

# Fossil record of *Ephedra* in the Lower Cretaceous (Aptian), Argentina

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**Abstract** Fossil plants from the Lower Cretaceous (upper Aptian) of the La Cantera Formation, Argentina, are described. The fossils studied represent a leafy shooting system with several orders of articulated and striated axes and attached leaves with unequivocal ephedroid affinity. We also found associated remains of ovulate cones with four whorls of sterile bracts, which contain two female reproductive units (FRU). Ovulate cone characters fit well within the genus *Ephedra*. Special characters in the ovulate cones including an outer seed envelope with two types of trichomes, allowed us to consider our remains as a new *Ephedra* species. Abundant dispersed ephedroid pollen obtained from the macrofossil-bearing strata also confirms the abundance of Ephedraceae in the basin. The co-occurrence of abundant fossil of *Ephedra* (adapted to dry habitats) associated with thermophilic cheirolepideacean conifer pollen (*Classopollis*) in the unit would suggest marked seasonality at the locality during the Early Cretaceous. Furthermore, the floristic association is linked to dry sensitive rocks in the entire section. The macro- and microflora from San Luis Basin are similar in composition to several Early Cretaceous floras from the Northern Gondwana floristic

province, but it may represent one of the southernmost records of an arid biome in South America.

**Keywords** Central western Argentina · Early Cretaceous · *Ephedra* · Gnetales

## Introduction

Gnetales is an order of gymnosperms of considerable interest because of its much debated relationship to other seed plants (e.g., Bowe et al. 2000; Crane et al. 2004; Hajibabaei et al. 2006; Ickert-Bond and Renner 2016). Today it is represented by three monogeneric families, Ephedraceae (*Ephedra* L.), Gnetales (*Gnetum* L.), and Welwitschiaceae (*Welwitschia* Hook. f.). The three extant genera are morphologically different from each other, but each genus is morphologically homogenous. In the fossil record, several fossil taxa show a higher morphological variation within each family during the Mesozoic (see Friis et al. 2014; Liu and Wang 2016; Rydin et al. 2006a; Yang and Ferguson 2015). Several molecular studies and morphological characters support the monophyly of the group (Bowe et al. 2000; Chaw et al. 2000; Goremykin et al. 1996; Magallón and Sanderson 2002; Rydin et al. 2002), and Ephedraceae has retained many ancestral morphological characters that indicate it is the earliest diverging clade within the Gnetales: e.g., presence of archegonia, development patterns of male and female gametophytes, embryo without a feeder (Bierhorst 1971; Chamberlain 1935; Chaw et al. 2000; Crane 1985; Friedman 1990; Gugerli et al. 2001).

Some Gnetalean fossils were attributed to the *Gnetum*-*Welwitschia* clade (Crane and Upchurch 1987; Dilcher et al. 2005; Guo et al. 2009; Rydin et al. 2003), and many others share characters with *Ephedra* (Cao et al. 1998; Cladera

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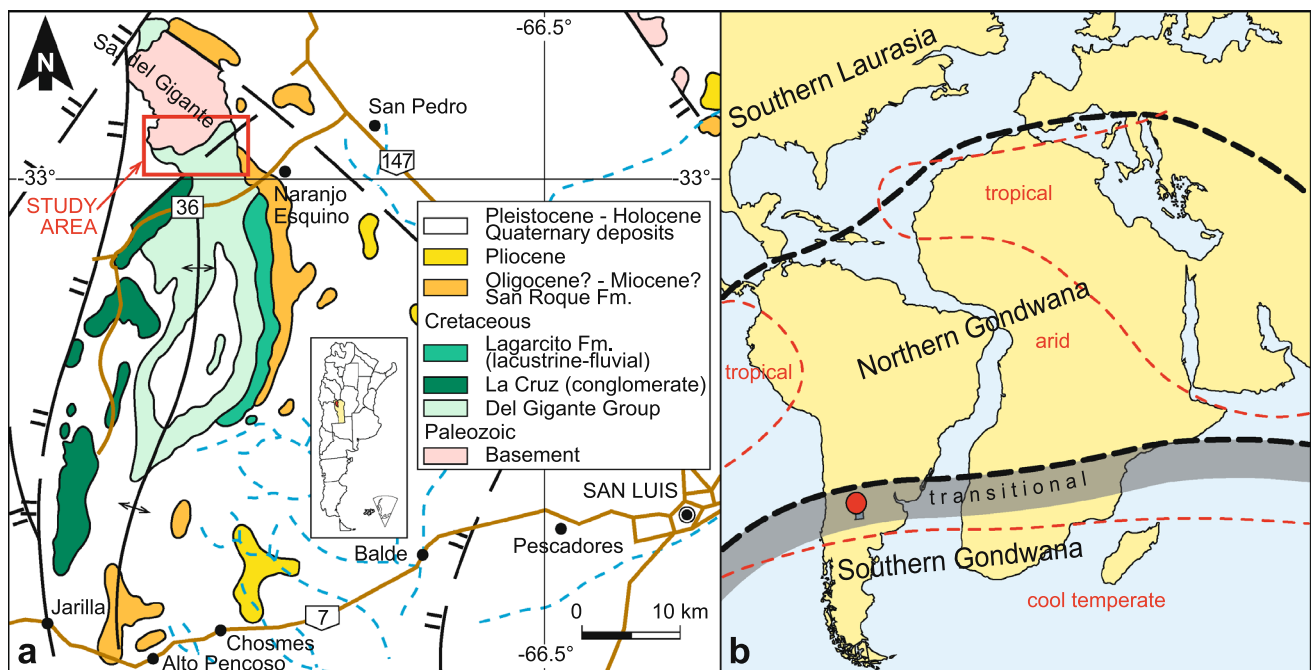
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et al. 2007; Guo and Wu 2000; Krassilov 1982; Krassilov et al. 1998; Ricardi-Branco et al. 2013; Rydin et al. 2004, 2006a, b; Sun et al. 2001; Tao and Yang 2003; Wang and Zheng 2010; Wu et al. 1986, 2000; Yang et al. 2005).

*Ephedra*, comprises ~54 extant species (Kubitzki 1990; Rydin et al. 2010; Ickert-Bond and Renner 2016) inhabiting arid and semiarid environments of the Northern Hemisphere and South America, mostly distributed in dry extra-equatorial areas (except for occurrences in the equatorial Andes). All extant species are quite similar in overall morphology. They are herbs, sub-shrubs or shrubs and vines with opposite or whorled phyllotaxis and reduced leaves. Ovulate cones have fleshy or dry bracts generally in three—sometimes 2 to 6—whorls (Yang et al. 2005). Gnetales seeds have a unique structure among all other gymnosperms; the thin integument is surrounded by an outer envelope formed by fusion of bract-like structures (Ickert-Bond and Rydin 2011; Judd et al. 2008; Yang 2004). The pollen in *Ephedra* is also very distinctive, ellipsoidal polyplicate grains with alternating thick and thin regions of exine (Bolinder et al. 2016; El-Ghazaly and Rowley 1997; Osborn 2000; Steeves and Barghoorn 1959). The characteristic pollen morphology of the *Ephedra* pollen grains allows for easy recognition of Ephedraceae in the fossil record, with records dating back to the latest Paleozoic (Azéma and Boltenhagen 1974; De Lima 1980; Pocock and Vasanthi 1988; Wang 2004; Wilson 1962), and becoming

more common since the Triassic (Rydin and Hoorn 2016; Traverse 1988). Furthermore, polyplicate pollen grains resembling *Ephedra* and *Welwitschia*, usually referred to “gnetophyte pollen grains”, are frequent and geographically widespread in the Mesozoic record. This type of pollen grain is frequently found in Cretaceous sediments around the world (especially at mid-low latitudes), represented by three fossil genera: *Ephedripites* Bolkh ex Pot, *Steevesipollenites* Stover, and *Gnetaceaepollenites* Thierg. Based on molecular clocks analyses, it was proposed that extant species of *Ephedra* should have evolved during the Oligocene–Miocene (Huang and Price 2003; Ickert-Bond et al. 2009). Nevertheless, the presence of vegetative and reproductive mega- and mesofossils from Lower Cretaceous strata from China, central Asia, Australia, Europe, Brazil, and Patagonia show that Ephedraceae were already diverse and widespread during the Early Cretaceous (Crane 1996; Crane and Lidgard 1989; Osborn et al. 1993).

Here we describe a new fossil species of *Ephedra* from the upper Aptian, La Cantera Formation, San Luis Basin, central western Argentina (Fig. 1). The fossil material includes ovulate cones, seeds, associated axes and leaves. The preservation of the specimens allow recognition of several morphological and anatomical features for the assignment of the fossils to Ephedraceae. Furthermore, the ovulate cones and seeds can be unequivocally attributed to a new fossil species of *Ephedra*. The occurrence in



**Fig. 1** **a** Geological map showing the La Cantera Formation type locality at the Sierra del Gigante (ridge), with the arrangements of Cretaceous and Cenozoic units as they crop out in the region. **b** Relative situation of the continents during the Early Cretaceous

( $100 \pm 10$  M.y.); boundaries between palynofloristic provinces with paleoclimatic reconstruction (following Brenner 1976, Herengreen et al. 1996; Scotese et al. 1999). The point shows the location of the study area

the same sediments with both abundant and diverse polyplicate pollen grains with ephedroid affinity indicates that this group was common within the plant community during the Early Cretaceous in the San Luis Basin. This new Argentinean record provides additional information related to the southernmost limits of the arid biomes in subtropical Gondwana during the mid-Cretaceous and adds important information for the reconstruction of South American plant diversification.

## Materials and methods

### Locality

The macro and microfossils studied here come from the type locality of the La Cantera Formation (32°59'25"S, 66°52'48"W), from the El Gigante Group (Flores and Criado Roque 1972), exposed at the San Luis Basin, Argentina (Fig. 1). The El Gigante Group consists of six units (Flores 1969; Rivarola and Di Paola 1992), which are named from the base to the top: Los Riscos, El Jume, La Cantera, El Toscal, La Cruz, and Lagarcito formations (Fig. 1).

The La Cantera Formation is mainly composed of fine laminated green-grey mudstones, siltstones and claystones, with red-brown sandstone and gypsum interbedded at the top of the succession. It was interpreted as being deposited in ephemeral lakes related to a fluvial environment with some periods of a quiet lacustrine system allowing the preservation of delicate fossil structures (Criado Roque et al. 1981; Prámparo 1989). Prámparo (1999) interpreted a shallow freshwater eutrophic environment with some evaporitic episodes based on diverse and abundant phytoplankton (*Scenedesmus* Meyen, *Tetrastrum* Chodat, *Tetraedron* Kützing, *Botryococcus* Kützing and *Leiosphaeridia* Eisenack) from the La Cantera Formation.

The palynological association (*Stephanocolpites* Van der Hammen, *Huitrinipollenites* Volkheimer and Salas, *Afropollis* Doyle, Jardiné and Doerenkamp) and the 107.4–109.4 K/Ar ages from the overlying basalt of the La Cruz Formation, restricts the age of the La Cantera Formation to the late Aptian (Prámparo 1990, 1994; Prámparo et al. 2007).

The pollen associations are dominated by polyplicate grains (*Ephedripites* Bolkhovitina, *Steevesipollenites* Stover) with minor representation of *Classopollis* (Pflug) Pockock and Jansonius, *Cycadopites* Wodehouse and *Monosulcites* Couper. Angiosperms, ferns and bryophytes are less common and are interpreted as the vegetation surrounding the lacustrine basin. The macroflora include

angiosperms, monilophytes and bryophytes (Puebla 2004, 2009, 2010; Puebla et al. 2012). The La Cantera Formation also preserved fishes, ostracods, conchostracans and insects (Arcucci et al. 2015).

In *Ephedra*, ovulate units of ovulate cones are referred to by many different names; e.g., ovules, flowers, and female reproductive units (Eames 1952; Pearson 1929; Yang 2001, 2013). In this paper, we follow Yang (2001) and call the reproductive units of the ovulate cones “female reproductive unit” (FRU). The terminology used for reproductive structures description follows Rydin et al. (2010).

For epidermis observation and bract margin delimitation, we used an Olympus microscope BX51 with an Olympus fluorescent Unit connected to an Olympus Evolt E-330 camera housed at the INFIVE (Instituto de Fisiología Vegetal, Universidad Nacional de La Plata). Best results were obtained with an Endow GFP BP filter (440–640 nm) and a RGB PE filter (500–700 nm). Specimens were observed under scanning electron microscopy (SEM) using a JEOL (JSM-6490LV) from the MEBYM (Microscopía Electrónica de Barrido y Microanálisis, CCT-Mendoza-CONICET), and a LEO 1450VP of the LABMEM (Laboratorio de Microscopía Electrónica y Microanálisis, Universidad Nacional de San Luis).

Fossil specimens are housed at the Museo Interactivo de Ciencias (MIC) of the Universidad Nacional de San Luis (UNSL), San Luis Province, Argentina, under the acronym MIC-P.

Palynological samples were processed using standard techniques of maceration in HF and HCl following Volkheimer and Melendi (1976). England Finder coordinates are provided for the illustrated specimens. All slides are stored in the paleopalynological collection of the Instituto Argentino de Nivología, Glaciología y Ciencias Ambientales (IANIGLA), CCT-Mendoza CONICET, under the acronym MPLP.

### Systematic paleontology

#### Spermatophyta

Gymnospermae

Order Gnetales Blume

Family Ephedraceae L.

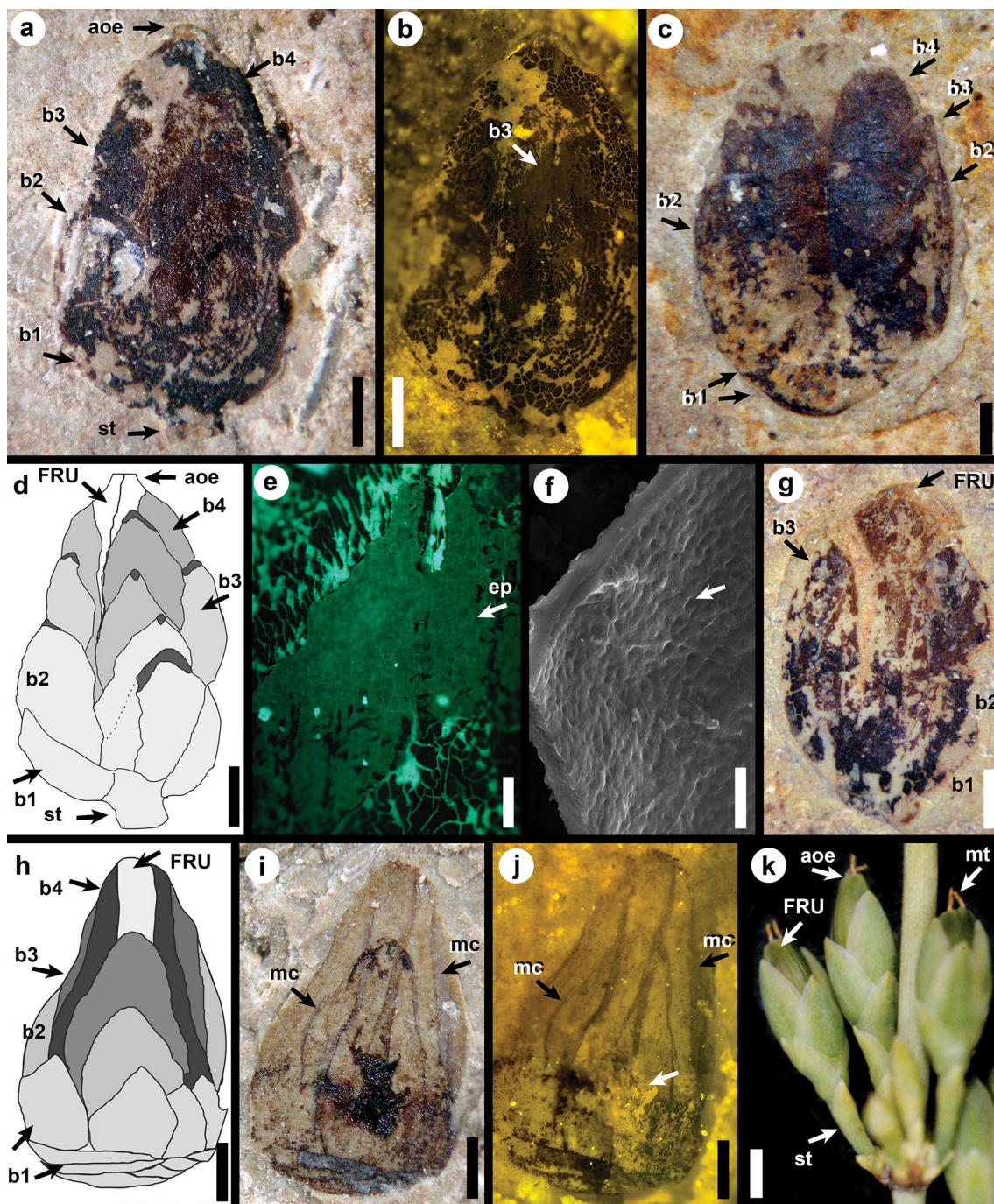
Genus *Ephedra* L.

Type species *Ephedra distachya* L.

*Ephedra canterata* Puebla, Iglesias, Gómez and Prámparo sp. nov. (Figs. 2a–j, 3).

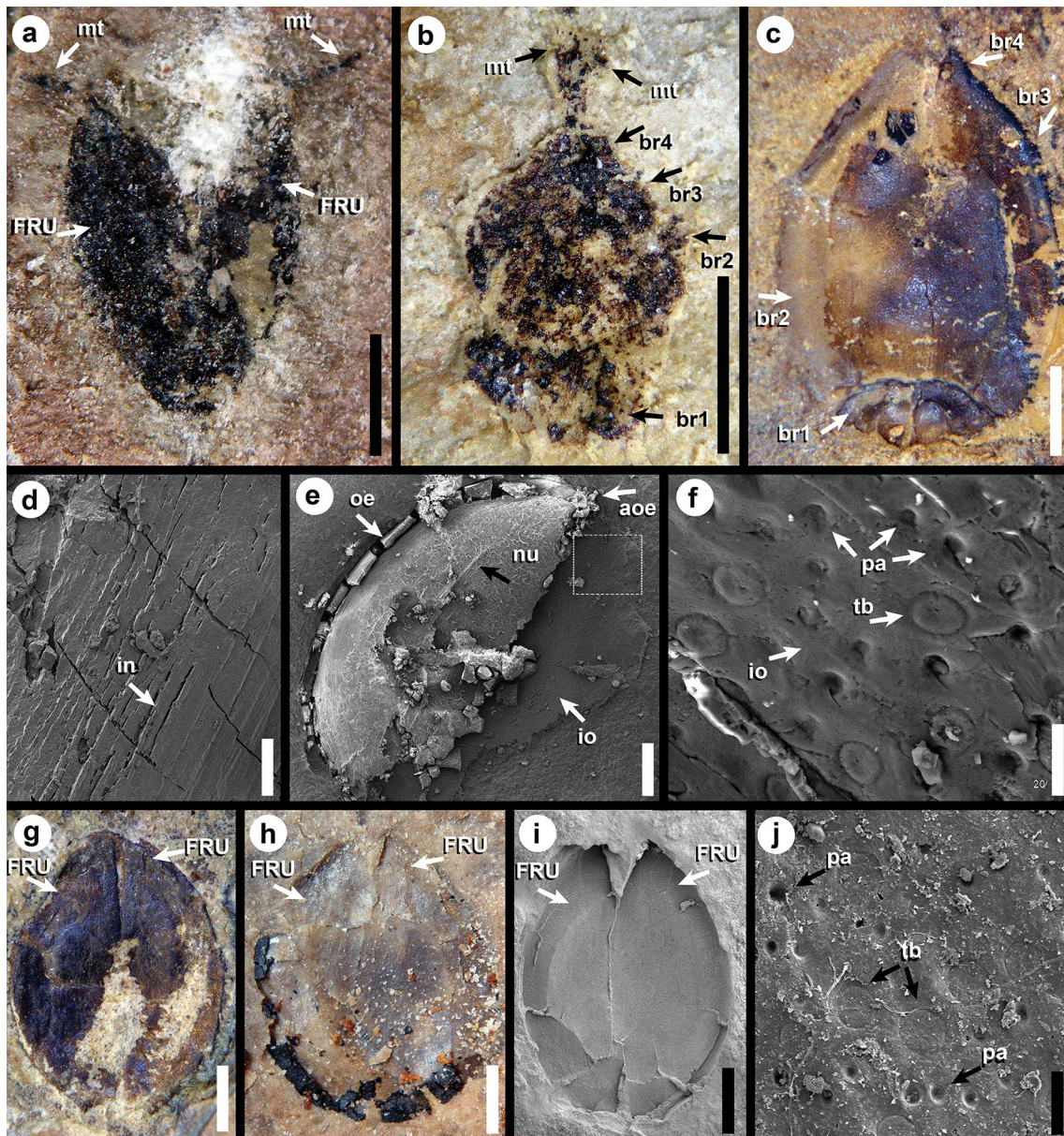
*Holotype* MIC-P672 (Fig. 2a–f).

*Paratype* MIC-P673; MIC-P675; MIC-P677a; MIC-P677b; MIC-P679; MIC-P683; MIC-P684; MIC-P687; MIC-P688; MIC-P689; MIC-P691 (Fig. 2g–j).



**Fig. 2** *Ephedra canterata* sp. nov. ovulate cones preserving both whorls of bracts and female reproductive units (FRU); and extant *Ephedra* cones. **a, b** Specimen MIC-P672 scale 500  $\mu\text{m}$ . **a** Photo under stereoscope. **b** Picture resulted from a stacking of pictures under fluorescence light (RGB Phycoerythrin PE filter), note the fluorescence of epidermis in bracts (white arrow), magnified in **e**. **c** Ovulate cone with basal bracts removed (*b1* scars). Scale 500  $\mu\text{m}$ . MIC-689. **d** Reconstruction of the ovulate cone showing the arrangement of bracts in four whorls. **e** Specimen MIC-P672 under fluorescence light (Endow GFP BP green filter). Note anticlinal walls of hexagonal epidermal cells (*ep*). Scale 200  $\mu\text{m}$ . **f** SEM picture of a cone bract showing internal cuticle surface with papillae impressions (arrow).

Scale 5  $\mu\text{m}$ . MIC-P672. **g** Ovulate cone preserving bracts in four whorls. Scale 500  $\mu\text{m}$ . MIC-P691. **h–j** Ovulate cone MIC-P673 preserving both whorl of bracts and FRU. Scale 500  $\mu\text{m}$ . **h** Reconstruction of the ovulate cone showing the arrangement of bracts in four whorls. **i** Photo under stereoscope and white light. **j** Photo stacking under fluorescence light (RGB Phycoerythrin PE filter), note the bract marginal cells (*mc*) and highly fluorescence of bract cuticle (white arrow). **k** Stalked seed cone in extant *Ephedra viridis*. *aoe* apical outer envelope, *b1* bract from the first cone world, *b2* bract from the second cone world, *b3* bract from the third cone world, *ep* epidermal cell, *FRU* female reproductive unit, *mt* micropylar tube, *mc* bract marginal cells, *st* cone stalk



**Fig. 3** *Ephedra canterata* sp. nov. female reproductive units (FRU). **a–c** FRU under stereoscope. *Scale* 500  $\mu$ m. **a** specimen MIC-P679. **b** Specimen MIC-P675. **c** Specimen MIC-P688. **d–g** SEM of the specimen MIC-P677a. **d** Coalified smooth to lined integument surface at seed half way. *Scale* 50  $\mu$ m. **e** FRU general view, note nucellar keel (black arrow). *Scale* 500  $\mu$ m. **f** External surface of seed envelop showing papillae (*pa*) and large trichome bases (*tb*). *Scale* 30  $\mu$ m. **g** SEM closer view of thick coalified cell walls of the outer envelope.

*Scale* 20  $\mu$ m. **h** FRU under stereoscope. *Scale* 500  $\mu$ m. specimen P338. **i** SEM picture from specimen P340 showing two FRU. *Scale* 500  $\mu$ m. **j** Closer view of the impression of the outer envelope in **i** *Scale* 30  $\mu$ m. *aoe* apical outer envelope, *b1* bract from the first cone whorl, *b2* bract from the second cone whorl, *b3* bract from the third cone whorl, *FRU* female reproductive unit, *in* integument, *io* impression of hexagonal cells from the outer envelope, *mt* micropylar tube, *nu* nucellus, *oe* outer envelope, *pa* papilla, *tb* trichome base

**Type locality** Type section of the La Cantera Formation (32°59'25"S, 66°52'48"W), San Luis Province, Argentina (Fig. 1a).

**Stratigraphy** La Cantera Formation (upper Aptian), El Gigante Group, San Luis Basin.

**Etymology** The specific epithet refers to the La Cantera Formation where the fossils were found.

**Specific diagnosis**

Ovulate cones ovoid in three-dimensional shape, with four whorls of thin sterile bracts. Several small and broad bracts comprise the basal whorl, several (six?) small bracts found in the second whorl, three large bracts comprise the third whorl, and two or three larger bracts are found in the fourth

whorl. The fourth whorl of bracts partially cover the two fertile reproductive units. Bracts are thin, with a narrow margin, 2–5 cells wide. Two ovoid seeds per ovulate cone are found, which are completely covered by a seed envelope, protruding as a long and narrow micropylar tube. The outer surface of the seed envelope papillate, with two types of trichomes. Most epidermal cells show a central prominent papilla; other scattered cells exhibit a single flat trichome base, occupying most of the cell surface. Epidermal cells of the seed envelope are polygonal in shape.

## Description

### *Ovulate cones*

Cones ovoid in shape, 2.58–3.63 mm long and 1.66–2.50 mm wide. Thin flat bracts in whorls, imbricated at the base. Strong peduncle (stalk), 0.51 mm long and 0.41 mm wide (Fig. 2a). Each ovulate cone has four whorls of sterile bracts. The basal whorl is a condensed whorl of small, somewhat broad bracts with an imbricate arrangement at the basal 0.5 mm of the ovulate cone. In most cases only basal scars are visible (Fig. 2h–j). We have counted up to six basal bracts in one face from a compressed cone (Fig. 2a–d), several (probably six) bracts at the second whorl, three larger bracts at the third whorl, and two or three large bracts at the fourth whorl (Fig. 2d, h). Bracts have smooth margins with lateral extensions, with two to three rows of thinner and darker epidermal cell walls at the margin (Fig. 2e, i, j). The bract margins have up to five rows of cells at the bract apex (Fig. 2i, j). Bracts in the basal whorl are 0.70–1 mm long and 0.4–0.8 mm wide, bracts in the second whorl are 0.70–1.38 mm long and 0.33–0.5 mm wide, bracts in the third whorl are 0.79–1.5 mm long and 0.35–0.72 mm wide, and bracts in the fourth whorl are 0.75–1.67 mm long and 0.30–0.63 mm wide. General thickness of the bracts measures 2.5  $\mu$ m (Fig. 2f). Bract epidermal cells on the abaxial surface are hexagonal, 100  $\mu$ m in diameter, and oriented in longitudinal rows toward the apex (Fig. 2e). A papillate texture can be observed on the inner epidermis of bracts (Fig. 2f). Each ovulate cone contains two elliptical FRU projecting toward the apex (Fig. 2a, d), and a long and narrow micropylar tube is preserved (Fig. 3a, b).

### *Female reproductive units (FRU)*

Beside FRU attached to ovulate cones, several isolated FRU were also found in the same stratigraphic bed (Fig. 3e–i). The isolated FRU vary from 2.5 to 8 mm in length to 1.5–3 mm in width. Bracts cover the FRU almost completely, but several specimens show two exerted FRU at the tip of the ovulate cone (Fig. 2a, i). They are ovoid

in shape, with a rounded base and pointed apex, although often they are preserved as flat compressions (Fig. 3g, h).

### *Organization and structure of the FRU*

The FRU are composed of three distinct layers: a thick outer seed envelope, a thin coalified integument, and the nucellus (Fig. 3e). The outer envelope consists of an outer epidermis, mesophyll (preserved as an undifferentiated coalified layer), and an inner epidermis. The outer seed envelope thickness measures 50  $\mu$ m at the half seed (oe at Fig. 3e). The outer epidermal cell shapes are preserved as impressions (Fig. 3e, g). Outer epidermal cells are polygonal, isodiametric in shape, and 20–25  $\mu$ m in diameter (Fig. 3f). Most outer epidermic cells are papillate, but scattered among these papillate cells other epidermal cells possess a central large rounded protuberance with bordered edges, interpreted as a trichome base. The papillae are 10  $\mu$ m in diameter and 6  $\mu$ m high. The trichome bases are 20  $\mu$ m in diameter, with a central depression and bordered ring, raised less than 3  $\mu$ m above the epidermal cell surface (Fig. 3f, j).

The outer envelope is 50  $\mu$ m thick and no anatomical structures were observed, other than spherical cavities of 10  $\mu$ m with a possible diagenetic origin (Fig. 3g). The inner epidermis of the outer envelope was not well preserved, although it does seem to have a similar papillate surface than the outer envelope.

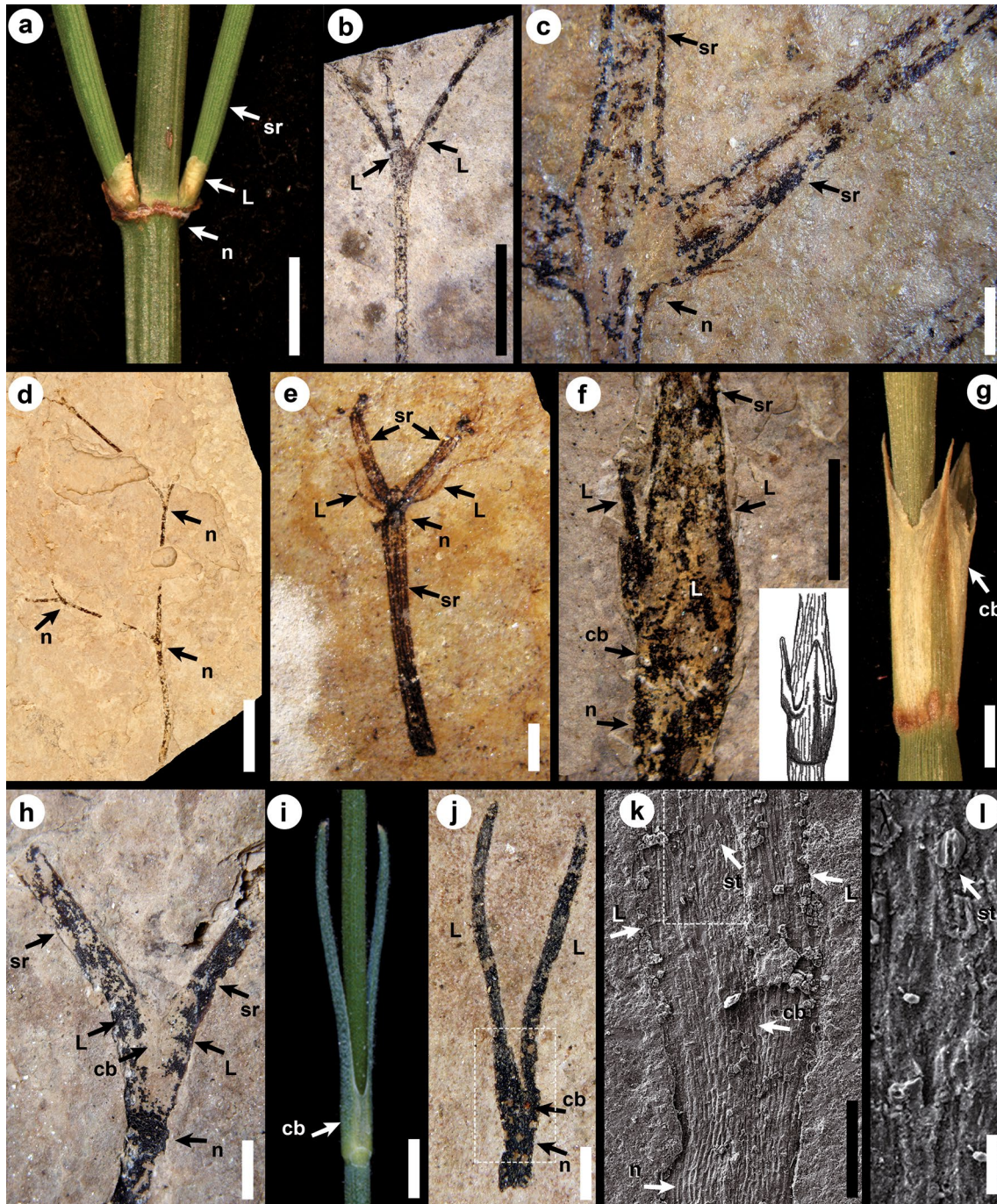
The integument is thin and further extended from the outer envelope into a long, narrow micropylar tube (Fig. 3a, b). In most cases, the micropylar tube is not preserved beyond the seed apex (Figs. 2a, 3c, e). The integument is evident at the distal half of the seed, consisting of a thin coalified layer (Fig. 3e). The coalified integument is interpreted as originally lignified cell walls (Fig. 3a, b). The long micropylar tube measures 800  $\mu$ m in length and 90  $\mu$ m in width.

The nucellus is ovoid in shape with a rounded base and acuminate apex. There is one longitudinal keel in each external seed surface (Fig. 3e) giving a three-radiated symmetry in cross section.

### *Associated vegetative structures*

**Stems** Erect and striate stems divided into nodes and internodes, branching at angles of 26°–69°. Striate stems with at least four longitudinal ribs slightly swollen at the nodes. The axes in the internodes usually between 0.3 and 2 mm wide, and up to 35 mm long (Fig. 4a–e).

**Leaves** Leaves are inserted in whorls of 2 or 3 per node, with connate bases (Fig. 4b, c, e, f, h). We recognized two distinct leaf types at the nodes: one, with two long leaves



**Fig. 4** *Ephedra* living (**a**, **g**, **i**) and fossil (**b–f**, **h**, **j–l**) vegetative structures (stems, branches and leaves). **a** Extant *E. boelckei*. Scale 1 mm. **b** Fossil stem showing trifurcated branching with attached scale leaves at node. Scale 1 cm. MIC-P283. **c** Node detail from MIC-P686 with two opposite branches arising at acute angle. Scale 2 mm. **d** Fossil stem with branching MIC-P685. Scale 2 cm. **e** Fossil stem with two linear leaves in opposite arrangement MIC-P670. Scale 1 mm. **f** Closer view at a fossil node with three scale leaves in a whorl. Scale 1 mm. MIC-P669 (line drawing detailed in box). **g** Extant *E. triandra* node detail showing three scale leaves, simi-

lar to the fossil specimen illustrated in **f**. Scale 1 mm. **h** Fossil stem with branching and connate base of leaves scale 1 mm. MIC-P685. **i** Extant *E. aspera* with two large linear leaves. Scale 1 mm. **j** Two linear leaves in opposite arrangement with sheathing bases. Scale 1 mm. MIC-P699. **k** SEM image showing rectangular epidermal cells in leaves and more detail in the rectangle. Scale 200  $\mu$ m. MIC-P699. **l** SEM image showing scattered stomata in the epidermis of the stem. Scale 50  $\mu$ m. MIC-P669. *cb* connate base of leaves, *L* leaf, *n* stem node, *sr* stem, *st* stomata

in opposite arrangement, measuring 5.5 mm in length and 0.5 mm in width at the base (Fig. 4h, j). Other nodes bear scale-like leaves, with tristichous arrangement, the leaves have fused bases and a triangular apex (Fig. 4f). The epidermal cells of both leaf types are polygonal, narrow-elongate (55–89  $\mu\text{m} \times 17\text{--}20 \mu\text{m}$ ), and arranged in longitudinal rows (Fig. 4k, l). Sunken stomata were observed in the abaxial cuticle of the larger leaves (Fig. 4l).

### Comparison with extant *Ephedra* species

#### Reproductive characters

The fossils described here include associated (but not attached) vegetative and reproductive plant remains that were recovered from the same strata of the La Cantera Formation. The association of the fossil organs with the presence of distinctive morphological characters allows a partial reconstruction of a plant with similarities to extant *Ephedra* (fourth reliability grade from Anderson and Anderson 1985) (Table 1). Among the reproductive structures, the most important characters identified are: (1) two large seeds in the apex of each ovulate cones, covered with bracts in whorls, (2) FRUs with two seed envelopes (an outer envelope, plus the inner integument that formed the micropylar tube), (3) long micropylar tube, and (4) a papillate outer seed envelope. Furthermore, we recognized associated vegetative characters such as: (1) nodes with 2–3 whorled leaves, (2) scale-like leaves, (3) connate leaf bases, (4) striate and erect stems with nodes and internodes, and (5) and sunken stomata (Table 1). Furthermore, pollen grains isolated in the same fossiliferous bed confirm the presence of Ephedraceae for the paleoenvironment.

The extant *Ephedra* as well as the new fossil species have orthotropous ovules, and each ovulate cone can have one to three ovules (always two in the new fossil species) positioned in the axils of the distalmost whorl of sterile bracts (Rydin et al. 2010; Yang 2004, 2007). In extant *Ephedra* species the micropylar tube is hollow throughout, a character that was not possible to determine in the compressed ovulate cones of *E. canterata* sp. nov.

In the new fossils, the integument is coalified (Fig. 3d), which is likely caused by the lignified nature of cell walls. In most extant *Ephedra* species, that lignification is only present at the tangential and radial walls of the inner epidermis cells (Rydin et al. 2010).

In many extant *Ephedra* species, cone bracts become dry and papery, but in other species the bracts become fleshy and colored at seed maturity, and aid in attracting seed dispersers. Free portions of cone bracts in most extant *Ephedra* species have a hyaline margin (Rydin et al. 2010). The hyaline margin and the amount of mesophyll tissue are characters used for recognition of dry or fleshy bracts (Hollander et al. 2010; Ickert-Bond 2003; Rydin et al. 2010; Stapf 1889; Yang and Wang 2013). In *Ephedra* cone bracts, epidermal cells can be tanniferous with undulate walls and the cuticle can be thin (e.g. *E. californica* S. Watson) or thick (e.g. *E. major* Host). In *E. canterata* sp. nov. the cone bracts show differentiated marginal cells that consist of two to three rows of epidermal cells with a thicker wall (Fig. 2i, j) which is uncommon in wind-dispersed *Ephedra* cone bracts (Hollander et al. 2010).

The number of bracts and seeds per cone are characters that vary among and within species in extant *Ephedra* (El-Ghazaly and Rowley 1997; Foster 1972; Huang et al. 2005; Ickert-Bond 2003). In *E. canterata* sp. nov. four whorls of bracts are clearly distinguishable. They have 3–6 small

**Table 1** Comparison between *Ephedra canterata* sp. nov. and extant *Ephedra* species

Morphological and anatomical characters	<i>Ephedra canterata</i> sp. nov	Extant <i>Ephedra</i> species
Reproductive characters		
Orthotropous ovules	Yes	Yes
Number of ovules/seed	2	1–3
Ovules position	Axillary	Axillary
Marginal cells of cone bracts	2–5 rows of thick epidermal cells	Thin or thick epidermal cells
Number of bracts	Four whorls of bracts	Variable
Long micropylar tube	Yes	Yes
Sculpture of seed envelope	Papillate	Pluspapillate, transverse
	Large trichome bases <sup>a</sup>	llamellar, smooth
Vegetative characters		
Leaves	Scale-like and foliose	Scale-like, foliose, linear or triangular
Stems	Striate with nodes and internodes	Striate with nodes and internodes
Stomata	Sunken	Sunken

<sup>a</sup>New character of *E. canterata* not found in extant *Ephedra* species



bracts in the basal whorl, three broad bracts in the second whorl, three larger bracts in the third whorl, and two or three large bracts in the fourth whorl (Fig. 2d, h). In all cones examined, there were only two well-developed seeds.

*Ephedra canterata* sp. nov. has a stalked cone, with basal bracts that partially covered its base, as in *Ephedra viridis* Coville (Fig. 2k) which was historically placed in Section Asarca Stapf, although not resolved as a monophyletic group (Ickert-Bond 2003).

The seed envelope of extant *Ephedra* species can show three different micromorphological patterns on the outer surface including papillae, transverse lamellar protuberances, and smooth surfaces (Ickert-Bond and Rydin 2011; Yang 2011). The inner epidermis of the most distal part of the seed envelope shows a papillate zone in all species of *Ephedra* (Rydin et al. 2010). The eleven extant *Ephedra* species from South America have a smooth outer seed envelope, meanwhile in *E. canterata* sp. nov. the outer envelope is papillate, as was recorded in others Cretaceous fossil species (Ickert-Bond and Rydin 2011; Rydin et al. 2006a). The rare basal trichomes found in the new fossil species are totally new for the genus, although Rydin et al. (2010) note similar size papillae on the epidermis of the seed envelope, near the micropyle. Seeds with papillate sculpture in extant species of *Ephedra* are present in: *E. equisetina* Bunge, *E. major* and *E. pachyclada* Boiss. from Asia and *E. fasciculata* A. Nelson and *E. funerea* Coville et Morton from North America (Yang 2011).

Similarly, outer epidermal cells with a polygonal isodiametric outline shape were recorded in the seed envelopes of several extant *Ephedra* species, this characteristic does not appear to be useful in clade recognition (Majumder et al. 2014; Rydin et al. 2010).

#### *Vegetative morphological and anatomical characters*

In addition to the fossil ovulate cones and isolated seeds (FRU) recovered at the La Cantera Formation, several vegetative organs were also recovered, that provide further information for reconstructing *Ephedra* in the Cretaceous San Luis Basin. The size and shape of leaves and stems also vary within living *Ephedra* species (Gifford and Foster 1989; Ickert-Bond 2003). In most species, the leaves are minute (less than 10 mm long), foliose, linear or ovate to triangular in shape, and they have 2–3 parallel veins (Dorken 2012; Kubitzki 1990; Rydin et al. 2006a; Yang et al. 2015). The scale-like leaves are common in most extant *Ephedra* species, e.g. *E. trifurca* Torrey ex S. Watson (Fig. 4g). Further development of leaves has only been cited in a few species, e.g. *E. aspera* Engelm. ex S. Watson (Fig. 4i) and *E. nevadensis* S. Watson, showing that presence of both leaf types can be found on the same plant. The

last, it could also be interpreted in the new fossil described here, otherwise associated vegetative structures could indicate the presence of more than one *Ephedra* species in the La Cantera Formation.

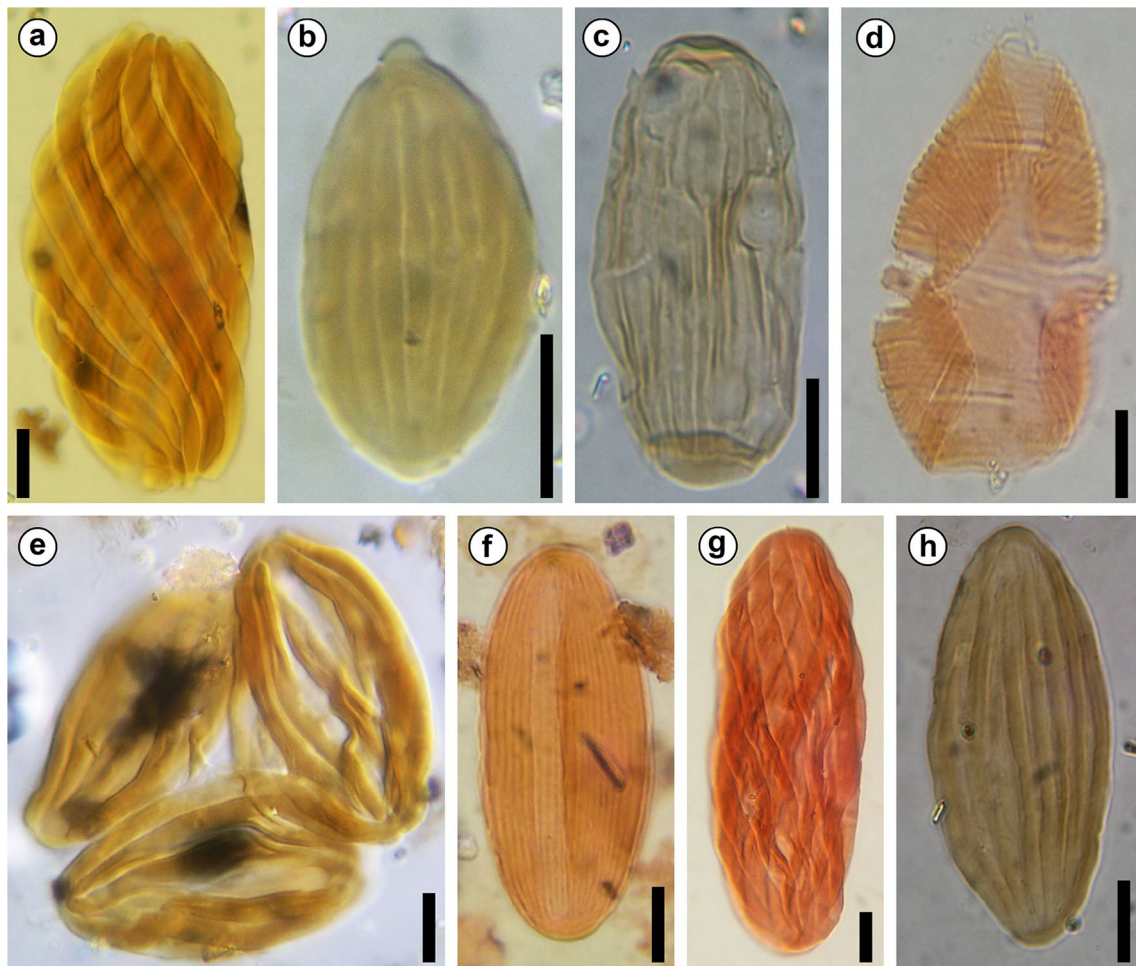
#### *Polylicate pollen grains from the La Cantera formation*

Up to date, no pollen cones were recognized in the La Cantera Formation. However, the abundant polylicate pollen grains recovered from the same strata with plant remains confirms the presence of diverse gnetaleans, and the presence of the ephedroid pollen type (Fig. 5).

Plicate pollen grains are very abundant and diverse in the studied microflora with an abundance of 3.9% in the less represented associations to 46.8% in those highly represented. Different polylicate pollen types belonging to three different fossil genera were recognized from the studied section: *Ephedripites* spp., *Steevesipollenites* spp. and *Gnetaceaepollenites* spp. In general, ephedroid pollen grains are elliptical and polylicate with a wide range of variation in pollen size and plicae (twisted spiral or longitudinal plicae). The sculpture of pollen grain exines in *Gnetum* is spinose or spinulose, while it is polylicate in *Ephedra* and *Welwitschia*.

The smaller type of these pollen grains measures 22–26 µm in length (*Steevesipollenites pygmeus* Azema & Boltenhagen, Fig. 5b) and the larger type is 40–67 µm in length (*Ephedripites*, *Gnetaceaepollenites* Thiergart, Fig. 5g, h). Pollen grains with ellipsoidal to fusiform shape, parallel ridges which converge at the tips forming a thickened cap-like area, are placed in the fossil taxon *Steevesipollenites* (Fig. 5c). In the palynoflora from the La Cantera Formation, we also found dispersed shed pollen exines (germinated pollen grains) curled up in a characteristic transverse striation (Fig. 5d), identical to those recovered and illustrated by Rydin et al. (2004) from Early Cretaceous *Ephedra* seeds in Buarcos, Portugal. Some grains with a longitudinal aperture found in the fossil association (Fig. 5f) could probably best be described as the *Welwitschia* pollen type. Pollen of living *Welwitschia* are similar to *Ephedra*, but *Welwitschia* grains have a sulcus and the exine remains on the micro gametophyte that differentiates it from *Ephedra* (Rydin and Friis 2005; Rydin et al. 2006a).

Recently, Bolinder et al. (2016) published the results of a comparative study on the pollen morphology of *Ephedra* and discussed its evolutionary implications. They recognized two distinct forms of ephedroid pollen in the fossil record, an ancestral type with unbranched pseudosulci, and a derived type with branched pseudosulci. All ephedroid pollen types from the La Cantera Formation possess unbranched pseudosulci.



**Fig. 5** *Ephedra* pollen types from the La Cantera Formation, San Luis Basin, Argentina. Scale 10  $\mu\text{m}$  on all photomicrographs. **a** *Gnetaceapollenites barghoornii* (Pocock) de Lima; 2243a: Q31/0. **b** *Steevesipollenites pygmeus* Azema and Boltenhagen, 3049c:Q32/4. **c** *Steevesipollenites* sp. 2243a:S33/0. **d** *Gnetaceapollenites* sp. (prob-

ably *Ephedra* shed pollen exines) 8263c:N24/4. **e** *Ephedripites* sp. (three specimens together) 3049c: U37/0. **f** Probable *Welwitschia* pollen type. Polyplcate grain with a sulcus; 2245a:S26/4. **g** *Ephedripites* sp. 8267H: M25/4. **h** *Ephedripites* sp. 3049c:H25/2

### Fossil distribution and comparison

During the Mesozoic, the ephedroid pollen record rapidly increase in both abundance and diversity during the Cretaceous, reaching a peak around 100 Mya (Crane and Lidgard 1989). Ephedroid cretaceous macrofossils are abundant and diverse in China (e.g. Cao et al. 1998; Rydin et al. 2006b; Sun et al. 2001; Wu et al. 1986; Yang et al. 2013; Yang and Wang 2013), central Asia (Krassilov 1982; Krassilov and Bugdaeva 1982), and Australia (Krassilov et al. 1998).

In South America, the Cretaceous record with ephedroid affinity includes pollen grains and macrofossils. Numerous ephedroid polyplcate pollen grains were recovered in several basins (e.g. Archangelsky and Llorens 2009; Baldoni 1992; Brenner 1968; Lima 1978; Narvaez et al. 2014; Papú 2002; Prámparo 1988a, b, 1989; Prámparo and Volkheimer

1999; Quattrocchio et al. 2006; Vallati 2006; Volkheimer et al. 1977; Volkheimer and Prámparo 1984).

Macrofossil of Ephedraceae in South America are known only from two sites: one of them of Aptian age in the Araripe Basin (Brazil) based on isolated stems, ovulate cones and seeds but without a detailed description for comparison (Bernardes de Oliveira et al. 2003; Mohr et al. 2004) and *Ephedra verticillata* Cladera et al., which is known based on organic connection of stems, ovulate cones and seeds from the Aptian Baqueró Group, southern Argentina (Cladera et al. 2007). *Ephedra verticillata* has acuminate FRUs with a striate surface and only two large triangular bracts that are widely set apart at the cone apex (Cladera et al. 2007), clearly differing from *E. canterata* sp. nov.

Additionally, also from the Araripe Basin (Brazil) *Itajuba yansanae* Ricardi-Branco et al. is known based on

striate and trifurcate branching system bearing terminal ovulate cones (Ricardi-Branco et al. 2013). The very peculiar reproductive structures of *I. yansanae* do not correspond to *Ephedra* or any other gnetalean genus and was placed as *incertae sedis* among Gnetales.

Up to date, the world-wide macrofossil record of *Ephedra* consists of the following species: *E. sotzkiana* (Unger) Schimper, *Ephedra* sp. Engelhardt, and *E. miocenica* Wodehouse, which were established based only on vegetative organs. *Ephedra paleozoique* Renault was described from a longitudinal section of a reproductive organ, but lacks more diagnostic characters. *Ephedra portugallica* Rydin et al. from the Buarcos locality (Portugal) and *E. drewriensis* Rydin et al. from the Potomac Group sequence at the Drewry's Bluff locality (eastern USA), were based on the ultrastructure of seeds with in situ pollen grains (Rydin et al. 2006a). Both are distinguished from *E. canterata* by their small size (0.6–1.6 mm), four-angled nature and also the fossil described here lack in situ pollen grains (Rydin et al. 2006a). The seed *Ephedrispermum* Rydin et al. shares similarities to recent *Ephedra*, although it differs from *E. canterata* in the absence of papillate surface in the seed envelop (Rydin et al. 2006a).

Five fossil species are known with preservation of the articulate branches, leaves and reproductive structures: *Ephedra nudicaulis* Saporta from the Miocene flora of d'Aix-en-Provence, France, possesses articulate branches, with striations on the surface, and ovulate cones with bracts containing “seeds”; the FRU is kidney-shaped while the lobes of the leaf sheath were triangular, features that rule out any similarity with *E. canterata* sp. nov. and the associated fossil vegetative material. *Ephedra cheniae* (Cao and Wu) Liu et al. (2008) ≡ *Liaoxia cheniae* (Guo and Wu), from the Barremian in the Liaoning Province, China, has typically ovate to spatulate ovulate cone scales. *Ephedra xingxuei* (Tao and Yang) Liu et al. (2008), from Aptian-Albian of Jilin of China has branched stems asymmetrical and spike-like ovulate cones with oblong FRU and spatulate cone bracts. *Ephedra archaeorhytidosperra* Yang et al. from China has FRU that resembles *E. canterata* sp. nov. in their size, ovoid shape with pointed apex and the presence of an extended micropyle (Yang et al. 2005), but clearly differ in having a prominent, longitudinal, dorsal ridge and transverse, lamellar and undulating protuberances. *Ephedra hongtaoi* Wang and Zheng from The Yixian Formation (China) has a pair or single FRU in each ovuliferous cone, but the last are poorly defined: the bracts are rarely seen and their seeds are unknown (Wang and Zheng 2010) which make further comparison difficult with the new fossil species. Also from northeast China *Ephedra carnosa* Yang and Wang has triovulate cones with only one whorl of bracts and the bracts are thickened (Yang and Wang 2013) which clearly differ from *E. canterata*.

*Ephedra multinervia* Yang and Lin (Yang et al. 2015) is another Yixian Formation fossil, this species has leaves with multiple dichotomizing veins and ovuliferous cones with a single pair of fertile bracts forming a cupule enclosing two inner seeds, these characters differ from those of *E. canterata*.

Based on the above, the new fossils here described from the La Cantera Formation cannot be assigned to any other previously described fossil or any recent *Ephedra* species, supporting the delineation of a new species, by the presence of cone bracts in four whorls and an outer seed envelope with two types of trichomes. The new record corresponds to one of the oldest macrofossil records of *Ephedra* in the Southern Hemisphere.

## Final conclusions

Based on the evidence presented here, the new fossil described from the La Cantera Formation, San Luis Basin, can be unequivocally placed within the genus *Ephedra*. The analysis of reproductive and associated vegetative plant organs preserved in the same stratigraphic level, allowed a partial reconstruction of a new *Ephedra* species whole plant.

Most of the previously assembled fossil data available for *Ephedra* come from pollen. In the Cretaceous, ephedroid pollen grains were more diverse in morphology and much more abundant in the paleofloras than they are today (Rydin and Hoorn 2016; Traverse 1985). Existing *Ephedra* species are adapted to dry environments, and the abundance in Cretaceous sediments could be related to warmer and dryer environments that were developed into tropical and subtropical regions during the Cretaceous (Scotese et al. 1999; Wang and Zheng 2010), similar environmental conditions were interpreted based on different evidences for the La Cantera Formation.

The abundance of characteristic ephedroid polyplicate pollen grains are also recorded in some other Cretaceous floras from northern and central South America (Brenner 1968; De Lima 1980; Herngreen 1973; Herngreen and Dueñas Jimenez 1990; Narvaez et al. 2014; Pons 1988). Furthermore, the rich variety of polyplicate palynomorph species with straight or twisted ridges belonging to the ephedroid type is a characteristic feature of the African–South American (ASA) microfloral province (Herngreen and Dueñas Jimenez 1990).

As noted by Prámparo (1990), the presence of basal angiosperms with strikingly distinctive morphologies and affinities (mainly *Afropollis*), abundant and diverse plicate grains, but also abundant *Classopollis* place the microflora of the La Cantera Formation as transitional between the Northern Gondwana (*Dicheiropollis etruscus*/*Afropollis*)

and Southern Gondwana (*Trisaccites* Cookson and Pike) Cretaceous palynofloral provinces (Fig. 1b). The Northern Gondwana province was interpreted as a savanna-type vegetation thriving under arid to semi-arid conditions (Brenner 1976; Chumakov et al. 1995; Dino et al. 1999). The abundant record of *Ephedra* in the La Cantera Formation (indicative of dry, even arid conditions) in association with abundant thermophilic pollen (*Classopollis*), climatic sensitive rocks, primary gypsum and frequent desiccation cracks, suggest a marked arid season (Arcucci et al. 2015; Prámparo 1999). The fossil record of *E. canterata* in the San Luis Basin, corresponds to a new element that gives light into the Early Cretaceous paleobiogeographical reconstruction of South America. Based on the latitudinal position (Fig. 1b) this flora may represent one of the southernmost records of the Cretaceous arid biome for Southwestern Gondwana.

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