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The rise of herbaceous diversity at southeastern margin of the Tibetan Plateau: first insight from fossils

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Abstract: The Hengduan Mountains region (HMR) on southeastern Tibetan Plateau, supports a high diversity of herbs, particularly in its subalpine to alpine ecosystems due to high altitude and cool temperate climate. Current understandings on the formation of such herbaceous richness is based chiefly on molecular phylogenies, while direct geological evidence is lacking because herbs are rarely preserved as macroscopic fossils. In this study, we present abundant fossil fruits and seeds of herbs from the late Pliocene Heqing Basin in the southern HMR. Our systematic analysis shows the presence of at least 18 species belonging to 11 genera, i.e., Ranunculus, Corydalis, Rumex, Polygonum, Chenopodium, Stellaria, Fragaria, Astragalus, Aster, Carex and Schoenoplectus, of which Polygonum is most abundant followed by Astragalus. This finding throws the first light from fossil evidence on the rise of herbaceous diversity in the region. We interpret the local assembly of these herbs as resulting from rapid pre-Pliocene species diversifications of many herbaceous groups in HMR. As nowadays most of these herbs grow primarily in meadows and a few occur as subaquatic plants, we suggest an open meadow hosting some scattered shrubs in the vicinity of a vegetated wetland in the Heqing Basin during the late Pliocene. This provides the first direct evidence of past treeless open vegetation within the HMR and thus improves our knowledge of vegetation evolution in the region. We suggest that the uplift-induced climate cooling and monsoon-associated precipitation seasonality are potentially the key driving forces for the opening of meadow vegetation in the HMR.
Graphical Abstract

Fossil fruits and seeds of herbs from the late Pliocene Heqing Basin, southern Hengduan Mountains.


Keywords: fossil carpology; Hengduan Mountains; late Pliocene; monsoon; meadow; Tibetan Plateau
1. Introduction

The Hengduan Mountains region (HMR), a global biodiversity hotspot on southeastern edge of the Tibetan Plateau, has long been renowned for supporting an extremely high diversity of plant species, commonly associated with its complex relief and dynamic climate (Myers et al., 2000; Boufford, 2014; Sun et al., 2017; Yu et al., 2020). It is particularly rich in herbs that comprise subalpine to alpine meadows (Sherman et al., 2008; Liu et al., 2014; Ding et al., 2020). Many herbaceous groups, such as Delphinium L. (Ranunculaceae) (Jabbour & Renner, 2012), Saxifraga Tourn. ex Linn. (Saxifragaceae) (Gao et al., 2015; Zhang et al., 2019), and Primula L. (Primulaceae) (Ren et al., 2015), have their diversity centers in this region (Sun et al., 2017; Yu et al., 2020).

Current understandings on the formation of the exceptional herbaceous diversity in the HMR are derived mainly from the study of living species using molecular phylogeny (Chen et al., 2005; Jabbour & Renner, 2012; Fan et al., 2013; Nie et al., 2013; Gao et al., 2015; Ren et al., 2015; Luo et al., 2016; Hauenschild et al., 2017; Xing & Ree, 2017; Zhang et al., 2019; Ding et al., 2020). For examples, rapid species differentiation in Persicarieae (Polygonaceae) is considered to have begun at about 10 million years ago (Ma) (Fan et al., 2013), and species diversification in Saxifraga is thought to have accelerated after 9.48 Ma (Gao et al., 2015). It is generally accepted that the Miocene epoch is a crucial

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period for many herbs to acquire their modern diversity in the region, coincidental with a rise of environmental heterogeneity in both time and space, essentially orogenic activity, drainage incision, climate cooling and seasonality amplification (Xing & Ree, 2017; Ding et al., 2020; Spicer et al., 2020). In contrast to the broad molecule-based efforts, fossil evidence as an alternative proxy for understanding the evolution of herbaceous diversity in the HMR is lacking among its nevertheless rich fossil floras (Huang et al., 2016b). Although some pollen fossils of herbs have been recognized from the sediments, the shortcoming is that they are often identified at higher categories, e.g., family (Xu et al., 2004; Kou et al., 2006; Wu et al., 2019). So far, macroscopic fossils of herbs with better taxonomic resolution remain scarce (Huang et al., 2019, 2020), largely attributed to the fact that herbs possess limited recalcitrant tissues to be preserved in sediments through time. However, their fruits and seeds, if coated with a woody or leathery integument, may have a higher potential of preservation, especially when they are charred by wildfire given the chemical inertness of charcoals (Scott, 2010).

Recently, a fossil assemblage containing abundant fruits and seeds of herbs was recovered from the Upper Pliocene Sanying Formation of Heqing Basin in the southern HMR (Zhu et al., 2016; Huang et al., 2019). In this study, we identified these fossils based on morphological examinations and comparisons, evaluated the floristic importance of each element, and determined the general vegetation
character. Our study provides the first fossil-based insight into the rise of herbaceous diversity and the establishment of open meadows in the HMR.

2. Materials and method

2.1. Fossil site, sampling layer and geological horizon

The Heqing Basin is a sedimentary basin filled with Neogene and Quaternary deposits in the southern HMR (Fig. 1). At Nanbanbang Village on the western border of the basin (26°31’ N, 100°10’ E; 2200 m a.s.l.), there are discontinuous profiles exposed along a gentle mountain slope. Based on our field observations and previous considerations (Huang et al., 2016a, 2019; Zhu et al., 2016), these profiles belong to the Sanying Formation. The Sanying Formation has been broadly identified from several sedimentary basins in the southern HMR (Ge & Li, 1999; Li et al., 2013; Zheng et al., 2014). It has a lower age limit of late Miocene and extends to the early Pleistocene, but its time span differs from one basin to another due to independent sedimentations (Ge & Li, 1999; Li et al., 2013; Zheng et al., 2014). In the Heqing Basin, the Sanying Formation, with a total thickness of 300 m, is dominated by unconsolidated mudstone, siltstone and sandstone (Zhu et al., 2016; Huang et al., 2019). It lies unconformably over the Eocene Lijiang Formation and is overlain by Pleistocene deposits (Zhu et al., 2016; Huang et al., 2019). In its middle to lower part, there is a thick layer of mudstone that has yielded a leaf fossil assemblage dominated by *Quercus* sect.
*Heterobalanus*, a group of sclerophyllous evergreen alpine oaks (Huang et al., 2016a). Existing fossil archives show that *Quercus* sect. *Heterobalanus* occurred widely as a dominant community within Pliocene strata of the Sanying Formation (Tao & Kong, 1973; Tao, 1986; Zhou et al., 2007; Su et al., 2015; Huang et al., 2016b) as well as those of the Yangyi Formation in Baoshan Basin to the south (Xiao et al., 2006; Li et al., 2009; He et al., 2014). This biostratigraphic feature can therefore be used to symbolize the Pliocene phase among Neogene basins in the HMR (Huang et al., 2020). In the upper part of the formation, there is a two-meter-thick succession of finely layered siltstone, suggesting lacustrine lithological facies. It therefore likely represents late Pliocene sedimentation (Zhu et al., 2016; Huang et al., 2019). The siltstone is carbonaceous, containing abundant plant materials that include both charred and uncharred organs.

### 2.2. Fossil preparation, examination and identification

Fresh sediment approximating 50 liters in volume was previously quarried from the siltstone layer at the fossil site. The sediment was immersed in water until it became a slurry, which was then filtered successively through sieves of 4 mm, 1 mm and 0.5 mm to exclude small clay particles. Residues on the 4 mm sieve were checked with unaided eyes, while those on the 1 mm and 0.5 mm sieves were checked with the aid of a Leica binocular microscope (S8APO). Intact and fragmentary fruits and seeds, as well as many other plant organs such as...
conifer needles and shoots, were isolated from the mixture. They were gently cleaned using an ultrasonic bath (GT-1620QTS) to remove clay particles adhering on their surface. Air dried, the fossil fruits and seeds were observed once more with the Leica binocular microscope, and 732 specimens belonging to 11 genera of herbs were recognized. Most of them were intact, but some were fragmentary.

The fossils were initially photographed using a Zeiss digital camera (AxioCam HRc) anchored on a Zeiss binocular microscope (Stereo REO Discovery V20). Well-preserved specimens of each morphotype were further examined using a scanning electron microscope (SEM; Zeiss EVO LS10). All these fossils are accessible at the palaeobotanical collection in the Key Laboratory for Plant Biodiversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). To improve their taxonomic attribution, the fossils were compared extensively with extant species from East Asia, chiefly from the modern HMR. Comparative morphological information was obtained by studying herbarium specimens in the Herbarium of Kunming Institute of Botany and by referring to published sources (Yin & Yan, 1996; Decraene et al., 2000; Nakayama et al., 2000; Guo et al., 2009; Jimenez-Mejias & Martinetto, 2013; Du & Yang, 2015).
3. Systematics, descriptions and remarks

Family: Ranunculaceae Jussieu, 1789

Genus: Ranunculus L., 1753

Species: Ranunculus cf. tachiroei Franch. et Sav., 1876 (Figs. 2, A; 9, A, B)

Materials: 27 achenes (NBB 121–NBB147)

Description: achenes compressed, elliptic to ovate; length 1.6–2.2 mm, and width 1.2–1.8 mm; marginal area thickened, and central area depressed; surface obliquely reticulate except for the marginal area, with the reticulum broad-fusiform in outline.

Surface character of Ranunculus achenes exhibits great interspecific variation, including shallowly pitted (e.g., R. tachiroei), transversely ridged (e.g., R. kazusensis Makino), and tuberculate to spinose types (e.g., R. parviflorus L.) (Reid & Reid, 1915; Yin & Yan, 1996; Nakayama et al., 2000; Field, 2013). Previous studies noted that the exterior surface of Ranunculus achenes may be damaged during fossilization (Field, 2013). The surface of our fossils is distinctively reticulate, which does not occur obviously in living Ranunculus. This reticulate pattern likely represents an underlying structure after the removal of the outmost layer. It corresponds well to the shallowly pitted achene surface of

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Family: Fumariaceae Marquis, 1820

Genus: *Corydalis* Candolle, 1805

Species: *Corydalis* spp. (Figs. 2, B, C; 3)

Materials: 64 seeds, mostly intact but cracked (NBB 148–NBB 211)

Description: seeds lenticular, elliptic to circular; dorsal margin rounded, and ventral margin relatively straight; elaiosome well-developed and incurved; surface brightly lustrous and weakly striate; surface cells circular, polygonal to rectangular, arranged in an alveolate form; seed coat 25–30 µm thick, weakly palisade-structured in the cross section.

Seeds of *Corydalis* can be confused with those of several genera in Amaranthaceae, e.g., *Amaranthus* L., *Celosia* L. and *Chenopodium* L., which share overall lenticular shape and lustrously striate surfaces. The most striking feature that distinguishes *Corydalis* from them is the development of elaiosome. Additional differences also exist, e.g., seed marginal area of *Corydalis* is roundly inflated while that of *Amaranthus* is often depressed. *Corydalis* is a large genus of up to 500 species, and in China alone there are approximately 357 species (Wu & Raven, 1995). Our observations from herbarium materials show that different

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species have seed characters in common, including the lenticular shape, lustrous and striate surface, and a large and incurved elaiosome, which makes it difficult to differentiate between species using only seed characters. However, it is easy to recognize at least two morphotypes among the fossils that likely represent two different species. *Corydalis* sp. 1 (NBB 148–NBB 163) has elliptic and larger seeds (Figs. 2, B; 3, A–C), while *Corydalis* sp. 2 (NBB 164–NBB 211) is distinguishable by its circular and smaller seeds (Figs. 2, C; 3, D–F).

Family: Polygonaceae Jussieu, 1789

Genus: *Rumex* L., 1753

Species: *Rumex* sp. (Figs. 2, D; 9, C, D)

Material: one achene (NBB 212)

Description: achene prismatoidal, about 2.3 mm long and about 1.5 mm wide; apical view of the achene triangular in outline; three longitudinal arrises extending from the base to the apex; surface smooth in general view, but weakly striate in microscopic view.

Our observations and available sources (Yin & Yan, 1996; Nakayama et al., 2000; Guo et al., 2009; Du & Yang, 2015) show that *Rumex* achenes appear morphologically uniform though their size may vary among species. This makes
species delimitation difficult for our fossil which is known only from a single specimen.

Genus: *Polygonum* L., 1753

Species: *Polygonum* cf. *nepalense* Meisn (Figs. 2, E; 4, 5, A–F)

Materials: 327 achenes and achene fragments (NBB 213–NBB 539)

Description: achenes roundly to triangularly pyramidal; both base and apex narrowed sharply; length (1.1–1.8 mm) slightly greater than width (1.0–1.6 mm); sepal mostly missing, leaving a prominent circular hole-like scar at the base; three longitudinal traces (arrises) covering the full length of the achene; surface reticulate formed by papillate ridges.

The roundly to triangularly pyramidal shape characterizes achenes of most *Polygonum* species. Generally, they bear two surface types due to different thickenings of cell walls: smooth surface and reticulate, papillate surface (Decraene et al., 2000). As is shown (Fig. 5, A–F), our fossils evidently belong to the second type. By surveying achene morphologies of the genus from the herbarium and literature (Yin & Yan, 1996; Nakayama et al., 2000; Guo et al., 2009; Du & Yang, 2015), we found the fossils are morphologically closer to *P. nepalense* based on its smaller size, round inflation and smaller length-to-width ratio.

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Species: *Polygonum* sp. (Figs. 2, F; 5, G–I)

Materials: 15 achenes and achene fragments (NBB 540–NBB 554)

Description: achenes small, roundly to triangularly pyramidal; base rounded while apex narrowed relatively gradually; length (1.0–1.5 mm) almost equals width (1.0–1.5 mm); sepal missing, leaving a circular hole-like scar at the base; surface smooth; three longitudinal traces extending from the basal scar to the apex.

The fossils evidently belong to the smooth-surfaces achene type in *Polygonum* as described in Decraene et al. (2000), while their taxonomy at the species level is left undetermined due to their limited characters.

Family: Amaranthaceae Jussieu, 1789

Genus: *Chenopodium* L., 1753

Species: *Chenopodium gracilispicum* Kung, 1978 (Figs. 2, G; 9, E, F)

Materials: 12 seeds and seed fragments (NBB 555–NBB 566)

Description: seeds round, lenticular, small, 1.0–1.3 in diameter; hilum indistinctive; ventral margin near the hilum weakly concave, with a radially-oriented short groove formed by the curved embryo; surface lustrous, radially pitted and bumpy.

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According to our own and previous (Yin & Yan, 1996; Nakayama et al., 2000; Du & Yang, 2015) observations, most species of Chenopodium produce smooth-surfaced or sometimes radially striate seeds. However, *C. gracilispicum* is distinctive in having a radially pitted seed surface as seen in our fossils, which justifies the species assignment.

**Family:** Caryophyllaceae Jussieu, 1789

**Genus:** *Stellaria* L. 1753

**Species:** *Stellaria media* (L.) Vill., 1789 (Figs. 2, H; 6, A–C)

**Material:** one seed (NBB 567)

**Description:** seed compressed, dorsally fan-shaped to almost circular, small, about 0.8 mm in diameter; ventral side abruptly sunken, and dorsal side rounded; seed margin distinctively undulate to spiny; surface cells radially arranged, with deeply lobed walls that interfinger tightly with adjacent cell walls.

*Stellaria* often has similar seed morphologies to the related genus, *Silene* L., e.g., the undulate, fan-shaped dorsal margin. However, the deeply lobed walls of the seed surface cells make our fossils much closer to *Stellaria*. Additional characteristics of the fossils, in particular the almost round shape, small size, undulate to spiny margin and radially arranged surface cells, collectively resemble
the seeds of *Stellaria media* as illustrated in Yin & Yan (1996) and Nakayama et al. (2000).

Species: *Stellaria petiolaris* Hand.-Mazz., 1940 (Figs. 2, I; 6, D–F)

Materials: 10 seeds, mostly intact (NBB 568–NBB 577)

Description: seeds compressed, elliptic, small, 0.8–0.9 mm long and 0.6–0.7 mm wide; ventral side concave, and dorsal side rounded; seed margin distinctively undulate to tubercular; surface cells distinctive, with deeply lobed walls that interfinger tightly with lobes of adjacent cells.

By studying seed morphology of 14 species from the herbarium and literature (Yin & Yan, 1996; Nakayama et al., 2000; Guo et al., 2009), we found that our fossils closely resemble seeds of *S. petiolaris*, which encourages an inclusion of the fossils to this living species.

Family: Rosaceae Jussieu, 1789

Genus: *Fragaria* L., 1753

Species: *Fragaria* sp. (Figs. 2, J; 9, G, H)

Materials: 19 achenes, mostly intact (NBB 073–NBB 091)
Description: achenes small, abaxially rounded; length from the attachment scar to the abaxial margin 0.4–0.7 mm; surface smooth in general view, but weakly reticulate formed by circular to elliptic surface cells in microscopic view.

These fossil achenes have already been reported as an undetermined species of *Fragaria* in Huang et al. (2019). Their distinction from the related genera with similar achene morphologies, *Duchesnea* Smith and *Potentilla* L., has been discussed previously (Huang et al., 2019).

Family: Leguminosae Jussieu, 1789

Genus: *Astragalus* L., 1753

Species: *Astragalus* sp. (Figs. 7; 8)

Materials: 182 seeds, with seed coat removed to different extent (NBB 578–NBB 759)

Description: seeds elliptic, adaxially curved; length 3.5–4.5 mm, and width 2.5–3.5 mm; hilar scar unobservable due to the damaged seed coat; embryo curved, with a large, raised radicle; cotyledons large and roundly swollen, with a generally surface; seed coat 0.4–0.5 mm thick, displaying a palisade structure in the cross section.

As one of the largest families of flowering plants, Leguminosae is a challenge regarding identification among its diverse genera using seed characters alone. Our
fossils are closest to seeds of *Astragalus* and *Oxytropis*, especially *Astragalus*,
given their elliptic shape, curved outline and medium size. Here we have
attributed the fossils tentatively to *Astragalus*, yet species placement within this
diverse genus based only on seed characters seems almost impossible.

Family: Compositae Giseke, 1792

Genus: *Aster* L., 1753

Species: *Aster* sp. (Figs. 2, K; 9, I, J)

Materials: two achenes (NBB 760, NBB 761)

Description: achenes oblong, small, 0.8–1.0 mm long and 0.3–0.4 mm wide;
upper part narrowed sharply while lower part narrowed gradually; longitudinal
ribs weakly developed; apex truncate, and protuberate at the center.

The protuberance on the truncate apical surface, where hairs have been
attached, typifies achenes of almost all genera, except a few such as *Xanthium* L.,
in Compositae. Moreover, longitudinal ribs are common in most genera of the
family, but much less inconspicuous in some such as *Anaphalis* DC., *Artemisia* L.
and *Aster*. Our fossils exhibit similar achene sizes and morphologies to several
species in *Aster*, e.g., *A. ageratoides* Turcz., *A. scaber* Thunb., and *A. tataricus* L.
f. as illustrated in the literature (Nakayama et al., 2000; Guo et al., 2009), which
warrants the genus placement.

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Family: Cyperaceae Jussieu, 1789

Genus: *Carex* L., 1753

Species: *Carex* spp. (Figs. 2, L–P; 10, A–J)

Materials: 69 achenes, mostly intact (NBB 762–NBB 830)

Description: achenes ranging widely in shape, from oblong to broad elliptic and from compressed to roundly prismatoidal; style long, attenuate, or absent; surface reticulate, with surface cells in longitudinal orientation.

*Carex* is a species-rich, taxonomically troublesome genus of approximately 2000 species worldwide (Jimenez-Mejias & Martinetto, 2013), with more than 500 species in China (Wu & Raven, 1995). Its achene morphology has been extensively surveyed, which facilitates fossil identification (Jimenez-Mejias & Martinetto, 2013). Our fossils include at least five morphological types that likely represent five different species. Here we abandon their identifications at the species level due to limited characters associated with their small size combined with significant morphological similarities among species. Despite the uniformity of their surface sculptures, other features may vary among the five potential species. Achenes of *Carex* sp. 1 (NBB 762–NBB 776) differ from the other four morphotypes described here by greater length, larger length to width ratio, and finely mucronate apex (Figs. 2, L; 10, A, B); those of *Carex* sp. 2 (NBB 777–NBB

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are characterized by the roundly prismatoidal shape (Figs. 2, M; 10, C, D); those of *Carex* sp. 3 (NBB 786–NBB 793) have a distinctive long style (Figs. 2, N; 10, E, F); those of *Carex* sp. 4 (NBB 794–NBB 828) are broad elliptic with rapidly narrowed apex and base (Figs. 2, O; 10, G, H); and those of *Carex* sp. 5 (NBB 829, NBB 830) are elliptic with a broader base (Figs. 2, P; 10, I, J). This suggests the presence of a somewhat high species diversity of *Carex* at the fossil site during sedimentation.

**Genus: Schoenoplectus** (Rchb.) Palla, 1888

**Species: Schoenoplectus** sp. (Figs. 2, Q; 10, K, L)

**Materials:** three achenes (NBB 831–NBB 833)

**Description:** achenes obovate to roundly triangular; length 1.3–1.5 mm, and width 0.9–1.0 mm; apex narrowed rapidly, forming a short protuberance, and base narrowed gradually; surface with broad, transverse ridges; surface cells oblong, undulate-margined and longitudinally orientated.

**4. Discussion**

4.1. Herbaceous diversity and its implications

Previous works on molecular phylogeny have sought to disentangle diversifications of various herbaceous groups in the HMR (Chen et al., 2005; Jabbour & Renner, 2012; Li et al., 2014; Hauenschild et al., 2017; Xing & Ree, 2018).
2017; Zhang et al., 2019; Ding et al., 2020). Such works are limited by the molecular data preserved in surviving lineages. Our fossil finding provides the first direct evidence demonstrating the historical rise of herbaceous diversity in the region, thereby complementing molecular phylogenetic studies.

Our systematic results show the presence of at least 11 genera of herbs from 9 families in the fossil assemblage, i.e., *Ranunculus, Corydalis, Rumex, Polygonum, Chenopodium, Stellaria, Fragaria, Astragalus, Aster, Carex* and *Schoenoplectus* (Table 1). As far as we know, most of them are recorded here for the first time from the ancient HMR, including *Ranunculus, Corydalis, Rumex, Stellaria, Astragalus, Aster* and *Schoenoplectus*. Although families to which they belong, such as Polygonaceae and Compositae, have been documented in the pollen record (Xu et al., 2004; Kou et al., 2006; Wu et al., 2019), taxonomic resolution from the fossil pollen was insufficient to confirm the identification of these genera. Here, species assignment is made for some genera, e.g., *Ranunculus* and *Stellaria*, but remains unresolved for others, e.g., *Corydalis* and *Carex*, largely because their achenes alone do not allow for reliable species delimitation in these species-rich genera. However, one thing can be certain: different species are represented within a same genus based on large morphological distinctions among their fossils. For an example, the *Carex* achenes encompass at least five distinctive morphotypes that most likely represent five different species. In this, we estimate at least 18 species of herbs belonging to 11 genera in the fossil assemblage. A
species with close affinities to *Polygonum nepalense* is dominant in terms of fossil occurrences, followed by an unassigned species of *Astragalus* (Table 1; Fig. 11). As aforementioned, the studied fossils are mostly intact though many of them are fragile as charcoals, meaning that they must be of local origin without having undergone much pre-depositional transportation. For this reason, we infer that all the 18 herbaceous species occurred locally, suggesting a high diversity of herbs in the Heqing Basin during the late Pliocene. Such a local assembly of various herbs, a feature now widespread among subalpine to alpine ecosystems in the HMR (Sherman et al., 2008), can probably be traced back to at least the late Pliocene.

Available results of molecular phylogenetic studies consistently point to the Miocene as the period for major species diversification of many herbaceous lineages in the HMR (Jabbour & Renner, 2012; Fan et al., 2013; Gao et al., 2015; Hauenschild et al., 2017; Xing & Ree, 2017; Ding et al., 2020), although the diversification began in the Oligocene due to the Paleogene rise of this region (Ding et al., 2020). This timing estimate is obviously earlier than the age of our fossils. We interpret that the wide pre-Pliocene species differentiation might have greatly increased the likelihood of occupation of different herbs within geographically small areas like the Heqing Basin. In this respect, our finding stands in line with the previous molecule-based results.

4.2. Open meadow vegetation and its implications

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As is shown (Table 1), most of the identified herbs nowadays grow mainly in open environments such as meadows on mountain slopes or in flatlands, though sometimes they also seek cover on the forest edge or in the understory (Wu & Raven, 1995). A few of these herbs, e.g., Carex and Schoenoplectus, prefer wetland habitats due to their subaquatic habit (Wu & Raven, 1995). All these suggest an open ecosystem composed of a meadow and a wetland in the Heqing Basin during sedimentation. Since Polygonum is represented best in terms of fossil occurrences, we infer that it might have dominated this open ecosystem. In addition to herbs, the fossil assemblage also encompasses some woody elements, including Picea A. Dietrich, Pinus L., Myrica L., Eurya Thunb., Rubus L., Hypericum L. and Rhododendron L., of which Eurya, Rubus and Rhododendron mainly as shrubs are relatively well-represented (Zhu et al., 2016; Huang et al., 2019). We infer that the reconstructed meadow was probably scattered with some shrubs. Forest cover, however, might be limited in the immediate vicinity of the deposition site because tree components are only poorly represented in the fossil assemblage. The reconstructed wetland possibly represents the depositional environment that evidently received plant remains from surroundings of the basin as well as from the wetland itself. This inference is supported by not only the deduced in situ preservation of the fossils, but also the lacustrine lithological facies enveloping the fossil-bearing sediment (Zhu et al., 2016; Huang et al., 2019).
The southern portion of the HMR underwent dramatic vegetation changes during the Neogene. Previous studies using fossil floras suggested that during the Miocene subtropical broadleaved forests dominated (Jacques et al., 2014), while during the late Pliocene temperate types such as evergreen sclerophyllous forest (Tao & Kong, 1973; Tao, 1986; Su et al., 2015; Huang et al., 2016a) and coniferous forest (Kou et al., 2006; Wu et al., 2019; Huang et al., 2020) expanded. It seems that hitherto our knowledge of vegetation evolution in the southern HMR is confined to closed forest, whereas open types remain poorly known. The newly reconstructed meadow and wetland represents the first report of open type of vegetation ecosystem from the Neogene HMR. It therefore sheds important light on vegetation evolution in the region.

Today, open meadows in the HMR occur mainly in subalpine and alpine zones that experience a relatively cool climate (Liu et al., 1984; Sherman et al., 2008; Boufford, 2014). This modern analogy suggests a high altitude in the basin during the late Pliocene, probably close to the modern level that approximates 2200 m above sea level. With a warmer late Pliocene, the reconstructed meadow could have occupied an even higher altitude. All these suggest surface uplift of the basin in prior to the late Pliocene, consistent with observations from areas nearby, e.g., Eryuan Basin (Wu et al., 2019) and Lanping Basin (Huang et al., 2020).
4.3. Causal relationship for the opening of meadow

Climate change is closely linked to biome alteration, e.g., cooling and drying as a driving force for the spread of steppes in central Asia (Barbolini et al., 2020). In the HMR, much of the surface rose during the late Paleogene, fundamentally as a result of the India-Asia collision (Hoke et al., 2014; Gourbet et al., 2017; Spicer et al., 2020; Zhang et al., 2020). Recently, multiple lines of evidence have showed that the northern part of the region uplifted much earlier, e.g., during the Eocene, and the rise was rapid (Su et al., 2019; Xiong et al., 2020). Although the uplift in its southern part could be relatively late in time and moderate in extent (Gourbet et al., 2017), a close-to-modern surface height is thought to have been reached by the Miocene (Hoke et al., 2014), which might be the case for the Heqing Basin as well. We interpret that the raised surface, combined with global cooling (Zachos et al., 2001; Mosbrugger et al., 2005), probably cooled the local climate and thus favored open meadows. This accords with repeated statements on the combined role of the uplift-induced cooling and secular cooling for stimulating radiations of herbs in the HMR (Chen et al., 2005; Fan et al., 2013; Gao et al., 2015; Ren et al., 2015; Hauenschild et al., 2017; Ding et al., 2020).

In addition to lowered temperatures, other climate factors might also have played a role. As the Asian monsoon systems strengthened in the Paleogene (Spicer et al., 2016, 2017, 2020), rainfall in the wet season increased while that in...
the dry season decreased. Quantitative palaeoclimate estimates indicate that the southern HMR began to experience greater wet and dry seasonality by the late Pliocene (Xie et al., 2012; Su et al., 2013; Huang et al., 2015). In the wet season, heavy and frequent rainfall could bring disturbance or even damage to local vegetation probably by causing floods and landslides. Annual and biennial herbs, due to their shorter lifecycles (Holmes & Matlack, 2019), might occupy the disturbed areas as pioneers and consequently established their communities. In the dry season, moisture deficiency could be a key factor restricting the growth and generation of plants. Perennial herbs, however, might be favored as compared to trees in that they are capable of reducing their vegetative parts but retaining reproductive roots or stems underground for new growth (Axelrod, 1985). Thus, the intensified precipitation seasonality might have been instrumental in further opening up of meadow environments in the HMR.

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References


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**Figures**

**Table 1.** Identified herbaceous elements from the fossil assemblage, abundance of their fossil occurrences, and the natural habitats of their modern equivalents based on descriptions in the Flora of China (Wu & Raven, 1995).

Fig. 1. Fossil site, stratigraphy and the studied sedimentary horizon. A. Maps, created with GeoMapApp 3.6.10 (Ryan et al., 2009), showing the location of the fossil site in the western Heqing Basin of the southern HMR. B. Field pictures showing the sampling layer at the fossil site. C. The stratigraphic position of the sampling layer.

Fig. 3. Fossil seed and seed fragments of *Corydalis* shown by SEM. A–C. *Corydalis* sp. 1. A. NBB 149, showing the gross shape. B. Close-up of surface sculpture of NBB 149, showing circular to polygonal cells in an alveolate arrangement. C. NBB 150, cross section of the seed coat showing weakly developed palisade. D–F. *Corydalis* sp. 2. D. NBB 165, showing the gross morphology. B. Close-up of the marginal area of NBB 165, showing rectangular surface cells in a regular arrangement. C. Inner view of
NBB 165, showing cross section and interior surface cells of the seed coat. Scale bars = 0.5 mm for images A, D; 50 µm for images B, C, E, F.

Fig. 4. Fossil achenes of *Polygonum* cf. *nepalense* Meisn.
Fig. 5. Fossil achenes of *Polygonum* shown by SEM. A–F. *Polygonum* cf. *nepalense* Meisn. A. NBB 215, lateral view showing the triangular conical shape and reticulate surface. B. NBB 216, oblique view showing the triangular conical shape, three longitudinal traces (arrises) and the persistent sepal (arrowed). C. NBB 217, basal view showing the reticulate surface, a circular basal scar formed by the decay of the sepal and three longitudinal traces that terminate at the scar. D. NBB 218, apical view showing the reticulate surface and three longitudinal traces that terminate at the apex. E. Close-up of the achene surface of NBB 218, showing papillate ridges of the reticulation. F. Close-up of the ridges, showing closely packed papillae. G–I. *Polygonum* sp. G. NBB 542, lateral view of the fragmentary exocarp, showing the smooth surface and the circular basal scar. H. NBB 541, basal view showing the smooth surface, three longitudinal ribs and a prominent circular basal scar. I. Close-up of the achene surface of NBB 541. Scale bar = 0.5 mm for images A–D, G, H; 50 µm for images E, F and I.

Fig. 6. Fossil seeds of *Stellaria* shown by SEM. A–C. *Stellaria media* (L.) Vill. A. NBB 567, showing the gross shape. B. Close-up of the surface of NBB 567, showing cells with deeply lobed walls that interfinger closely with adjacent cell walls. C. Close-up of the dorsal part of NBB 567, showing cells with tubercles and lobes that This article is protected by copyright. All rights reserved.
interfinger with adjacent cell walls. D–F. Stellaria petiolaris Hand.-Mazz. D. NBB 568, showing the gross shape. E. Close-up of the surface of NBB 568, showing cells with deeply lobed walls that interfinger closely with adjacent cell walls. F. Close-up of the dorsal part of NBB 568, showing cells with outward swollen walls and sharp lobes that interfinger with adjacent cell walls. Scale bars = 0.5 mm for images A, D; 50 µm for images B, C, E, F.

Fig. 7. Fossil seeds of Astragalus sp. A. NBB 578. B. NBB 579.
Fig. 8. Fossil seeds of *Astragalus* sp. shown by SEM. A. NBB 580, side view showing the gross shape, and the large and curved embryo (arrowed). B. NBB 581, adaxial view showing the large, raised radicle (arrowed) and the middle suture of two cotyledons, with the hilum absent due to damage of the seed coat. C. NBB 582, abaxial view showing two large, smooth-surfaced cotyledons separated by the straight, longitudinal middle suture. D. NBB 583, showing a seed with most seed coat retained. E. Close-up of the seed coat of NBB 583, showing fractured tissues. F. Cross section of the seed coat, showing a fine palisade structure. Scale bars = 1 mm for images A–D; 50 µm for images E, F.

Fig. 9. Fossil fruits and seeds of other dicotyledonous herbs shown by SEM. A, B. *Ranunculus* cf. *tachiroei* Franch. et Sav. (NBB 122), showing the gross shape and a closer view of the reticulate surface. C, D. *Rumex* sp. (NBB 212), showing the gross shape and a closer view of the weakly striate surface. E, F. *Chenopodium gracilispicum*

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Kung (NBB 556), showing the gross shape and a closer view of the pitted, bumpy surface. G, H. *Fragaria* sp. (NBB 090), showing the gross shape and a closer view of the weakly reticulate surface. I, J. *Aster* sp. (NBB 761), showing the gross shape and a closer view of the apex (arrowed) where hairs were formerly attached. Scale bars = 0.5 mm for images A, C, E, G, I, K; 50 µm for images B, D, F, H, J, L.

Fig. 10. Fossil fruits of monocotyledonous herbs shown by SEM. A, B. *Carex* sp. 1 (NBB 763), showing the gross shape and a closer view of the reticulate surface. C, D. *Carex* sp. 2 (NBB 778), showing the gross shape and a closer view of the reticulate surface. E, F. *Carex* sp. 3 (NBB 787), showing the gross shape and a closer view of the reticulate surface. G, H. *Carex* sp. 4 (NBB 795), showing the gross shape and a closer view of the reticulate surface. I, J. *Carex* sp. 5 (NBB 830), showing the gross shape and a closer view of the reticulate surface. K, L. *Schoenoplectus* sp. (NBB 832), showing the gross shape and a closer view of the surface with longitudinally oriented and

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undulate-margined cells. Scale bars = 0.5 mm for images A, C, E, G, I, K; 50 µm for images B, D, F, H, J, L.
Fig. 11. Graph showing the respective proportions of herbaceous elements in the fossil assemblage in terms of fossil occurrences.

![Graph showing the respective proportions of herbaceous elements in the fossil assemblage in terms of fossil occurrences.](image)

Figs. 1-3, 5, 6, 8-10, Table 1, double columns; Figs. 4, 7, 11, single column

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Waterside, wet valley
Meadow, grassy slope, waterside, wetland
Forest edge, grassy slope, watershed
Meadow, grassland, forest edge, waterside
Forest edge and understory, shrubland
Meadow, forest edge and understory, grassy slope
Meadow, steppe, desert, grassy
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