RESEARCH ARTICLE

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Asclepiadospermum gen. nov., the earliest fossil record of Asclepiadoideae (Apocynaceae) from the early Eocene of central Qinghai-Tibetan Plateau, and its biogeographic implications

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PREMISE: Apocynaceae is common in the fossil record, especially as seed remains from the Neogene of Europe and North America, but rare in Asia. Intrafamilial assignment is difficult due to the lack of diagnostic characters, and new fossil and modern data are needed to understand the paleobiogeography of this group.

METHODS: We studied three Apocynaceae seed impressions from the Lower Eocene Niubao Formation, Jianglang village, Bangor County, central Qinghai-Tibetan Plateau. Morphological data from living and fossil species were phylogenetically mapped to enable systematic assignment.

RESULTS: We describe a new genus, *Asclepiadospermum* gen. nov., and two new species, *A. marginatum* sp. nov. and *A. ellipticum* sp. nov. These species are characterized by an elliptical seed, a margin surrounding the central part of the seed, and polygonal, irregular, and small epidermal cells, and differ mainly in terms of the size of the margin and the shape of the apex. All these characters indicate that this new genus belongs to the subfamily Asclepiadoideae (Apocynaceae).

CONCLUSIONS: These fossils represent the earliest fossil seed records of Asclepiadoideae. *Asclepiadospermum* indicates a humid tropical to subtropical flora during the early Eocene in central Tibet. Moreover, our discoveries indicate a close floristic connection between Eurasia and Africa during the early Eocene, which expands our knowledge of the floristic linkage between Tibet and other regions at that time.

KEY WORDS Asclepiadoideae; biodiversity; biogeography; fossil; paleobotany; paleoenvironment; phylogeny; Qinghai-Tibetan Plateau; seed.

Apocynaceae Juss. is widely distributed in subtropical and tropical regions. It includes 366 genera and ~4500 species (Endress et al., 2014; Fishbein et al., 2018), mainly lianas, herbs, and trees. The family was first described by Jussieu (1789) and subsequently was divided into two closely related families, Apocynaceae (subfamilies Rauvolfioideae and Apocynoideae) and Asclepiadaceae (subfamilies Periplocoideae, Secamonoideae, and Asclepiadoideae), based on pollen characters (Brown, 1810). However, recent molecular phylogeny studies show that Asclepiadaceae is nested within the Apocynaceae, based in particluar on the finding that Periplocoideae is closer to Apocynoideae than other Asclepiadaceae subfamilies (Senblad and Bremer, 1996; Potgieter and Albert, 2001). Based on the latest molecular and morphological data, the reunification of the two families was proposed by Endress and Bruyns (2000), resulting in a family (Apocynaceae) containing ~370 genera in five subfamilies—Rauvolfioideae, Apocynoideae, Periplocoideae, Secamonoideae, and Asclepiadoideae (Endress and Bruyns, 2000; Endress et al., 2014)—and included in the order Gentianales (APGIV, 2016). The subfamilies Rauvolfioideae and Apocynoideae are considered paraphyletic, whereas Periplocoideae, Secamonoideae, and Asclepiadoideae remain monophyletic (Rapini et al., 2007; Fishbein et al., 2018; Nazar et al., 2019). In particular, Secamonoideae and Asclepiadoideae form a monophyletic group (Nazar et al., 2019).

This family is common in the fossil record (Martínez-Millán, 2010). The earliest of the remains assigned to the Apocynaceae, namely *Paraapocynaceoxylon barghoorni* Wheeler, Lee et Matten, is a late Cretaceous wood from the McNairy Formation, USA (Wheeler et al., 1987). However, the affinity has not been fully confirmed and the assignment is considered dubious (Wheeler et al., 1987). A leaf, *Apocynophyllum helveticum* Heer, and a pollen form, *Tabernaemontana* cf. *T. coronaria* Leopold and Clay-Poole, 2001, were recovered from middle and late Eocene sediments of Germany and the United States, respectively (Wilde, 1989; Leopold and Clay-Poole, 2001). However, most of the fossil records are from Oligocene and Neogene sediments of North America and Europe (Martínez-Millán, 2010).

Fossil seeds attributed to Apocynaceae represent the most important records in terms of numbers of species and occurrences (Table 1). Fossil Apocynaceae seeds are mainly recognizable by a fusiform or spatulate shape and the presence of a coma (or tuft of hairs) at the apex. According to the seed record, during the middle Eocene to the middle Miocene, Apocynaceae was distributed only in North America (Brown, 1929; Macginitie, 1969; Manchester, 2001) and Europe (Unger, 1850; Heer, 1859; Saporta, 1889; Reid and Chandler, 1926; Weyland, 1938; Rüffle, 1963; Kvaček and Walther, 1995; Wilde and Frankenhäuser, 1998; Hably et al., 2000; Sakala, 2000; Collinson et al., 2012). However, for most of these seed fossils, a lack of diagnostic characters makes it difficult to give them systematic assignment. In Asia, only one Apocynaceae-like leaf has been recorded from the early Cenozoic of central Asia (Aktyubinsk, Kazakhstan; Vassiljev, 1976). As far as Asclepiadoideae is concerned, only one seed, namely *Tylophora antiqua* Reid & Chandler from the early-middle Oligocene of Europe, has been described (Reid and Chandler, 1926). The leaf-and-seed genus *Acerates* from the middle Miocene of Germany is also considered to represent the Asclepiadoideae (Heer, 1859).

The Qinghai-Tibetan plateau (QTP) is the highest and largest plateau in the world, with a total surface of ~2.5 million km^2 (Zhang et al., 2014). The elevation of the central plateau exceeds, on average, 4500 m. In general, modern vegetation in the center of the QTP comprises alpine desert, steppe, and meadow (Scientific Expedition Team to the Qinghai-Xizang Plateau, 1988; Ni and Herzschuh, 2011). However, newly discovered fossils from the QTP evidence a succession of different environments throughout the Cenozoic (Wu et al., 2017; Su et al., 2019) influenced by plateau-orogeny (Harris, 2006; Song et al., 2009; Spicer, 2017; Su et al., 2019). Thus, newfound paleobotanical data for the QTP are pivotal for understanding the paleoenvironmental and biogeographic history of Southwest China.

The Eocene is a key period of diversification and intensive exchange of flora in the Northern Hemisphere (Wolfe, 1985; Manchester, 1999). The study of fossils from the Eocene of the central QTP is crucial to understanding the biogeographic connections and contribution of Tibet to other regions early in the orogeny of Tibet. However, our understanding of Paleogene plant diversity in central Tibet is still far from sufficient, with only a few taxa having been reported recently from the Lower Eocene Niubao Formation (Liu et al., 2019; Tang et al., 2019) and the Upper Oligocene Dingqing Formation (Jia et al., 2018; Jiang et al., 2019; Su et al., 2019).

Here, we report well-preserved fossil seeds from the Lower Eocene Niubao Formation in the central QTP, including a new genus, *Asclepiadospermum* gen. nov., and two new species, *A. marginatum* sp. nov. and A. *ellipticum* sp. nov. These discoveries attest the presence of a tropical to subtropical climate during the Eocene in Tibet. Moreover, these seeds are the earliest fossil record for the Asclepiadoideae and are important in documenting the floristic connection between Africa and Eurasia during the Eocene.

MATERIALS AND METHODS

Geological setting

The fossil seeds presented here are from Jianglang village, Bangor County, Lunpola Basin, which today is at an altitude of ~4800 m (Fig. 1; 31°37.5′N, 90°1.5′E). The Lunpola Basin is located along the Bangong-Nujiang Suture in the central QTP, and the Cenozoic strata in the basin consist of the Niubao Formation and Dingqing Formation (Rowley and Currie, 2006; Hetzel et al., 2011; Liu et al., 2019; Tang et al., 2019). The Niubao Formation is composed of reddish clastic deposits, dominated by mudstone, sandstone, and gravel that indicate a fluvial to lacustrine paleoenvironment (Rowley and Currie, 2006; Hetzel et al., 2011; Wu et al., 2016). The age of the Niubao Formation ranges from Paleocene to Eocene, based on ostracods, insects, and paleobotanical evidence (Xia, 1982; Szwedo et al., 2015; Tang et al., 2019; Wang et al., 2019) as well as stratigraphic correlation (Wu et al., 2016). The fossil seeds described here were excavated from lacustrine strata within the middle section of the Niubao Formation, which is assigned to the early Eocene on the basis of current knowledge (Bureau of Geology and Mineral Resources of Xizang Autonomous Region, 1997; Liu et al., 2019; Tang et al., 2019).

Morphological observations

The fossils are deposited in the Paleoecology Collections of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. The specimens were observed using a stereo microscope (Leica S8APO) and photographed with a smart digital microscope (Zeiss Smart Zoom 5). *Asclepiadospemum* seeds are described following the terminology of Reid and Chandler (1926). Measurements were taken using ImageJ version 1.8 (Rasband, 2016).

A detailed examination of the morphology of the seeds of genera within Apocynaceae allows us to place the fossils more precisely within the family. Comparisons with the living species in Apocynaceae were made using specimens obtained from the Xishuangbanna Tropical Botanical Garden (XTBG) herbarium (HITBC), living collections in XTBG, and the Muséum national d'Histoire naturelle de Paris (P). We examined 105 species representing the five main subfamilies in order to have an overview of seed shape diversity in Apocynaceae (Appendix S1). We here illustrate one species from each of 18 genera, representative of the diversity of seed morphologies found in the five subfamilies of Apocynaceae. Epidermal studies were carried out using living samples of *Cosmostigma* and *Dregea*. The epidermal observations, within an area of approximately 0.5 × 1 cm^2, were made from the center and periphery of the seeds. First, we applied clear nail polish directly to the selected area of the seed surface and then waited for ~15 min. When it was dry enough, we carefully removed the nail polish from the surface of the seed and put it on slides for observation. Images were photographed using a light microscope (Leica DM1000) attached to a camera (Leica DFC295).

A literature review of fossil seeds of Apocynaceae was made in order to compare existing taxa with our fossils (Table 1). We evaluated this record using the criteria of Martínez-Millán (2010), such

TABLE 1. Occurrence and acceptance status of Apocynaceae fossil seeds.

Species	Organ	Locality	Age	References	Accepted?
Asclepiadospermum marginatum	Seed	Jianglang, China	E Eocene	This study	Yes
Asclepiadospermum ellipticum	Seed	Jianglang, China	E Eocene	This study	Yes
Acerates firma Heer	Seed and leaf	Oeningen, Germany	M Miocene	Heer, 1859	Needs revision
Acerates veterana Heer	Seed and leaf	Oeningen, Germany	M Miocene	Heer, 1859	Needs revision
Apocynospermum coloradensis Brown	Seed	Green River	M Eocene	Brown, 1929; Macginitie, 1969	Yes
Apocynospermum dubium Reid et Chandler	Seed	Bembridge, England	E-M Oligocene	Reid and Chandler, 1926	Yes
Apocynospermum elegans Reid et Chandler	Seed	Bembridge, England	E-M Oligocene	Reid and Chandler, 1926	Yes
Apocynospermum rostratum Reid et Chandler	Seed	Bembridge, England	E-M Oligocene	Reid and Chandler, 1926	Yes
Apocynospermum sp.	Seed	Florissant, Colorado	L Eocene-E Oligocene	Manchester, 2001	No
Apocynospermum sp.	Seed	Budapest-Obuta	E Oligocene	Hably et al., 2000	Yes
Apocynospermum striatum Reid et Chandler	Seed	Bembridge, England	E-M Oligocene	Reid and Chandler, 1926	Yes
Apocynospermum striatum Reid et Chandler	Seed	Northern Bohemia	Oligocene	Kvaček and Walther, 1995	Yes
Apocynospermum striatum Reid et Chandler	Seed	Northern Bohemia	L Miocene	Sakala, 2000	Yes
Cypselites angustus Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites aquensis Saporta	Seed	Aix, France	L Oligocene	Saporta, 1889	Yes
Cypselites bisulcatus Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites brachypus Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites cincinnatus Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites costatus Heer	Seed	Rott, Germany	L Oligocene	Weyland, 1938	Yes
Cypselites costatus Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites deletus Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites dubious Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites ellipticus Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites fischeri Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites fractus Saporta	Seed	Aix, France	L Oligocene	Saporta, 1889	Yes
Cypselites grandis Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites lessingi Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites naegelii Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites regeli Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites rostratus Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites schulzii Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites sp.	Seed	Messel, Germany	M Eocene	Collinson et al., 2012	Yes
Cypselites sp.	Seed	Lenningen, Germany	E Miocene	Rüffle, 1963	Needs revision
Cypselites sp.	Seed	Eifel, Germany	M Eocene	Wilde and Frankenhäuser, 1998	Needs revision
Cypselites spoliatus Saporta	Seed	Aix, France	L Oligocene	Saporta, 1889	Yes
Cypselites striatus Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites tenuirostratus Saporta	Seed	Aix, France	L Oligocene	Saporta, 1889	Yes
Cypselites tenuis Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites truncatus Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cypselites ungeri Heer	Seed	Oeningen, Germany	M Miocene	Heer, 1859	Yes
Cyspelites trisulcatus Saporta	Seed	Aix, France	L Oligocene	Saporta, 1889	Yes
Echitonium microspermim Ung	Seed and leaf	Croatia	?	Unger, 1850	Needs revision
Echitonium obovatum Ung	Seed and leaf	Croatia	?	Unger, 1850	Needs revision
Echitonium superstes Ung	Seed and leaf	Croatia	?	Unger, 1850	Needs revision
Phyllanthera vectensis Reid et Chandler	Seed	Bembridge, England	E-M Oligocene	Reid and Chandler, 1926	Yes
Tylophora antiqua Reid et Chandler	Seed	Bembridge, England	E-M Oligocene	Reid and Chandler, 1926	Yes

as the availability of diagnostic characters and adequate illustrations. We classified the occurrences as "accepted," "not accepted" (only one occurrence, *Apocynospermum* sp., based on the opinion of Martínez-Millán, 2010), or "in need of revision."

Phylogenetic mapping

We used a simplified topology of the molecular phylogenetic tree from the latest Apocynaceae phylogeny (Nazar et al., 2019) as a backbone for mapping the morphological characters of seeds. We selected 34 genera corresponding to the genera present in the molecular phylogeny analyses (Nazar et al., 2019) and in our morphological study (Appendix S1): 19 in Asclepiadoideae, two in Secamonoideae, six in Apocynoideae, five in Periplocoideae, and two in Rauvolfioideae. These genera cover all the subfamilies in the Apocynaceae and generally represent the range of variation in seed morphology in the family. The two genera in Rauvolfioideae are considered the outgroup in our analysis, taking into account the very different shapes of the

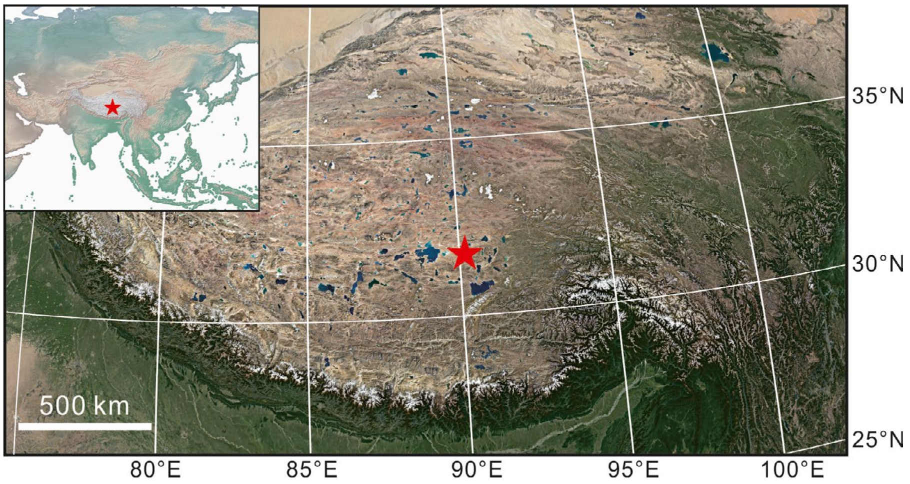


FIGURE 1. Location of the Jianglang section, Qinghai-Tibetan Plateau, China.

seeds compared to the fossils described here and the basal position of this group. We defined eight morphological characters based on seeds (Table 2). The morphological matrix contains 272 combinations (Appendix S2) with three unknown character states (1.1%) and 14 inapplicable character states (5.1%). Mapping of characters was done using Mesquite version 3.6 (Maddison and Maddison, 2001) with the parsimony ancestral states option. The raw data from Mesquite are presented in Appendix S3.

SYSTEMATICS

Family—Apocynaceae Juss.
Genus—*Asclepiadospermum* C. Del Rio, T. Su & Z.-K. Zhou gen. nov. (Figs. 2 and 3)
Type species—*Asclepiadospermum marginatum* C. Del Rio, T. Su & Z.-K. Zhou sp. nov.
Genus diagnosis—Seed elliptical or pear-shaped, tapering rapidly toward a blunt to slightly round apex, and rounded at the base; an oval to elliptical central part surrounded by a margin; a straight median line corresponding to the trace left by the raphe runs from the apex to the center of the seed (Fig. 2: 5; Fig. 3: 2). Cells polygonal and irregularly arranged, but often arranged in rows at the margin (Fig. 2: 6, 7; Fig. 3: 4).

TABLE 2. Morphological characters and character states used for phylogenetic mapping.

Character/state of character	0	1	2
1. Size of the seed (length)	<10 mm	>10 mm	
2. Shape of the seed	Elliptical	Spatulate	Elongate
3. Margin surrounding the central part of the seed	Absent	Present	
4. Coma at the apical part of the seed	Absent	Present	
5. Central ornamentation of the seed	No	Rugulate	
6. Relative size of the margin	Narrow	Wide	
7. Seed compressed laterally	No	Yes	
8. Position of the raphe	Apical to central part	Crossing the seed length	

Other species—*Asclepiadospermum ellipticum* C. Del Rio, T. Su & Z.-K. Zhou sp. nov.
Etymology—*Asclepiadospermum* refers to the affinity of the seeds with the subfamily Asclepiadoideae.
Species—*Asclepiadospermum marginatum* C. Del Rio, T. Su & Z.-K. Zhou sp. nov. (Fig. 2)
Diagnosis—Seed pear-shaped, tapering rapidly toward a blunt to slightly round apex with a small bump, and rounded at the base; central part oval and smooth, surrounded by a wide margin; a straight median line corresponding to the trace left by the raphe runs from the apex to the center of the seed. Cells are polygonal and irregularly arranged.
Etymology—The specific epithet *marginatum* refers to the exceptionally wide margin that characterizes this species.
Holotype—XZBGJL5-0432 (Fig. 2: 1, 2)
Reposition—Paleoecology Collections, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences
Stratigraphy—The Lower Eocene Niubao Formation
Type locality—Jianglang village, Bangor County, Central Qinghai-Tibetan Plateau, China (31°37.5′N, 90°1.5′E)
Paratype—XZBGJL5-0116 (Fig. 2: 3)
Description—Seed pear-shaped (Fig. 2: 1–3), compressed laterally, length 6.0–7.1 mm, width 4.0–4.3 mm; tapering rapidly toward a blunt to slightly round and asymmetrical apex, ~0.8 mm wide, with a small bump (Fig. 2: 4; corresponding to the attachment zone of a coma, not preserved here), and rounded at the base; central part oval and smooth, length 2.6 mm, width 1.6–1.8 mm, surrounded by a wide margin, 1.2–1.6 mm laterally and in the basal part, 1.7–2.6 mm in the apical part; a straight median line (Fig. 2: 5) goes from the apex to the beginning of the central part, corresponding to the trace left by the vascularization of the seed or raphe; cells (Fig. 2: 6, 7) 22–40 μm wide (mean 29.8 μm wide), polygonal, and irregularly arranged, but usually arranged in rows at the margin (Fig. 2: 6).
Species—*Asclepiadospermum ellipticum* Del Rio, T. Su & Z.-K. Zhou sp. nov. (Fig. 3)
Diagnosis—Seed elliptical, tapering rapidly toward a blunt to slightly round and asymmetrical apex and rounded at the base; central part elliptical and irregularly spotted, surrounded by a thin margin with a straight median line corresponding to the trace left by the raphe from the apex to the center of the seed. Cells small (~30 μm wide), polygonal, and irregularly arranged.
Etymology—The specific epithet *ellipticum* refers to the elliptical shape of the seed.
Holotype—XZBGJL5-0459, XZBGJL5-0514
Reposition—Paleoecology Collections, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences
Stratigraphy—The Lower Eocene Niubao Formation
Type locality—Jianglang village, Bangor County, Central Qinghai-Tibetan plateau, China

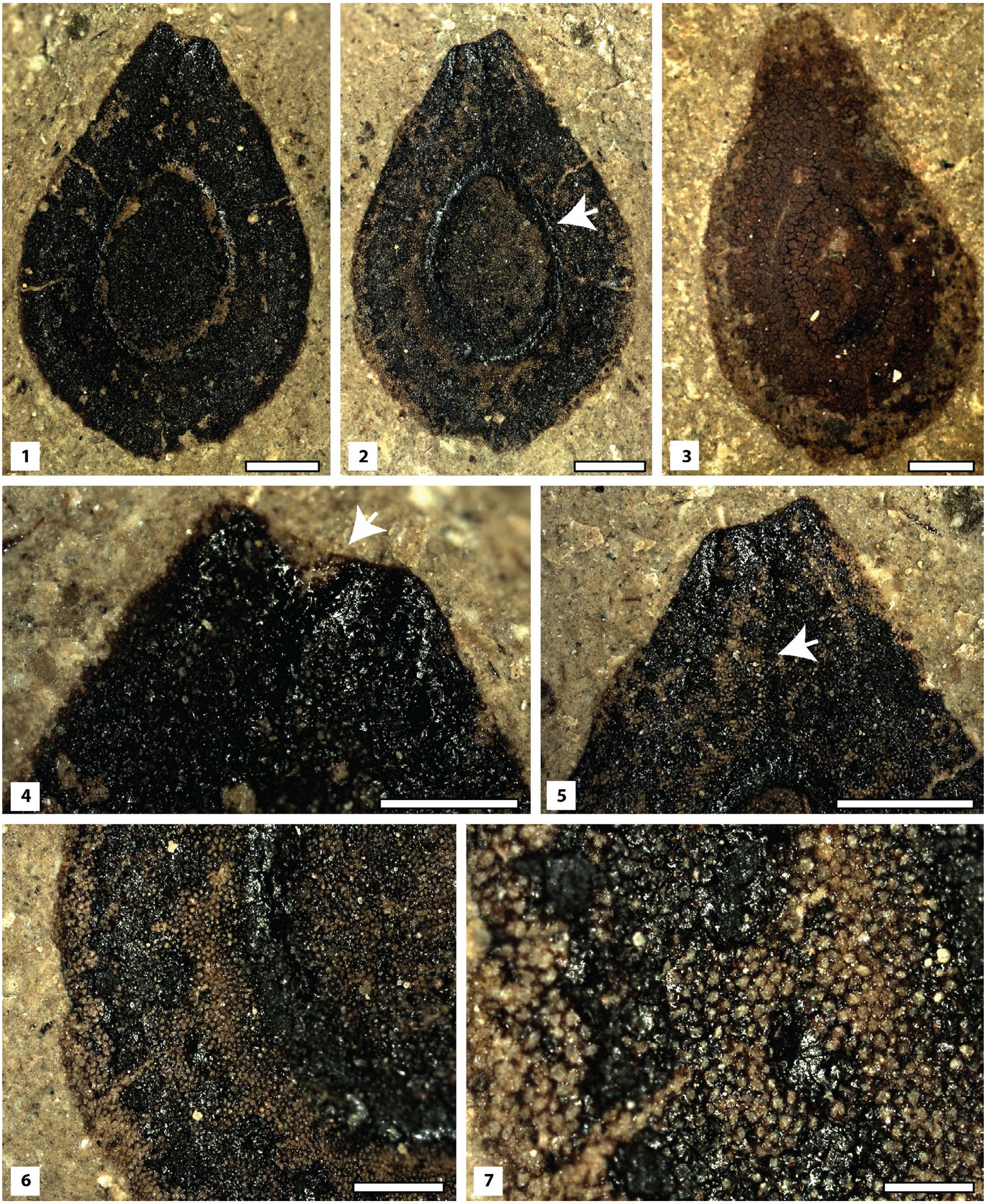


FIGURE 2. *Asclepiadospermum marginatum* C. Del Rio, T. Su & Z.-K. Zhou sp. nov. (1, 2) Holotype (XZBGJL5-0432); arrow = center of the seed surrounded by a wide margin. (3) Paratype (XZBGJL5-0116). (4) Apex (XZBGL5-0432 A); arrow = straight apex with a small bump. (5) Straight line corresponding to the raphe (XZBGJL5-0432 B). (6) Detail of the margin (XZBGJL5-0432 B). (7) Detail of the cell's organization (XZBGJL5-0432 B). Scale: (1–3, 5) = 1 mm; (4, 6) = 500 µm; (7) = 200 µm.

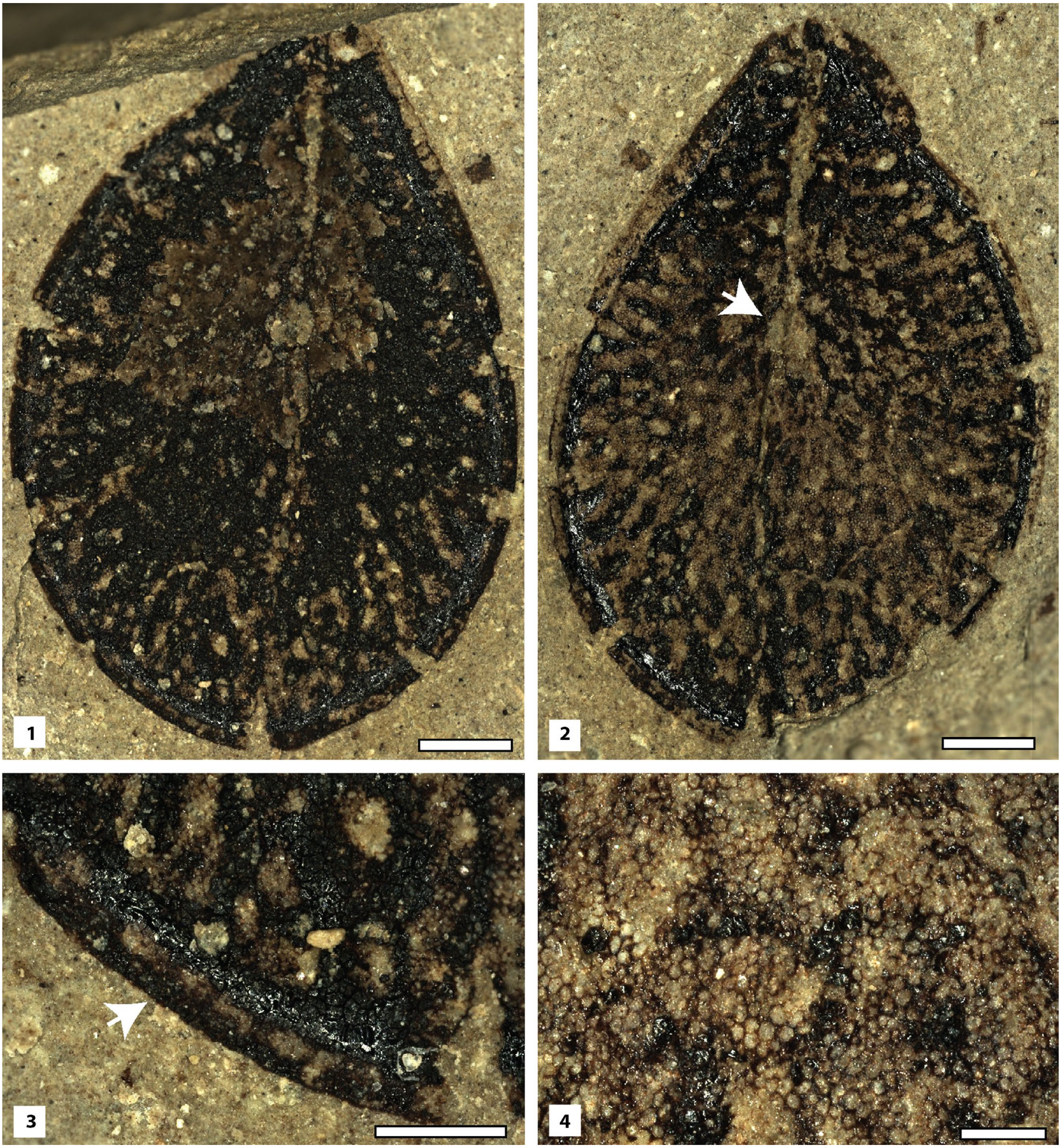


FIGURE 3. *Asclepiadospermum ellipticum* C. Del Rio, T. Su & Z.-K. Zhou sp. nov. (1, 2) Holotype (1 = XZBGJL05-0459, 2 = XZBGJL05-514); arrow = raphe with the central chalaza. (3) Small margin surrounding the central part (arrow, XZBGJL05-0459). (4) Detail of the cell's organization (XZBGJL05-514). Scale: (1, 2) = 1 mm; (3) = 500 µm; (4) = 200 µm.

Description—Seed elliptical (Fig. 3: 1, 2), compressed laterally; length 8 mm, width 5.4 mm; tapering rapidly toward a blunt to slightly round and asymmetrical apex, width ~1 mm (Fig. 3: 1, 2) and rounded at the base; central part elliptical, irregularly spotted, length 7.2 mm, width 4.9 mm, surrounded by a thin margin (Fig. 3: 3), 0.2–0.3 mm laterally and basally, ~0.5 mm in the apical part; a straight median line (Fig. 3: 1, 2) goes from the apex to the central part, corresponding to the trace left by the vascularization of the seed or raphe, and diverges at the central area (which corresponds to the chalaza; Fig. 3: 2, arrow); cells (preserved only in the central part) 24.7–47.6 µm wide (mean 32.8 µm wide), polygonal, and irregularly arranged (Fig. 3: 4).

Phylogenetic character mapping—Twenty-eight steps were necessary to reconstruct the evolutionary history of Apocynaceae using morphological characters (CI = 0.32, RI = 0.67). In Apocynaceae, subfamilies except for Rauvolfioideae are characterized by the presence of a coma (Fig. 4: character 4, state 1 [ch. 4-1]). Only basal genera belonging to Rauvolfioideae and some genera in Apocynoideae do not have laterally compressed seeds (Fig. 4: ch. 7-1). The presence of a margin is diagnostic for the clade Asclepiadoideae-Secamonoideae (Fig. 4: ch. 3-1). The presence of the raphe running from the apex to the center of the seed is diagnostic to Asclepiadoideae (Fig. 4: ch. 8-1). Small seed size (<10 mm) is found in three indirectly related clades (Fig. 4: ch. 1-0). Seeds with an elliptical shape appear to be diagnostic for the tribe Asclepiadeae (Fig. 4: ch. 2-0) but also occur in genera in the two other tribes (i.e., *Dregea* and *Caralluma*). Granular or spotted ornamentation in the center of the seed (Fig. 4: ch. 5-1) as well as a wide margin (Fig. 4: ch. 6-1) are found only within Asclepiadoideae, but across indirectly related genera.

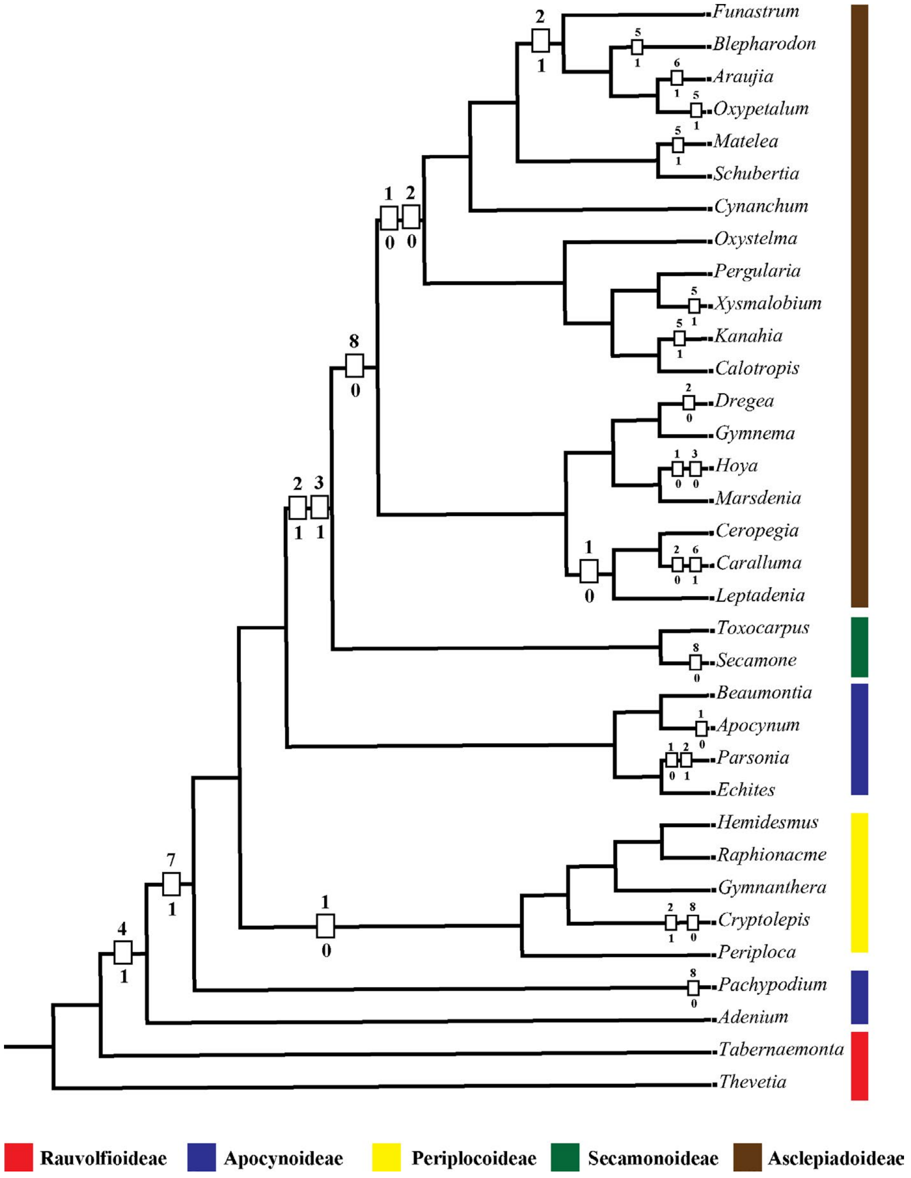


FIGURE 4. Simplified phylogeny of the Apocynaceae from Nazar et al. (2019) and mapping of morphological characters using Parsimony ancestral states in Mesquite version 3.6. White rectangle indicates synapomorphy or autapomorphy.

DISCUSSION

Systematic affinity

Asclepiadospermum is characterized by the elliptical shape of the seed, with a straight apex, a center surrounded by a margin, the presence of the raphe running to the apical part from the center of the seed, and polygonal small cells irregularly arranged (Figs. 2 and 3). To our knowledge, these characters are found only in the Apocynaceae. The family Cucurbitaceae also has seeds with elliptical shape and a small margin; however, the raphe is not apparent and the apices are more rounded, with no bump. The same cell shape, size, and arrangement were found in modern Apocynaceae species (Fig. 5). The straight apex, with the small bump in *A. marginatum*, attests the presence of a coma, not preserved here. In fact, the coma tends to detach from the seed when the fruit is mature and seed drops on the ground (C. Del Rio et al., personal observation). In the latest phylogeny of Apocynaceae (Nazar et al., 2019), a coma is present in all the subfamilies except the basal Rauvolfioideae (Fig. 4; Endress and Bruyns, 2000; Collinson et al., 2012). The presence of a margin surrounding the central part of the seed is shared by both subfamilies Secamonoideae and Asclepiadoideae (Fig. 6: 1–20; Fig. 4), and is mostly absent in Periplocoideae and Apocynoideae (Fig. 6: 21–32). However, the pear-like or elliptical seed shape is diagnostic for the tribe Asclepiadeae (Fig. 4) and more generally is found in all tribes and in several genera of Asclepiadoideae (e.g., *Dregea* E. Mey., *Cosmostigma* Wight, *Pachycymbium* L.C. Leach, *Pentarrhinum* E. Mey., and *Xysmalobium* R. Br.; Fig. 6). A few species in Secamonoideae (e.g., *Pervillaea tomentosa* Decne and *Secamone perrieri* Choux) have a very similar shape (Fig. 6: 17, 18). But in these, the apical part is flared and the length/width ratio (~1.8) is quite wide compared to our fossils (~1.5). The size of the

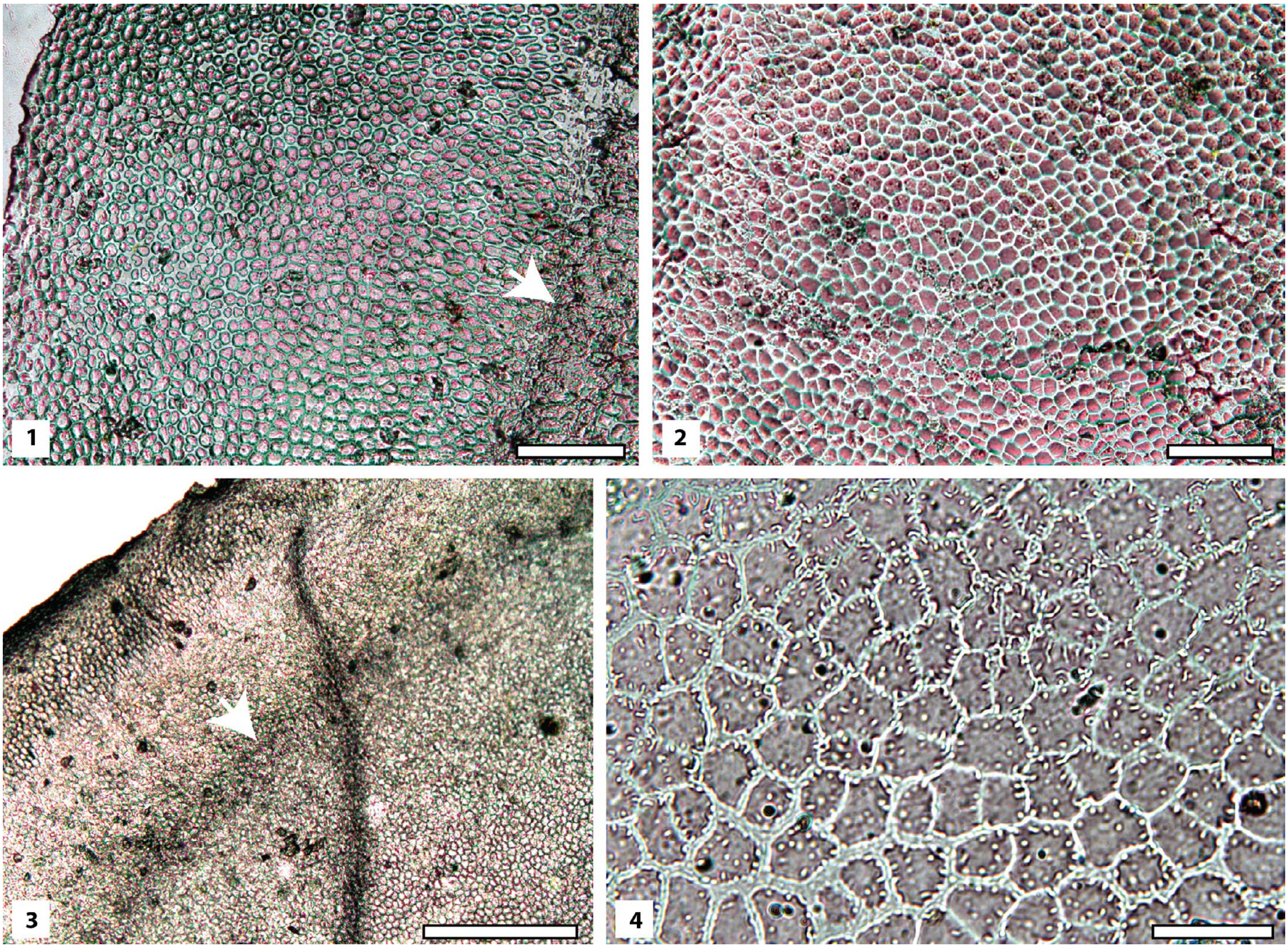


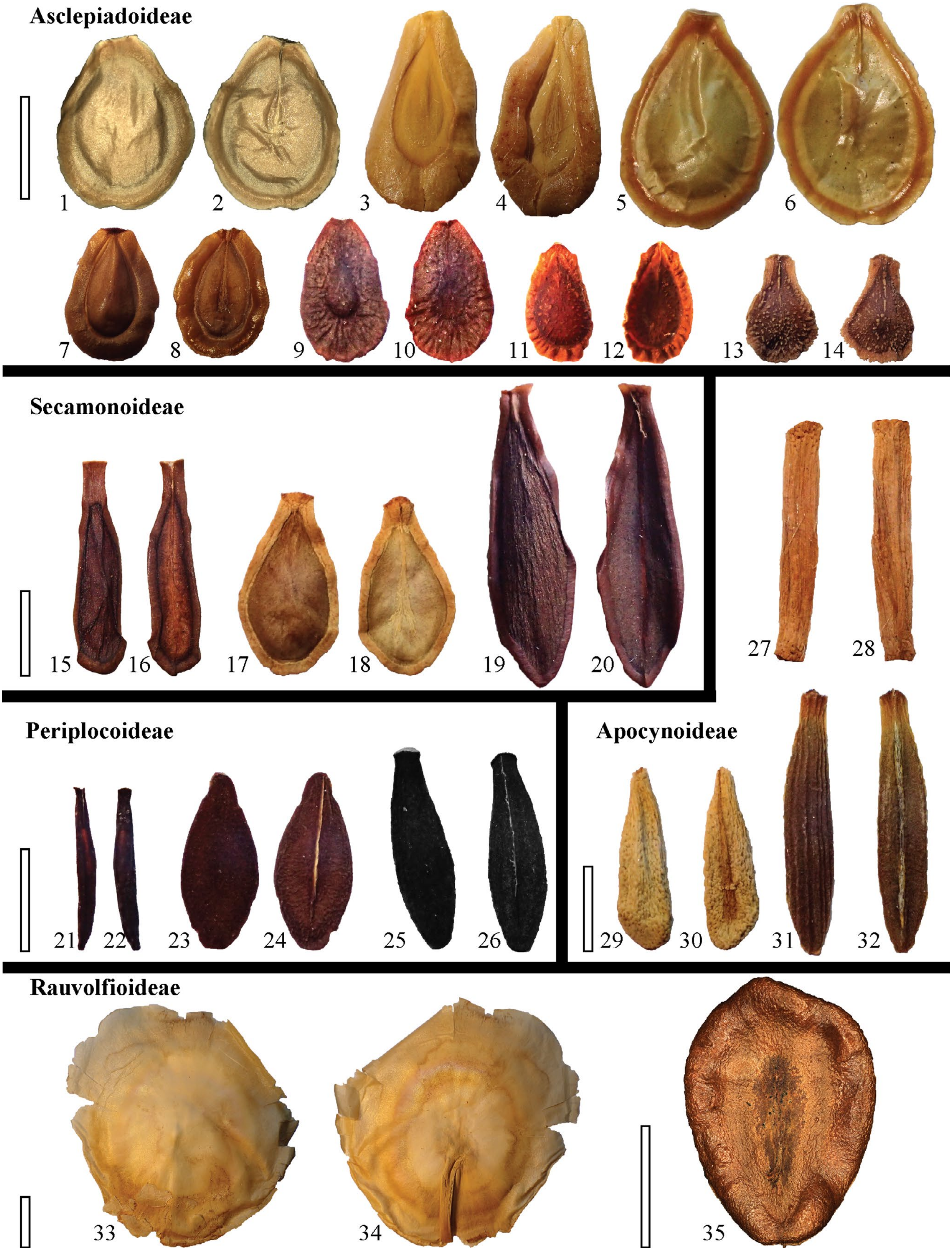
FIGURE 5. Tegument of two modern Asclepiadoideae species. (1) Cells of the margin of *Cosmostigma cordatum* (Poir.) M.R.Almeida (living collection XTBG). (2) Same, with a focus on cells from the central part. (3) Cells of the margin and the central part of *Dregea volubilis* (L.f.) Benth. Ex Hook.f. (living collection XTBG). (4) Same, with a focus on cells from the central part. Arrows = limit between the margin and the central area. Scale: (1) = 200 µm; (2) = 100 µm; (3) = 500 µm; (4) = 50 µm.

seeds included in *Asclepiadospermum* also corresponds to the range seen in Asclepiadoideae, unlike Secamonoideae (Fig. 4). Moreover, the presence of the raphe running from the apex to the central part of the seed, and not crossing the seed, is diagnostic for the Asclepiadoideae. Thus, we include *Asclepiadospermum* in subfamily Asclepiadoideae.

Given the uncertainty about the tribe or genus assignment of these fossils, we describe a new morphological genus named *Asclepiadospermum*. In the fossil record, *Cypselites* Heer (= *Apocynospermum* Reid & Chandler) refers to seeds that could be assigned to Apocynaceae (Heer, 1859; Reid and Chandler, 1926). However, this genus encompasses fusiform and spatulate (more elongate and narrower) seeds that are more common in Apocynoideae and Periplocoideae (Figs. 4 and 6) than in Asclepiadoideae. The genera *Acerates* Heer and *Echitonium* Unger contain fossils similar to our species (especially *Acerates*); however, both those genera were reported as leaves and seeds, without a detailed description of the seeds (i.e., only the overall shape was described; Unger, 1850; Heer, 1859).

Asclepiadospermum marginatum and *A. ellipticum* are characterized by exhibiting an elliptical to pear shape, the presence

FIGURE 6. The morphological diversity of modern seeds in Apocynaceae. (1, 2) *Dregea vollubilis* (L. f.) Benth. ex Hook. f. (living collection, XTBG). (3, 4) *Caralluma flava* N.E. Br. (P03895898). (5, 6) *Raphistemma pulchellum* (Roxb.) Wall. (P04918819). (7, 8) *Pachycymbium decaisneanum* (Lem.) M.G. Gilbert (P03859902). (9, 10) *Pentarrhinum insipidum* E. Mey. (P04225372). (11, 12) *Xysmalobium heudelotianum* Decne. (P00519971). (13, 14) *Oxypetalum capitatum* Mart. (P03903235). (15, 16) *Genianthus laurifolius* (Roxb.) Hook. f. (Li Yanhui 2139, HITBC). (17, 18) *Pervillaea tomentosa* Decne. (NA, P). (19, 20) *Toxocarpus villosus* (Blume) Decne. (Tao Guoda 7176, HITBC). (21, 22) *Baroniella camptocarpoides* Costantin & Gallaud (P04215915). (23, 24) *Cryptolepis volubillis* (Balf.f.) O.Schwartz (P03902170). (25, 26) *Periploca laevigata* Aiton, (P04221700). (27, 28) *Adenium multiflorum* Klotzsch (P04238651). (29, 30) *Pachypodium lamerei* Drake (NA, P). (31, 32) *Alafia barteri* Oliv. (P00088313). (33, 34) *Aspidosperma macrocarpon* Mart (Ratter & Ramos 341, P). (35) *Allamanda schottii* Pohl. (living collection, XTBG). Scale: (1–32) = 5 mm; (33–35) = 10 mm.


Asclepiadoideae
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Secamonoideae
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Periplocoideae
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Apocynoideae
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Rauvolfioideae
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of a margin surrounding a central part, a straight-tapered apex, a straight median line running from the apex to the center of the seed, and cells that are polygonal, irregularly arranged, and 30 μm wide. However, *A. marginatum* differs from *A. ellipticum* in terms of its larger margin, its slightly smaller size, and its more pear-shape outline (Figs. 2 and 3). In addition, *A. ellipticum* has a central part irregularly spotted or granular, which is not found in *A. marginatum*.

In modern Apocynaceae species, only a few have a large margin comparable with *A. marginatum* (e.g., *Caralluma flava* N.E. Br. & *Pentarrhinum insipidum* E. Mey.; Fig. 4: ch. 6-1). *Caralluma flava* occurs in the high-rainfall part of the Arabian Peninsula (Bruyns and Jonkers, 1993), whereas *P. insipidum* occurs in shrubland and savanna of the Sudano-Zambesian region, Africa (Liede and Nicholas, 1992). However, these genera are not closely related and these characters seem to represent a morphological convergence (Fig. 4). Spotted seeds like *A. ellipticum* were found in five modern genera (Fig. 4: ch. 5-1), among which the tropical African genus *Xysmalobium* R. Br. (Fig. 6: 11, 12) and the Neotropical genus *Oxypetalum* R. Br. (Fig. 6: 13, 14) are the most similar. However, this character again seems to be a convergence and is not appropriate for placing these fossils more precisely in the current phylogeny (Fig. 4).

Fossil record of Asclepiadoideae

Among all seed fossil records attributed to Apocynaceae (Table 1), only a few have clear affinity. This is mainly due to the fact that fusiform seeds with a coma are found in both Apocynoideae and Periplocoideae and represent a plesiomorphic character for the group (Fig. 4; Fig. 6: 21–32). Moreover, Apocynoideae is now a paraphyletic concept (Nazar et al., 2019). Only seeds with a margin surrounding a central body and with an elliptical shape can be confidently assigned to the monophyletic subfamily Asclepiadoideae. Only *Tylophora antiqua* from the early-middle Oligocene of England (Reid and Chandler, 1926) can be attributed with confidence to the Asclepiadoideae.

Previously, there was a gap between the estimated age of the origin of the Asclepiadoideae and that inferred from fossil records. According to molecular data, the origin of the Asclepiadoideae could date back to the early Eocene (55 Ma; Fishbein et al., 2018), but the ages of all existing fossil records are much younger than the early Eocene. However, the newly discovered early Eocene *Asclepiadospermum* from the central QTP clearly belongs to Asclepiadoideae; our discoveries thus reconcile the fossil record and molecular estimations and represent the earliest fossil record for the subfamily. Based on current knowledge, *Asclepiadospermum* could represent an example of early diversification of Apocynaceae in Asia, with subsequent diversification in the Northern Hemisphere (Table 1).

Floristic affinity of Eocene Tibet with other regions

The subfamily Asclepiadoideae contains 164 genera (Endress et al., 2014), mainly distributed in Africa, Asia, and South America. It exhibits diverse seed shapes (Fig. 6: 1–14). The origin center of the subfamily seems to be in Africa (Rapini et al., 2007), as suggested by the African distribution of genera representing basal taxa in the molecular phylogeny (Rapini et al., 2003; Nazar et al., 2019). A split between Secamonoideae and Asclepiadoideae with subsequent radiations and dispersions from Africa has been inferred to have taken place during the Eocene, Oligocene, and Neogene (Goyder, 2006; Rapini et al., 2007; Livshultz et al., 2011). However, the ages inferred in those studies need to be reinvestigated (Fishbein et al., 2018). Results of the present study suggest that the dispersal of Apocynaceae from Africa had occurred by at least the early Eocene.

Few paleobotanical data show that African taxa contributed much to the Northern Hemisphere's floral composition during the early Eocene. Only 27% of the species described for the early Eocene London Clay flora have an African affinity (Reid and Chandler, 1933), and <15 genera in the North American Clarno Formation have an African affinity (Manchester, 1994). This lack of an African affinity is probably due to the wide Neo-Tethys seaway, which may have limited exchanges between Africa and the Northern Hemisphere in the Eocene. Recently, however, some studies on mammals (embrithopods, rodents, and primates) have shown that Paleocene-Eocene exchanges were possible between Africa and Eurasia (Tabuce and Marivaux, 2005; Sen, 2013). In particular, the distribution of embrithopods during the early Eocene spanned both sides of the Neo-Tethys. During the early Eocene, an island network with "sweepstake dispersal routes" was hypothesized to explain the dispersal of the embrithopods from Africa to Eurasia (Sen, 2013).

Our Tibetan fossils represent the earliest record of the subfamily, which raises the question of an Asian origin center for the Asclepiadoideae. However, in the absence of other paleobotanical data, especially from Africa, and taking into account the phylogenetic results (Rapini et al., 2003; Nazar et al., 2019), we consider this hypothesis less likely than an early dispersion from Africa to Eurasia. In either case, the discovery of *Asclepiadospermum* in the QTP provides paleontological evidence that is consistent with this apparently infrequent connection between Eurasia and Africa during the early Eocene. Together with the previous published hypothesis of a connection between Tibet and North America, Europe, and India (Liu et al., 2019; Tang et al., 2019), this new African connection hypothesis allows us to regard Tibet as an important, but lesser-known, paleogeographic pathway within the Northern Hemisphere during the early Eocene.

The early Eocene is characterized by a much warmer climate than nowadays (Greenwood and Wing, 1995; Zachos et al., 2008), which caused the expansion of the tropical zone (Wing et al., 2005). A largely homogeneous tropical forest (or boreotropical forest) was present during the Eocene in North America and Europe (Wolfe, 1975). The recent finding of *Lagokarpos tibetensis* H. Tang, T. Su & Z.K. Zhou from the Jianglang site, closely related to *Lagokarpos lacustris* McMurran & Manchester from the Eocene Green River Formation (McMurran and Manchester, 2010), argues for a biogeographic connection between North America and Tibet during the early Eocene (Tang et al., 2019), and it is becoming increasingly clear that Tibet may have played an important role in the diversity and dispersion of the boreotropical flora in the Northern Hemisphere during the Eocene.

The environment of central Tibet in the Paleogene

The Jianglang site is now situated at an altitude of ~4800 m and hosts cold alpine vegetation dominated by grassland (Ni and Herzschuh, 2011). However, our fossil finding shows that the early Eocene climate and biodiversity were profoundly different. Asclepiadoideae is now present in Asia and widespread in tropical to subtropical areas (Li et al., 1995; GBIF database). In particular, Asclepiadoideae is an important component of the tropical regions in South China and Malaysia (Zhu et al., 2006). Assuming that the modern tolerances of *Asclepiadospermum* persisted through time, it seems that a tropical to subtropical climate existed at the Jianglang site during the early Eocene. This type of climate is also supported by other taxa from the same flora. An extinct genus, *Lagokarpos* supposedly occurred in the warm and humid subtropical to tropical climates of fossil sites in North America and Europe (Tang et al., 2019). Moreover, *Ailanthus maximus* J. Liu, T. Su et Z.-K. Zhou from the Jianglang site is close to *A. triphysa* (Dennst.) Alston, which is present in lowland humid tropical forests in southern Asia (Liu et al., 2019). Collectively, all the fossil discoveries at the Jianglang site suggest a tropical to subtropical flora in central Tibet during the early Eocene.

CONCLUSIONS

A new Apocynaceae genus, *Asclepiadospermum*, is described, based on well-preserved fossil seeds from the Jianglang section of the Niubao Formation, Bangor County, central QTP. We recognize two new species, *A. marginatum* and *A. ellipticum*. Comparison with modern seeds and mapping of the seed characters on a phylogeny of the family Apocynaceae allow us to recognize these fossils as part of the subfamily Asclepiadoideae. These fossils represent the earliest fossil seed record for Asclepiadoideae and the first finding in Asia. Our discoveries lead us to infer a biogeographic connection between Eurasia and Africa by the early Eocene, and expand previous knowledge of the floristic connection between Tibet and other regions worldwide. Furthermore, *Asclepiadospermum* supports the emerging hypothesis of a humid tropical to subtropical flora inhabiting central Tibet during the Eocene.

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AUTHOR CONTRIBUTIONS

T.S., Z.-K.Z., R.A.S., and F.-X.W. designed the research. C.D.R., T.-X.W., S.-Q.L., and J.L. contributed to data acquisition. C.D.R. and T.S. analyzed and interpreted the results. C.D.R. and T.S. led the writing. All authors revised and approved the manuscript.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Specimens checked for morphological seed studies.

APPENDIX S2. Morphological matrix used in the phylogenetic mapping analysis.

APPENDIX S3. Results of mapping of eight characters on a simplified (34-genera) molecular phylogenetic backbone of the Apocynaceae (Nazar et al., 2019) using Parsimony ancestral reconstruction option in Mesquite version 3.6.

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