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Review of Palaeobotany and Palynology 124 (2003) 325–334
Review of Palaeobotany & Palynology
www.elsevier.com/locate/revpalbo

Pollen dimorphism in *Ephedra* L. (Ephedraceae)

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Received 5 March 2002; received in revised form 3 December 2002; accepted 12 December 2002

Abstract

The pollen of three *Ephedra* taxa, *Ephedra torreyana*, *E. trifurca* and *E. funerea* ⇔ *E. torreyana*, showed a marked pollen dimorphism when examined using scanning electron microscopy. Typical pollen grains in all of these taxa have straight ridges, but the variant forms exhibit a highly folded ectexine. In addition, previously used characters such as the presence or absence of bifurcating valley structure do not appear to be uniform, even within a single microsporangium, suggesting that their value to taxonomic study should be reassessed.

Keywords: pollen dimorphism; *Ephedra*; section *Alatae*

1. Introduction

Ephedra is the largest of the three extant genera included in the gymnospermous Gnetales and, based on the plesiomorphic character states of ovule, embryo and gametophyte development, the most basal genus (Crane, 1984; Doyle and Donoghue, 1986, 1992; Doyle et al., 1994; Doyle, 1996; Hickey and Taylor, 1996; Price, 1996). The genus comprises about 50 species, about equally distributed between the Old World and New World. The taxonomy of *Ephedra*, an arid-adapted group of plants, has always been controversial. This is due to the highly reduced morphology, the small number of usable character states, and the convergent evolution of species occurring in xeric habitats. The pollen of *Ephedra* has sometimes been included among characters selected for their potential taxonomic value (Welten, 1957; Steeves and Barghoorn, 1959; Kedves, 1987).

Ephedra pollen is generally described as ellipsoidal, polyplicate and inaperturate (Erdtman, 1952; Bernhard and Meyer, 1972; Ueno, 1973; El-Ghazaly et al., 1998; Osborn, 2000). Pollen size ranges from 20 to 80 μm in length and from 16 to 50 μm in width (Osborn, 2000). The ectexine is three-layered, and consists of an undulated solid tectum, a granular infratectum and a narrow solid footlayer (Kurman, 1992; El-Ghazaly and Rowley, 1997; Osborn, 2000).

Pollen morphological features that have been used systematically include the number of ridges, patterns of the valleys and size variation (Welten,

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doi:10.1016/S0034-6667(03)00002-2

Table 1
Specimens used for SEM study of pollen micromorphology

Taxon	Collection	Locality information	Herbarium Acronym
E. trifurca	Worthington 24587	New Mexico: Luna Co., Cedar Mts.	NY
E. torreyana ⇔ *E. funerea*	Beatley 12202	Nevada: Clark Co., Indian Springs	NY
E. torreyana var. *torreyana*	Clokey 7816	Nevada: Clark Co., S of Indian Springs	NY

1957; Steeves and Barghoorn, 1959; Kedves, 1987). However, the consistency of some of these characters has been questioned. For example, in *Ephedra foliata* Boissier, El-Ghazaly and Rowley (1997) showed that great variability exists in pollen shape and ridge morphology within one plant and even within a single microsporangium.

A number of reports describing fossil ephedroid pollen have relied on characters of the valleys and ridges to ascertain affinities with the pollen of extant species (Lang, 1951; Tynni, 1959; Fritz, 1963; Mayer, 1964; Crane, 1988, 1996; Kedves, 1995). Polyplicate ephedroid pollen is documented from the Lower Permian to the Recent with great abundance during the Mid-Cretaceous (Hesse et al., 2000; Osborn, 2000). While the polyplicate pollen type is also known from several angiosperm orders (e.g. Arales, Laurales and Zingiberales), Hesse et al. (2000) conclusively demonstrated that these grains are structurally and chemically different from ephedroid polyplicate grains.

The current study is part of a taxonomic revision of New World *Ephedra* (Ickert-Bond and Cranfill, 2001). From a total of 18 species of New World *Ephedra* investigated (Ickert-Bond, unpublished), a unique type of pollen dimorphism occurs in three species. This report describes the dimorphism and discusses pollen morphological variation and its usefulness to taxonomic study.

2. Materials and methods

Pollen was removed from herbarium sheets (Table 1), and acetolyzed (Erdtman, 1960), OTOTO coated (Kelley et al., 1973; Chissoe et al., 1994, 1995), freeze fractured (Skvarla et al., 1988), dried with HMDS (Nation, 1983; Chissoe et al., 1994), mounted on stubs with double-sided tape, coated with approximately 200 Å of gold in a Denton Vacuum Desk II vacuum evaporator or sputter coated with a gold/palladium target (60/40) in a Hummer VI Sputter Coating System (Chissoe and Skvarla, 1996), and viewed with a JEOL JSM-880 scanning electron microscope (SEM) at 10–15 kV. Size measurements were generated from SEM micrographs. Thirty pollen grains were counted for each species from five fields of view. All measurements were averaged ($\bar{x}$). Means and ranges are provided (Table 2). The terminology used follows the Glossary of Pollen and Spore Terminology (Punt et al., 1994).

3. Results

3.1. *Ephedra trifurca* Torrey ex S. Watson, 1871 (Plate I, figs. 1–7; Table 2)

Grains are ellipsoidal and inaperturate with no protrusions visible at the poles (figs. 1 and 2). The polar axis ranges from 28 to 38 µm ($\bar{x}$32 µm), the

Table 2
Comparison of micromorphological characters of studied *Ephedra* pollen

Taxon	Polar axis	Equatorial axis	L/W ratio	Number of ridges	Valleys
E. trifurca	28–38 ($\bar{x}$32)	14–20 ($\bar{x}$19)	1.4–2.1:1	13–16	s
E. torreyana	28–35 ($\bar{x}$31)	17–21 ($\bar{x}$19)	1.3–1.9:1	12	s, b
E. torreyana ⇔ *E. funerea*	22–36 ($\bar{x}$28)	12–21 ($\bar{x}$17)	1.4–2.1:1	12–14	s, b

Measurements in µm. Abbreviations: s, straight; b, branched.

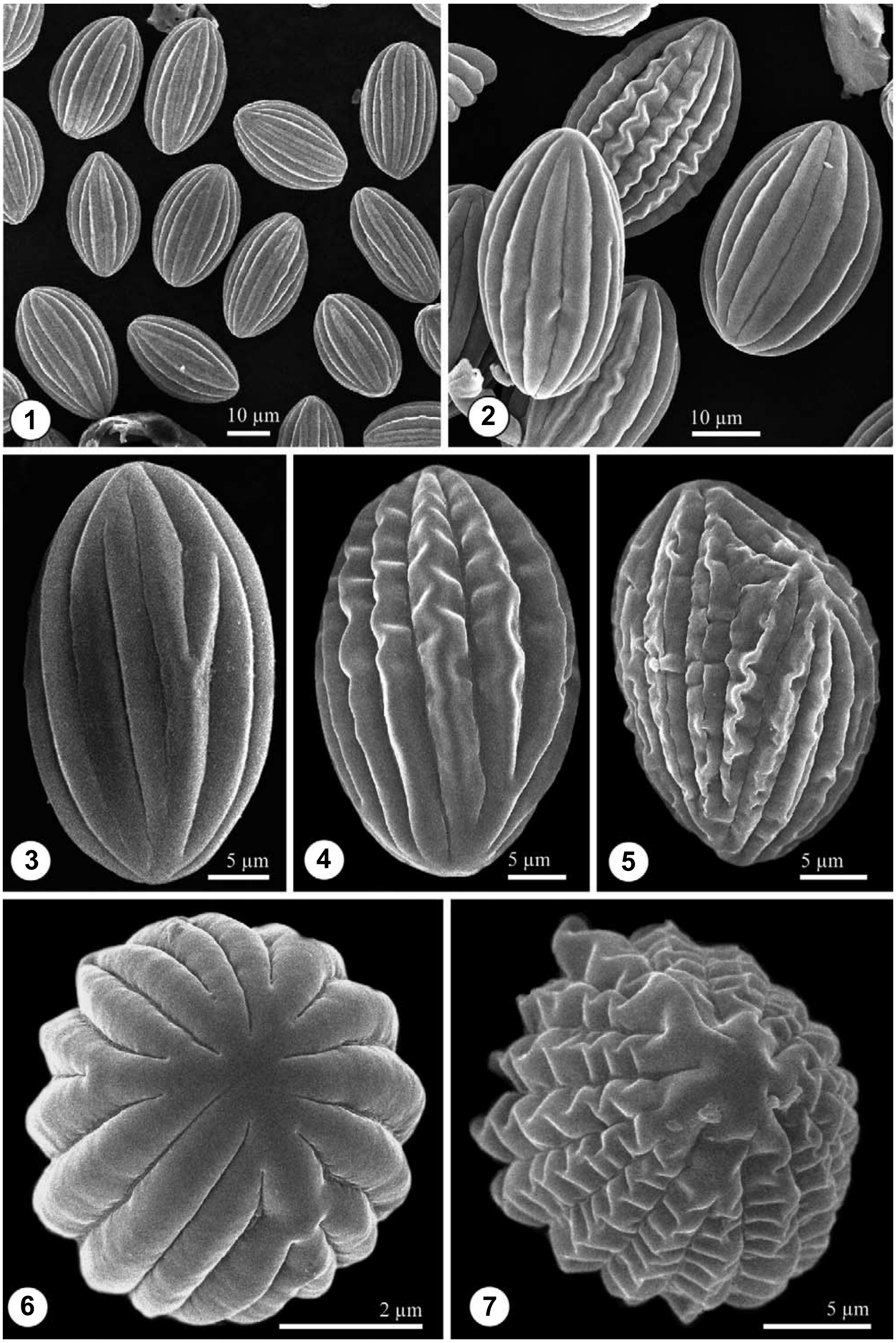

Plate I (*Caption overleaf*).

equatorial diameter is 14–20 μm ($\bar{x}$19 μm). The length to width ratio (L/W) ranges from 1.5:1 to 2.1:1 ($\bar{x}$1.7:1). The number of ridges varies between 13 and 16. Ridges are commonly straight and of sharp descent, separated by valleys, which sometimes bifurcate (figs. 2 and 3). The ectexine is composed of a solid tectum measuring 0.5 μm in thickness. Pollen grains with a marked dimorphism in the patterning of the ridges can be found within the same microsporangium (figs. 1, 2, 4, 5 and 7). While typical grains have a straight ridge (fig. 3), variant forms show a highly folded zig-zag patterning of the ectexine (figs. 4, 5 and 7).

3.2. Ephedra torreyana *S. Watson, 1879* ⇔ Ephedra funerea *Coville and Morton, 1935 (Plate II, figs. 8–13; Table 2)*

Grains are ellipsoidal and inaperturate with small polar apices that protrude (figs. 8, 9 and 11). The polar axis ranges from 22 to 36 μm ($\bar{x}$28 μm), the equatorial diameter is 12–21 μm ($\bar{x}$17 μm). The L/W ratio ranges from 1.4:1 to 2.1:1 ($\bar{x}$1.7:1). The number of ridges is commonly 12–14. Ridges are commonly straight and of sharp descent, separated by valleys that bifurcate (figs. 10 and 12) or less often unbranched (fig. 13). The ectexine is composed of a solid tectum. Pollen grains with a marked dimorphism of the patterning of the ridges can be found within the same microsporangium. The ridges in variant forms exhibit a highly folded zig-zag pattern (figs. 9, 11 and 13) which is in sharp contrast to those of typical grains with straight ridges (figs. 8, 10, and 12).

3.3. Ephedra torreyana *S. Watson, 1879 (Plate III, figs. 14–19; Table 2)*

Grains are ellipsoidal and inaperturate with small polar apices that protrude (figs. 14 and 17). The polar axis ranges from (20–) 28 to 35 μm ($\bar{x}$31 μm), the equatorial diameter is 17–21 μm ($\bar{x}$19 μm). The L/W ratio varies from 1.3:1 to 1.9:1 ($\bar{x}$1.6:1). The number of ridges is typically 12. The ridges are commonly straight and of sharp descent, separated by valleys that are slightly sinuous in outline (figs. 15 and 16) or sometimes branched (fig. 19). Pollen with a marked dimorphism of the patterning of the ridges occurs within the same microsporangium (figs. 14, 17–19). In these grains the central portion of the ridges is thinner than the surrounding ectexine and shows a zig-zag folding pattern. The ectexine is composed of a solid tectum, below which infratectal granules are apparent (fig. 19).

4. Discussion

The first worldwide study of *Ephedra* pollen was by Steeves and Barghoorn (1959) with transmitted light microscopy. They examined pollen of 43 species representing all three sections, *Alatae*, *Asarca*, and *Ephedra* and all subsections of Stapf's (1889) original classification system. Steeves and Barghoorn (1959) noted four distinct types of pollen, 'Types A–D', based primarily on the number and structure of the ridges and the sculpture of the intervening valleys. 'Type A' represents pollen grains with a steep ridge, ridge numbers ranging from 5 to 9, and both undulating ridges and val-

Plate I. *Ephedra trifurca.*

1. Overview showing the morphology of typical pollen.
2. Normal grain with straight and branched valleys of ectexine and variant grain showing ectexine folding (upper middle).
3. Normal grain showing branched valleys and detail of normal ectexine.
4. Variant grain showing details of ectexine folding.
5. Variant grain that is apparently the result of a non-disjunction during microsporogenesis (note two apical poles).
6. Polar view of normal grain showing number of ridges.
7. Polar view of variant grain showing details of highly folded ectexine.

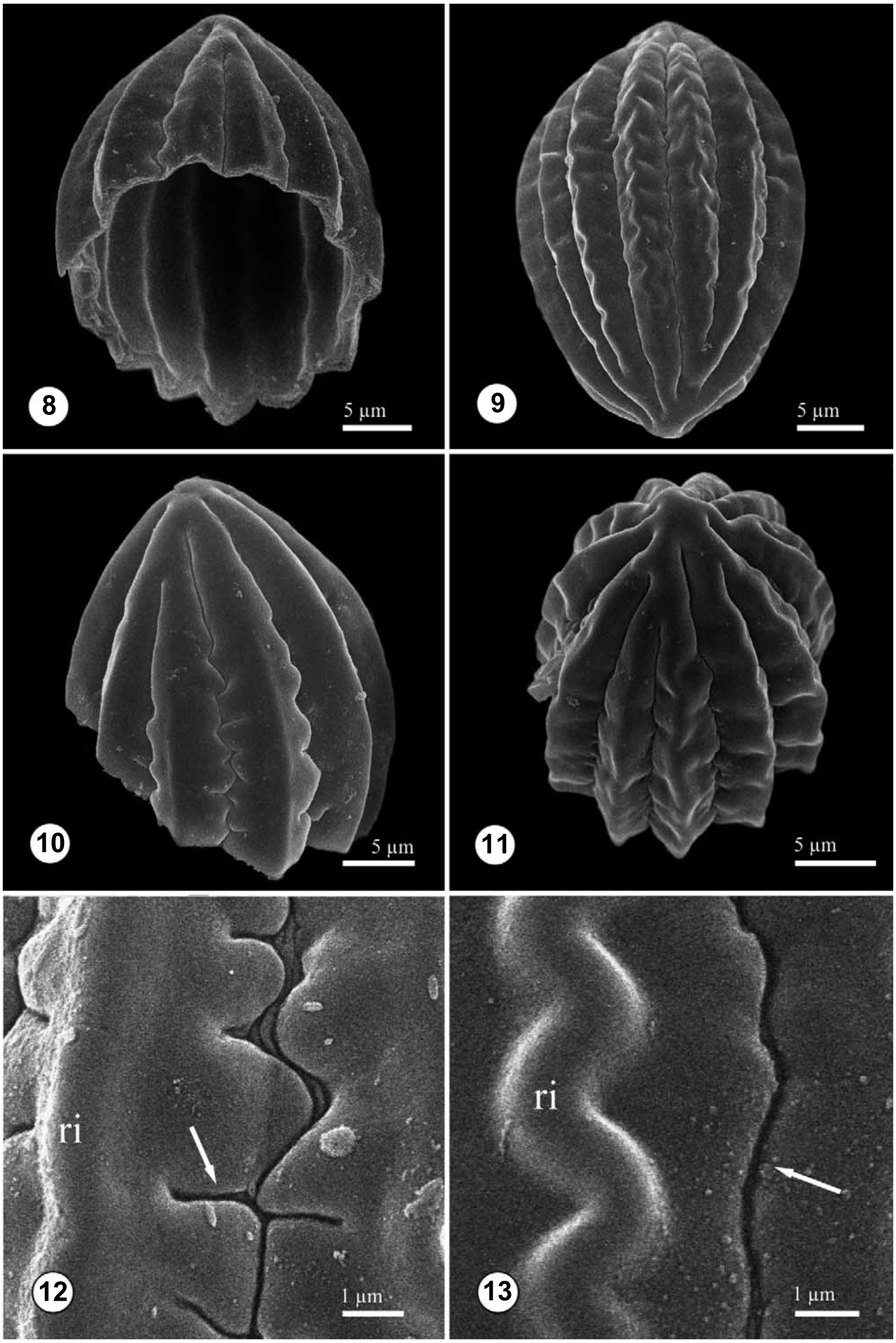

Plate II (*Caption overleaf*).

leys, which also bifurcate. 'Type B' has a greater number of ridges than 'Type A', the valleys are undulating, but do not bifurcate. 'Type C' has straight ridges, but otherwise 'Types B and C' are closely related. 'Type D' is considered as a distinct form with gradual ridges and possesses the greatest number of ridges (up to 20). When compared to the classification of *Ephedra* by Stapf (1889), there was little or no correlation. Zhang and Xi (1993) used Steeves and Barghoorn's pollen types to classify 11 species of Chinese *Ephedra*. While they classify pollen into 'Type A' and 'Type D', they also include a new 'Type BC' with 10–13 ridges, hyaline lines distinct or indistinct, but unbranched, merging Steeves and Barghoorn's 'Type B' and 'Type C'.

Another attempt in classifying *Ephedra* pollen was by Kedves (1987) who noted three distinct pollen morphological types: 'Type 1' with smooth surfaces of both ridges and valleys, 'Type 2' with undulating ridges, and 'Type 3' with hyaline to zig-zag patterns in the valleys. Similarly, Kedves' pollen types did not correlate with the existing classifications of *Ephedra*.

Most recently, Freitag and Maier-Stolte (1994) used pollen types in a conspectus of 18 species of *Ephedra* from Southwest Asia and adjacent regions. Only two pollen types, the '*Fragilis*-type' with straight valleys and the '*Distachya*-type' with branched valleys, are mentioned. The '*Fragilis*-type' occurs in three of their four 'natural groups' reflecting sections *Alatae* and *Ephedra* of Stapf, and the '*Distachya*-type' is restricted to species from their 'Group *Distachyae*', part of Stapf's section *Ephedra*. Species from section *Asarca* were not considered since they are strictly North American in distribution.

Few studies have addressed interspecific variability in *Ephedra* pollen. El-Ghazaly and Rowley (1997) showed that considerable variability existed in both pollen shape and ridge morphology within a single microsporangium. These authors compared the pollen variation to Steeves and Barghoorn's (1959) classification and found all four types (A–D) within a single microsporangium of *Ephedra foliata* Boissier. Pollen dimorphism has been well studied in heterostylous angiosperms (Dulberger, 1975; Köhler, 1976; Ganders, 1979; Goldblatt and Manning, 1989), but little is known about its extent in gymnosperms. Variability in pollen morphology, including size variation and morphological differences, is often associated with hybrids among angiosperm groups (Chaturvedi et al., 2000). It may be that the relatively low incidence of hybridization among gymnosperms accounts for the less frequent occurrence of pollen dimorphism. One case of unreduced and abortive pollen has been reported for *Cupressus dupreziana* A. Camus, from the Mediterranean (El Maataoui and Pichot, 2001).

Several other incidences of pollen dimorphism have been observed for *Ephedra*. Beug (1956) described both normal grains and grains considerably larger and having a greater number of ridges as occurring together. This size difference was attributed to an incomplete separation of pollen mother cells during meiosis. The larger grains could also represent macropollen, possibly with unreduced gametes and be indicative of polyploidy. In a second study of normal grains of *Ephedra helvetica* C.A. Meyer, either straight or undulating ridges occurred along with some 'abnormal' double grains with straight ridges (Chaturvedi, 1978a). In a study of *Ephedra intermedia* Schrenk and C.A. Meyer, 'normal' externally thickened ridges as well as unusual grains with internal thickenings (Chaturvedi, 1978b) were observed. A combination of both size and ridge pat-

Plate II. *Ephedra torreyana* ⇔ *Ephedra funerea*.

8. Top portion of typical grain showing straight ridges and small polar apices that protrude.
9. Variant grain showing undulating ridge and exine folding.
10. Typical grain with straight ridges and valleys that show broad bifurcations.
11. Top portions of variant form showing highly folded ectexine patterning and less prominent bifurcations.
12. Typical grain showing details of the straight ridge (ri) and prominent brifurcations at arrow.
13. Variant grain showing details of sinuous ridge (ri) and unbranched valleys at arrow.

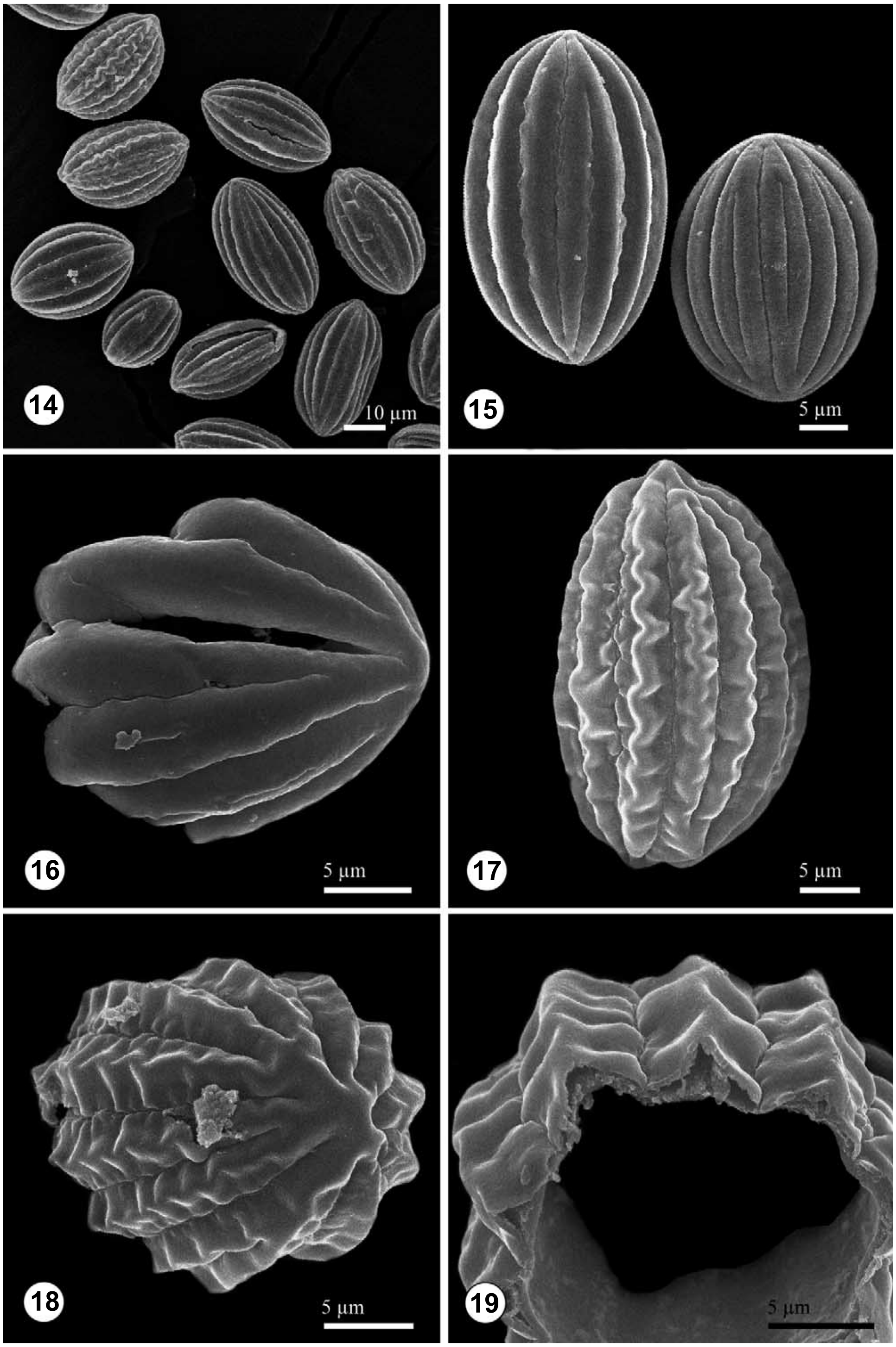

Plate III (*Caption overleaf*).

terning differences has also been observed in several species of *Ephedra*, although no explanation for this phenomenon was offered (Kedves, 1987).

In the present study, the observed dimorphism is of a somewhat different nature in that grains of the same plant showed marked differences in the structure of the ridges. Typical grains have straight ridges, but the variant forms (approximately less than 10% variant grains) exhibit a highly folded ectexine. In addition, previously used characters such as the presence or absence of bifurcating valley structure (Steeves and Barghoorn, 1959; Kapp et al., 2000) do not appear to be uniform, even within a single microsporangium (Plate II, figs. 10–13).

Of 18 species examined (Ickert-Bond, unpublished), the only three taxa with a marked pollen dimorphism profiled here, are all of Stapf's section *Alatae*, the 'wing-bracted' ephedras. Furthermore, two of these species are related to *Ephedra torreyana*. This is the most widespread species in North America, and most frequently involved in hybridization, in fact the two hybrids described by Cutler (1939) for North America involve *E. torreyana*. Wendt (1993) further concluded that *E. torreyana* could be the putative parent of more hybrids with other species, e.g. *E. aspera* Engelm. ex S. Watson and *E. funerea*. One of the examples of pollen dimorphism from the current study is from a herbarium specimen that is morphologically intermediate between *E. funerea* and *E. torreyana* (designated as *E. torreyana* ⇔ *E. funerea*) and derives from an area where both putative parent species occur sympatrically in Nevada (Table 1). It may be that the observed pollen dimorphism is the result of introgression of populations and that, even though some specimens appear morphologically distinct as *E. torreyana* or *E. funerea*, they may indeed be the result of introgression, which is manifested in the genotype. Pollen from allopatric populations of *E. funerea* from Death Valley, California, separated by about 200 km from the nearest *E. torreyana*, lack the unique pollen dimorphism (Ickert-Bond, unpublished) which is observed in the sample from Nevada used in this study (Table 1). Further studies, for example examinations of percentages of aborted grains, generally considered a good indicator of hybridity, are needed to confirm these assumptions.

Acknowledgements

This study was funded in part by an American Dissertation Fellowship from the American Association of University Women Educational Foundation, a Sidney Karling Graduate Student Award from the Botanical Society of America, scholarship funds from the Phleps Dodge Foundation and the National Council of State Garden Clubs, made available to S.M.I.-B. The use of the scanning electron microscope at the Samuel Roberts Noble Microscopy Laboratory, University of Oklahoma, is greatly appreciated. We are grateful to useful comments made by M.M. Harley and W. Punt.

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Plate III. *Ephedra torreyana.*

14. Overview showing a mixture of pollen types. Note two variant grains in the upper left corner.
15. Prominent size difference within typical grains with straight ridges and intervening straight to slightly sinuous valleys.
16. Typical grain showing details of straight ridges.
17. Variant grain with highly sinuous ridge and exine folding.
18. Polar view of variant grain showing number of ridges and exine folding.
19. Variant form showing details of ectexine folding and bifurcating valleys.

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