Locomotive implication of a Pliocene three-toed horse skeleton from Tibet and its paleo-altimetry significance

Tao Deng^{a,b,1}, Qiang Li^a, Zhijie Jack Tseng^{c,d}, Gary T. Takeuchi^c, Yang Wang^e, Guangpu Xie^f, Shiqi Wang^a, Sukuan Hou^a, and Xiaoming Wang^{a,c}

^aKey Laboratory of Evolutionary Systematics of Vertebrates, Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing 100044, China; ^bDepartment of Geology, Northwest University, Xi'an, Shaanxi 710069, China; ^cDepartment of Vertebrate Paleontology, Natural History Museum of Los Angeles County, Los Angeles, CA 90007; ^dIntegrative and Evolutionary Biology Program, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089; ^eDepartment of Earth, Ocean, and Atmospheric Science, Florida State University and National High Magnetic Field Laboratory, Tallahassee, FL 32306; and [†]Department of Natural Science, Gansu Provincial Museum, Lanzhou, Gansu 730050, China

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The Tibetan Plateau is the youngest and highest plateau on Earth, and its elevation reaches one-third of the height of the troposphere, with profound dynamic and thermal effects on atmospheric circulation and climate. The uplift of the Tibetan Plateau was an important factor of global climate change during the late Cenozoic and strongly influenced the development of the Asian monsoon system. However, there have been heated debates about the history and process of Tibetan Plateau uplift, especially the paleo-altimetry in different geological ages. Here we report a wellpreserved skeleton of a 4.6 million-y-old three-toed horse (Hipparion zandaense) from the Zanda Basin, southwestern Tibet. Morphological features indicate that H. zandaense was a cursorial horse that lived in alpine steppe habitats. Because this open landscape would be situated above the timberline on the steep southern margin of the Tibetan Plateau, the elevation of the Zanda Basin at 4.6 Ma was estimated to be ~4,000 m above sea level using an adjustment to the paleo-temperature in the middle Pliocene, as well as comparison with modern vegetation vertical zones. Thus, we conclude that the southwestern Tibetan Plateau achieved the present-day elevation in the mid-Pliocene.

vertebrate paleontology | paleoecology | stable isotope | tectonics

F ossils of the three-toed horse genus *Hipparion* that have been found on the Tibetan Plateau have provided concrete evidence for studying the uplift of the plateau (1–3), including a skull with associated mandible of *Hipparion zandaense* within the subgenus *Plesiohipparion* from Zanda (Fig. 1 and Fig. S1). In August 2009 a *Hipparion* skeleton (Fig. 2) was excavated from Zanda Basin, with IVPP (Institute of Vertebrate Paleontology and Paleoan-thropology, Chinese Academy of Sciences) catalog number V 18189 (see Table S1 for a composite list of vertebrate taxa from the Zanda strata). Its dental morphology confirmed its assignment to *H. zandaense*, and its postcranial morphology is very similar to another species of the *Plesiohipparion* group, *Hipparion houfennese* (see *SI Text* and Figs. S2–S5). Paleomagnetic dating showed that the Zanda Formation was deposited 6.15–3.4 Ma, in which the fossiliferous bed-bearing *H. zandaense* has an age of about 4.6 Ma, corresponding to the middle Pliocene (*SI Text*).

Geological Setting

The Zanda Basin is a late-Cenozoic sedimentary basin located just north of the high Himalayan ridge crest in the west-central part of the orogen (32° N, 80° E). The Sutlej River has incised through to the basement, exposing the entire basin fill in a spectacular series of canyons and cliffs (4). The Zanda Basin stretches in a NW-SE direction, and is 150 km long and 20–50 km wide. The almost horizontal strata of the Zanda Basin, superposed on Jurassic and Cretaceous shale and limestone, consist of weakly consolidated clastic rocks of up to 800 m in thickness (5). A single unit, the Zanda Formation, is used for the entire Neogene sequence in this basin (*SI Text*). The *Hipparion* skeleton was discovered in the eastern bank (Fig. 1) of the main wash of Daba Canyon west of the Zanda county seat and south of the Sutlej River.

Description

Because both morphology and attachment impressions on fossilized bones can reflect muscular and ligamentous situations, they can provide evidence for the type of locomotion that extinct animals use when they lived. The skeleton of *H. zandaense* preserved all limb bones, pelvis, and partial vertebrae (Fig. 2), which provide an opportunity to reconstruct its locomotive function.

A greatly hypertrophied medial trochlear ridge (MTR, black arrows in Fig. 2, c1-c3) of the femur serves to "snag" the medial patellar ligament, or parapatellar cartilage, and the patella when the knee joint is hyperextended (6), forming a passive stay-apparatus or "locking" to reduce muscular activity in the knee extensors during long periods of standing. The well-developed MTR is an indicator of the presence of this locking mechanism (7). The femur MTR of H. zandaense is greatly enlarged relative to the lateral trochlear ridge (Fig. 2, c2). Like modern horses (Fig. 2, c3), which may stand erect for over 20 h a day, even in their sleep (8), H. zandaense could remain on its feet for long periods of time without fatigue. The femur MTR in Hipparion primigenium (Fig. 2, c1) is obviously smaller than in H. zandaense. The ratio between the maximum depth of the MTR and the maximum length of the femur is 0.27 in H. primigenium (9), whereas the ratio is 0.3 in H. zandaense.

Gracile limb bones are a marker for cursorial ability, which is most clearly exhibited on metapodials of ungulates (10). The gracility of the metapodial shaft is represented by diminished breadth relative to its length. In Fig. 3, above the zero line are the comparatively larger measurements and below it are the smaller ones. The ratio between the maximum length and the minimum breadth indicates that *H. zandaense*, *Hipparion* sp. from Kirgiz Nur, Mongolia, and the extant Tibetan wild ass have relatively slender metapodials (measurement 3 is smaller or slightly larger than measurement 1), but the primitive *H. primigenium* and *Hipparion xizangense* have very robust metapodials (measurement 3 is obviously larger than measurement 1), and the subgenus *Proboscidipparion* (*Hipparion sinense* and *Hipparion pater*) and *H. houfenense* in the North China Plain also show increased robustness (Fig. 3).

During the evolution of increased cursoriality in horses, the posterior shifting of the lateral metapodials relative to the third

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¹To whom correspondence should be addressed. E-mail: dengtao@ivpp.ac.cn.

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Fig. 1. Location map showing the study site (C) of the Zanda Basin (B) in Tibet, China (A).

metapodial is not only an evolutionary change toward functional monodactyly, but also a better adaptation for running, usually accompanied by a deepening of the whole bone and an effacement of the distal supra-articular tuberosity. As a result, the width of the distal tuberosity appears reduced relative to the articular width (11). The width of the distal tuberosity of the metapodials is much smaller than the width of the distal articulation in *H. zandaense*, whereas the former is larger than the latter in *H. primigenium*

(Tables S2 and S3). The distal articulation of metapodials in *H. zandaense* is wider than those in *H. primigenium* and *H. xizangense* (Fig. 3, measurement 11 is comparatively larger), but the width of the distal tuberosity in *H. zandaense* is narrower than in the other two species (Fig. 3, measurement 10 is comparatively smaller).

The well-developed sagittal keel on the distal extremity of the metapodial is another character to enhance pendular movement of limb bones and an adaptation for running (12). The development of the keel is relative to the deepening of the distal lateral groove, and is accompanied with the thickening of the medial condyle. These changes diminish lateral mobility and create better conditions for anteroposterior movements (13). The sagittal groove of the first phalanx III contains the keel of the distal articulation of the metapodial to avoid dislocation and sprain of the joint in lateral orientation, especially during rapid turning (11). The ratio between the depth of the lateral groove and the thickness of the keel on the distal extremity of metacarpal III is 0.84 in H. zandaense, but 0.88 in H. primigenium, and the ratio between the dorsal length and the total length of the first phalanx III is 0.92 in H. zandaense, but 0.94 in H. primigenium, which reflects a stronger keel for H. zandaense, so it can better minimize the lateral movement of the foot articulation, thereby strengthening the anteroposterior movement more effectively.

In *Hipparion*, the increase in size of the oblique ligaments on the proximal and central phalanx may have allowed the central toe to stand more vertically, thus causing the side toes to be lifted from the ground and become nonfunctional in locomotion, allowing the animal to run faster by supporting the fetlock and by adding



Fig. 2. Hipparion zandaense skeleton (IVPP V 18189) and comparisons of forefeet (A), femora (C), and hind first Ph III (B). Reconstruction of skeleton showing preserved bones in dark gray. a1, Hipparion zandaense; a2, H. primigenium (9); b1, H. xizangense; b2, H. zandaense; b3, Equus caballus; c1, H. primigenium (9); c2, H. zandaense; c3. E. caballus.



Fig. 3. Ratio diagrams of metapodials of *H. zandaense* and other equids. *H.* sp.: *Hipparion* sp. from Kirgiz Nur, Mongolia. Measurement numbers: 1, maximal length; 3, minimal breadth; 4, depth of the shaft; 5, proximal articular breadth; 6, proximal articular depth; 10, distal maximal supra-articular breadth; 11, distal maximal articular breadth; 12, distal maximal depth of the keel; 13, distal minimal depth of the lateral condyle; 14, distal maximal depth of the medial condyle. The *y* axis is the logarithm (base 10) of ratios between the measurements of each species and the reference species (Asiatic wild ass *Equus hemionus onager*, zero line).

bounce (14). The V-scars of *H. xizangense* (Fig. 2, b1) (2) and *H. primigenium* (9) are less developed on both fore and hind phalanx III, whereas that of *H. zandaense* is much wider and flatter (Fig. 2, b2), more similar to *Equus* (Fig. 2, b3).

In hipparionine horses, each foot has three toes (digits II to IV); digits I and V are absent. The reduction of the side toes (digits II and IV) in horses is a marked evolutionary trend toward better running ability (15). *H. primigenium* has evolved to use the unique functional central toe (digit III) during running, but all three toes may be used during slow walking, the latter being the more deliberate movement of *H. primigenium* (9). The side toes of *H. zandaense* are more distinctly reduced. For example, the total length of the three fore phalanges II of *H. zandaense* is 67.4 mm, whereas that of *H. primigenium* is 78.8 mm. Digit III of *H. zandaense* is also slightly longer than that of *H. primigenium*, so the side toes of the former have a larger suspending extent (Fig. 24). This character indicates that the side toes of *H. zandaense* have completely lost locomotive function, a characteristic related to faster running.

If distal elements of a limb are lengthened relative to proximal ones, the whole limb will be lengthened, yet keep its center of mass situated proximally and reduce its inertia, which allows for a long, rapid stride; speed is the product of stride length and stride frequency (16). Lengths of distal elements of fore- and hindlimbs (i.e., metapodials and first phalanges), relative to proximal elements in *H. zandaense* are much longer than in *H. primigenium* (Fig. 4), which indicates the stronger running ability of the former. Both the advanced *H. houfenense* and *H. sinense* have these characteristics.

Discussion

The preceding analysis of locomotive function shows that *H. zandaense* had the ability to run fast and stand persistently, which is beneficial only on open habitats, because close forests would encumber running. Hipparionine horses are typical hypsodont ungulates, and the tooth crowns of the subgenus *Plesiohipparion* are especially high (17), which indicate that they are grass-grazing specialists (10). Because grazing is inefficient in terms of nutritional intake, a great amount of food is required to obtain adequate nutrients (18). Grazing horses spend a large portion of the



Fig. 4. Proportions of limb bones in H. zandaense and other equids.

day standing and eating in open habitats with mainly herbaceous plants, so that they can keep watch for potential predators. The well-developed MTR of the femur in *H. zandaense* is a fitting adaptation for this ecosystem (7). The vestigial side toes of *H. zandaense* also reflect its adaptation for open environments instead of forests. The running ability of *H. primigenium* is weaker and more suitable to slower movement in closed habitats (i.e., woodland or forest) (9, 11), and its locomotive function stands in contrast to the inferred ecosystem and behavior of *H. zandaense*. Other mid-Pliocene mammalian forms from Zanda also indicate an open landscape (19).

The Tibetan Plateau is the youngest and highest plateau on Earth, and its elevation reaches one-third of the height of the troposphere, with profound dynamic and thermal effects on atmospheric circulation and climate (20, 21). The uplift of the Tibetan Plateau was an important factor of global climate change during the late Cenozoic and strongly influenced the development of the Asian monsoon system (22, 23). However, there have been heated debates about the history and process of Tibetan Plateau uplift, especially the paleo-altimetry in different geological ages (24-27). The Tibetan Plateau has gradually risen since the Indian plate collided with the Eurasian plate at about 55 Ma. Regardless of the debates over the rising process and elevation of the plateau (26-28), there is no doubt that the Himalayas have appeared as a mountain range since the Miocene, with the appearance of vegetation vertical zones following thereafter (29). Open grasslands per se have no direct relationship to elevation, because they can have different elevations in different regions of the world, having a distribution near the sea level to the extreme high plateaus. Controlled by the subduction zone, on the other hand, the southern margin of the Tibetan Plateau has been high and steep to follow the uplift of this plateau so that the open landscape must be above the timberline in the vegetation vertical zones. Because the Zanda Basin is located on the south edge of the Tibetan Plateau, its vegetation ecosystem is tightly linked to the established vertical zones along the Himalayas. In the Zanda area, the modern timberline is at an elevation of 3,600 m between the closed forest and the open steppe (30). Our locomotive analysis indicates that H. zandaense was more suited to live in an open environment above the timberline, as opposed to a dense forest. The inference of high-elevation open habitat is supported by the carbon isotope data. The δ^{13} C values of tooth enamel from modern and fossil herbivores indicate that the mid-Pliocene horses, like modern wild Tibetan asses, fed on C_3 vegetation (Fig. S6A). Although carbon isotope analysis of fossil plant materials in the basin showed that C_4 grasses (warm climate grasses) were present in local ecosystems in the latest Miocene and Pliocene (4), our enamel δ^{13} C data show that C₄ grasses must have been a minor component of local ecosystems since the mid-Pliocene because they were insignificant in the diets of local herbivores. The pure C₃ diets

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indicate that grasses ingested by these animals were cool-season grasses commonly found in high-elevation ecosystems.

The mid-Pliocene global climate was significantly warmer than the Holocene, whereas crucial boundary conditions, such as the placement of continents, were about the same as today (31). Therefore, it was likely that temperature (32), instead of longitude and latitude, was the main factor in determining the timberline of the Himalayas in the Pliocene when global surface temperatures were between 2 °C and 3 °C warmer than present (33). Based on the marine record (34), the temperature of the mid-Pliocene was ~2.5 °C warmer than today, and consequently the elevation of the timberline in the Zanda area at 4.6 Ma was 400 m higher than the modern one of 3,600 m, assuming a temperature lapse rate of 0.6 °C/ 100 m applies to the past. This finding suggests that the Zanda Basin had achieved an elevation comparable to its present-day elevation by 4.6 Ma.

The material of *H. xizangense* from Biru, Tibet includes limb bones, especially distal elements, with an age of early Late Miocene at about 10 Ma (2). The metapodial proportions of *H. xizangense* are nearly identical to those of *H. primigenium* (Fig. 3), indicating their common locomotive function, which means that *H. xizangense* was a woodland-forest horse and lived in a habitat with a lower elevation. *Hipparion forstenae* from Gyirong, Tibet is represented by skulls and mandibles, but lacking limb bones, with an age of late Late Miocene at 7.0 Ma (1, 17). *H. forstenae* was widely distributed in Gansu and Shanxi provinces in eastern China with a lower elevation, so this species would have lived in similar environments in Gyirong (27). Therefore, *Hipparion* fossils of different ages from three localities in Biru, Gyirong, and Zanda have been clear to reflect the progress and magnitude of the uplift of the Tibetan Plateau since the Late Miocene.

The limb bones of the Tibetan wild ass, which lives in the Tibetan Plateau today, are very close in proportion to *H. zandaense*, especially the gracility of their metapodials. Both of *Equus kiang* and *H. zandaense* are different from the open plain adapted *H. houfenense*, and more distinct from the forest adapted *H. primigenium* and *H. xizangense* (Figs. 3 and 4). Judged from this situation, *H. zandaense* and *E. kiang* took a convergent evolutionary path in morphological function, having both lived in the same plateau environment. These shared features further support our conclusion that the paleo-environment and paleo-elevation estimations for *H. zandaense* are reasonable.

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Supporting Information

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SI Text

Geologic Setting of Zanda Basin. Zanda Basin is a late Cenozoic basin (Fig. S1A) located just north of the high Himalayan ridgecrest in the west-central part of the orogen (\sim 32° N, 80° E; elevation 3,700–4,500 m). The basin fill consists of ~800 m of fluvial, lacustrine, eolian, and alluvial fan deposits (1).

Since the initial establishment of the Zanda Formation as a lithological unit (2), additional formation (such as the Tuolin and Xiangze formations) or even group names (Zanda Group) were proposed (3), often based on a perceived depositional hiatus that later proved to be false (1, 4). Here we use a single unit name, Zanda Formation, for the entire basin sequence.

The fossils were collected from the eastern bank of the main wash of Daba Canyon in the Zanda Basin (ZD0918. GPS: $31^{\circ}25'$ 27.9''N, 79°45'31.1'' E; elevation: 3,937 m above sea level) (Fig. S1A) in a grayish-green and brownish-yellow lacustrine sandstone of the lower part of the Zanda Formation (Fig. S1B). A skeleton [Institute of Vertebrate Paleontology and Paleoanthropology (IVPP) V 18189] of a single individual of *Hipparion zandaense* with only the limbs, broken pelvis, and lumbar, sacral, and some caudal vertebrae in situ was collected in an area of 1.5 m × 1 m (Fig. S1B). The skull, vertebrae, and ribs had weathered out and were found as fragments down slope. The crano-caudal trend of the *Hipparion* skeleton is northeast-southwest. No other associated mammalian fossils were found at this locality.

Magnetochronology and Biochronology. There have been no fewer than four independent attempts at paleomagnetic age determination of the Zanda strata during the past 12 y, although only three of these provided enough documentation to be evaluated here (1, 4, 5). All three studies measured an 800+ m section for the total thickness of Zanda sediments, and arrived at roughly similar magnetic reversal patterns. On the other hand, none of the fossils in these paleomagnetic sections are well preserved or well studied enough to offer real constraints within their proposed age range of late Miocene through Pliocene.

Our own mammalian faunas are unique in offering the most restrictive age constraints so far known (6). Critically, in the lower part of the sequence, we have recovered a small mammal assemblage (IVPP localities ZD0609 and 0904) that falls in the 174- to 186-m level of the south Zanda section (1) within the top part of an alternating greenish sandstone and silt unit and just below the fine-grained lacustrine mudstone with fine laminations. The most age-diagnostic element of this small mammal assemblage is Mimomys, which is most comparable in level of crown heights to Mimomys (Aratomys) bilikeensis from the Early Pliocene Bilike locality of Inner Mongolia, which is the earliest representative of arvicoline rodents in China (7). The appearance of these rodents thus is a highly age-diagnostic event throughout northern continents. IVPP localities ZD0609 and 0904 fall in a relatively long normal chron that we interpret to be C3n.4n (i.e., 4.997–5.235 Ma in ATNTS2004) (8) (Fig. S1C).

Large carnivorans, such as *Chasmaporthetes* (IVPP locality ZD0908), *Vulpes* (IVPP locality ZD1001), *Nyctereutes* (IVPP locality ZD0624), and *Meles* (IVPP locality ZD1001), offer additional chronologic constraints, even though most of these are stratigraphically 30 m to more than 200 m higher than the *Mimomys* horizon. The Asiatic first occurrences of these genera are mostly confined to the Pliocene, although occasional Late Miocene records have been suggested elsewhere (9–12). Collectively, they have a distinctly Pliocene characteristic.

Overall, the fossiliferous middle Zanda sequence yields characteristic Pliocene faunas (Table S1), although the upper alluvial conglomerates and lower fluvial sandstones, from which few vertebrate fossils are found thus far, range into the Pleistocene and Miocene, respectively. Based on our paleontologic constraints, we reinterpreted previously published paleomagnetic columns from various parts of the basin, and our new age estimates (Fig. S1*C*) of the Zanda section spans ~400 ka to 6.1 Ma in GPTS of ATNTS2004 (8). Our alternative interpretation is closest (but not identical) to those proposed by Qian (5) and takes into account the fast deposition in the upper conglomerates and lower fluvial sandstones.

ZD0918 is approximately at 267 m level of leg 6 in the South Zanda section (1), and it falls near the lower end of a magnetically normal interval correlated to Chron C3n.2n (4.493–4.631 Ma, ATNTS2004). Our correlation yields an age of 4.6 Ma for ZD0918.

Description and Comparison of the Limb Bones of *Hipparion zandaense* (Figs. S2–S5; Tables S2 and S3). The postcranial bones of *H. zandaense* are close to those of *Hipparion houfenense* within the same subgenus, *Plesiohipparion* in morphology, but smaller than the latter in size (Fig. 3, Figs. S2–S5, and Tables S2 and S3) (13–18). *H. houfenense* is a typical representative in the North China Plain during the Pliocene. The forelimb of a mammal consists of the humerus, radius and ulna, carpals, metacarpals, and phalanges, and the hindlimb consists of the femur, tibia and fibula, tarsals, metatarsals, and phalanges. The representative characters of the main elements of the fore- and hindlimbs of *H. zandaense* (IVPP V 18189) are described in the following sections.

Humerus. A groove is indistinct below the characteristic lateral epicondyle crest in Hipparion, which is oblique postero-inferiorly. The lower part of the lateral epicondyle (i.e., the part behind the trochlea and the lateral ligament fossa) forms a narrow concave surface, the posterior margin of which forms a distinct concave line. The latero-superior side of the coronoid fossa is surrounded by a very prominent crest that is nearly parallel to the upper margin of the trochlear articulate surface and makes this part of the coronoid fossa relatively narrow (Fig. S2, 1d). The above characters are identical with those of H. houfenense (15). The difference between the two species is that the lateral epicondyle crest of H. zandaense is enlarged downward and forms a scar, the margin of which is surrounded by interrupted ridges. Measurements: minimal breadth, 28.6 mm; diameter perpendicular to, and at the level of the minimal breadth, ~36 mm; maximal depth at the level of the median tubercule, 68.4 mm; distal maximal depth, 73.7 mm; maximal trochlear height, 46.7 mm; minimal trochlear height, 33.8mm; trochlear height at the sagittal crest, 40.4 mm.

Radius. On the proximal articular surface, the lateral diameter is much smaller than the medial one, and the synovial fossa at the posterior part of the sagittal ridge is relatively large (Fig. S2, 2e). The radial tuberosity on the proximal anterior surface is weak, lateral to which there is not a distinct concave surface (Fig. S2, 2a); the proximal medial tuberosity does not exceed the medial margin of the elbow articular surface; the upper margin of the medial rough surface for ligament attachment is much lower than the round opening of the radius-ulna interosseous space (Fig. S2, 2c); the posterior margin of the proximal articular surface is straight, the latero-posterior corner of which lacks a clear sharp prominent angle posteriorly (Fig. S2, 2e); on the distal anterior surface there are two distinct ridges and three grooves, among them the lateral ridge is located at the boundary between the distal facets for the

scaphoid and lunate (Fig. S2, 2a). These characters are the same as those of *H. houfenense*. Measurements: maximal length, 281.2 mm; medial length, 272.2 mm; minimal breadth, 36 mm; depth of diaphysis at level of the minimal breadth, 23.5 mm; proximal articular breadth, 64.8 mm; proximal articular depth, 33.8 mm; proximal maximal breadth, 68 mm; distal articular breadth, 53.1 mm; distal articular depth, 31 mm; distal maximal breadth, 63 mm; breadth of the radial condyle, 22.4 mm; breadth of the ulnar condyle, 13.6 mm.

Ulna. The medial end of the proximal facet for the radius distinctly exceeds the posterior end of the sagittal ridge, and the ulna shaft does not reach the lateral side of the radius shaft; the proximal lateral tuberosity is well-developed, and exceeds the lateral border of the elbow articular surface (Fig. S2, 2b); the medial ridge-like rough surface is weak, which is located on the lower one-third of the volar surface and attaches the superficial digital flexor muscle (Fig. S2, 2c). These characters are different from those of H. houfenense, in which the ulna is placed more laterally relative to the radius; the medial end of the facet for the radius slightly exceeds the posterior end of the sagittal crest; the shaft exceeds the lateral side of the radius shaft so that the radius looks more robust; the proximal lateral tuberosity is weak; the ridge-like rough surface on the volar surface attached by the superficial digital flexor muscle is better developed, lateral to which is a groove, and then a ridge that separates from the medial surface of the radius. Measurements: maximal length, 349.5 mm; length of the olecranon, 68.6 mm; maximal articular breadth, 39.9 mm; minimal depth of the olecranon, 43.5 mm; depth across the processus anconaeus, 59.5 mm.

Third metacarpal. On the proximal surface, the facet for the magnum has a highly rising sharp angle on its postero-medial corner, the posterior margin of which is approximately parallel to the volar surface of the shaft; the angle between facets for the unciform and the magnum on the proximal anterior margin is large, nearly 160°, identical with that of H. houfenense. The whole proximal articular surface is a sector in shape, but its posterior end is not constricted to a point, and its anterior and posterior parts face in approximately the same direction (Fig. S3, 1e). Above the lateral ligament fossa, the distal rough surface for the long ligament attachment is located anteriorly, at which the contact facets for the side phalanxes have a distinct bending (Fig. S3, 1d). On the distal anterior side, there is a smooth surface with a clear border above the articular surface, but the antero-superior end of the sagittal keel does not become low (Fig. S3, 1a). Measurements are in Table S2. Fore first phalanx III. The fore first phalanx III is very similar to that of H. houfenense, proportionally slender and flattened, and constricted in middle (Fig. S3, 2a and 2b). The proximal articular surface is wider than thick. The proximal rough surface extends posteriorly to a small degree (Fig. S3, 2e). The side depressions for ligamentous attachment are low, and the impressions for the attachment of the superficial digital flexor muscle are large (Fig. S3, 2c and 2d). Differently in H. zandaense, the proximal sagittal groove is deeper, which makes the central depression on the anterior margin more distinct (Fig. S3, 2a); the distal articular surface is more undulate (Fig. S3, 2f); the volar V-scar is rough and wide, at the basal center of which is a marked bulge. The bottom of the V-scar is rounded and extends to reach the middle level of the bone (Fig. S3, 2b). Measurements: maximal length, 67 mm; anterior length, 61.8 mm; minimal breadth, 27 mm; proximal breadth, 40.8 mm; proximal depth, 30.5 mm; distal breadth at the tuberosities, 34.4 mm; distal articular breadth, 34.8 mm; distal articular depth, 21 mm; minimal length of the V-scar, 26.8 mm; medial supratuberosital length, 53 mm; lateral supratuberosital length, 55.3 mm; medial infratuberosital length, 10.8 mm; lateral infratuberosital length, 10.9 mm.

Fore second phalanx III. The common character with *H. houfenense* is a weak sagittal ridge of the proximal articular surface (Fig. S3, 3e), and the different character is a relatively large distal and medial impression for ligamentous attachment in *H. zandaense*

(Fig. S3, 3c and 3d). Measurements: maximal length, 41 mm; anterior length, 32.7 mm; minimal breadth, 34.6 mm; proximal maximal breadth, 42.1 mm; proximal maximal depth, 25.9 mm; distal articular maximal breadth, 41.6 mm.

Fore third phalanx III. The dorsal slope is a low angle (34°) (Fig. S3, 4c and 4d), compared with a 45–50° angle in the extant *Equus.* The anterior margin has a central groove. The palmar process is weak, and the dorsal groove is faint (Fig. S3, 4e and 4f). The flexor surface of the volar surface is large and distinctly prominent (Fig. S3, 4f). The grooves for attachment of the collateral ligaments are deep (Fig. S3, 4c and 4d). The proximal articular surface is trapezoidal (Fig. S3, 4b). Measurements: length from the posterior edge of the articular surface to the tip of the phalanx, 57 mm; anterior length, 57.4 mm; maximal breadth, 69.7 mm; articular breadth, 42.7 mm; articular depth, 21.9 mm; maximal height, 43.2 mm; angle between the sole and the dorsal line, 34°; circumference of the sole, 180 mm.

Comparatively, the side toes (digits II and IV) of *H. zandaense* are too short to touch the ground during movement, so they cannot assist in distributing the animal's body weight and have completely lost locomotive function, which is related to faster running speeds.

Femur. The medial epicondyle is a strongly prominent ridge. There is not a clear ridge above the medial ridge of the distal trochlea, which is wide, and the proximal rough line for the medial vastus muscle is well-developed (Fig. S4, 1c). The transition from the trochlea to the intercondyloid fossa is very steep (Fig. S4, 1f). These characters are similar to those of Equus but different from most species of Hipparion. In Hipparion primigenium and Hipparion sinense, for example, the medial epicondyle has no a ridge, and the ridge above the medial ridge is clear (15, 18). The minor trochanter is well developed, with a wide and thick rough surface, and without a sharp ridge to connect to the femoral head (Fig. S4, 1a-1c). The position of the distal supracondyloid fossa is very low, the upper border of which is only 63.5 mm from the upper margin of the condyle (Fig. S4, 1b and 1d). The shaft inferior to the anterior part of the major trochanter is straight (Fig. S4, 1a). The connecting line between the medial condyle and the trochlea is low and flat (Fig. S4, 1c). The posterior part of the major trochanter is rotated medially (Fig. S4, 1b). The distal extensor fossa is a large and deep triangle (Fig. S4, 1d). These characters are much different from those of Equus, but identical with those of H. houfenense (15). The medial and lateral trochlear ridges are divergent craniodorsally, and the former has a sharp hook on its antero-dorsal aspect (Fig. S4, 1a), which are similar to those of Equus. The unique character of H. zandaense is a sharp and prominent upper end of the medial ridge of the distal trochlea, which is much higher than the bony surface (Fig. S4, 1a). Measurements: maximal length, 346.5 mm; length from caput femoris to lateral condyle, 320.3 mm; minimal breadth, 30.6 mm; diameter perpendicular to, and at the level of the minimal breadth, 42.4 mm; proximal maximal breadth, 101.1 mm; proximal maximal depth, 66.7 mm; distal maximal breadth, 80.5 mm; distal maximal depth, 104.3 mm; maximal breadth of the trochlea, 52.3 mm; maximal depth of caput femoris, 47.3 mm. *Tibia.* The tibia of *H. zandaense* is smaller than most specimens of H. houfenense, but identical with those of small individuals of the latter in size (15). Tibiae of the two species are very similar to each other in morphology, such as the lower part of the tibia crest produces a medial slant due to the attached area of the semitendinosus tendon (Fig. S4, 2a); the medial and lateral tubercles are widely separated from each other, with a distance of 14 mm (Fig. S4, 2b and 2e). The anterior depression for the cruciate ligamentous attachment is slightly larger than the posterior one, and both of them are shallow. The medial end of the ridge that separates the two depressions connects to the posterior end of the medial spine, and the lateral end connects to the middle part of the lateral spine (Fig. S4, 2e). The differences

between the two species include: the upper half of the proximal groove for the middle patellar ligament is rough and the lower half is smooth in H. zandaense (Fig. S4, 2a), whereas the rough surface for ligamentous attachment is indistinct in *H. houfenense*; the muscular sulcus is deep in *H. zandaense* (Fig. S4, 2e), but shallow in H. houfenense (15). The tuberosity for the posterior cruciate ligamentous attachment medial to the popliteal notch is lowly prominent (Fig. S4, 2e). Lateral to the distal lateral condyle, the lateral extensor tendon groove is deep and narrow, and the tuberosity behind the condyle is strong (Fig. S4, 2d). There is a wide and shallow medial flexor tendon groove on the posterior one-third of the medial surface of the medial condyle (Fig. S4, 2c). These two characters are similar to those of H. houfenense. The distal articular surface extends to an enlarged area to the medial condyle in H. zandaense (Fig. S4, 2f), which differs in H. houfenense. Measurements: maximal length, 333 mm; medial length, 323.4 mm; minimal breadth, 34.2 mm; minimal depth of the diaphysis, 33 mm; proximal maximal breadth, 87.2 mm; proximal maximal depth, 79.1 mm; distal maximal breadth, 62 mm; distal maximal depth, 41.3 mm; length of the fossa digitalis, 47.6 mm.

Astragalus. The trochlea is unsymmetrical, with a wider lateral ridge than the medial ridge; the lateral wall of the medial ridge is nearly vertical, and the medial wall has a wide wing-like surface that extends downward to reach above the distal deep fossa; the distal end of the medial ridge is weak, curved medially; the medial wall of the lateral ridge is moderately oblique, and the distal end is 10 mm apart from the distal articular surface (Fig. S5, 1a); the upper margin of the trochlear surface extends the most posteriorly at the central groove (Fig. S5, 1e). The distal articular surface is comparatively thick, with a transverse nonarticular impression on the central part of the lateral half and a rounded posterior angle (Fig. S5, 1f). These characters are identical with those of *H. houfenense*. The four facets for the calcaneum on the plantar surface (Fig. S5, 1b) are similar to those of H. houfenense in morphology. Both proximal and distal tuberosities on the medial surface are strong (Fig. S5, 1c), which are different from *H. houfenense* whose distal tuberosity is strong but proximal one weak (15). Measurements: maximal length, 53 mm; maximal diameter of the medial condyle, 54 mm; breadth of the trochlea, 25 mm; maximal breadth, 52 mm; distal articular breadth, 42.2 mm; distal articular depth, 33.5 mm; maximal medial depth, 44.3 mm.

Calcaneum. The calcaneum is elongated as a whole. The facets for the astragalus are corresponding to those on the planter surface of the astragalus in shape (Fig. S5, 2a and 2f). The lower part of the dorsal margin of the anterior process is oblique posteriorly, with a right angle to the distal articular surface in lateral view (Fig. S5, 2d). The plantar border of the bone is robust, but constricted in middle. The tarsal groove postero-superior to the sustentaculum tali is shallow (Fig. S5, 2b). The cochlear process is small (Fig. S5, 2c and 2d). The dorsal part of the medial surface of the sustentaculum tali lacks a clear vertical groove, which is similar to H. houfenense, but the medial surface is widely and shallowly concave in H. zandaense (Fig. S5, 2c), but flat in H. houfenense. The distal articular surface is narrowly mushroom-like and obviously constricted at middle in *H. zandaense* (Fig. S5, 2f), but straightly strip-like in H. houfenense (15). Measurements: maximal length, 103 mm; length of the proximal part, 62 mm; minimal breadth, 18.6 mm; proximal maximal breadth, 31 mm; proximal maximal depth, 46 mm; distal maximal breadth, 47 mm; distal maximal depth, 46.2 mm.

Third metatarsal. The medial facet for the first and second cuneiforms and the lateral facet for the cuboid have angles of 160° and 170°, respectively, to the middle facet for the third cuneiform at the proximal anterior margin, and two ridges separating the three facets are high (Fig. S5, 3e). On the plantar surface of the shaft, the side rough surfaces become close to each other at the upper half of the shaft, between which the concave surface does not extend to the lower one-third of the shaft. The lower end of the

lateral rough surface ends at the position where the artery groove turns medially (Fig. S5, 3b). These characters are similar to those of *H. houfenense*. The plantar margin of the facet for the third cuneiforms projects posteriorly as a circular arc, obviously separating from the small lateral facet for the metatarsal IV, the direction of the latter is nearly vertical (Fig. S5, 3e). The sagittal keel of the distal trochlea is distinctly prominent at the upper margin of the dorsal surface, above which is a large and deep depression (Fig. S5, 3a). These characters are different from those of *H. houfenense*. In *H. houfenense*, the plantar margin of the facet for the metatarsal IV, the latter faces latero-superiorly; the sagittal keel markedly becomes low at the upper margin of the dorsal surface, above which is a faint depression (15). Measurements are in Table S3.

Log ratio diagrams (Fig. 3) clearly show the differences in morphology and size between different horse species. The sagittal keel on the distal extremities of the metapodial is weak in *H. primigenium*. As a result, the lateral movement of the metapodial has not been completely diminished, and the lateral mobility is beneficial on uneven ground, such as a forested environment with trees (19). The metapodial of *H. primigenium* are more robust and shorter than those of *H. zandaense* (Fig. 3: comparative relationship between measurements 1 and 3, and Tables S2 and S3), and the distal elements of limb bones in the former is comparatively shorter than those in the latter (Fig. 4).

Hind first phalanx III. Most characters are similar to those of the fore first phalanx III, but its differences include: it is more robust and shorter (Fig. S5, 4a and 4b); the proximal medial tuberosity for ligament attachment is well developed (Fig. S5, 4e); the plantar rough V-scar lacks a basilar tubercle, but there is a marked tubercle on each side of its lower end (Fig. S5, 4b). Measurements: maximal length, 62.8 mm; anterior length, 59 mm; minimal breadth, 28 mm; proximal breadth, 41.2 mm; proximal depth, 33 mm; distal breadth at the tuberosities, 34 mm; distal articular breadth, 33 mm; distal articular depth, 21.1 mm; minimal length of the V-scar, 24 mm; medial supratuberosital length, 49.2 mm; lateral supratuberosital length, 12.7 mm.

Hind second phalax III. The distal width is smaller than the proximal width, so that the whole shape is a superiorly wide and inferiorly narrow trapezoid (Fig. S5, 5a and 5b). The distal ligament fossae face laterally and medially, respectively, and they are nearly rounded (Fig. S5, 5c and 5d). The sagittal ridge of the proximal articular surface is weak (Fig. S5, 5e), which is similar to *H. houfenense* (15). Measurements: maximal length, 40.4 mm; anterior length, 30.4 mm; minimal breadth, 33.9 mm; proximal maximal breadth, 42.1 mm; proximal maximal depth, 25.9 mm; distal articular maximal breadth, 36.7 mm.

Based on a reconstruction of the Zanda skeleton limb bones, *H. zandaense* is a large three-toed horse with a shoulder height of 1.45 m in life. This reconstructed body size is similar to the extant Przewalsky's horse (*Equus przewalskii*, 1.26–1.48 m) and the Tibetan wild ass (*Equus kiang*, 1.53–1.59 m), but larger than the earliest Eurasian *Hipparion* (*H. primigenium*, 1.36 m) (18).

Paleoenvironment Estimates Based on Stable Isotopes. Carbon and oxygen isotope ratios of fossil tooth enamel contain valuable information about the diet and water composition (20–23). We analyzed the carbon and oxygen isotopic compositions of 110 serial and bulk enamel samples from 25 teeth or tooth fragments from a diverse group of middle Pliocene mammals and 60 bulk and serial enamel samples from nine teeth from eight modern Tibetan wild asses (*E. kiang*) from Zanda Basin.

The δ^{13} C values of enamel samples from modern wild Tibetan asses from Zanda Basin are $-8.8 \pm 1.7\%$ (n = 60 enamel samples from eight individuals), which indicate a C₃-based diet and are consistent with the current dominance of C₃ vegetation in the area. The enamel- δ^{13} C values of fossil horses, rhinos and bovids for the time period of 3.1–4.0 Ma are –9.6 ± 0.8‰ (n = 110enamel samples from 25 teeth), indicating that these ancient herbivores, like modern Tibetan asses, fed primarily on C₃ vegetation and lived in an environment dominated by C₃ plants (Fig. S6A). Enamel samples from the mid-Pliocene large herbivores yielded δ^{18} O values that are generally lower than those of modern Tibetan asses (Fig. S6B), likely indicating a shift to more arid conditions in the basin after the mid-Pliocene.

Paleo-Altimetry Estimates Based on Locomotive Functions. Scott (24) has suggested very early in his studies of evolution that everything has been killed to speed in the horses, making the animal a "cursorial machine." The locomotive function of animals is tightly linked to their musculoskeletal systems. A clear picture of how animals move is essential to understanding many of their adaptations and life strategies (25). Our locomotive analysis and comparison of limb bones show that *H. zandaense* lived in an open alpine steppe habitat and spent a significant amount of time to graze, so it evolved to have an ability of fast running and long-time standing. The timberline is a boundary between two

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extremely distinct ecosystems of the closed forest and the open grassland in vegetation vertical zones, is sensitive to global and regional climate changes, and is regarded as a result of long-term climate patterns. The unique environmental conditions of temperature, heat, and moisture in alpine areas restrict tree growth, and form an upper limit for forests. Factors that determine the elevation of the timberline are numerous, including latitude, longitude, and climate, among which, temperature is the most decisive climatic factor to influence the timberline (26). The distribution of vegetation vertical zones is directly related to the atmospheric temperature, and elevation of the timberline increases as temperature ascends in the growing season (27). As a result, the open alpine steppe habitat, where H. zandaense lived, must indicate an exact elevational range in vertical zone classification. Because a temperature increase of 2.5 °C during the mid-Pliocene (28) would make the boundaries of the vegetation vertical zones about 400 m higher, and the present elevation of the fossil locality is close to 4,000 m, the timberline in the Zanda area, by extrapolation, would have been at a maximum elevation of 4,000 m at that time.

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Fig. S1. (*A*) Map showing important fossil localities and important geographic locations of the Zanda Basin in Ngari, Tibet, China, including the horse skeleton locality ZD0918. (*B*) Exposures of fluvial and lacustrine sediments of the Zanda Formation, where (ZD0918) the skeleton of *H. zandaense* (IVPP V18189) was excavated. (*C*) Correlation of three published paleomagnetic sections and stratigraphic positions of key Zanda fossil mammal localities. Red star indicates *Hipparion* skeleton locality (ZD0918) and red circle indicates key fossil sites (ZD0609, 0904) for biochronologic constraints. Ages for magnetic chrons in the Geomagnetic Polarity Time Scale (GPTS) are based on ATNTS2004 (8).



Fig. S2. Right humerus (1), and left radius and ulna (2) of *H. zandaense* from Zanda: a, anterior view; b, posterior view; c, medial view; d, lateral view; e, proximal view; f, distal view. The proximal part of the humerus is absent.



Fig. S3. Right metacarpal (Mc) III (1), and fore first (2), second (3), and third (4) Ph III of *H. zandaense* from Zanda: a, anterior view; b, posterior view; c, medial view; d, lateral view; e, proximal view; f, distal view.



Fig. 54. Left femur (1) and right tibia (2) of *H. zandaense* from Zanda: a, anterior view; b, posterior view; c, medial view; d, lateral view; e, proximal view; f, distal view.

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Fig. S5. Right astragalus (1), calcaneum (2), metatarpal (Mt) III (3), and hind first (4), and second (5) Ph III of *H. zandaense* from Zanda: a, anterior view; b, posterior view; c, medial view; d, lateral view; e, proximal view; f, distal view.



Fig. S6. (A) δ^{13} C values of bulk and serial enamel samples from herbivores; (B) the mean enamel δ^{18} O values of obligate drinkers in the Zanda Basin.

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Table S1. Composite list of vertebrate taxa from the Zanda strata

Vertibrate taxa	IVPP locality number				
Osteichthyes					
Cyprinidae					
Mammalia					
Insectivora					
Soricidae indet.	ZD0609, 1001				
Carnivora					
Nyctereutes cf. N. tingi	ZD0624				
Vulpes sp. nov.	ZD1001				
Panthera (Uncia) sp. nov.	ZD1001				
Meles sp. nov.	ZD1001, ZD1004				
Mustela sp.	ZD1001				
Chasmaporthetes sp.	ZD0908, 1029, 0636				
Perissodactyla					
Hipparion zandaense	ZD0701, 0918, and others				
Coelodonta thibetana	ZD0740				
Artiodactyla					
Cervavitus sp. nov.	ZD0624				
?Pseudois sp. nov.	ZD0712				
Antilospira/Spirocerus sp.	ZD0701, 1001				
Qurliqnoria sp.	ZD0604, 0745				
Bovidae gen. A	ZD1001				
Bovidae gen. B	ZD1001				
Proboscidea					
Gomphotheriidae indet.	ZD0746, 1015, 1033, 1036, 1046, 1048				
Rodentia					
Aepyosciurus sp.	ZD1001)				
Nannocricetus sp.	ZD0609, 1001				
Cricetidae gen. et sp. nov.	ZD1001				
Prosiphneus cf. P. eriksoni	ZD1001				
Mimomys (Aratomys) bilikeensis	ZD0609, 0904				
Apodemus sp.	ZD0609, 0904				
Lagomorpha					
Trischizolagus cf. T. mirificus	ZD0609, 0904				
Trischizolagus cf. T. dumitrescuae	ZD0726, 1001				
Ochotona sp. 1	ZD0609, 0904				
Ochotona sp. 2	ZD0609, 0902, 0904				
Ochotona sp. 3	ZD1001				
Ochotona sp. 4	ZD0726				

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Table S2.	Measurements and	l comparison	of Mc III between	Hipparion	zandaense an	d other	equid	species
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Measurement	<i>H. zan.</i> V 18189 (mm)	H. hou. n = 19–23 (mm)	H. sin. n = 15–17 (mm)	<i>H. pat.</i> n = 6–8 (mm)	<i>H. xiz.</i> n = 2 (mm)	<i>H. pri.</i> n = 10–16 (mm)	<i>H.</i> sp. n = 2 (mm)	<i>E. hem.</i> n = 14–16 (mm)	<i>E. kia.</i> n = 4 (mm)
1	225.5	249.8	274.5	234.3	213	212.8	219.5	212	242
2	217.3	_	_	_	210	207.4	213.5	206	234
3	26.3	32.8	32.9	29.1	30.5	31.7	26.3	25.9	27.8
4	22.5	27.2	29.3	24.1	23.4	22.5	23	21.1	23.4
5	41.3	49.1	50.6	43.4	~42	39.9	40.6	43.2	46.3
6	27.4	34.3	34.8	30.7	29.5	27.9	26.5	27.1	30.1
7	34.7	_	_	_	32	34.6	33.5	34.2	37.4
8	11.9	_	_	_	11.8	11.7	12.7	12.3	13.8
9	4.5		_	_		7.5		1.9	1.9
10	37.3	43.4	44.1	39.7	38	39.5	37.3	38.7	43.3
11	38.4	43.9	42.4	38.8	38.2	37.1	35.3	38.5	41.3
12	30	36.1	36.9	32.8	28.5	28.3	30	29.4	30.9
13	25.3	30	30.6	28.5	24.3	24.8	~25.2	~24.1	~25.8
14	27.7	~33.3	~34.1	~30.3	28	26.4	26.2	25.9	28.5
15	82	_	_	_	110.3	_	_	_	_
16	3.8	_	_	_	3.5	_	_	_	_

Abbreviations and sources: E. hem., Equus hemionus onager (14); and E. kia., Equus kiang (14); H. hou., Hipparion houfenense (15); H. pat., Hipparion pater (15); H. pri., Hipparion primigenium (18); H. sin., Hipparion sinense (15); H. xiz., Hipparion xizangense (13); H. zan., Hipparion zandaense; H. sp., Hipparion sp. from Khirgiz Nur, Mongolia. Measurements: 1, maximal length; 2, medial length; 3, minimal breadth; 4, depth of the diaphysis at level of 3; 5, proximal articular breadth; 6, proximal articular depth; 7, maximal diameter of the articular facet for the third carpal; 8, diameter of the anterior facet for the fourth carpal; 9, diameter of the articular facet for the second carpal; 10, distal maximal depth of the keel; 13, distal minimal depth of the fourth carpal (16).

Measurement	<i>H. zan.</i> V 18189 (mm)	H. hou. n = 18–24 (mm)	<i>H. sin.</i> n = 14–18 (mm)	H. pat. n = 8–9 (mm)	H. xiz. n = 2–4 (mm)	<i>H. pri.</i> n = 16–24 (mm)	<i>E. hem.</i> n = 14–16 (mm)	<i>E. kia.</i> n = 4 (mm)
1	253.2	273.8	320.3	266.1	247.7	242.5	247.5	279.3
2	248.4	_	_	_	242.6	237.2	242	274.5
3	25.6	31.7	33.8	27.3	30.4	31.4	25.1	26.6
4	28	31.5	34.7	28.3	29.4	28.6	25.3	27.3
5	39	47.8	50.7	42.1	42.1	41.8	40.5	44.1
6	36	37.7	40.2	34.1	37.5	34.3	35	40.4
7	37.1	_	_	_	38.7	39.5	36	39.6
8	7.9	_	_	_	9.9	9.9	8.7	10.5
9	8.6	_	_	_	7.5	6.5	6.2	5.6
10	38.1	43.8	46.7	39.2	39.3	39.7	38.2	42.5
11	38.5	42.9	42.5	37.8	37.9	37.8	37.4	40.5
12	30	35	38.5	31.7	32.6	30.7	30.1	32.4
13	24	~28.4	~30.8	~25.1	26.1	25.3	~23.7	~26.1
14	27.2	~31.7	~34.9	~28.7	30.1	27.3	26.2	28.9
15	90	_	_	_	100	_	_	_

Table S3. Measurements and comparison of Mt III between Hipparion zandaense and other equid species

Abbreviations and sources: E. hem., Equus hemionus onager (14); and E. kia., Equus kiang (14); H. hou., Hipparion houfenense (15); H. pat., Hipparion pater (15); H. pri., Hipparion primigenium (18); H. sin., Hipparion sinense (15); H. xiz., Hipparion xizangense (13); H. zan., Hipparion zandaense; H. sp., Hipparion sp. from Khirgiz Nur, Mongolia. Measurements: 1, maximal length; 2, medial length; 3, minimal breadth; 4, depth of the diaphysis at level of 3; 5, proximal articular breadth; 6, proximal articular depth; 7, maximal dismeter of the articular facet for the third tarsal; 8, diameter of the articular facet for the fourth tarsal; 9, diameter of the articular facet for the second tarsal; 10, distal maximal supra-articular breadth; 11, distal maximal articular breadth; 12, distal maximal depth of the lateral condyle; 14, distal maximal depth of the medial condyle; 15, angle measuring the dorso-volar development of the keel (16).