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Full Length Article

New Fossil Record of a Subspecies of *Bubalus* from the Weihe Area, Shaanxi, China

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Abstract

Bubalus brevicornis (water buffalo) fossils are rare in China. They have only been found in Early-Middle Pleistocene sites and are thought to extinct long before the Holocene epoch in China. Recently, we discovered two specimens of *B. brevicornis*. Their characteristics include: small size; short and stout horn cores; both the superior face of the horn core and the skull *facies frontal* in the same plane; horn cores extend moderately backwards and sligshtly curved inward; cross-sections of the horn cores almost isosceles triangle; anterior face and inferior face are flat and occipital region projects backward. Furthermore, clustering analysis showed that the specimens have high similarity with *B. brevicornis* and represent a new subspecies. Additionally, the results of AMS^{14}C dating showed that the age of specimens are from 3959~4050a before present (BP). The result suggest that the *B. brevicornis* could have survived from Pleistocene to Holocene and there are two extinct *Bubalus* species (*B. brevicornis* and *B. mephistopheles*) during the Holocene in China not just *B. mephistopheles* as previously reported.

Keywords: *Bubalus*; Weihe area; Holocene

Introduction

As a representative species of the extra-tropical environment, *Bubalus* plays a significant role in the reconstruction of palaeo-environment (Tong, 2007). Research shows that the *Bubalus*, living in all Quaternary periods has been found in wide areas from north to south of China (Young, 1932; Chow and Hsu, 1957; Guo, 2008; Dong *et al*., 2014). There were ten species of *Bubalus* buffalos reported from China: *B. Mephistopheles* (Hopwood, 1925; Wei *et al*., 1989), *B. teilhardi* (Young, 1932; Dong, 1999; Hu, 2005), *B. wansjocki* (Boule *et al*., 1928; Takai,1941; Institute of Vertebrate Paleontology, 1959; Zhou *et al*., 1990; Ding and Liu, 1994; Nie *et al*., 2008), *B. brevicornis* (Young, 1936; Young and Liu, 1950), *B. tingi* (Bohlin, 1938), *B. youngi* (Chow and Hsu, 1957; Xue and Li, 2000), *B. guzhensis* (Liu and Zhen, 1981), *B. triangulates* (Liu and Zhen, 1981), *B. fudi* (Guo, 2008) and *B. brevicornis chowi* (Dong *et al*., 2014). The research data revealed that all fossil buffaloes became extinct before the Holocene epoch except *B. mephistopheles* (Tong *et al*., 2015). The earliest fossil of water buffalo is *B. brevicornis* which was only discovered in a few strata from the lower and middle Pleistocene (Fig. 1). Therefore, it is generally believed that *B. brevicornis* is an extinct and quite ancient species of fossil water buffalo (Dong *et al*., 2014; Tong *et al*., 2015). *B. mephistopheles* from the Holocene may have evolved from the *B. brevicornis*, but fossil evidence is lacking.

In recent years, two skulls of *Bubalus* were found in sandpits of Xianyang and Gaoling area in the Weihe basin. They were preliminarily identified as a subspecies of *B. brevicornis* based on their morphological characteristics. In addition, radiocarbon dating shows that they are from the middle Holocene about 3959 to 4050a BP. This probably suggests that *B. brevicornis* could have survived from the Pleistocene to Holocene in China.

Materials and Methods

The studied materials in this paper were two incomplete skulls (Fig. 2; Table 1). The terminology and measurement procedures were those of Von Den Driesch (1976), Colbert and Hooijer (1953), Hooijer (1958), Xue and Li (2000) and Tong (2015). Lengths are measured in millimeters (mm) (Table 2). The research methods included direct observation, morphological description and measurements using the vernier caliper and tape. Photographs were taken using the digital camera and image processing using Photoshop, Corel Painter Essential and CorelDraw. Horn core index was calculated using the formula:

Horn core index = (Breadth of the back face/length along the convex border) × 100

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Table 1: Studied fossil specimens of *Bubalus*

Specimens	Taxonomic assignment	Elements	Locality	Horizon	Repository
NWUV1403.1	*Bubalus brevicornis*	Broken skull and cores	Gaoling	4050a	Northwest University
NWUV1403.2		Broken skull and cores	Xianyang	3959a	Northwest University

NWUV 1403, Specimens number of Institute of Cenozoic Geology and Environment, Northwest University

Table 2: Measurements of *Bulalus* from Weihe area and comparison with others in China (mm)

Types Characteristics		NWUV1403.1	NWUV 1403.2	*B. brevicornis*	*B. mephistopheles*	*B. teihardi*	*B. bubalus*	*B. bubalus*
Diameters of horn core at base	Anterior surface	70	77	79	74	85	55	58
	Upper surface	125	116	162	108	105	86	96
	Ventral surface	123	103	142	103	130	85	96
Circumference of horn-core at base		340	282	405	300	340	236	250
Length of the horn core along the convex border		311*	-	412	282	550	300	290
Length of the horn core along the concave border		240*	-	350	225	470	240	250
Spread of horn-cores, tip to tip		578*	-	665	430	840	560	588
Width between bases of horn-cores	At the anterior edge	133	-	93	145	100	175	207
	At the posterior edge	88	60	42	93	99	140	157
Breadth of skull across the orbits		211	-	230	255	210	167	218
Breadth at the post-orbital constriction		140	-	160	155	180	165	212
Distance between the supra-orbital foramina		79	-	77	85	95	97	111
Minimum distance between the posterior ends of the temporal fossae		110	91	-	79	85	-	86
Distance(along the skull) between the posterior line of the horn cores and the occipital crista		76	72	70	78	93	53	69
Maximum breadth of the muzzle (between the first molars)		-	-	-	171	170	-	155
Basal length from the occipital condyles to the fore-border of P2		-	-	-	521	350	-	487
Depth of occipital crest to top of foramen magnum		84	82	114*	-	-	-	71
Depth of occipital crest to lower border of foramen magnum		130	130	150*	-	-	80	97
Anterior-posterior diameter of the orbit		-	-	-	66	69		70
Vertical diameter of the orbit		-	-	-	68	68		66
Maximum breadth between the occipital condyles (from outside)		114	122	112*	-	115	97	98
Maximum breadth of the occipital wall		254	215	250	248	200	170	-
Minimum distance between the orbits and the core at the base		49	-	55	58	49	42	-
Maximum breadth of the parietal		90	83	-	-	116*	105	
Length of the parietal		92	82	-	-	94*	100	
Horn core index		40*		39.5	36	19	28.6	38.4
Source of data		Author	Author	Young 1936	Hopwood 1925	Young 1932	Xue and Li 2000	Tong *et al.* 2015

To get the age of these *Bubalus*, the two specimens were sent to Xi'an Center of Accelerator Mass Spectrometry, Institute of Earth Environment, Chinese Academy of Sciences and to American Beta Analytic, both of which use Accelerator Mass Spectrometry (AMS) to date the specimens. The results accept customary age after using $\delta^{14}C$ values to adjust separation fluid effect.

Cluster analysis utilized SPSS software. In Windows XP system, SPSS17.0 software performs statistical analysis on the data of classifying ten parameters from typical *Bubalus* in China and uses "Z" value standardization to remove the numerical range between individuals, adopts Euclidean distance to conduct matrix analysis and wield methods of links between groups to judge their relationship or similarity (Zhang, 2006).

Results

Systematic Paleontology of the Samples

Class Mammalia Linnaeus, 1758
Order Artiodactyla Owen, 1848
Suborder Ruminantia Scopoli, 1777
Superfamily Bovidae Gray, 1821
Family Bovinae Gray, 1921
Subfamily Bovini Gray, 1821
Tribe Bovini Gray, 1821
Genus Tribe *Bubalus* Smith, 1827
Bubalus brevicornis guanzhongensis subsp. Nov.

Sampled Material

One incomplete skull with horn cores (NWUV1403.1, specimen number of Institute of Cenozoic Geology and Environment, Northwest University the same hereafter). Another (NWUV1403.2) is a young individual with broken brain case, occiput and fragment of cores preserved.

Localities: Sandpits at Gaoling and Xianyang near Weihe River, Shaanxi.

Horizon: Middle Holocene, about 3959a~4050a BP.

Holotype: An incomplete skull with two horn cores (NWUV1403.1). It's a small skull with the parts bebind the orbits, nearly complete top of brain case and occiput, and broken tip of two cores.

Included specimens: An young individual with broken brain case, occiput and the base parts of two horn cores (NWUV1403.2).

Etymology: *Guanzhongensis* (Guanzhong area) is named after the local region where the *Bubalus brevicornis guanzhongensis* were found.

Diagnosis: A small water buffalo with following characteristics: short and stout horn core; both the upper surface of horn core and the forehead surface are nearly in a same plane; horn cores are moderately backwards and slightly curved inward; the cross-section of the horn core is almost an isosceles triangle; the anterior surface and upper surface are flat; Ventral surface slightly curved and the occipital region projects backward.

Description

As given in Fig. 2A the skull is small-sized; the frontal bone between the post-orbits is narrow with a slightly depressed area and gradually bulges at the base of the cores. Both the area between the base of the horns and the supra-orbital foramina appear humpy. The sagittal suture is remarkable in the upper frontal area and raised in between the orbits, while it slants downward to two sides between the supra-orbital. The supraorbital sulcus is clear. The supra-orbital foramina are slightly in front of the edge of postorbital. The distance between the orbits and the core at the base is short. The parietal bone is vestigial, short and shrinks out at the top of cranium, while it projects backwards behind the horn core. The angle between the parietal bone and the occipital bone was about 145°. The distance from the posterior line of the horn cores to the occipital crista was long. The occipital crest was moderately developed and the depth from the occipital crest to lower border of foramen magnum is a long distance. The temporal fossae is broad. The occipital condyles and mastoid processes are well preserved. Outside pillow nodules are not developed. The occipital wall is broad and nearly vertical. The mastoid process is strong and bent. The top edge of foramen magnum is convex.

The horn cores are short overall and stout at the base, but broken at the tip. They are sharply trihedral. The upper face (the broadest of the three) and anterior face are quite flat, while the lower face (Ventral) is rather curve. The cross section of the horn cores is almost an isosceles triangle. There are plentiful longitudinal grains, microscopic holes at the surfaces of horn cores, while the upper surface is rougher and bear some over-hanging bony tubercles.at the base of hore core. The superior face of horn core is almost in the same plane as the *facies frontalis*. The distance between bases of the horn-cores near the parietal diminishes distinctly. The angle between the horn cores and the sagittal plan is about 60° and extends moderately backward and curves gently inward forming an extensive "V" shaped crescent on the plane of the superior border of the frontal.

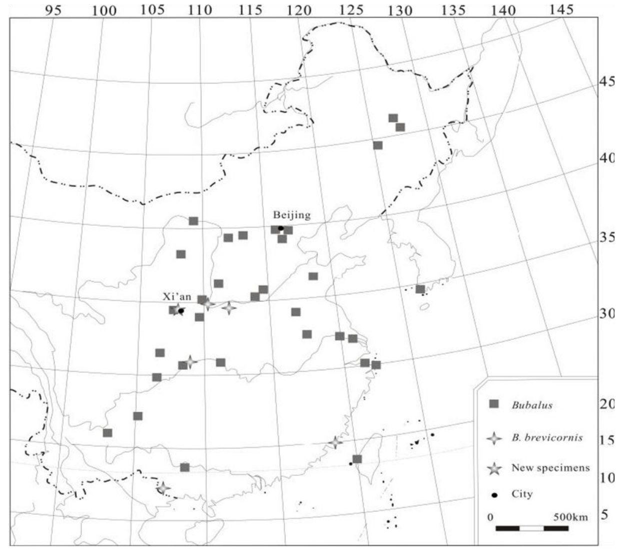

Fig. 1: Geographic distribution of fossil *Bubalus* from China (Updated from Dong *et al*., 2014). Square represent *Bubalus* sites that have been discovered, Shuriken represent *B. brevicornis* sites, pentacle represents specimen site. Dots represent cities

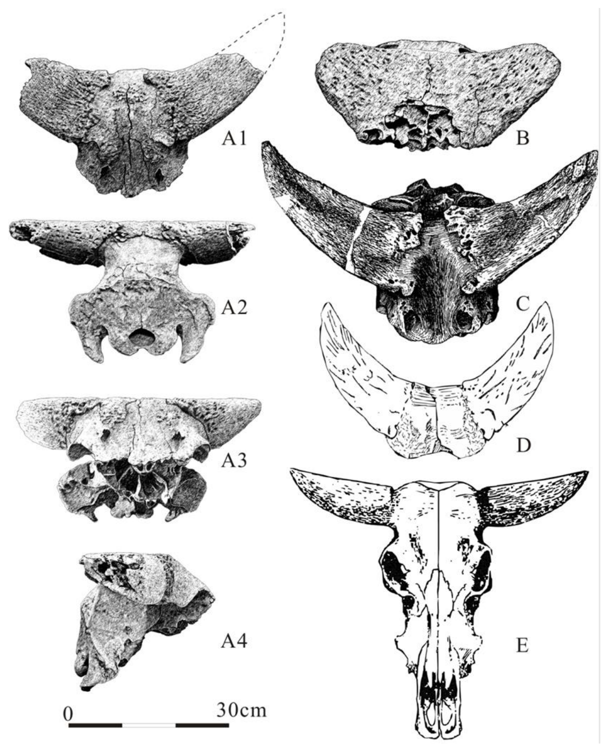

Fig. 2: Bubalus fossils. A1, dorsal views (NWUV1403.1). A2, occipital views. A3, frontal views. A4, lateral views. B, dorsal views (NWUV1403.2). C, dorsal views (holotype species of *B. brevicornis*). D, dorsal views (holotype species of *B. mephistopheles*). E, dorsal views (Modern water buffalo). Scale bar equals 30 cm

Comparison and Measurement

Compared to other *Bubalus* spp, the skulls from the Weihe are distinctly smaller than *B. teilhardi* and *B. youngi* and very close to the Pleistocene *B. brevicornis*, Holocene *B. mephistopheles* and the modern water buffalo of Asia.

In *B. brevicornis* (Fig. 2C) the core at the base is a medium stout horn that extends backward and the tip is gently curve inward. The horn of *B. mephistopheles* is short, directed upward, while the horn of modern buffalos is fine, extend to the back and downward (Fig. 2D). The horn of the new specimens from Weihe are medium shout, extend slight outward and backward (Fig. 2A, B). In top view, both horn cores form a broad crescent in the new specimens from Weihe and the *B. brevicornis* (Fig. 2A1, B and C), while the crescent in *B. mephistopheles* is obviously narrower (Fig. 2D).

Comparing the cross-section of the horn-core, *B. brevicornis*'s is shaped like an isosceles triangle the trihedral shape gradually lost outward; *B. mephistopheles*'s is almost an isosceles triangle but the trihedral shape slowly rounds to the tip; the modern water buffalo's is a flat triangular shape; for the new specimens from Weihe it is an arc-isosceles triangle.

For the uplift of the frontal between bases of horn-cores the frontal of *B brevicornis* is slightly uplifted back of the base of the horn-cores the area between the base of the horn-cores and the supraorbital foramina has an obvious dent. The frontal between the base of *B. mephistopheles*'s horn-core is slightly concave and between two orbits is distinctly sunken. The frontal between modern buffalos's horn cores is clearly uplifted, while the area between supraorbital foramina is gently hollow. Similarly, the frontal at the back of the base of the horn cores in the new specimens from Weihe is uplifted and the area between of horn-core and supraorbital foramina is indented, which is analogous to *B brevicornis*'s, only the degree of sunkenness is relatively weak.

The occipital wall of *B. mephistopheles* is wide and strong, projects backward and the occipital crista is developed. The occipital region of modern buffalo does not project backward with weak developed occipital crista. The occipital region of *B. brevicornis* projects backward, the occipital crista is moderately developed. The characteristics of new specimens from Weihe are close to *B. brevicornis*'s, the occipital region is projected backward and the occipital crista is moderately developed (Fig. 2A4).

Distinct differences can be observed in measured characteristics of different *Bubalus* species (Table 2). The length of the horn core along the convex border of modern water buffalo is 290–300 mm; for *B. mephistopheles* is 282 mm; *B. brevicornis* is 412 mm and the new specimen from Weihe is 311 mm. For the breadth of the upper surface of the horn core at the base, the modern water buffalo's is 86–96 mm; *B. mephistopheles*'s is 108–110 mm; *B. brevicornis*'s is 162 mm and the new specimen's from Weihe is 116–125 mm. And for the width between bases of horn-cores, the width is the shortest in *B. brevicornis* (the anterior edge is 93 mm and the posterior edge is 42 mm), while the modern water buffalo's is the largest (anterior edge is 175–207 mm, posterior edge is 140–157 mm). *B. mephistopheles*'s anterior edge is 145 mm, posterior edge is 84–93 mm; The anterior edge of the new specimen from Weihe is 133 mm, the posterior edge is 60–80 mm. It is clear that the characteristics of measurements of the new specimens from Weihe are closest to those of *B. brevicornis*.

Comparing the shapes of horn cores, features of horn base, width between base of the horn-core, forms of cross section and measurements described above the new specimens from Weihe are closer to *B. brevicornis* than to either *B. mephistopheles*, modern *B. bubalus*, *B. teilhardi* or *B. youngi*.

Although the new specimens are similar to *B. brevicornis* in morphology and other features, it is not exactly the same. They have some remarkable differences. The new specimen have a wide distance between bases of the horn cores, a relatively weakly developed bony tubercles in the area between bases of the horn cores, a close distance between supraorbital foramina and a weakly sunken area on the frontal. Considering the differences of the morphology, geography and age the new specimens from the Weihe area should be classified as a new subspecies, *B. brevicornis guanzhongensis*.

Discussion

Water buffalo fossils are reported from the early Pleistocene to Holocene in China. Ten species of water buffalos failed to survive in the Holocene epoch except for *B. mephistopheles*r, which means that there were two species of *Bubalus* in China, *B. mephistopheles* and *B. bubalus* (modern buffalos) were living in Holocene epoch (Xue and Li, 2000; Liu *et al*., 2006; Hu *et al*., 2007; Tong *et al*., 2015). *Bubalus brevicornis* was named by Young (1936) in Mianchi, Henan. After that, some fragments of horn cores were found in Yanjinggou, Sichuan and Changlu, Shandong, respectively (Young, 1936; 1939). There, however, were few related reports since then.

There are two reports about *B. brevicornis* in recent years. One by Fan and Zheng (2006) simply described some materials including a short and stout horn core, two pieces of broken horn cores, a broken jaw and a single tooth from Shishi waters, Fujian Province and classified them as the *B. brevicornis*. However, there was lack of detailed data on the horn-core of the incomplete specimens and most of them were collected from fishermen. Their locations and stratigraphic positions were unclear. The age was inferred during the late stage of the Late Pleistocene epoch. Furthermore, the upper surface at the base of horn cores is almost vertical to the anterior surface, which is incompatible with the isosceles triangle cross section of *B. brevicornis*. So, we suggest that these specimens should be classified as *B. bubalus*.

Another report by Dong *et al*. (2014), classified a new subspecies named *B. brevicornis chowi*. But some scholars deem that the species should be classified as *B. tingi* rather than *B. brevicornis* because the angle between the extending long axis of the horn cores and the sagittal plane of the skull is only about 30°, which happens to be consistent with *B. tingi*. It is a species with the smallest angle of *Bubalus* in China (Dong *et al*., 2014; Tong *et al*., 2015). In addition, the *B. brevicornis chowi* and *B. tingi* have other very similar features. Their horn-cores are slender than other species. So, we think that *B. brevicornis chowi* should be classified as *B. tingi* rather than *B. brevicornis*.

Based on the morphology comparison above, we deal with them using clustering analysis deeply. It is easy to distinguish the massive *Bubalus,* such as *B. youngi* and *B. teilhardi* from the relatively smaller *Bubalus,* such as *B. brevicornis*, *B. mephistopheles* and modern buffalos. But it is hard to reflect the similarity of the species each other in the smaller *Bubalus*. While the reported fossils are rare, the preserved fossils are different and the typical characters of all remaining fossils are difficult to compare with each other. Therefore, we choose the key morphological data from *B. brevicornis*, *B. mephistopheles*, modern buffalos (*B. bubalus*), *B. teilhardi* and our new specimen to conduct statistical cluster analysis (Tables 2–3; Fig. 3).

We referenced published metrical data of modern buffalos from two scholars at the same time (Table 2), because there is not always a complete agreement about the division of modern buffalos species which include *B. bulalis*, *B. arnee*, *B. depressicornis*, *B. mindorensis*, *B. quarlesi* etc. (Groves and Grubb, 2011) and it probably have some difference in data of the modern buffalos reported by different scholars (Xue and Li, 2000; Tong *et al*., 2015).

Modern buffalos (including two sets of data from different scholars) have greater differences than the extinct fossilized *Bubalus*. At the same time, they still approximate to each other most closely, although modern biologists have no consensus on division about the modern water buffalos and different scholars show the different measurement data from modern buffalos. They distinctly differ from other fossil records of *Bubalus* species. In all of the *Bubalus* spp, the massive and typical *B. teilhardi* is distinct from other small buffalos, like *B. brevicornis*, *B. mephistopheles* and modern buffalos (*B. bubalus*). The new specimens from Weihe and *B. brevicornis* were well placed under one class (Fig. 3).

The distance result of Euclidean distance matrix gives an index of similarity between water buffalos: lower numbers represent the higher similarity between two buffalos. The new specimen from Weihe is 0.656 distance from the *Bubalus brevicornis*; is 0.813 distance from *B. mephistopheles*; is 0.988~1.128 distance from the modern buffalos (*B. bubalus*); and is 1.111 distance from the *B. teilhardi*. The result shows that the new specimens from Weihe were most similar to the *B. brevicornis* named by Young (1936) and dissimilar from *B. teilhardi,* which were completely consistent with the morphological analysis results. Thereby, we believe that the new Holocene specimens (about 3959a–4050a BP) from Weihe area are of a species close to *B. brevicornis* and probably evolved from early *B. brevicornis*.

Table 3: The similarity index of approximate matrix from different *Bubalus*

Case	Euclidean distance					
	1*	2	3	4	5	6
1*	0.000	0.656	0.813	1.111	0.988	1.128
2	0.656	0.000	1.004	0.897	1.347	1.542
3	0.813	1.004	0.000	1.435	1.308	1.294
4	1.111	0.897	1.435	0.000	1.084	1.375
5	0.988	1.347	1.308	1.084	0.000	0.418
6	1.128	1.542	1.294	1.375	0.418	0.000

1. *B. brevicornis* from Weihe area; 2. *B. brevicornis* from Mianchi, Henan Province (Young, 1936); 3. *B. mephistopheles* from Chang-de-hu, Henan Province (Hopwood, 1925); 4. *B. teihardi* from Chouk'outien, Beijing (Young, 1932); 5. Modern Buffalo (*B. bubalus*) from Shaanxi (Xue and Li, 2000); 6. Modern Buffalo (*B. bubalus*) from Beijing (Tong, 2015)

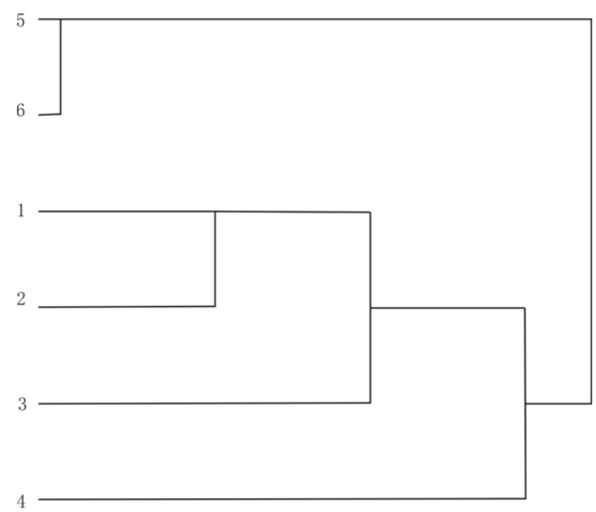

Fig. 3: Dendrogram of fossil *Bubalus*. 1. specimen; 2. *B. brevicornis* (Young, 1936); 3. *B. mephistopheles* (Hopwood, 1925); 4. *B. teilhardi* (Young, 1932); 5. Modern water buffalo (*B. bubalus*) (Xue and Li, 2000); 6. Modern water buffalo (Tong, 2015)

Conclusion

From fossil record we have classified the new specimens from Weihe as a new subspecies of *Bubalus* and named it as *B. brevicornis guanzhongensis*. The results of AMS^{14}C dating show that the ages of specimen are 3959~4050a BP. The results of this research suggest that *B. brevicornis* could have survived from Pleistocene into the Holocene.

There are two extinct *Bubalus* species (*B. brevicornis* and *B. mephistopheles*) during the Holocene in China, not just one (*B. mephistopheles*) as previously proposed.

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