Nat. Hist. Bull. Siam. Soc. 59(1): 57–60, 2013

Pure Banteng *Bos javanicus* Persist in Southern Preah Vihear Province, Central Cambodia, Despite Apparent Hybridisation with Domestic Cattle

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Cambodia supports globally important populations of Banteng *Bos javanicus* (Timmins *et al.,* 2008). However, interspecific hybridization may have occurred and may still occur in some areas. Hassanin & Ropiquet (2007) have revealed that Cambodian populations of Banteng acquired a mitochondrial genome of Kouprey *Bos sauveli* by natural introgressive hybridisation during the Pleistocene epoch, at 1.34 ± 0.45 Myr ago. Wharton (1957) found evidence of interbreeding with domestic cattle. More recently, several cases of hybridisation between wild and domestic cattle have been identified in Cambodia. For instance, a male hybrid obtained from a cross between a female Gaur *Bos gaurus* and a domestic bull is illustrated in Figure 1A. In addition, a specimen preserved at the Museum of Bourges (France) was the result of a cross between a female Kouprey and a domestic bull (Fig. 1B; Hassanin *et al.,* 2006), as recently confirmed by the analysis of nuclear DNA sequences (A.H., unpublished data; Table 1). Thus, hybridisation occurs between domestic and wild cattle in Cambodia. Since populations of wild cattle have seriously declined in Cambodia over the last decades due to ongoing habitat destruction and degradation, hunting, and human expansion into the last remote areas (Timmins *et al.,* 2008), wild potential sexual partners have become rarer while populations of domestic cattle have grown, thus facilitating sexual encounters with wild cattle.

According to locals in southern Preah Vihear province, starting in 1973, the Khmer Rouge (KR) regime collected villagers in the area to work in labour camps and KR soldiers took control of their semi-free-ranging domestic cattle. With the Vietnamese invasion of Cambodia in 1979, most of the KR troops moved, leaving everything behind, including the cattle. Following months of general confusion, many villagers were finally allowed to return to their homes and round up some of their original cattle. However, in this province alone, probably thousands of domestic cattle got away. Most of these animals, that subsequently became feral, were gradually shot during the 1980s and 1990s, but until then they shared the forests with wild cattle and may have hybridised with these.

A hunter and ex-KR soldier, who had been hunting throughout Preah Vihear province since the early 1980s, estimated that in the southern part of the province c. 50 % of the adult Banteng he has seen up to present did not show the characteristic purely white markings, but had interspersed brown colour, especially on the legs, but less frequently also on the rump.

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Received 21 November 2012; accepted 25 June 2013.

These pelage characters may be indicative of domestic cattle introgression. According to the same hunter, in currently still more remote areas in northern Preah Vihear province (where human and thus domestic cattle populations were previously very low) most Banteng show the characteristic pure white markings.

On 17 Dec 2009, we learnt about a newborn Banteng calf captured by local villagers c. 25 km east of Rovieng district town in southern Preah Vihear province, north-central Cambodia, close to the Preah Vihear-Kampong Thom provincial border. We were able to convince the villagers to keep the animal alive, and on 19 Dec 2009 it was handed over to the Angkor Centre for Conservation of Biodiversity (ACCB), a wildlife rescue and nature conservation centre in Siem Reap province. Upon arrival at the centre, the female calf weighed 24.5 kg. Unfortunately, the animal died on 25 Dec 2009 due to septicaemia caused by necrosis and infection of an injury in the left front leg that had resulted from its capture.

The calf showed the following characteristics of neonatal/juvenile Banteng (only partially visible in Figures 2A and 2B): white markings on lower and upper legs, especially on the insides (with purely white area on the inside of the thighs and lower belly, and white patches on the inside of the upper front legs), pale rump, pale markings around eyes and snout, marked withers, and a dark stripe along the back. However, in the light of locals' accounts of apparent Banteng–domestic cattle hybrids in the area where the calf had been caught, doubts remained regarding the animal's purity and a genetic analysis of muscle tissue preserved in alcohol was conducted by A.H. at the *Muséum national d'Histoire naturelle* in Paris. Four molecular markers were sequenced: the complete mitochondrial cytochrome b gene and three nuclear introns (FGB, SPTBN1 and TG) (Table 1). The comparisons with the data analyzed in HASSANIN & ROPIQUET (2007) showed that the animal was a pure Banteng. Indeed, the sequences were found to be identical to those previously generated for a specimen of Cambodian Banteng preserved in the collections of the Natural History Museum in Paris (voucher MNHN 1876-535; HASSANIN & ROPIQUET, [2007]). By contrast, these sequences were different from those published for domestic cattle (>5 differences in each of BFG, SPTBN1, and TG; see Figure 3 in HASSANIN & ROPIQUET [2007] for comparison).

Therefore, pure Banteng offspring are still being produced in this area where Banteng and domestic cattle are frequently encountered and where local people suggest high levels of hybridisation.

However, this Banteng population of unknown size in central Cambodia is threatened: The area where the calf had been captured has only been made more easily accessible during 2009, when a road was constructed to access a large area of forest granted to a Vietnamese company for conversion into a rubber plantation.

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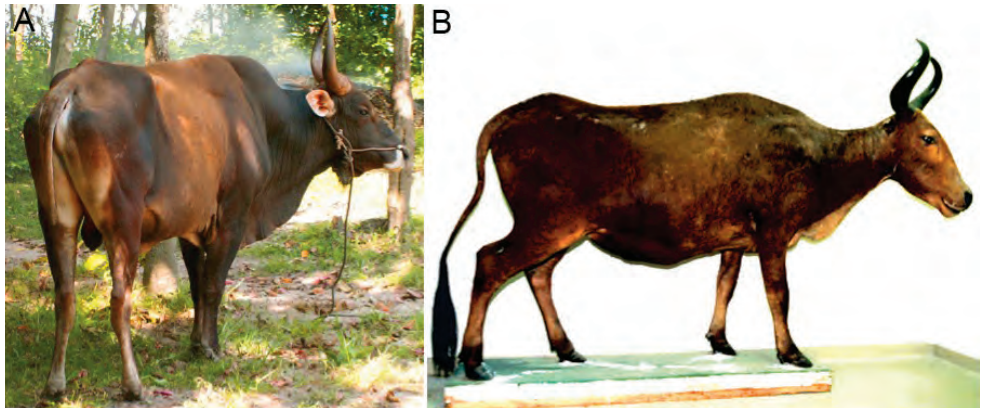

Figure 1. Examples of hybrids between wild and domestic cattle in Cambodia.
A: Adult male hybrid between a female Gaur (*Bos gaurus*) and a domestic bull. Phnom Tamao Wildlife Rescue Center, Cambodia, 2005. Photo: Alexandre Hassanin.
B: Adult male hybrid between a female Kouprey (*Bos sauveli*) and a domestic bull. Museum of Bourges, France, 2004. Photo extracted from Hassanin *et al.* (2006).

Figure 2. Female Banteng calf, central Cambodia (Photos: Markus Handschuh).
A: During transport to a wildlife rescue centre, 19 Dec. 2009.
B: Post-mortem at the Angkor Centre for Conservation of Biodiversity, Siem Reap province, 25 Dec. 2009.

Table 1. GenBank accession numbers of the sequences analyzed in this study.

Specimen	Tissue code	Origin	Cytochrome b	Nuclear markers	Taxonomic conclusion
Banteng ♀ (Fig. 2)	T2146	Rovieng, Preah Vihear, Cambodia	KF193888	FGB : KF193890 SPTBN1 : KF193891 TG : KF193892	*Bos javanicus*
Museum of Bourges ♂ (Fig. 1B)	1871-576	Cambodia	DQ275470*	FGB : KF193886 SRY : KF193889 TG : KF193887	Hybrid *Bos sauveli* ♀ X *Bos taurus* ♂

* sequence published in HASSANIN *ET AL*. (2006)