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

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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 well-preserved 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

Fossils 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 Paleoanthropology, 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 houfenense* (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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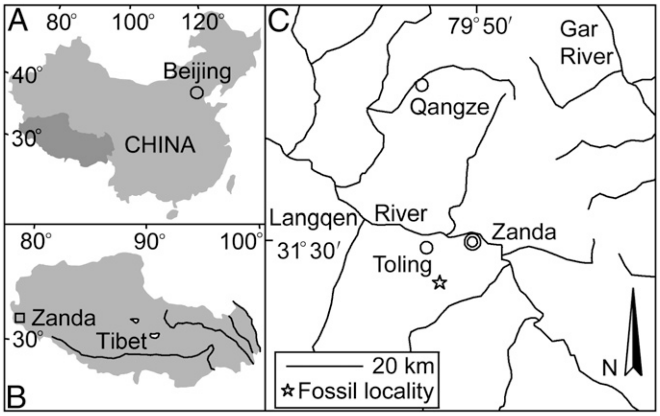

Fig. 1. Location map showing the study site (*C*) of the Zanda Basin (*B*) in Tibet, China (*A*).

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

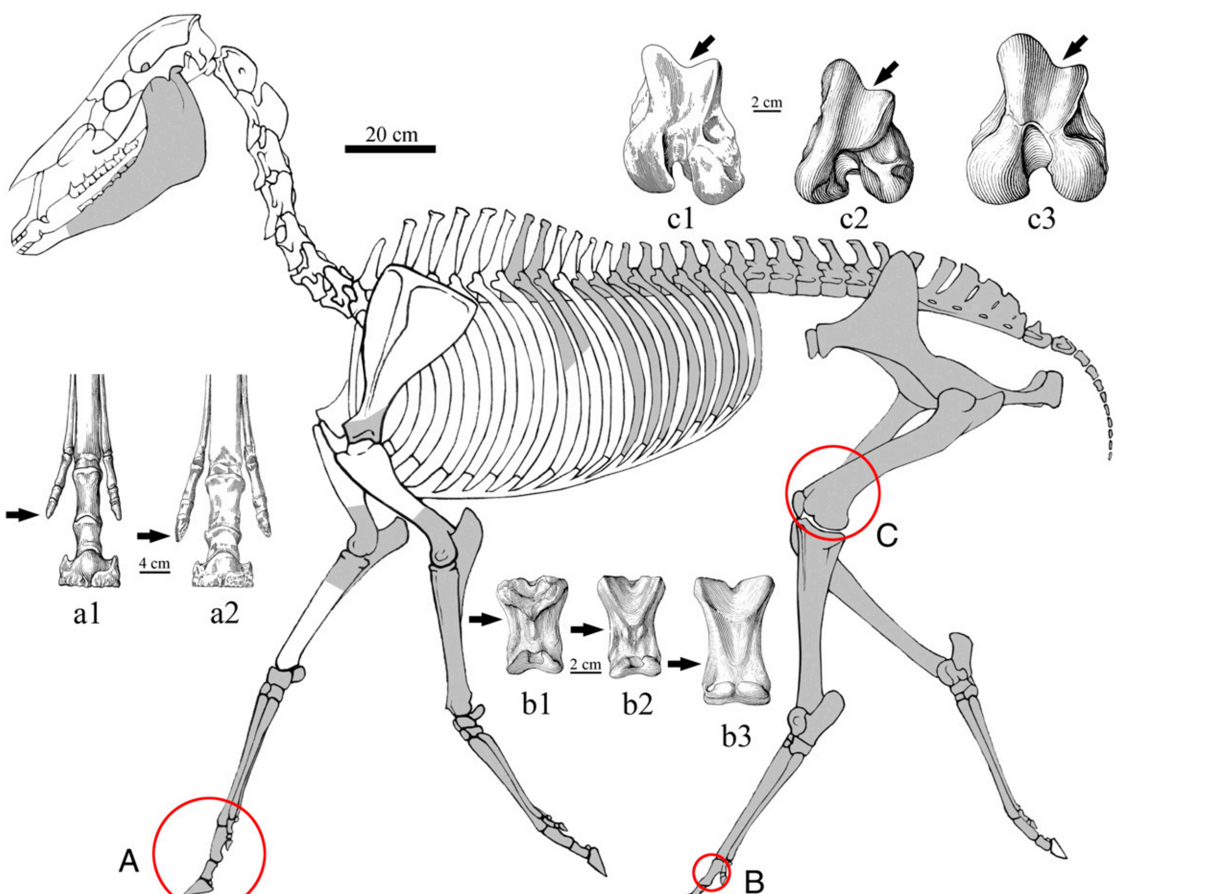

Fig. 2. *Hipparion zandaense* skeleton (IVPP V 18189) and comparisons of forefeet (*A*), femora (*C*), and hind first Ph III (*B*). Reconstruction of skeleton showing preserved bones in dark gray. a1, *Hipparion zandaense*; a2, *H. primigenium* (9); b1, *H. xizangense*; b2, *H. zandaense*; b3, *Equus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

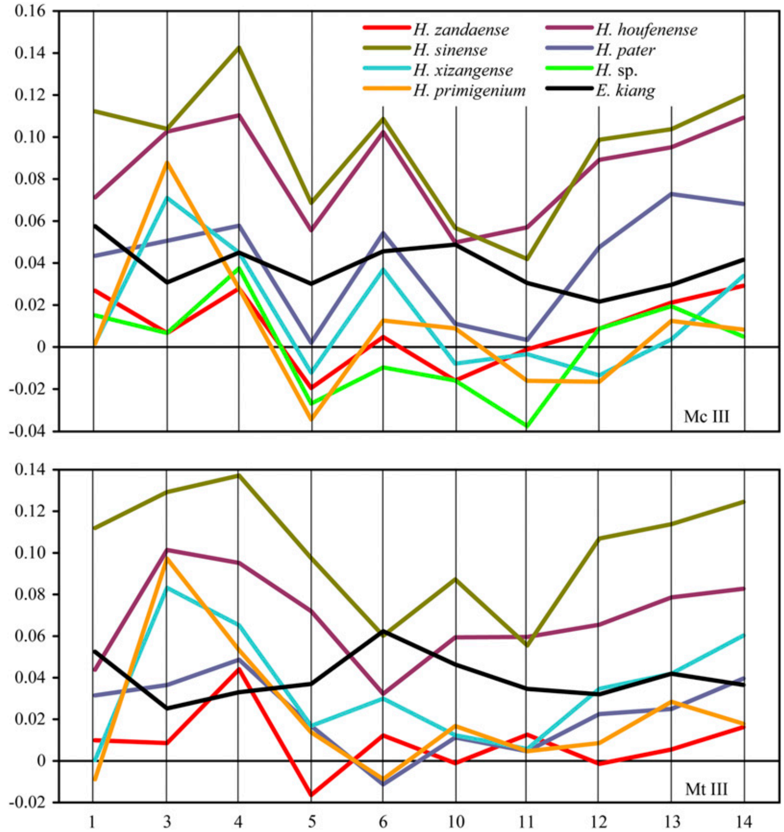

Fig. 3. Ratio diagrams of metapodials of *H. zandaense* and other equids. *H.* sp.: *Hipparion* sp. from Kirgiz Nur, Mongolia. Measurement numbers: 1, maximal length; 3, minimal breadth; 4, depth of the shaft; 5, proximal articular breadth; 6, proximal articular depth; 10, distal maximal supra-articular 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. 2*A*). 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

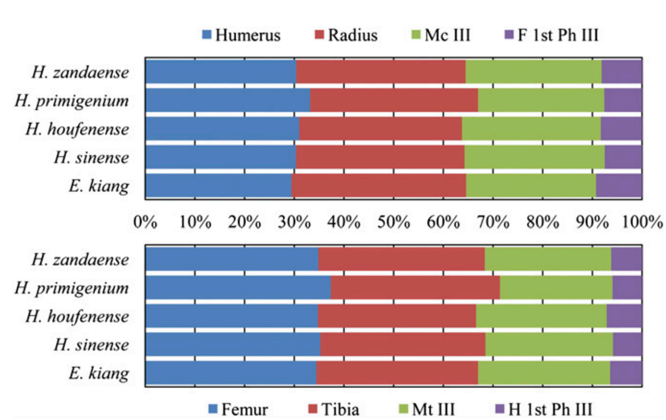

Fig. 4. Proportions of limb bones in *H. zandaense* and other equids.

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 $\delta^{13}C$ 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. S6*A*). 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 $\delta^{13}C$ data show that C_4 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 C_3 diets 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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