chao et al. 1993, Wu 1998, Cheng et al. 2000, Yang et al. 2007, Mohapatra & Panda 2014). But, occasionally two young are born among Indian pangolins according to Prater (2005), and hunters in China (L. Xu, pers. comm.) have stated that they have seen two equally-sized Chinese pangolin young riding on their mother's back in the wild. In addition, when they dug out a Chinese pangolin's burrow, they found one adult pangolin and two young ones suggesting, this species may have two young on occasion (H. Liu, pers. comm.).

Weaning age

It was observed that the weaning age for the three captive Sunda pangolin young MJ85, MJ-X1b1, and QFY-34 was 115, 105, and 136 day (118.67 ± 15.82 days, around four months), respectively; and their weaning weight was 1.88, 0.93, and 0.75 kg (1.19 ± 0.50 kg), respectively. We observed that, with growth, young pangolins gradually learned the skills of eating an artificial diet from their mothers, gradually broke away from their mothers, and increasingly spent time in independent activities. Gradually, the young reduced the frequency of suckling, the duration of suckling decreased, and the frequency and quantity of eating artificial food increased until the young fed on artificial food exclusively and could be considered weaned. After weaning, the young lived independently, completely separated from their mothers, no longer holding onto the dorsal part of their mother's tail, accompanying or having contact with their mothers, and sleeping in a place completely separated from their mothers.

Lim & Ng (2008) studied the mother-infant relationship and home range of a mother Sunda pangolin and its young in the wild using radio-tracking technology, and estimated the weaning age to be three to four months old, supporting our observations (four months). In addition, Challender et al. (2012) reported a social behavior, "wrestling" between a mother pangolin and its four months old young in captivity, and concur with Lim & Ng (2008) on the age of weaning. Some notable behavioural observations were found during the weaning period in PRB-SCNU, such as when the young tried to ride the mother's tail, the mother turned her back to scratch the young with its forelimbs to prevent it from doing so and would flee to move away from the juvenile.

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