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Research Article

Late Miocene *Palaeocarya* (Engelhardieae: Juglandaceae) from Southwest China and its biogeographic implications

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Abstract Fossil fruits of *Palaeocarya* (Juglandaceae) are described from late Miocene sediments of southeastern Yunnan, China. The fruits present a tri-lobed wing consisting of an intact oblong-ovate middle lobe and two lateral lobes. The lobes are apically obovate, and have pinnate venation. The middle lobe is thicker at the base and gradually tapers to the apex. The nutlet, located at the base of the winged fruit, is round and hispid, and is subdivided by a septum into two compartments. Based on extensive morphological comparisons to previously documented fossil fruits, we found that the fossil fruits align most closely with members of the genus *Palaeocarya*, but have a unique combination of characters. Thus, we describe the fossils as a new species, *Palaeocarya hispida* sp. nov. This species represents an important range expansion for low-latitude occurrences of *Palaeocarya* in the late Miocene and therefore substantially improves our understanding of the biogeographic history of the genus. We propose that the wide distribution of *Palaeocarya* and relatively narrow distributions of close relatives, *Engelhardia*, *Alfaropsis*, and *Oreomunnea*, might be associated with a stepwise cooling and a major ice sheet expansion in the Antarctic and Arctic from the late middle Miocene to early Pliocene. In particular, the climatic oscillations during the Quaternary, such as the last glacial maximum, might have led to a decrease in the geographic distribution of Engelhardieae.

Key words: biogeography, Engelhardieae, fossil fruit, Juglandaceae, late Miocene, *Palaeocarya*.

The tribe Engelhardieae (Juglandaceae) is characterized by tri-lobed, bracteate winged fruits, and comprises four extant genera, *Engelhardia* Leschenault ex Blume, *Oreomunnea* Oerst., *Alfaropsis* Iljinsk, and *Alfaroa* Standl., which are widely distributed in tropical and subtropical regions of the Southern and Northern Hemispheres (Manchester, 1987; Manos & Stone, 2001; Manos et al., 2007; Stone, 2010). *Engelhardia* and *Alfaropsis* are endemic to tropical and subtropical regions of southern and southeastern Asia (Lu, 1982; Stone, 2010), whereas *Oreomunnea* and *Alfaroa* are distributed in tropical Central America (Fig. 1) (Stone, 1972; Manning, 1978). Paleobotanists have explored the evolution and radiation of the Juglandaceae with the help of the family's distinctive morphological characters and rich Cenozoic fossil records (Manchester, 1987; Manchester et al., 1994; Manos & Stone, 2001; Manos et al., 2007; Stone, 2010).

Fossil records of tribe Engelhardieae consist of three genera, *Palaeocarya*, *Paraengelhardtia*, and *Paleooreomunnea*, which were well-documented from the Northern Hemisphere (Manchester, 1987). *Paraengelhardtia* and *Paleooreomunnea* can be differentiated from *Palaeocarya* by their nutlet size and wing shape. Fossil fruits of *Palaeocarya* have a deeply tri-lobed wing with a sinus depth of 65%–85% of the fruit, whereas fossil fruits of *Paraengelhardtia* and *Paleooreomunnea* have a shallowly lobed wing with a sinus depth of 12%–20% of the fruit (Manchester, 1987). *Palaeocarya* was originally documented by Saporta (1873), who noted the similarity to Engelhardia or in the Engelhardian complex of the Juglandaceae (Manning, 1978; Lu, 1982) based on fruit characteristics such as a deeply tri-lobed wing, a deep sinus, and main veins along each lobe of the wing. As *Alfaroa* fruits are wingless and generally have large endocarps (Manchester, 1987), the tri-lobed winged fruits of *Palaeocarya* are similar to the extant genera *Engelhardia*, *Alfaropsis*, and *Oreomunnea* in regards to their tissue venation and nut morphology (Stone, 2010; Xie et al., 2010). *Palaeocarya* has been considered the nearest relative of *Engelhardia* (Saporta, 1873; Jin, 2009). Fossil fruits can be more easily identified than the remains of other organs (e.g., foliage and pollen) and can provide additional evidence on the taxonomy and evolution of the Engelhardieae.

Palaeocarya has been emended based on the uncertainty of assigning fossil winged fruits to the extant genera *Engelhardia*, *Alfaropsis*, or *Oreomunnea* (Manchester, 1987; Manos et al., 2007; Stone, 2010). Fossil fruits of *Palaeocarya* are abundant in Cenozoic sediments in the Northern Hemisphere, from sites in Europe, North America, and Asia (Fig. 1). The fruits of *Oreomunnea* are like those of *Engelhardia* in that they are tri-lobed wing nutlets, but they are different from

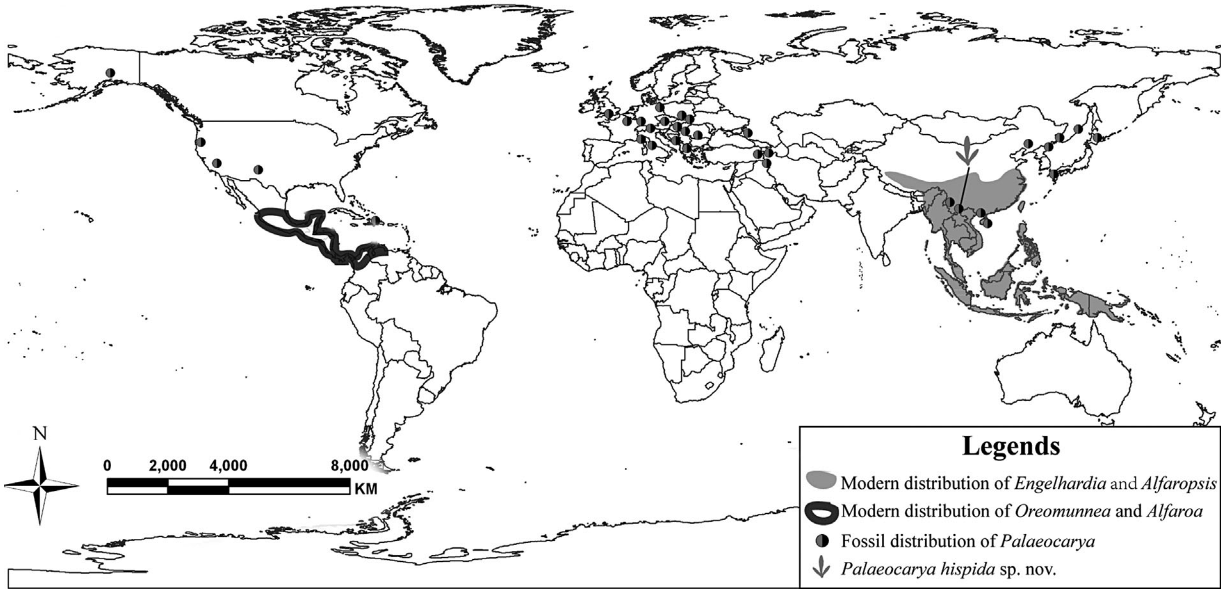

Fig. 1. Map showing modern and fossil fruit distribution range of Engelhardieae (adapted from Manchester, 1987).

Engelhardia in their foliage, germination, and fruit wing venation and nutlet morphology (Stone, 1972). The nutlet of *Oreomunnea* is partitioned by thin septa into eight compartments, whereas that of *Engelhardia* is partitioned into two or four segments (Stone, 1972; Manchester, 1987). *Alfaropsis*, with fruits that are similar to *Engelhardia*, is monotypic, and is widespread in southeastern Asia (i.e., *Alfaropsis* Iljinsk = *Engelhardia roxburghiana* Wall.) (Stone, 2010). *Alfaropsis* is recognized as the sister clade to all Engelhardieae from the phylogeny of extant and fossil Juglandaceae (Manos et al., 2007). The new fossil species of *Palaeocarya* cannot be assigned to any extant species in *Engelhardia, Oreomunnea*, or *Alfaropsis* because the inner structure of the nutlets have not been well preserved by the fossilization process (Manchester, 1987; Li et al., 2003).

Palaeocarya fossils have also been reported from Eocene and Pliocene strata in western, central, and southeastern Europe, Asia, and North America (Tanai & Uemura, 1983; Jähnichen et al., 1984; Manchester, 1987; Guo & Zhang, 2002). In North America during the early Eocene to Neogene, *Paleocarya* was distributed across a wide expanse from Alaska, south along the West coast and across the continent to southeastern North America (Leopold & MacGinitie, 1972; Manchester, 1987). In Asia, *Palaeocarya* were preserved in Oligocene–Pliocene strata from the Far East, North Korea, to Hokkaido and Honshu in Japan (Tanai & Uemura, 1983; Li et al., 2003). In China, fossil records of *Palaeocarya* have been relatively rare, with reports from only four localities, including Longjing (Jilin), Ningming (Guangxi), Tengchong (Yunnan), and Changchang (Hainan) in strata dated to Paleocene, Oligocene, Pliocene, and Eocene, respectively (Guo & Zhang, 2002; Li et al., 2003; Xie et al., 2006; Jin, 2009; Xie et al., 2010).

In this paper, we describe fossil fruits of *Palaeocarya* from the Upper Miocene Xiaolongtan Formation of Wenshan County, southeast Yunnan, China. These fossil materials present *Palaeocarya* nutlets with clear septa and hispid hair. The fossil materials of *Palaeocarya* are the first late Miocene fossil records of the genus in China; the occurrence in Wenshan may provide new evidence for understanding the evolutionary and biogeographic history of the genus. We compare these new fossils to previously published fossils, extant *Alfaropsis*, *Oreomunnea*, and *Engelhardia* in detail. Southern China is recognized as the center of diversification of modern *Engelhardia* and *Alfaropsis*, while Central America is the center of diversification of *Oreomunnea*, where there have been well-documented fossil records of *Palaeocarya*. We analyze the newly discovered *Palaeocarya* fossil fruits and the modern distributions to address the biogeographic history of these genera.

Material and Methods

Geological setting

Fourteen tri-lobed, *Engelhardia*-like fruit fossils were collected in Wenshan County, southeast Yunnan, China. These fossil-bearing strata belong to the Xiaolongtan Formation (Zhang, 1997) which is widely distributed in central and southeastern Yunnan (CGRSTY, 1978). Its age is assigned to the late Miocene according to stratigraphic correlations (YBGMR, 1990), pollen data (Wang, 1996), mammal fossils, and plant macrofossils (Zhou, 1985). Specimens were well preserved as compressions in mud stone and shaley siltstone from several layers of the same strata. Among the collection, fruits of *Ailanthus confucii* Unger and leaves of *Bauhinia wenshanensis* H. H. Meng & Z. K. Zhou have been reported from the same strata recently (Su et al., 2013; Meng et al., 2014).

Morphological study

General views of the fossil fruits were photographed using a digital camera (D700; Nikon, Tokyo, Japan), and detailed anatomic morphology was observed with a stereo microscope (S8APO; Leica, Wetzlar, Germany). Morphological comparisons followed diagnostic characters described in the published reports of *Palaeocarya* fossil fruits (Manchester, 1987; Guo & Zhang, 2002; Li & Chen, 2002; Jin, 2009; Xie et al., 2010), including 16 published fossil fruit descriptions and illustrated plates (Table 1). Fruits of eight extant species of *Engelhardia*, *Oreomunnea*, and *Alfaropsis* were from previous reports, Xishuangbanna Tropical Botanical Garden (Mengla, China), and Kunming Botanical Garden, Chinese Academy of Sciences (Kunming, China) for a morphological comparison (Table 1). The descriptive terminology of fruit fossils follows Manchester (Manchester, 1987).

Systematics

Family Juglandaceae De Candolle ex Perleb (1818)

Genus *Palaeocarya* Saporta, 1873, emend. Manchester, 1987

Species *Palaeocarya hispida* H. H. Meng & Z. K. Zhou sp. nov.

Holotype *DMS-0297*, Fig. 2A (designated here). *DMS-0296* (the counterpart of the same specimen of the holotype *DMS-0297*, Fig. 2B).

Paratypes *DMS-0308, 0306, 0305, 0298, 0303, 0302, 0299* (Figs. 2C–2I); *DMS-0300, 0301, 0304, 0307, 0309* (designated here).

Repository Paleoecology Research Group, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

Type locality Dashidong village, Wenshan County, southeast Yunnan, China (23°20′50″N, 104°17′19″E, alt. 1277 m). All voucher specimens of *P. hispida* were collected from the same locality and stratum.

Stratigraphy Upper Miocene Xiaolongtan formation.

Etymology The specific epithet "*hispida*" describes the hairs on the nutlet of the fossil fruits (Fig. 3).

Diagnosis Fossil fruits consisting of a nutlet subtended by a large tri-lobed wing; deep sinuses between lobes, extending almost to the nutlet; lobes oblong to oblanceolate, 20–50 mm long, 10–30 mm wide; middle lobes usually longer than the lateral lobes; venation of lobes variable, from simple pinnate to tri-veined; nutlet rounded, subdivided by septa into two or more compartments.

Description Fossil fruits in the study are tri-lobed and winged (Fig. 2). The fruit wing has an oblong-ovate middle lobe up to 36 mm in length and two lateral lobes 7–10 mm. The width of the middle lobe is approximately 20 mm in its upper part and approximately 6 mm near the base. The apex of the lobe is obovate. The lateral lobes have pinnate venation, with a midvein that is thicker at the lobe base, gradually tapering to the apex of the lobe. The secondary veins are at angles of 50–70° to the midvein, and the branching angle is narrower at the base, becoming wider toward the apex. The secondary veins extend towards the margin of the lobes, and connect to adjacent veins, forming loops. The tertiary veins comprise an irregular net inside these loops, forming smaller irregular loops. The nutlet is at the base of the winged fruit, hispid, and rounded, with a diameter of approximately 3 mm, and is subdivided by a septum into two compartments (Figs. 2G–2I).

Results and Discussion

Comparison with extant and fossil species

Engelhardia, *Oreomunnea*, *Alfaropsis*, and *Alfaroa* all retain tri-lobed primary bracts on their mature fruits, which are either enlarged to form a wing with three lobes, or persist as tri-lobed scales at the base of a wingless nut. *Engelhardia*, *Alfaropsis*, and *Oreomunnea* have tri-lobed wings, but differ in features such as wing venation, stylar configuration, and nutlet septation. The fruits of *Alfaroa* are large nuts without wings, which are distinguished from *Engelhardia*, *Alfaropsis*, and *Oreomunnea* by their lack of wings and pinnate venation. The fossil fruits from Wenshan have nuts with tri-lobed wings, and pinnate venation. Thus, the new fossil fruits differ from *Alfaroa*, and most resemble fruits of *Engelhardia*, *Alfaropsis*, and *Oreomunnea*.

The genus *Oreomunnea* was established in 1856, but some taxonomists consider it to be a section of *Engelhardia* (e.g., Manning, 1959, 1960) due to their similar tri-lobed, winged nutlets. *Oreomunnea* was resurrected as a genus by Stone (1972). *Alfaropsis* was proposed in 1993 (Iljinskaya, 1993), and it was not generally recognized until the phylogeny of extant and fossil Juglandaceae was examined (Manos et al., 2007). Fossil fruits placed in *Palaeocarya* might in some cases represent one of the extant genera, *Engelhardia*, *Alfaropsis*, and *Oreomunnea*, but cannot be distinguished to a specific modern species without information from the inner structure of the nutlet. Some key phenotypic traits (e.g., the inner structure of the nutlet, the stylar configuration) are seldom preserved in fossils (Manchester, 1987; Li et al., 2003). The fossil genus "*Palaeocarya*" was emended and allowed us to assign the winged fruits to *Palaeocarya* instead of the modern genera *Engelhardia*, *Alfaropsis*, or *Oreomunnea* (Manchester, 1987; Li et al., 2003).

The external morphological traits of *Engelhardia*, *Alfaropsis*, and *Oreomunnea* fruits are similar, but the inner nutlet structures are different. We compared the new fossils with *Engelhardia* and *Alfaropsis* species due to the similarity of external features with both species. Previously, the systematic relationships in *Engelhardia* were uncertain; for example, *E. fenzelii* and *E. unijuga* were combined with *Alfaropsis roxburghiana* (Wall.) Iljinskaya (Manos & Stone, 2001); also, *E. roxburghiana* was transferred to *Alfaroa* by Iljinskaya (1990), and later it was recognized as the distinct genus, *Alfaropsis* (Iljinskaya, 1993). Additionally, *Alfaropsis roxburghiana* (Wall.) Iljinskaya was recognized as a synonym of *E. roxburghiana* in *Flora of China* (Lu et al., 1999). However, subsequent research on the phylogeny of extant and fossil Juglandaceae recognized that Engelhardieae contain four genera, that is, *Engelhardia*, *Oreomunnea*, *Alfaropsis*, and *Alfaroa* (Manos & Stone, 2001; Manos et al., 2007; Stone, 2010). *Engelhardia*, including six species, is distributed in southwestern, southern, and southeastern China (Lu et al., 1999). *Engelhardia* has been considered as the extant relative of *Palaeocarya* (Li et al., 2003; Jin, 2009). After comparing our fossil fruits with the fruits of modern *Engelhardia* and *Alfaropsis* plants in China, including *Alfaropsis roxburghiana* and *Engelhardia fenzelii* Merrill, *E. spicata* Leschen. ex Bl., *E. spicata* var. *aceriflora* (Reinwardt) Koorders & Valeton, *E. spicata* var. *colebrookiana* (Lindley) Koorders & Valeton, and *E. serrata* Blume (Table 1; Fig. 4) and previously published *Palaeocarya* fossil fruits (Table 1), we focused on the characters of hairs and nutlet septa. The nutlets of *E. fenzelii* (Fig. 5B) and *Alfaropsis roxburghiana* (Fig. 5E) are not hispid, in contrast to the fossil fruits in Wenshan. *Engelhardia spicata* (Fig. 5F), *E. spicata* var. *aceriflora* (Fig. 5A), *E. spicata* var. *colebrookiana* (Fig. 5C), and *E. serrata* (Fig. 5D) have nutlets that are covered with hairs, similar to our fossil fruits.

Table 1 Morphological comparison between fruits of selected species of *Palaeocarya* and extant *Alfaropsis*, *Engelhardia*, and *Oreomunnea* (expanded from Xie et al., 2010)

	Species	Locality	Age	Morphological characters					References
				Wing size: mid- and lateral lobes	Venation of lobe	Nutlet (diameter)	Pedicel (length)	Indumentum	
Fossil	*P. nevadensis* (MacGinitie) Manchester	California, USA	Middle or late Eocene	Mid-lobe 30–60 mm long, 6–12 mm wide; lateral 20–30 mm long and 3–6 mm wide, diverging at angles of 50° from mid-lobe.	Consisting of a strong midrib flanked by a pair of weaker lateral veins connected with the midrib by a series of branching from the primaries and looping along the margin.	2 mm	Unknown	No	Manchester (1987)
	P. clarnensis Manchester	Wyoming, USA	Middle Eocene	Mid-lobe 30–45 mm long, 4–10 mm wide; lateral 20–32 mm long and 3–7 mm wide, diverging at angles of 30–50° from mid-lobe.	Midvein and a pair of lateral veins inside the margin, resulting in a tri-veined aspect; in the upper one-third of lobes, the lateral veins bowed outward intercepting secondary veins, resulting in weakly looped appearance.	3–5 mm	1.5–3 mm	Yes	Manchester (1987)
	P. wolfei Manchester	Washington, USA	Late Eocene	Mid-lobe 40 mm long, 7 mm wide; lateral 18–20 mm long and 5–6 mm wide, diverging at 35–65° from mid-lobes.	Midvein paralleled by two of ascending lateral veins, outer pair of ascending laterals thinner than the inner pair and enclosed by elongate tertiary loops.	4.5 mm	1.5 mm	No	Manchester (1987)
	P. willamettensis Manchester	Oregon, USA	Oligocene	Mid-lobe 20–40 mm long, 5–9 mm wide; lateral 22–32 mm long and 5 mm wide, diverging at angles of 30–50° from mid-lobes.	Midvein paralleled by two intramarginal veins formed by the coalescence of secondary loops.	3–3.8 mm	0.3–0.5 mm	No	Manchester (1987)
	P. olsoni (Brown) Manchester	Alaska, Idaho, USA	Miocene	Mid-lobe 25–30 mm long, 6–8 mm wide; lateral 20–22 mm long and 4 mm wide.	Midveinparalleled by two or four prominent ascending lateral veins; connected by arching tertiary veins.	2.5 mm	Unknown	No	Manchester (1987)

Continued

Table 1 Continued

Species	Locality	Age	Morphological characters					References
			Wing size: mid- and lateral lobes	Venation of lobe	Nutlet (diameter)	Pedicel (length)	Indumentum	
P. koreanica (Oishi) Manchester	Korea; Jilin, China	Oligocene	Mid-lobe 30 mm long, 5–8 mm wide; lateral 20–22 mm long and 4–7 mm wide, diverging at angles of 40–80° from mid-lobe.	Pinnate or tri-veined, primary vein more stout and straighter than lateral ones; secondary veins 5–8 pairs, fine and prominent, diverging at angles of 40–65° in the upper part joining with adjacent ones to make serial loops.	5–6 mm	Up to 10 mm	No	Manchester (1987) Guo & Zhang (2002)
P. mississippiensis (Berry) Manchester	Mississippi, USA	Eocene	Mid-lobe 65 mm long, −13 mm wide.	Simple pinnate venation. Secondary veins branching from the midvein at 70–80° angles.	7 mm	Unknown	No	Manchester (1987)
P. uintaensis (Mac Ginitie) Manchester	Armissan, France	Late Oligocene	Mid-lobe 25 mm long, 7–10 mm wide.	Pinnate but with weak ascending lateral veins intermeshing with the secondary vein loops.	3 mm	5 mm	No	Manchester (1987)
P. macroptera (Brongniart) Jähnichen, Friederick & Takáć	Messel Germany	Middle Eocene	Unknown	Unknown	Unknown	Unknown	No	Manchester (1987)
P. puryearensis (Berry) Manchester	Kentucky, Tennessee, USA	Middle Eocene	Unknown	Unknown	Unknown	Unknown	No	Manchester (1987)
P. oxyptera (Saporta) Manchester	Armissan, France	Late Oligocene	Unknown	Unknown	Unknown	Unknown	No	Manchester (1987)
P. guangxiensis Li & Chen Y. F.	Guangxi and Yunnan, China	Oligocene and Pliocene	Mid-lobe 25–37 mm long, 4–8 mm wide, lateral 15–25 mm long, 3–6 mm wide, diverging from the mid-lobe at ~50°.	Pinnate brochidodromous, secondary veins branching from the midvein at ~40–60°; lateral veins forming loops with adjacent ones.	3–4 mm	2.5–6 mm	No	Li et al. (2003) Xie et al. (2010)

Continued

Table 1 Continued

Species	Locality	Age	Morphological characters					References
			Wing size: mid- and lateral lobes	Venation of lobe	Nutlet (diameter)	Pedicel (length)	Indumentum	
P. ninmingensis Li & Chen Y. F.	Guangxi, China	Oligocene	Mid-lobe 25 mm long, 9 mm wide, lateral 15 mm long, 6 mm wide, departing at 70° from mid-lobe.	Pinnate eucamptodromous in lower part, brochidodromous in middle and upper parts; secondary veins diverging from the midvein at 30–35° at the base, 40° in the middle, and 60° at the top.	3 mm	Unknown	No	Li et al. (2003)
Palaeocarya sp.	Hainan, China	Late Eocene	Intact oblong-ovate left lateral lobe up to 20 mm long. The width of the upper middle is 7 mm, and the basal part is 6 mm.	Pinnate brochidodromous with a thicker midvein at the lobe base, gradually tapering to the apex of the lobe. Eight pairs of lateral veins at 50–65° to the midvein; branching angle is narrower at the basal part, wider toward the apex.	6 mm	Unknown	No	Jin (2009)
P. longialata San-Ping Xie & Bai-Nian Sun	Yunnan, China	Pliocene	Mid-lobe 49 mm long and 10 mm wide; lateral 25 mm long and 6 mm wide; diverging from the mid-lobe at 39°.	The midvein strong, straight to the apex; single midvein with ~13 pairs of secondary vein in median lobe, looping close to the lobe margin, diverging from the midvein at ~60°.	8 mm	2 mm	No	Xie et al. (2010)
P. yunnanensis San-Ping Xie & Bai-Nian Sun	Yunnan, China	Pliocene	Mid-lobe 17–27 mm long, 5–6 mm wide; lateral 11–19 mm long, 4–5 mm wide; diverging from the median at 38–50°.	Single midvein with 8–10 pairs of secondary veins in the median lobe, secondary veins branching from midvein 50–60°.	3 mm	Unknown	No	Xie et al. (2010)
P. hispida sp. nov.	Yunnan, China	Late Miocene	Mid-lobe up to 30 mm long, lateral 7–10 mm. The width of the upper middle is 20 mm, and the basal part is 6 mm.	Pinnate, with a midvein that is thicker at the lobe base, gradually tapering to the apex of the lobe. The lateral veins at angle of 50–70°.	3 mm	2–4 mm	Yes	This paper

Continued

Table 1 Continued

	Species	Locality	Age	Morphological characters					References
				Wing size: mid- and lateral lobes	Venation of lobe	Nutlet (diameter)	Pedicel (length)	Indumentum	
Extant	*A. roxburghiana* (Wall.) Iljinsk.	Yunnan, China	Living	Mid-lobe 20–35 mm long, 6–8 mm wide, lateral 15–22 mm long, 4 mm wide, diverging from mid-lobe at $\sim$50°.	Pinnate, $\sim$6 pairs secondary veins on mid-lobe, 4 pairs on lateral, secondary veins branching from midvein $\sim$30°.	3–4 mm	2–3 mm	No	This paper
	E. fenzelii Merrill	Yunnan, China	Living	Mid-lobe 40 mm long, 5–8 mm wide, lateral 12–28 mm long, 6 mm wide, diverging from mid-lobe at $\sim$30°.	Pinnate, $\sim$7 pairs secondary veins on mid-lobe and 5 pairs on lateral, secondary veins branching from midvein 30–40°.	3 mm	2.5 mm	No	This paper
	E. spicata Leschen. ex Bl.	Yunnan, China	Living	Mid-lobe 60 mm long, 7–10 mm wide, lateral 15–20 mm long, 5 mm wide, diverging from mid-lobe at $\sim$50°.	Pinnate, $\sim$11 pairs secondary veins on mid-lobe and 6 pairs on lateral, secondary veins branching from midvein 70–80°.	2.5 mm	4 mm	Yes	This paper
	E. spicata var. *aceriflora* (Reinwardt) Koorders & Valeton	Yunnan, China	Living	Mid-lobe 70 mm long, 7–12 mm wide, lateral 15–20 mm long, 5 mm wide, diverging from mid-lobe at $\sim$30–60°.	Lobe venation pinnate, $\sim$11 pairs secondary veins on mid-lobe and 6 pairs on lateral lobe, secondary veins branching from midvein 60–80°.	2 mm	3 mm	Yes	This paper
	E. spicata var. *colebrookiana* (Lindley) Koorders & Valeton	Yunnan, China	Living	Mid-lobe 60 mm long, 7–10 mm wide, lateral 15–20 mm long, 5 mm wide, diverging from mid-lobe at $\sim$50°.	Pinnate, $\sim$9 pairs secondary veins on mid-lobe and 5 pairs on lateral, secondary veins branching from midvein 60°.	2 mm	3 mm	Yes	This paper

Continued

Table 1 Continued

Species	Locality	Age	Morphological characters					References
			Wing size: mid- and lateral lobes	Venation of lobe	Nutlet (diameter)	Pedicel (length)	Indumentum	
E. serrata Blume	Yunnan, China	Living	Mid-lobe 45 mm long, 4–6 mm wide, lateral 15–25 mm long, 3–5 mm wide, diverging from mid-lobe at 40–60°.	Pinnate, ~10 pairs secondary veins on mid-lobe and 6 pairs on lateral, secondary veins branching from midvein 50–80°.	2 mm	2 mm	No	This paper
O. mexicana (Standl.) J.-F Leroy	Central America	Living	Mid-lobe of wing ~40 mm long.	Unknown	7 mm	Unknown	Unknown	Manchester (1987)
O. pterocaroa Oerst.	Central America	Living	Mid-lobe of wing ~120 mm long.	Unknown	12 mm	Unknown	Unknown	Manchester (1987)

Additionally, we compared our fossils with other fossil fruits (Table 1) of *Palaeocarya*. Two of the previously described fossil fruits have some characteristics that are similar to the new fossil. In particular, *Palaeocarya olsoni* (Brown) Manchester has one septum in the nutlet and *Palaeocarya clarnensis* Manchester has hair on the nutlet (Manchester, 1987). The septum of the nutlet is very important in the taxonomy of Engelhardieae fruits, and the well-preserved septum in fossils is of great significance because the inner structure of the nutlet can bridge our connection between the fossils and extant fruits. *Oreomunnea* has eight compartments partitioned by thin septa rather than two or four compartments in *Engelhardia* and *Alfaropsis*. Four large compartments in *Alfaropsis* are formed by the intersection of a secondary septum with the primary septum, and each of these compartments is subdivided basally by a septum of tertiary rank resulting in the "eight-cell" condition (Manchester, 1987). So, *Oreomunnea* can be distinguished from *Engelhardia* and *Alfaropsis* if the inner structure is well preserved. Our fossil fruits have the septum in the nutlet, but still cannot be identified as an extant species because there are undetermined numbers of septa and compartments.

It is worth noting that our fossil fruits preserve the primary septum and the hairs on the nutlet surface, which are unique morphological characteristics compared with previously described fossils. Importantly, the wings of the fossil fruits resemble the species of *Palaeocarya* sect. *Monocosta* based on their typical pinnate venation (Manchester, 1987).

Furthermore, it seems that there are some morphological features (i.e., prophyllum or bracteole) on the nutlet of the holotype (Fig. 2A). The bracteolar lobes (prophyllum) in Engelhardieae are variable in *Alfaroa*, bracteolar lobes are rudimentary or absent; *Engelhardia* has paired bracteoles partly fused to the lower half of the ovary on the adaxial side to form a moderately high lobe to protect the ovary; *Oreomunnea* extends far above the stigmas to encase the entire pistil (Stone, 1972; Manos & Stone, 2001). If these features can be more confidently identified as relict bracteoles, then that could potentially provide further evidence for assigning the fossils to an extant species. The prophyllum is clearly preserved in this fossil (Fig. 2A), and there are potentially some relict features of the pistil on the nutlet (Fig. 2F). These remnant external characters may align the fossil fruits with *Engelhardia*, not *Alfaroa* or *Alfaropsis*.

After comparing the fossil materials with specimens of extant species of the genus, we conclude that the fossil specimens represent a hitherto undescribed taxon of *Palaeocarya*. Therefore, a new species, *Palaeocarya hispida* sp. nov., is proposed.

The winged fossil fruits reported in this study are designated to a new fossil species in the fossil genus *Palaeocarya*. However, the occurrence in the late Miocene suggests that they might be closely related to modern species of *Engelhardia*, *Alfaropsis*, and *Oreomunnea*. These fossils occur in the Upper Miocene Xiaolongtan Formation, where *Engelhardia* can be found currently (Fig. 1). Moreover, the Eocene and Pliocene *Palaeocarya* fossils also suggest close relationships with living plants (Jin, 2009; Xie et al., 2010). If there was more evidence regarding the anatomy of the fossil nutlets (e.g., number of fruit partitions and septa), or additional details on the stigma or stylar configuration were

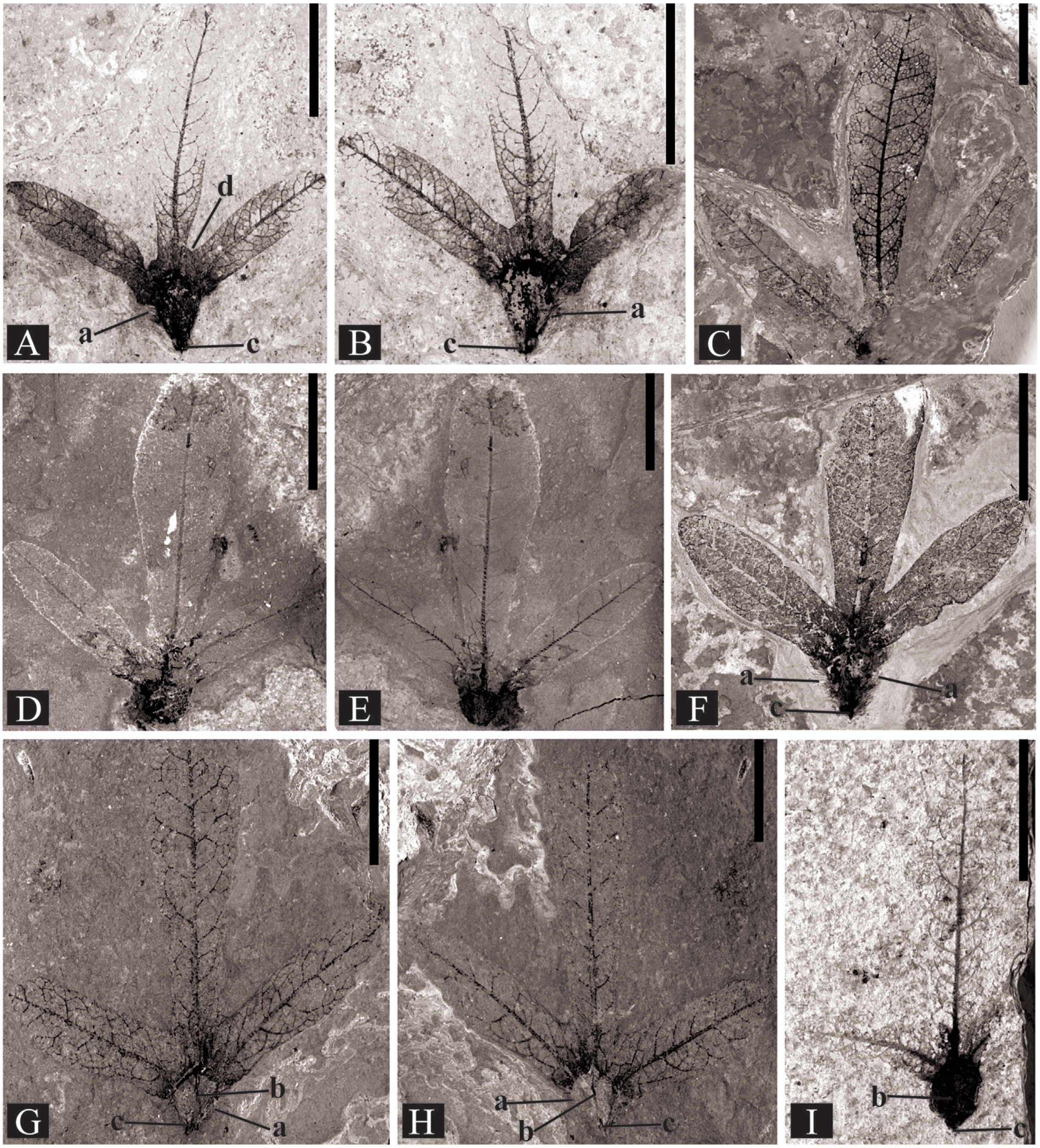

Fig. 2. Fossil fruits of *Palaeocarya hispida* from Wenshan, Yunnan, China. **A,** *DMS-0297*. **B,** *DMS-0296*. **C,** *DMS-0308*. **D,** *DMS-0306*. **E,** *DMS-0305*. **F,** *DMS-0298*. **G,** *DMS-0303*. **H,** *DMS-0302*. **I,** *DMS-0299*. a, Hispid hair (indumentum); b, Septum; c, Pedicle; d, Sinuses. Scale bars: A–I = 10 mm.

available, we could confirm the relationship among these fossil species and modern plants, *Engelhardia*, *Alfaropsis*, or *Oreomunnea* species.

Biogeographic implications

The biogeography of Engelhardieae remains unclear, although there are many hypotheses that addressed the biogeographic history of other members of the Juglandaceae (Stanford et al., 2000; Stone et al., 2009; Zhang et al., 2013). Fossil records indicate that *Palaeocarya* had a much wider distribution in the past than the current distributions of their modern relatives in *Engelhardia*, *Alfaropsis*, *Oreomunnea*, and *Alfaroa* (Fig. 1). The earliest fossil of *Palaeocarya* is recognized from the middle Eocene, approximately 46 Ma (Manos et al., 2007). The earliest fossil record of *Palaeocarya* in China is from the late Eocene at Changchang Basin, Hainan (Jin, 2009). Here, we address the biogeographic implications from the global fossil

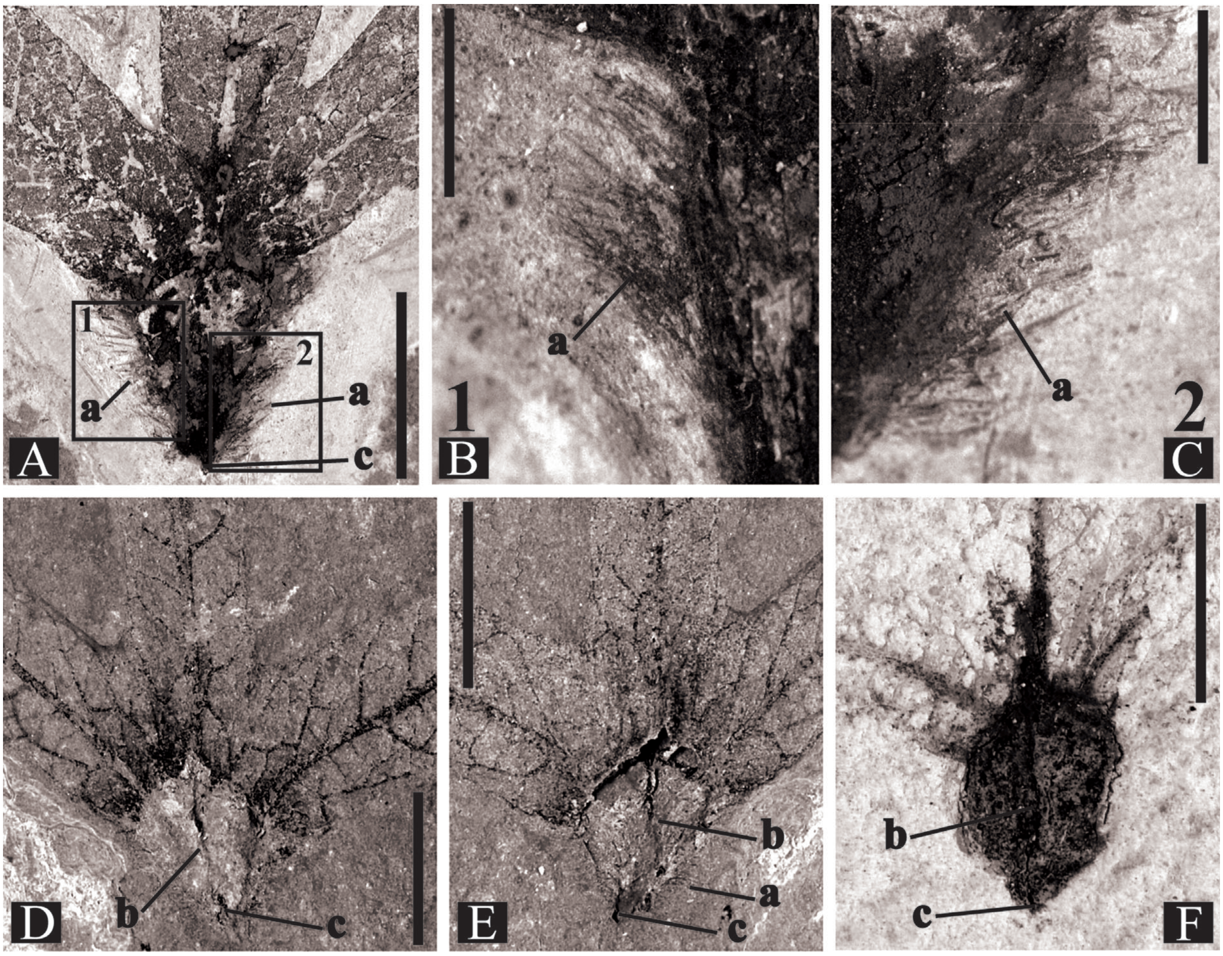

Fig. 3. Detailed characteristics of *Palaeocarya hispida*. **A,** Hispid hair on the nutlet, *DMS-0298*. **B,** Part 1 of A. **C,** Part 2 of A. **D,** Hispid hair and septum on the nutlet, *DMS-0303*. **E,** Hispid hair and septum on the nutlet, *DMS-0302*. **F,** Septum on the nutlet, *DMS-0299*. a, Hispid hair (indumentum); b, Septum; c, Pedicle. Scale bars: A, D–F = 50 mm; B, C = 500 μm.

records of *Palaeocarya* and its close relatives, the extant species of *Engelhardia*, *Alfaropsis*, and *Oreomunnea*.

The fossils illuminate the historic distribution and allow us to ask how geographic distributions have affected the evolutionary history of *Palaeocarya*, *Engelhardia*, *Alfaropsis*, and *Oreomunnea*. We will try to infer the biogeographic history based on fossil records and modern distributions.

The earliest known *Palaeocarya* fossil records are reported from the Eocene in North America, Europe and Hainan in China (Fig. 1; Table 1), indicating that *Palaeocarya* existed over a wide range across the Northern Hemisphere as early as the in Eocene. The nutlets of *Palaeocarya* were light and the trilobed wings contributed to the widespread dispersal of the seeds; the temperature in the Paleogene was warmer in the Northern Hemisphere (Li & Chen, 2002), which may also have contributed to the vast distribution range of *Palaeocarya*, even in high latitudes at that time.

We hypothesize that the local disappearance of *Palaeocarya* in Europe, North America, and the high latitudes of East Asia might have been the result of global cooling after the Eocene. During the Eocene, the Northern Hemisphere was dominated by a boreotropical flora (Wolfe, 1975; Mai, 1989), with the warmest climate during the Cenozoic (Tiffney & Manchester, 2001). *Palaeocarya* was presumably adapted to the warm climate and extended its largest geographic distribution across Europe and North America. After the Paleocene–Eocene Thermal Maximum (PETM), significant global cooling occurred over geological time (Zachos et al., 2001; Mosbrugger et al., 2005), and the Eocene–Oligocene cooling corresponded with a major decrease in thermophilic vegetation in northern latitudes (Tiffney & Manchester, 2001). After the PETM, the distribution of *Palaeocarya*'s relatives, that is, *Engelhardia*, *Alfaropsis*, and *Oreomunnea*, might have shrunk southward to favorable sites and survived in warmer regions, such as southeast and south Asia and Central America. We can speculate that *Palaeocarya* survived in temperate or subtropical floras of the Northern Hemisphere during the Eocene, along with other genera with this biogeographic pattern, such as *Ailanthus* (Simarubaceae), *Acer* (Aceraceae), *Alnus* (Betulaceae), *Bauhinia* (Leguminosae), *Carpinus* (Betulaceae), *Cornus* (Cornaceae), *Fagus* (Fagaceae), and *Zelkova* (Ulmaceae) (Mai, 1995; Denk et al., 2011; Su et al., 2013; Meng et al., 2014).

However, a younger fossil representative of *Palaeocarya*, *Palaeocarya olsoni*, was reported from the Miocene of western North America (Table 1), indicating that cooling had weak

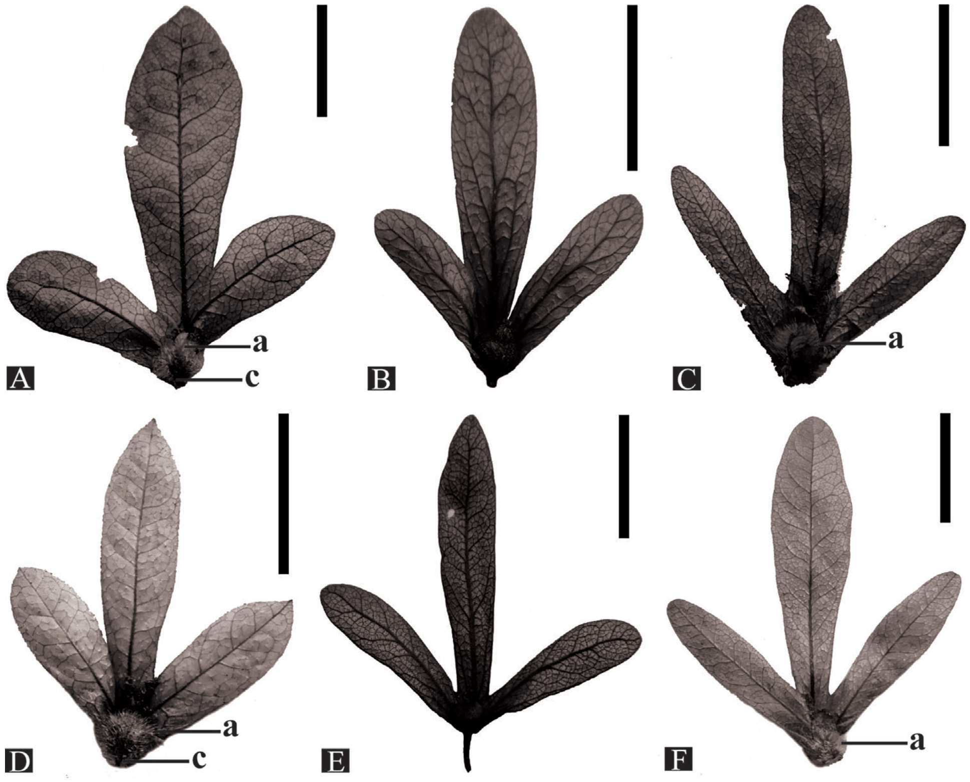

Fig. 4. Fruits of *Engelhardia* and *Alfaropsis*. **A,** *E. spicata* var. *aceriflora* (Reinwardt) Koorders & Valeton. **B,** *E. fenzelii* Merrill. **C,** *E. spicata* var. *colebrookiana* (Lindley) Koorders & Valeton. **D,** *E. serrata* Blume. **E,** *Alfaropsis roxburghiana* (Wall.) Iljinsk. **F,** *E. spicata* Leschen. ex Bl. a, Hispid hair (indumentum); c, Pedicle. Scale bars: A–F = 2 cm.

impacts on the distribution of *Palaeocarya*. An interval of relative warming during the early Miocene was short-lived and not as warm as the early and middle Eocene (Tiffney & Manchester, 2001), but it might have allowed *Palaeocarya* to expand northward to some extent. In addition, it is worth noting that global climate fluctuations were followed by a gradual cooling and a major ice sheet expansion in Antarctica and the Arctic from the late middle Miocene to early Pliocene (Zachos et al., 2001). The cooling following the middle Miocene progressed stepwise to the Pleistocene ice ages (Tiffney & Manchester, 2001), which might have caused *Palaeocarya*, along with other tropical or subtropical elements, to withdraw to low latitude zones to survive. A transition depicted from the early Miocene and Pliocene flora has illuminated a Miocene deciduous/conifer forest to a Pliocene Pinaceae-dominated flora richer in herbaceous forms and boreal forest (Fyles et al., 1994), which seems to support the disappearance of *Palaeocarya* at higher latitudes during the Miocene.

The remarkable climatic oscillations during the Quaternary instigated repeated cycles of habitat contraction–expansion and latitudinal–altitudinal shifts of species distributions, which have been described in many recent biogeographic studies of plant species, especially in temperate zones of the Northern Hemisphere (Petit et al., 1997; Hewitt, 2000; Meng et al., 2015). Although there were many climatic shifts from the Miocene to the Quaternary, we hypothesize that the geographic distribution patterns of *Palaeocarya* may have been associated with climatic oscillations during the Quaternary. The Last Glacial Maximum may have been a major factor contributing to the contraction of *Palaeocarya*'s distribution, rather than the drastic cooling after the PETM. Indeed, some plant phylogeographical research revealed that climatic oscillations during the Quaternary caused the geographical distribution ranges of other Juglandaceae plants to shrink, including *Platycarya strobilacea* Siebold & Zucc. and *Juglans mandshurica* Maxim. (e.g., Bai et al., 2010; Chen et al., 2012).

Further work is needed to combine molecular analyses with fossil records to test the biogeographic scenarios we have proposed and *Palaeocarya*'s response to paleo-climatic changes. Our biogeographic hypotheses are congruent with other paleo-geologic and paleo-climatic studies; however, limitations on our ability to test alternative scenarios also limit the robustness of inferences. Our scenarios should be cautiously considered as hypotheses and remain to be tested and refined by future studies integrating fossil records and molecular analyses.

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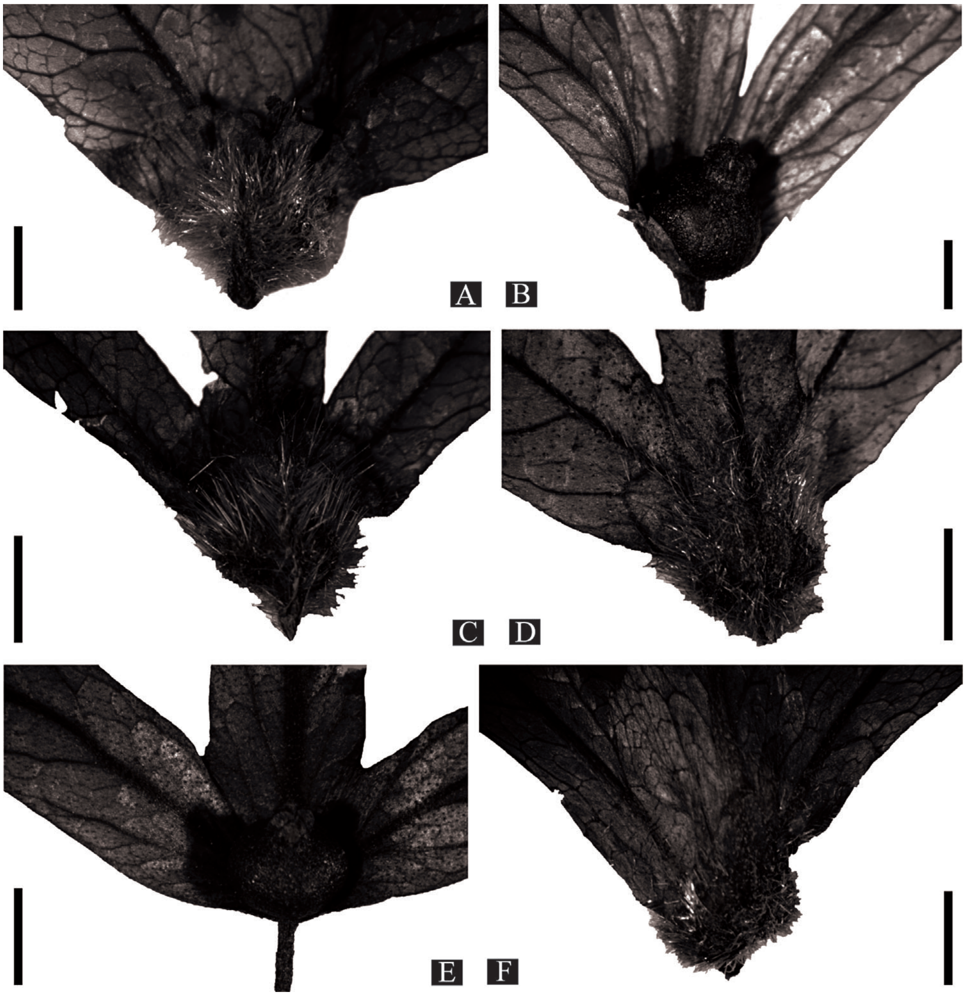

Fig. 5. Details of nutlet of *Engelhardia* and *Alfaropsis*. **A,** *E. spicata* var. *aceriflora* (Reinwardt) Koorders & Valeton. **B,** *E. fenzelii* Merrill. **C,** *E. spicata* var. *colebrookiana* (Lindley) Koorders & Valeton. **D,** *E. serrata* Blume. **E,** *A. roxburghiana* (Wall.) Iljinsk. **F,** *E. spicata* Leschen. ex Bl. Scale bars: A–F = 2 mm.

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