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麻黄属种子表面纹饰的多样性及其系统学意义

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摘要:麻黄属(*Ephedra*)的分子系统学研究结果明显不同于基于雌球果成熟苞片质地的传统分类,指出麻黄属的形态特征和分子特征存在冲突。对麻黄属53种植物的种子表面微形态特征进行了研究,结果表明麻黄属的种子表面纹饰包括3种主要类型,即横向片层型(T型)、乳突型(P型)和扁平型(S型)。麻黄属的种子表面纹饰特征既与宏观形态特征不相关,也与分子系统学的研究结果不相关,表明该属微形态特征、宏观形态特征和分子特征之间的更多冲突。在欧洲和东亚发现的与买麻藤类有关的几个大化石带有T型种子表面纹饰,暗示具有T型纹饰的麻黄类植物在早白垩纪就十分多样。

关键词:麻黄;买麻藤目;扫描电镜;种子表面纹饰;系统学
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Cuticular Diversity of the Seed Outer Envelope in *Ephedra* (Ephedraceae) with A Discussion on Its Systematic Significance

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Abstract: Recent molecular systematic studies have consistently given results markedly different from the traditional classifications of *Ephedra* based on such macromorphological characters as the bract nature of ripe female cones, suggesting a serious conflict occurs between molecular and morphological characters. The cuticular micromorphology of the seed outer envelope (SOE) of living *Ephedra* encompassing 53 extant taxa of *Ephedra* were investigated. The results indicates that sculpture of SOE of *Ephedra* bears useful characters and includes 3 major types, viz. the Transverse lamellar type (T-type), the Papillate type (P-type), and the Smooth-Striated-Reticulate type (S-type). Further analyses implied that sculptural characters of SOE show correlation neither with macromorphology nor with molecular within *Ephedra*, indicating additional conflicting characters between macromorphology, micromorphology, and molecular. Several megafossil species associated with the Gnetales from Early Cretaceous in both Europe and E Asia were found to bear the T-type sculpture of SOE, indicating that species of *Ephedra* with the T-type seed envelope were once highly diversified in Early Cretaceous.

Key words: *Ephedra*; Gnetales; Scanning electron microscopy (SEM); Seed outer envelope (SOE); Systematics

The family Ephedraceae are the most diversified family among the three extant families of the Gnetales, include one single living genus (viz. *Ephedra* L.) and ca. 50 living species that are widely distributed in the North Temperate Zone as well as some limited areas of South America[1-3].

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Phylogeny of the family remained arguable because on the one hand the wide distribution of the family makes it difficult to obtain a good sample of the family for systematic study[4–8], and the highly reduced morphology gives rise to few valuable taxonomic characters on the other hand[9]. This partially accounts for few monographs of the family hitherto, and many systematic problems are pending[1,3,10–17].

Two hundred and thirty six names of *Ephedra* have been published (refer to *Index Kewensis*). The genus is estimated to consist of ca. 50 species[3]. However, the 50 names listed by Price[3] still include some synonyms, i. e. *Ephedra lepidosperma* C. Y. Cheng is not a valid name and synonymous to *E. rhytidosperma* Pachomova. Further, four more new species were described since 1996[18–21]. As a result, there are at least 53 living species of *Ephedra* worldwide.

Morphological characters (e.g. habit, leaf and cone morphology, seed number per cone, pollen, shape of integument tube, and leaf cuticular morphology) were demonstrated to be variable either within a single species or evolved in a parallel way in different clade respectively[4,8,11]. These characters are thus limited in taxonomic delimitations. Recent molecular phylogenies suggested that once-believed 'good' taxonomic characters (e.g. bract nature of mature female cones either fleshy or membranous or coriaceous) is actually homoplasious[4–5,7–8,22].

Additional taxonomically valuable characters are thus needed to define groups of *Ephedra*. Micromorphological characters of seed surface were suggested to be stable and of great taxonomic significance[23–25]. Seeds of *Ephedra* are provided with an additional outer envelope besides the true, reduced, membranous seed coat. The micromorphology of the seed outer envelope was found to be diverse and useful in identification and taxonomy in classification of both fossil and modern plants[20,26–29], but only a few species in the living genus were investigated. This study is to investigate the sculptural diversity of the seed outer envelope of *Ephedra* with a worldwide sampling.

2 Materials and methods

The Ephedraceae have seeds bearing two envelopes, including one outer coriaceous envelope providing protection for the seed, and an inner membranous envelope giving rise to a micropylar tube to receive pollens at pollination. This study focuses on the external micromorphology of the seed outer envelope (SOE). It is addressed as so but not "seed coat" throughout this paper because the outer envelope was not derived from the true integument (viz. the inner envelope) but in fact from the two adaxials of three foliar organs of the ancestral secondary fertile shoots[30].

Fifty three living taxa (Table 1) from both the Old World and the New World were investigated. Seed materials were removed from female cones of standard herbarium specimens deposited at PE, MO, ASU, MSB, W, and KAS (Table 1). Basically 3 or more seed samples were examined for each species. For comparability of features generated from seeds, only those seeds from mature female cones are considered. Maturity of seeds of the genus *Ephedra* can be easily determined, the cone bract is an important indicator and it will become fleshy in sect. *Ephedra* but dry in sect. *Alatae* and sect. *Asarca*.

Seed materials were treated with 5% NaClO, rinsed with distilled water, dehydrated in alcohol series, dried, cochin. Observations and photographing were made under a Hitachi S-800 scanning electron microscope (SEM, Laboratory of Scanning Electron Microscopy, State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, the Chinese Academy of Sciences) and a ISI-SR-50 scanning electron microscope (University of Alaska Fairbanks).

3 Results

3.1 General morphology

Seeds of *Ephedra* are variable, their length ranges from 3.52～11 mm, width ranges from 0.84～4.66 mm,

Table 1 Vouchers of seed samples of *Ephedra* L.

Section	Species	Vouchers and storage	Distribution
Alatae	*E. alata* Decaisne	Africa, Mguebra, West Sea, Apr. 8, 1958, E. Cosson s.n., Apr. 8, 1958 (MO); Africa, S. Sinai, M. Abdullah 507 (PE)	N Africa to SW Asia
	E. alenda (Stapf) Andreanszky	Africa, Sahara Algeriensis, G. Andreanszky s.n., Apr. 22 1928 (PE); Africa, Sahara Algeriensis, G. Andreanszky s.n. Mar. 28, 1928 (PE)	N Africa
	E. boelkei Roeg.	Argentina, Mendoza, Stefanie Ickert-Bond 1253 (ASU)	S America
	E. multiflora Phil. ex Stapf	Chile, Atacama. Stefanie Ickert-Bond 1231 (ASU)	S America
	E. przewalskii Stapf	China, Gansu, Hexi Zoulang, Anxi Desert, Y. Z. Zhao 2000918 (PE); Y. X. Liu 81081 (Gansu); Z. D. Xia 90011 (Gansu); T. N. Liou 2198 (Gansu) (PE); China, Xinjiang, Xinzhun 348, June 19, 1959 (PE); China, Xinjiang, Qiemo County, Xindong 0133 (PE)	E & Central Asia
	E. strobilacea Bunge	0679: 1961 (PE-seed bank); Turcomania, June 15, 1912, A. Michelson 3369a (PE); Asia Media, Apr. 6, 1912, W. H. Lipsky 4181 (PE, Herb. No. 100203); Turkestania, May 11, 1905, N. Androssow s.n. (PE, Herb. No. 200087)	M Asia
	E. torreyana S. Watson	USA, SW Colorado, dry rocky hillside, 1800 m, May 26, 1914, E. Payson 353 (MO); USA, Nevada, I. W. Clokey 8224 (Nevada, PE); USA, New Mexico: Lincoln Co., Stefanie Ickert-Bond 1125 (ASU); USA, Arizona: Navajo County, Richard Spellenberg et al. 3290 (ASU); USA, Arizona: Apache County, Stefanie Ickert-Bond 666 (ASU)	N America
	E. trifurca Torrey ex S. Watson	USA, New Mexico, Luna Co., W. Hess, S. Vuono, K. Bolger 8006 (MO); USA, Arizona, A. E. Skjot-Pedersen s. n. (31. 3. 1928, Arizona, PE); Donald J. Pinkava 2100b (ASU); USA, Arizona: Mohave County, Stefanie Ickert-Bond 577 (ASU)	N America
Asarca	*E. aspera* Engelmann ex S. Watson	USA, S California, San Bernardino, S. B. & W. F. Parish s.n., June 1882 (MO); USA, Arizona, Mohave County, Gold Road, desert, 3200 ft, Mar. 11, 1940, L. S. Rose 40086 (MO); Mexico, Z. Debreczy, G. Biro, I. Racz & Y. H. Zhao 38986b (PE)	N America
	E. californica S. Watson	USA, California, B. Bartholomew & B. Anderson 4008 (PE); USA, California, San Bernardino County, May 29 1971, J. Henrickson 5559 (MO); USA, California: San Bernardino Co., James Hendrickson 559. May 29, 1971 (ASU # 54959); USA, California, L. Benson 15280 (PE); J. Hendrickson 8616 (ASU)	N America
	E. cutleri Peebles	USA, Utah: San Juan Co., June 7, 1997. Noel H. Holmgren 12744 (ASU); USA, Arizona, Apache Co., H. C. Cutler 2169 (MO); USA, Arizona, Oak Creek Canyon, May 23, 1935, A. Nelson & R. A. Nelson 2087 (MO)	N America
	E. clockeyi Cutler	USA, Arizona, M. E. Jones s.n., Mar. 17, 1932 (MO)	N America
	E. fasciculata A. Nelson	USA, California, Riverside County, L. M. Shultz & J. S. Shultz 8330 (MO)	N America
	E. funerea Coville & Morton	USA, S California, S. B. & W. F. Parish 1385 (MO); USA, N Clark Mt., San Bernardino County, R. F. Thorne, B. Prigge et al. 51414 (MO); USA, Arizona: Mohave County, S. Ickert-Bond 573 (ASU)	N America
	E. viridis Coville	USA, Nevada, Washoe County, June 22, 1979, M. S. Taylor 2048 (MO)	N America
Ephedra	*E. altissima* Desf.	2343: 1996 from Italy (PE-seed bank); Morocco, June 8, 1974, Reading Univ./B. M. Exped. 428 (MO); Tunisia, C. J. Pitard 640 (PE)	Mediterranean
	E. americana Humb. & Bonpl. ex Willd.	Ecuador, Prov. Pichincha, 1900 ~2000 m, Dec. 21, 1966, Benkt Sparre 13640 (MO)	S America

续表(Continued)

Section	Species	Vouchers and storage	Distribution
	E. antisyphilitica Berl. ex C. A. Meyer	USA, Texas, Live Oak Co., Apr. 16, 1964, D. S. Correll 29249 (MO)	N America
	E. breana Phil.	Chile, Prov. Antofagasta Dept. Taltal, Feb. 1926, E. Werdermann 1031 (MO); Chile, Atacama, Stefanie Ickert-Bond 1233 (ASU)	S America
	E. chilensis K. Presl.	Chile, Prov. Caulin, Feb. 1927, E. Werdermann 1250 (MO)	S America
	E. compacta Rose	Mexico, Z. S. Debreczy, G. Y. Biro, I. Racz & Y. H. Zhao 39069a (PE)	N America
	E. coryi E. L. Read	Wonock 10713; D. S. Correll 32762	N America
	E. dawuensis Y. Yang	China, Sichuan, Garzê Xian, W. K. Hu 13049 (PE)	E Asia
	E. distachya L.	3909: 1985, from France (PE); China, Xinjiang, Jeminay Xian, X. M. Shao 86065 (PE)	Europe
	E. equisetina Bunge	China, Nei Mongol, Mt. Helanshan, Y. Yang 2004003 (PE); China, Nei Mongol, Mt. Helanshan, Y. Yang NM06070502 (PE); China, Nei Mongol, Mt. Helanshan, Y. Yang 99016 (PE); China, Nei Mongol, Mt. Helanshan, Y. Yang NM08070503 (PE); China, Xinjiang, Fuyun (Koktokay) Xian, R. C. Ching (Xinjiang Exped.) 1691 (PE)	E Asia
	E. foeminea Forssk.	1526: 1983, from Italy (PE-seed bank); Dalmatia, ad muros prope Spalato, Pichler 2287 (PE); Dalmatia, A. De Degen s.n., July 23, 1905 (PE)	Mediterranean
	E. foliata Boiss. ex C. A. Meyer	Turcomania, N. Androssov 448 (PE, Herb. No. 209084, barcode PE00017451)	N Africa, W & Central Asia
	E. fragilis Desf.	3708: 1989, Italy (PE-seed bank); 5290: 1990, Italy (PE-seed bank); 1633: 1990, Germany (PE-seed bank); 1080: 1989, Italy (PE-seed bank); Spain: Almaria in Sierra Alhamilla, Joila & Rigo 219 (PE)	Mediterranean
	E. frustillata Miers	Patagonia, Nov. 18, 1928, A. Donat 42 (MO); Bolivia, J. Krach 7433 (MSB)	S America
	E. gerardiana Wallich ex C. A. Meyer	China, Xizang, Ge'gyai Xian, Qinghai-Xizang Exped. 76-8734 (PE); China, Xizang, Ge'gyai Xian, Qinghai-Xizang Exped. 76-8679 (PE)	E Asia, Himalayan
	E. glauca Regel	China, Xinjiang, on the way from Kaxgar to Akto, Xinjiang Exped. 666 (PE); China, Xinjiang, Baicheng, A. J. Li & J. N. Zhu 8225 (PE); Xinjiang, Urumqi Botanical Garden, Y. Yang XJ08070802 (PE)	E & Central Asia
	E. gracilis Phil. ex Stapf	Chile, L. R. Landrum & S. S. Landrum 7554 (MO)	S America
	E. intermedia Schrenk ex C. A. Meyer	China, Xizang, Baxoi Xian, Qinghai-Xizang Vegetation Exped. 8836; T. N. Ho, B. Bartholomew, M. Watson, M. Gilbert 1944 (Qinghai); Y. T . Zhang & K. Y. Lang 1728 (Xizang) (PE); Gansu, Lanzhou, Renshoushan Park, Y. Yang LZ060707, LZ08070601 (PE); China, Xinjiang, Urumqi Botanical Garden, Y. Yang XJ08070801 (PE)	Asia
	E. likiangensis Florin	China, Yunnan, Lijiang Nanshuibeidiao Exped. 6335 (Yunnan) (PE)	E Asia
	E. lomatolepis Schrenk	China, Xinjiang, Ili Diqu, Alt. 700 m, June 28, 1984, Coll. Unknown 84h027 (PE)	E Asia
	E. major Host	Africa, Imlilcasni, Haut Atlas, 2200 m, J. Lewalle 9642 (MO); Aveyron, Creissels pres de Millau, H. Coste 551 (PE)	Mediterranean
	E. minuta Florin	China, Sichuan, Dawu Xian, H. Smith 11822 (PE); Sichuan Exped. 1492 (Sichuan, Xiangcheng); X. Li 71811 (Sichuan); H. L. Tsiang 11002 (Sichuan, Pingwu); K. C. Kuan & W. T. Wang 787 (Sichuan, Kangding); C. W. Wang 69441 (Yunnan) (PE)	E Asia

(Continued)

Section	Species	Vouchers and storage	Distribution
	E. monosperma Gmel. ex C. A. Meyer	China, Sichuan, Shiqu Xian, Q. Q. Wang 7636 (PE); H. H. Tong 1-100 (Xizang); X. H. Wu 1-096 (Xizang); 3963: 1983; P.C. Tsoong 9095 (Gansu); Y. S. Lian et al. 96710 (Gansu); B. Z. Guo 3415 (Gansu); S. L. Hu & X. Z. Liu 1515 (Gansu) (PE); E Asia, China, Hebei, Zhuolu County, Mt. Donglingshan, B. Liu & Y. B. Sun 993 (PE)	E Asia
	E. nebrodensis Tineo	Turkey, Coll. Unknown s.n. (K)	Mediterranean to Asia
	E. nevadensis S. Watson	USA, Utah, Duchesne Co., Town of Mt. Emmons, B. F. Harrison & E. Larson 7747 (MO); USA, Arizona, Apr. 15, 1952, W. P. Cottam 12823 (MO); USA, Arizona, S. D. McKelvey 2253 (PE)	N America
	E. ochreata Miers	Argentina, Partido patagones Prov. Buenos Aires, Isla del Jabali, Rincon del Banco 13235 (MO)	S America
	E. pachyclada Boiss.	Afghanistan, Zakira Mt., 2300～2400 m, July 23, 1968, H. Freitag 3424 (KAS)	Mid Asia
	E. pedunculata Engelmann ex S. Watson	USA, Texas, Sonora, H. B. Parks 3199 (MO); S. D. McKelvey 1895 (Texas, PE)	N America
	E. aprocera Fisch. ex C. A. Meyer	Iran, Khovasan, 20 km W Goubad-e Kavus, Sept. 3, 1960, Stutz 626 (W); Iran, Elbrs Mountains, June 8, 1972, Reino Alava 10572 (W); Armenia, Unknown Coll. s.n. (PE, Herb. No. 1341644)	Mid Asia
	E. regeliana Florin	China, Xinjiang, K. C. Kuan 1067 (PE); China, Xinjiang, Urumqi, Hongshan Park, Y. Yang XJ08070803, XJ08070804, XJ08070805 (PE)	E Asia
	E. rhytidosperma Pachomova	China, Mt. Helanshan, Y. Yang 20060606, 2004002, 20060620, YC08070402, YC08070501 (PE)	E Asia
	E. rituensis Y. Yang & et al.	China, Qinghai, Gonghe Xian, J. Y. Ding 85004 (PE); China, Xizang, Zhag'yab Xian, Qinghai-Xizang Exped. 12981 (PE)	E Asia, Himalayan
	E. rupestris Benth.	Ecuador, Prov. Cotopaxi, 3800 m, H. Balslev, G. Pazymino & S. S. Renner 69131 (MO)	S America
	E. saxatilis (Stapf) Royle ex Florin	China, Xizang, Zhumulangma Mt. Exped. 592; China, Xizang, Qinghai-Xizang Exped. 750775 (Xizang); G. Forrest 5564 (Yunnan); R. C. Ching 31011 (Yunnan) (PE); E Asia, China, Xizang, Z. Debreczy & I. Racz 64200h, 64200I, 64201 (PE)	E Asia, Himalayan
	E. sinaica Riedl.	Egypt, Sinai, G. Musa, 2250 m, Oct. 16, 1989, H. Freitag 19.959a (KAS)	Mid Asia
	E. sinica Stapf	China, Ordos Sandland Ecosystem Research Station, Y. Chu 20060801 (PE); China, Hebei, Unknown Coll. s.n. (Herb. No. 00015747, PE); China, Hebei, Zhangjiakou, B. Liu 988 (PE); China, Ningxia, Yinchuan, Y. Yang 08070401 (PE); China, Nei Mongol, Togtoh Xian, Y. Yang 99771 (PE); China, Ningxia, Yinchuan, Y. Yang 2004001 (PE)	E Asia
	E. triandra Tul.	Bolivia, Prov. Tarija, 1900 m, J. West 8297 (MO); Bolivia, M. Nee & I. Vargas C. 44706 (Bolivia, PE); Argentina: Cordoba, Depto. Colan., Capitanelli 584 (ASU); R. Leal 15981 (ASU)	S America
	E. tweediana Fisch. ex C. A. Meyer	Uruguay, Monterideo, G. Herter 1010 (MO)	S America

and thickness ranges from 0.7～4.76 mm. The shape of *Ephedra* seeds varies between species (Table 2). The apex is either acute, or obtusely acute, or acuminate, or attenuate. In longitudinal section, they may be lance-ovoid (Plate I: A, e.g. *E. torreyana*), oblong ovoid (Plate I: D, *E. rhytidosperma*), ovoid (Plate I: G, *E. equisetina*), or subglobose (Plate I: B, *E. aspera*). In transverse section they are either triangular

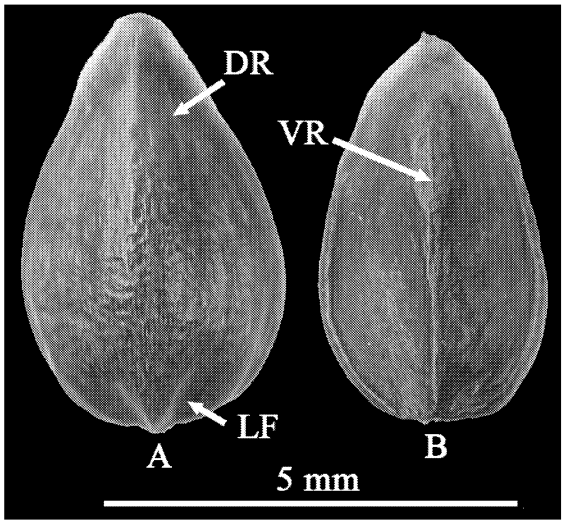

Fig. 1 Seeds of *E. intermedia* under LM

A. Dorsal view showing the dorsal median ridge (DR), and the lateral furrows (LF); B. Ventral view showing the ventral longitudinal ridge (VR).

(e.g., *E. sinica* and *E. triandra*), circular (e.g., *E. californica*), or 4-angled (e.g., *E. trifurca*). A dorsal median ridge is sometimes prominent (Fig. 1A, Plate I: D, Plate II: A, e.g., *E. rhytidosperma* and *E. cutleri*). Two abaxial, lateral furrows (Fig. 1A) are present in some species (e.g., *E. sinica* and *E. triandra*). In most cases the ventral side of the seed is flat (e.g. *E. sinica*, *E. triandra*, and *E. pedunculata*), except when it is convex (e.g., *E. equisetina*, *E. californica* and *E. dawuensis*), or has a median longitudinal ridge in 3-seeded cones (Fig. 1B; e.g., *E. intermedia*, and *E. przewalskii*). Mature seeds are typically larger and have longer epidermal cells than those at the pollination stage of development, but no distinct differences in shape or surface patterns were observed.

Table 2 Seed macromorphology and sculpture patterns of *Ephedra* L.

Section	Species	Seed macromorphology	SOE sculpture	General pattern
Alatae	*E. alata*	Ovoid, acuminate, middle part above sharply acuminate into a long beak, dorsal convex, ridge not clear, lateral furrows absent, ventral plane, brownish, not glossy	Smooth	S-type
	E. alenda	Ovoid, acuminate, middle part above abruptly acuminate into a long beak, dorsal convex, ridge clear, lateral furrows absent, ventral plane, brownish, not glossy	Smooth	S-type
	E. boelkei	Ovoid, acute, upper 1/3 abruptly contracted into a beak, dorsal convex, ridge clear, brownish in color, lower 2/3 oblong, , lateral furrows absent, ventral ridged, brownish purplish, not glossy, not glaucous.	Smooth to striated	S-type
	E. amultiflora	Triangular ovoid, acute or slightly acuminate, dorsal convex, ridge not clear, lateral furrows not clear, ventral plane or ridged, pale brown, not glossy, not glaucous	Smooth	S-type
	E. przewalskii	Narrow triangular ovoid to linear, lanceolate to attenuate, dorsal convex, ridge absent, lateral furrows absent, ventral longitudinally ridged, dark brown to black, not glossy, not glaucous	Smooth	S-type
	E. strobilacea	Narrow ovoid, lanceolate, dorsal convex, ridge clear, lateral furrows absent, ventral plane, yellowish brown, not glossy, not glaucous	Smooth to striated	S-type
	E. torreyana	Lance ovoid, acuminate, middle part above much narrower than the lower part, dorsal convex, ridge not clear, ventral plane, lower part brown, becoming brownish to the apex, not glaucous	Papillate to transverse unite type	T-type
	E. trifurca	Lanceolate ovoid, narrow, attenuate, or lanceolate, four angled, ventral and dorsal not clear, grayish brown, not glossy, not glaucous	Smooth to striated	S-type
Asarca	*E. aspera*	Ovoid, triangular, attenuate, dorsal convex, ridge clear, lateral furrows not clear, ventral plane, brownish purplish black, not glossy, not glaucous	Smooth to reticulate	S-type
	E. californica	Broad ovoid to subglobose, plumpy, obtusely acute, dorsal convex, ridge absent, lateral furrows absent, ventral convex and can not easily distinguished from the dorsal, purplish black, slightly glossy, not glaucous	Striated	S-type
	E. clockeyi	Narrow ovoid, four-angulared or triaungulared in cross section, obtusely attenuate to the apex, contracted near the base forming a basal disc, dorsal and ventral not clear, many fine longitudinal ridges, brownish or pale brownish, basal disc blackish, not glossy, not glaucous	Smooth	S-type

续表(Continued)

Section	Species	Seed macromorphology	SOE sculpture	General pattern
	E. cutleri	Ellipsoid, acute, dorsal convex, dorsal ridge present and soundly elevated, lateral furrows absent, ventral plane, brownish	Smooth	S-type
	E. fasciculata	Ovoid, attenuate or slightly acuminate, dorsal convex, ridge not clear, lateral furrows not clear, ventral convex, purplish black, not glossy, not glaucous	Smooth to striated	S-type
	E. funerea	Narrow ovoid, attenuate, dorsal convex, ridge not clear, lateral furrows absent, ventral plane, brownish black, not glossy, not glaucous	Papillate	P-type
	E. viridis	Oblong ellipsoid, acute, dorsal convex, ridge present, lateral furrows not clear, ventral plane, brownish, slightly glossy, not glaucous	Smooth	S-type
Ephedra	*E. altissima*	Ovoid, oblong ovoid, acute, dorsal convex, dorsal ridge absent, lateral furrows absent, ventral plane or convex, brown, not glossy	Striated to reticulate	S-type
	E. americana	Oblong ellipsoid, acute, dorsal convex, ridge not clear, lateral furrows present, ventral plane, grayish brown or brown, slightly glossy, not glaucous	Smooth	S-type
	E. antisyphilitica	triangular, ovoid, acute, dorsal ridge weak at the middle and below but prominent above the middle part, lateral furrows absent, ventral plane, brown, not glossy	Smooth to striated	S-type
	E. breana	Oblong ellipsoid, acute, dorsal convex, ridge not clear, lateral furrows absent, ventral plane, purpulish black, slightly glossy, not glaucous	Smooth to striated	S-type
	E. chilensis	Oblong elllipsoid, dorsal convex, ridge not clear, lateral furrows absent, ventral plane, purplish black, slightly glossy, not glaucous	Smooth to striated	S-type
	E. ciliata	Triangular, ellipsoid to oblong ovoid, obtusely acute, dorsal convex, ridge clear, blackish in color, lateral furrows	Smooth to striated	S-type
	E. compacta	Triangular, ovoid, attenuate, dorsal convex, lateral furrows not clear, ventral plane, grayish, not glossy, not glaucous	Smooth to striated	S-type
	E. coryi	Triangular ovoid, acute, ridge clear, lateral furrows present, ventral plane, brownish purple, not glossy, not glaucous	Striated	S-type
	E. dawuensis	Oblong ellipsoid, obtusely acute, dorsal convex, ridge absent, lateral furrows absent, ventral plane or convex, purplish black, slightly glossy, not glaucous	Smooth to striated	S-type
	E. distachya	Triangular ovoid, obtusely acute, dorsal convex, ridge absent, lateral furrows absent, ventral plane, purplish black, slightly glossy, not glaucous	Smooth	S-type
	E. equisetina	Ovoid, acuminate, dorsal convex, ridge not clear, lateral furrows present to not clear, ventral convex, grayish brown, not glossy, not glaucous	Papillate, occasionally transversely united of papillae	P-type
	E. foeminea	Broad ovoid, obtusely acute, dorsal convex, ridge clear, lateral furrows absent, ventral plane, purplish black, slightly glossy to not glossy, not glaucous	Striated to reticulate	S-type
	E. fragilis	Ellipsoid, attenuate, dorsal convex, ridge absent, lateral furrows absent, ventral plane, purplish black, slighly glossy or not glossy, not glaucous	Striated	S-type
	E. frustillata	Triangular ovoid, acute,dorsal convex, ridge not clear, lateral furrows absent, ventral plane, yellowish brown	Smooth	S-type
	E. gerardiana	Oblong ovoid to ellipsoid, acute, dorsal convex, ridge not clear, lateral furrows absent to weakly visible, ventral plane to convex, purplish black, slightly glossy, sometimes glaucous	Smooth to striated	S-type
	E. glauca	Narrow ovoid to oblong, acute, dorsal convex, ridge not clear, lateral furrows weak, ventral plane, yellowish to yellowish brown, glossy	Smooth	S-type
	E. gracilis	Obovoid, acute, dorsal convex, ridge not clear, lateral furrows absent, ventral plane, grayish black, not glaucous, not glossy		

续表(Continued)

Section	Species	Seed macromorphology	SOE sculpture	General pattern
	E. intermedia	Narrow ovoid, attenuate, dorsal convex, ridge not clear, lateral furrows absent or present, ventral ridged, purplish black, slightly glossy, not glaucous	Smooth	S-type
	E. likiangensis	Elliptic ovoid or oblong ovoid, attenuate or acute, dorsal convex, ridge not clear, lateral furrows absent, ventral usually convex but sometimes plane, purplish black, slight glossy, slightly glaucous	Striated to reticulate	S-type
	E. lomatolepis	Triangular ovoid, acute, dorsal convex, ridge not clear, lateral furrows weak, ventral plane and impressed inside the margin, purplish black, glossy, not glaucous	Smooth	S-type
	E. major	Oblong ellipsoid, obtusely acute, dorsal convex, ridge not clear, lateral furrows clear at the basal half but increasingly unclear to the apex, ventral convex, brown, not glossy, not glaucous	Papillate	P-type
	E. minuta	Oblong ellipsoid, obtusely acute, dorsal convex, ridge not clear, lateral furrows absent, ventral convex, purplish black, slightly glossy, glaucous	Reticulate	S-type
	E. monosperma	Ovoid, obtusely acute, dorsal convex, lateral furrows absent, ventral convex, brownish black, not glossy, not glaucous	Smooth to striated	S-type
	E. nebrodensis	Ovoid or frequently ellipsoid, obtusely acute, dorsal convex, ridge not clear, lateral furrows present to absent, ventral convex, not glossy, not glaucous	Papillate	P-type
	E. nevadensis	Ovoid, acuminate to attenuate, dorsal convex, ridge not clear, lateral furrows not clear, ventral convex or rarely plane, not glossy, not glaucous	Smooth	S-type
	E. ochreata	Oblong ellipsoid, acute, dorsal convex, ridge absent, lateral furrows absent, ventral usually ridged in triovulate cones but sometimes plane in biovulate cones, purplish black, slightly glossy, not glaucous	Striated with ambiguous cell boundaries	S-type
	*E. pachyclada**	—	Papillate	P-type
	E. pedunculata	Oblong ellipsoid, acute, dorsal convex, ridge clear, lateral furrows not clear, ventral plane, purplish black, slightly glossy, not glaucous	Striated	S-type
	E. procera	Ovoid, acute, dorsal convex, ridge not clear, lateral furrows not clear, ventral convex, grayish black, not glossy, not glaucous	Papillate	P-type
	E. regeliana	Narrow, ellipsoid, obtusely attenuate, dorsal convex, ridge not clear, lateral furrows present, ventral plane, purplish black, glossy, not glaucous	Smooth	S-type
	E. rhytidosperma	Obovoid, to ellipsoid, acute, dorsal convex, ridge present, pale in color, lateral furrows absent, ventral plane, grayish brown, not glossy	Transverse united type, lamellate	T-type
	E. rituensis	Ovoid, triangular, acute, dorsal convex, ridge not clear, lateral furrows not clear, ventral ridged, brownish, slightly glossy, ventral glaucous	Smooth	S-type
	E. rupestris	Obovoid, acute, dorsal convex, ridge not clear, lateral furrows not clear, ventral plane, purplish black, slightly glossy	Smooth	S-type
	E. saxatilis	Oblong ovoid, acute, dorsal convex, ridge not clear, lateral furrows absent, ventral plane, purplish black, slightly glossy, glaucous	Striated to reticulate	S-type
	*E. sinaica**	—	Smooth	S-type
	E. sinica	Ovoid, triangular, attenuate or obtusely acute, dorsal convex, ridge not clear, lateral furrows usually present but sometimes slightly visible, ventral plane, purplish black, slightly glossy	Smooth	S-type
	E. triandra	Oblong ovoid, acute, dorsal convex, ridge clear, lateral furrows clear at the basal, ventral plane, yellow, glossy, not glaucous	Reticulate to striated	S-type
	E. tweediana	Oblong ellipsoid, acute, dorsal convex, ridge not clear, lateral furrows present, ventral plane, purplish black, not glossy, glaucous	Reticulate	S-type

* Only broken seeds of these species were studied.

3.2 SEM observations and classification

Epidermal cells are linear to narrow oblong rectangular or sometimes angled at one or both end(s), they are arranged parallel to the longitudinal axis of seeds. Cell length is variable within and between species; they are much shorter in the apical region of the seed. Impressions between epidermal cells are commonly obvious or ambiguous. Outer periclinal walls of epidermal cells are compressed, impressed, uplifted, ornamented with many tiny papillae, or some of the adjoining epidermal cells grow outwards in a transversely united pattern. Three patterns of cuticular sculpture are recognized in the genus, they are the transverse united type (T-type), the papillate type (P-type), and the smooth-reticulate-striated type (S-type).

3.3 T-type

Seeds with T-type sculpture are lance ovoid (Plate I: A), or oblong ovoid (Plate I: D). Epidermal cells are longitudinally arranged. Cell boundaries are slightly depressed between adjacent epidermal cells. Periclinal walls of adjacent epidermal cells grow outwards and are united to form transverse ridges on both the ventral and the dorsal seed surface (Plate I: B, C, E, F). This pattern was observed in two species, *E. rhytidosperma* from E. Asia (Plate I: E, F), and in *E. torreyana* from N. America (Plate I: B, C). These two species differ in the extent of the transverse protuberances. In *E. rhytidosperma*, the combined outgrowths of periclinal walls of adjacent epidermal cells are well developed forming transverse or sometimes slightly slanted lamellae that are perpendicular to nearly adnate to the ventral surface. The lamellar outgrowths range from a few to over 40 cells. In contrast, in *E. torreyana*, the transverse combined outgrowths of periclinal walls of neighboring epidermal cells are less developed and are formed by two warty-like protuberances to over 20 epidermal cells.

3.4 P-type

Seeds with P-type sculpture are ovoid in the Old World (e.g. *E. equisetina*, Plate I: G), or lance ovoid in the New World *E. funerea*. Epidermal cells have clear cellular boundaries in most cases (Plate I: H, I), while they are less obvious in *E. pachyclada*. Prominent papillate structures are present on periclinal walls in the Old World taxa (e.g. *E. major*, Plate I: I), while papillae of the New World species are less pronounced (e.g. *E. funerea*, Plate I: H). These papillae are mainly arranged in one series along the longitudinal axis (Plate I: H, I). There may be one to several to numerous papillae per cell. Papillae in *E. equisetina* from Mt. Helan are sometimes transversely united showing pattern of outgrowths (Plate I: J) similar to that in *E. rhytidosperma* and *E. torreyana*.

3.5 S-type

Seeds with S-Type sculpture are narrow ovoid (e.g. *E. regeliana*), oblong ovoid with a dorsal median ridge (e.g. *E. cutleri*, *E. saxatilis* and *E. pedunculata*, Plate II: A), plump ovoid to subglobose (e.g. *E. californica* and *E. aspera*, Plate II: B), ovoid (e.g. *E. multiflora*, Plate II: C). Epidermal cells have clear cell boundaries. Most commonly periclinal walls of epidermal cells are flat forming a smooth overview (Plate II: D), sometimes they are convex forming a striated appearance (Plate II: E, H), or they are depressed forming reticulations (Plate II: G, I). Rarely, I also find irregular longitudinal striations (e.g. *E. aspera*, *E. californica*). Variation was also observed in the shape of the end walls, they are either straight (Plate II: D) or oblique (Plate II: E) and variously raised above the general epidermal cell surface (Plate II: F, I).

4 Discussion

Though the majority of *Ephedra* species have smooth seed outer envelopes there are several exceptions. The outer periclinal cell wall of the epidermis can be convex or depressed, which gives a striated or reticulate appearance respectively. The South American species bear only the S-type sculpture, the Mediterranean species complex are often striated or papillate, whereas the Asian species and the North American species include all three types, viz. the S-type, the P-type, and the T-type.

4.1 Evolutionary consideration

Transverse ridges on the surface of the seed outer envelope are present in two distantly related species: *E. rhytidosperma* from China, and *E. torreyana* from North America. In both species, the transverse ridges are variable in width within a single seed. The ridge is formed by the outer epidermis. In transverse sections, the outer tangential cell wall of one to several adjacent epidermis cells has extended outwards, forming a distinct protuberance[31]. The inner tangential cell wall of these cells is in line with that of remaining epidermis cells and the mesophyll is not involved in the formation of the protuberances[31].

These species belong to different clades and similar micromorphological patterns have evolved apparently independently in most major subclades of *Ephedra*. It is difficult to explain this parallelism by decoupling the ecology of these species, which is not notably different from that of most species of *Ephedra*. *Ephedra rhytidosperma* has fleshy cone bracts at seed maturity and seeds are probably dispersed by birds. In contrast, *E. torreyana* has membranous bracts at seed maturity and seeds are dispersed by wind[32].

The P-type seed envelope has arisen several times in the Asian clade (*E. equisetina, E. major, E. pachyclada*) and in the North American clade (*E. fasciculata* and *E. funerea*). Like the species with transversely ridged seeds, there is no ecological correlation between species with papillate seeds. *Ephedra equisetina* and *E. major* have fleshy cone bracts in the female cone, whereas *E. fasciculata* and *E. funerea* have dry winged bracts. Interestingly however, there is some support for an evolutionary link between papillae and transverse ridges. *Ephedra funerea* (papillate seeds) is sister to *E. torreyana* (transverse ridges); *E. equisetina* and *E. major* belong to the same (partly collapsed) clade as *E. rhytidosperma*[8]. *Ephdera pachyclada* from the Near East (papillate seeds) is, however, not closely related to *E. equisetina, E. major* and *E. rhytidosperma*. In one sample of *E. equisetina* from Mt. Helan I found a pattern of transverse ridges, with papillae fused into ridges (Plate I: J), thus combining the features of *E. rhytidosperma* and *E. equisetina*. One possible explanation is that the unique occurrence of transversely combined outgrowths of the papillae in *E. equisetina* might have been further modified to form the lamellate T-type sculpture typical of *E. rhytidosperma*. Otherwise, this intermediate pattern could have originated from hybridization, as these two species occur in close proximity in Mt. Helan.

4.2 Taxonomic significance

The P-type is useful for taxonomic purpose. *Ephedra major* and *E. equisetina* are difficult to distinguish based on morphology and have been considered conspecific by Freitag & Maier-Stolte[11]. Both *E. major* and *E. equisetina* have prominent woody stems as tall as 1.8 m, often thin and spreading green twigs, brown scale-like obtuse leaves, uni-ovulate cones bearing 3 ~ 4 pairs of bracts, the upper pair of bracts connate for ca. 1/2 of their length, seeds convex both ventral and dorsal side, apex tapering into a short beak. The conspecific opinion is further corroborated by new data that both of the two species share the papillate type. Molecular phylogeny, however, suggested that the sampled *E. major* may include two taxa, one is sister to *E. aphylla* embedded in a larger clade comprising *E. aphylla*, *E. alata*, *E. altissima*, *E. fragilis,* and *E. foeminea*, the other is close to *E. saxatilis*, *E. gerardiana*, *E. monosperma*, *E. rhtyidosperma* and *E. equisetina*[7]. A possible explanation for this erroneous placement could be a misidentification of the *E. major* material used in the molecular study.

In the taxonomic treatment of Freitag & Maier-Stolte[11], a few names are listed as synonyms of *E. major*, viz. *E. nebrodensis*, *E. procera*, *E. equisetina*, *E. botschantzevii*, *E. gerardiana*, *E. villarsii*, and *E. macedonica*. I concur with Freitag & Maier-Stolte[11] in placing *E. nebrodensis*, *E. procera*, and *E. equisetina* in synonomy to *E. major* supported by similar gross macromorphology and the papillate type, but it is clear from our observation that *E. gerardiana* is not a synonym of *E. major*. The 2-seeded strobili with oblong-ellipsoid, purplish black seeds in *E.*

gerardiana are in sharp contrast to the 1-seeded strobili with ovoid, grayish seeds in *E. major*. Furthermore, the S-type sculptural pattern in *E. gerardiana* is quite different from the P-type pattern shared by *E. nebrodensis*, *E. procera*, and *E. equisetina*.

Another example on the taxonomic importance of the seed envelope sculpture relates to *E. sinaica* and *E. pachyclada*. Riedl described *E. sinaica* Riedl in 1980, but Freitag & Maier-Stolte[33] reduced it to subspecies rank as *E. pachyclada* subsp. *sinaica* (Riedl) Freitag et Maier-Stolte. In our study *E. pachyclada* var. *pachyclada* shares the P-type sculpture with *E. equisetina*, *E. nebrodensis*, *E. procera*, and *E. major*, but shows an obviously different pattern from the S-type of *E. pachyclada* subsp. *sinaica*, suggesting that *E. sinaica* should remain a distinct species. It seems reasonable to put the name *E. sinaica* Riedl again into the checklist of accepted names while *E. pachyclada* subsp. *sinaica* should be treated as a new synonym to *E. sinaica* Riedl.

4.3 Taxonomic treatment

Ephedra sinaica Riedl in Notes Roy. Bot. Gard. Edinburgh **38**(2): 291. 1980. —— *E. pachyclada* subsp. *sinaica* (Riedl) Freitag & M. Maier-Stolte in Edinburgh J. Bot. **49**(1): 92. 1992. Syn. Nov.

4.4 Palaeobotanical importance

Reproductive morphology is important in classification of both modern and fossil plants. Gnetalean macrofossils were increasingly accumulated[7,27,34–46]. Affinities of those described Gnetalean macrofossils are ambiguous in most cases because most of them did not preserve detailed reproductive features, e.g. *Chaoyangia liangii* Duan and *Ephedrites cheniae* (Cao et al.) Guo & Wu. Both *Chaoyangia liangii* and *Ephedrites cheniae* show closely resemblance to modern *Ephedra* in many aspects, e.g. dichasial branch system, twigs finely striated, leaves opposite or ternately whorled at nodes, and ovulate cones terminal etc[36–37]. Both species are clearly associated with extant *Ephedra*, but they can not be treated as *Ephedra* in the current situation because they have no detailed reproductive features (e.g. seed morphology) compared with living representatives of *Ephedra* on the one hand, and *Chaoyangia liangii* even bears some unique features (e.g. the furcate appendages surrounding the ovulate cone) that are absent in extant *Ephedra* on the other hand. The recently described *E. hongtaoi* X. Wang & S. L. Zheng did not preserve clear reproductive features but was considered to be related to modern *E. sinica* O. Stapf[44]. Transverse outgrowths similar to those in *E. rhtyidosperma* and *E. torreyana* occurred in Early Cretaceous macro- and mesofossils from both the Old World and the New World[27–29,38,42]. These fossil seeds were either believed to be related to the Gnetales[27,38,42], or connective fossils of a 'BEG' clade comprising Bennettitales, Erdtmanithecales, and Gnetales[28–29]. Though further studies are needed to clarify relationships of these seed fossils and their living relatives, it is clear that species with T-type sculpture of the seed envelope are much more diversified in early lineages than in modern *Ephedra*.

4.5 Conflicting results from macromorphology, micromorphology, and molecular

Molecular evidence used to be considered as independent from morphology (external morphology, internal morphology, palynology) in phylogenetic assessments and can be used to test hypotheses based on evidence from other sources. Recent studies of *Ephedra* have consistently suggested conflicting results between molecular and external morphology[4–7]. Based on ripe bract features, Stapf's[1] classified *Ephedra* into 3 'Tribus (≡ sections)', viz. *E.* sect. *Alatae*, *E.* sect. *Asarca* and *E.* sect. *Ephedra*. Soskov[17], Pachomova[14–15], and Mussayev[13], Freitag & Maier-Stolte[11] basically followed Stapf's classification, but modified in a few minor aspects. In general, recent molecular phylogenies do not coincide with these morphological analyses but there are a few points need address. Within the Eurasian distributional group, Pachomova's[15] new section (viz. *E.* sect. *Monospermae* s.s.) is almost equal to Soskov's *E.* subsect. *Glabrae*[17] and to Freitag & Maier-Stolte's[11] Subgroup *Leptocladae* in composition and is

comparable with a clade in molecular analysis[4,7]. *Ephedra* subsect. *Scabrae* Soskov encompasses *E. intermedia* Schrenk ex C. A. Meyer, *E. tesquorum* Nikitin, *E. sinica* Stapf, *E. distachya* L., *E. regeliana* Florin, and *E. minuta* Florin that basically accord with a clade (*E. minuta* excluded) in molecular analysis[4,7-8]. In short, morphological hypotheses basically acquired no supports from molecular phylogenies and conflicting results between morphology and molecular are obvious[4-5].

This study shows that SOE of *Ephedra* is taxonomically valuable and encompasses 3 well-defined types. The result suggests that micromorphology is parallel neither to macromorphology nor to molecular within *Ephedra*. The P-type occurs in both *E.* sect. *Asarca* (*E. fasciculata* and *E. funerea*) and *E.* sect. *Ephedra* (*E. major*, and *E. equisetina*) and do not form a clade in molecular analysis, the T-type was placed in both the E. Asian clade and the North American clade[4,7-8]. This furthers the conflict of characters within the genus and how to reconcile it became a pressing problem to be resolved.

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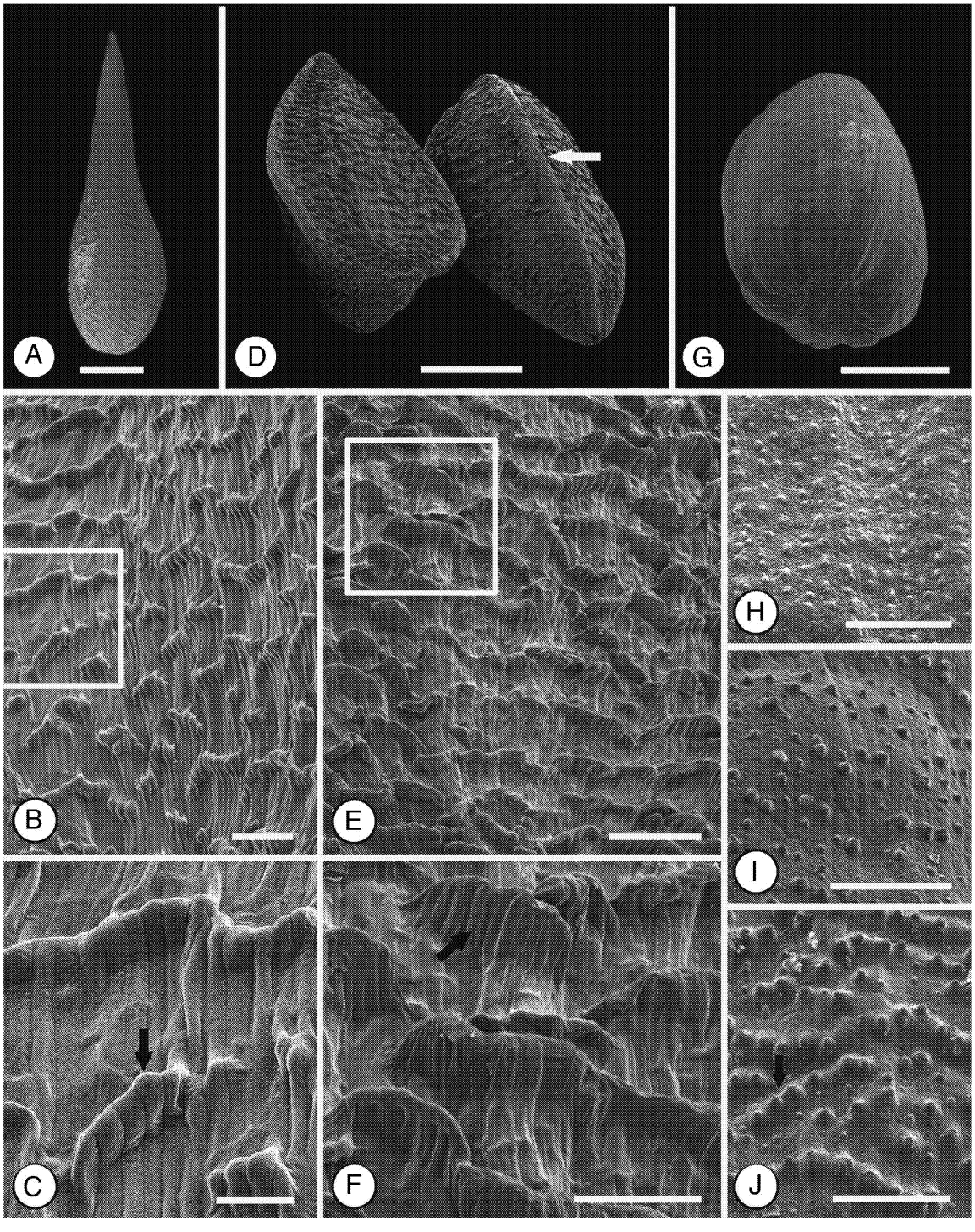

Plate I Seed overview and magnification under SEM

A. Lance ovoid shape of *E. torreyana*; B. Overview of the short transverse united outgrowths ranging from 2 to over 20 epidermal cells in *E. torreyana*; C. Partial magnification in Figure B (white box) showing the extent of the transverse united protuberances (black arrow); D. Flat ventral side and dorsal median ridge (white arrow) of *E. rhtyidosperma*; E. Overview of the seed envelope in *E. rhytidosperma* showing the transverse united lamellar protuberances; F. Partial magnification in Figure E (white box) showing strong transverse lamellar formation and striations (black arrow); G ~ J. Seed overview and magnification showing papillate sculpture of the seed envelope. G. Ovoid shape of *E. equisetina*; H. Tiny papillate protuberances longitudinally arranged in the median part of epidermal cells in *E. funerea*; I. The separate papillate protuberances and intercellular impressions of *E. major*; J. showing the transversely combination of papillae (black arrow) in *E. equisetina*.

A,D,G: bars = 2 mm; B: bar = 200 μm; E: bar = 400 μm; C,F,H ~ J: bars = 100 μm

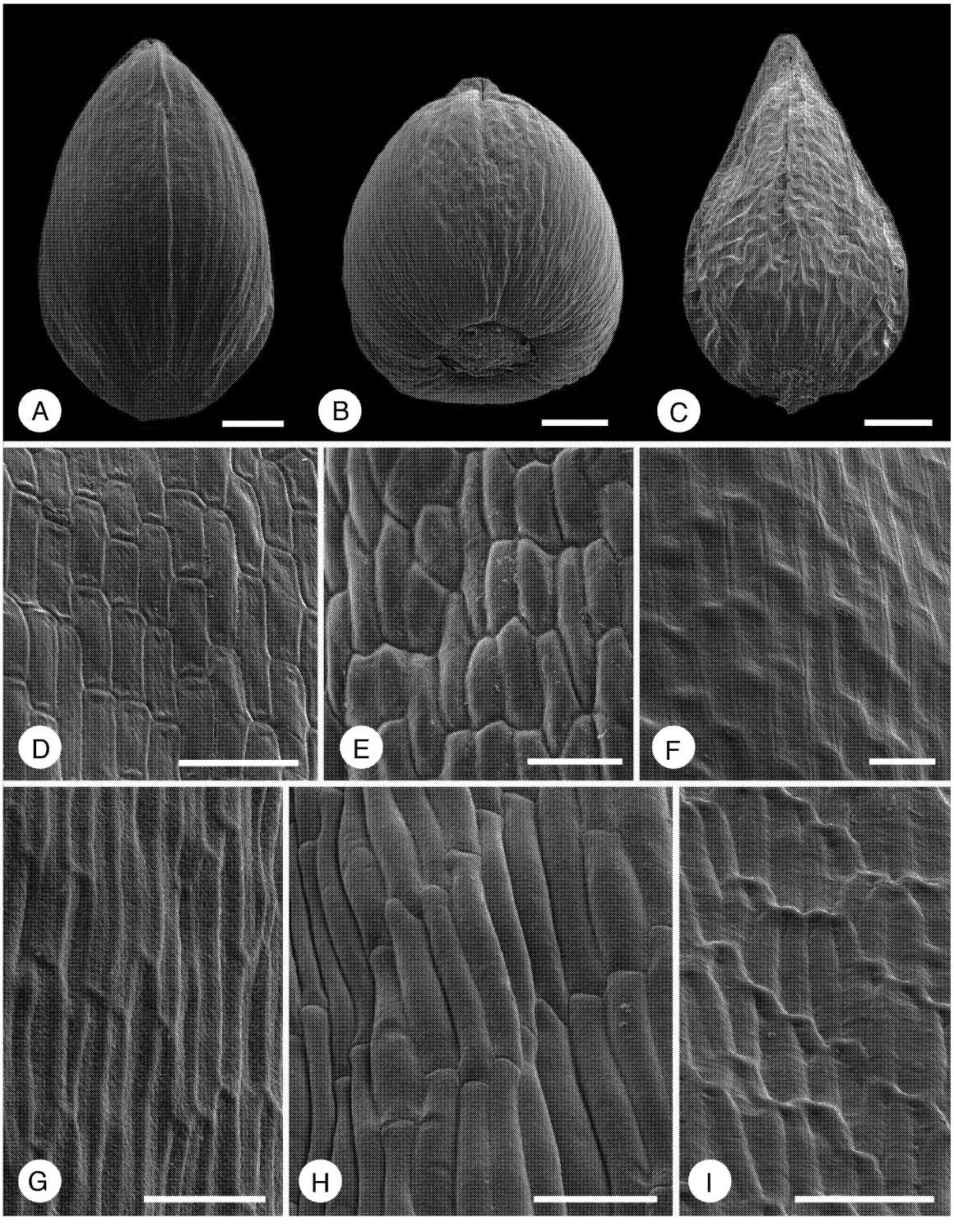

Plate II S-type seed sculpture and their general seed morphology

A ~ C. Seed overview under SEM; A. Oblong ovoid profile of *E. cutleri*; B. Plump seed shape of *E. aspera*; C. Ovoid seed shape of *E. multiflora*; D ~ I. Seed sculpture partial magnified under SEM; D. Smooth pattern with flat periclinal walls and clear epidermal cell boundaries of *E. cutleri*; E. Smooth pattern with convex periclinal walls with clear cell boundaries of *E. coryi*; F. Smooth pattern with slightly depressed periclinal walls and clear cell boundaries of *E. regeliana*; G. Reticulate pattern with depressed periclinal walls and clear cell boundaries of *E. tweediana*; H. Striated pattern with convex periclinal walls and clear cell boundaries of *E. trifurca*; I. Reticulate pattern with depressed periclinal walls of epidermal cells of *E. aspera*.

A ~ C: bars = 1 mm; D ~ E,G ~ I: bars = 100 μm; F: bar = 50 μm.