The LINNEAN SOCIETY of London BOTANICAL Journal of the Linnean Society

Botanical Journal of the Linnean Society, 2010, **163**, 387–430. With 5 figures

The female reproductive unit of *Ephedra* (Gnetales): comparative morphology and evolutionary perspectives

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Received 2 February 2010; revised 2 February 2010; accepted for publication 9 June 2010

Morphological variation in *Ephedra* (Gnetales) is limited and confusing from an evolutionary perspective, with parallelisms and intraspecific variation. However, recent analyses of molecular data provide a phylogenetic framework for investigations of morphological traits, albeit with few informative characters in the investigated gene regions. We document morphological, anatomical and histological variation patterns in the female reproductive unit and test the hypothesis that some Early Cretaceous fossils, which share synapomorphies with *Ephedra,* are members of the extant clade. Results indicate that some morphological features are evolutionarily informative although intraspecific variation is evident. Histology and anatomy of cone bracts and seed envelopes show clade-specific variation patterns. There is little evidence for an inclusion of the Cretaceous fossils in the extant clade. Rather, a hypothesized general pattern of reduction of the vasculature in the ephedran seed envelope, probably from four vascular bundles in the fossils, to ancestrally three in the living clade, and later to two, is consistent with phylogenetic and temporal analyses, which indicate that extant diversity evolved after the Cretaceous–Tertiary boundary. Notwithstanding striking similarities between living and Cretaceous *Ephedra*, available data indicate that the Mesozoic diversity went almost entirely extinct in the late Cretaceous causing a bottleneck effect in *Ephedra*, still reflected today by an extraordinarily low level of genetic and structural diversity.

ADDITIONAL KEYWORDS: anatomy – character evolution – Cretaceous – fossils – histology – reproductive morphology – seed plants.

INTRODUCTION

The phylogeny of seed plants is one of the major unresolved questions in evolutionary plant biology (see, for example Bateman, Hilton & Rudall, 2006; Doyle, 2008; Mathews, 2009). Studies based on molecular data have largely overturned what we thought we knew (based on morphology), but have also demonstrated strong conflicts among and within loci (e.g. Sanderson *et al*., 2000; Rydin, Källersjö & Friis, 2002; Burleigh & Mathews, 2007a, b; Rydin & Korall, 2009). Nearly all possible relationships among the six extant major clades have been suggested; for example, with Gnetales sister to angiosperms (Chase *et al*., 1993; Stefanovic *et al*., 1998), Gnetales sister to Pinaceae (Bowe, Coat & de Pamphilis, 2000; Chaw *et al*., 2000), Gnetales sister to all conifers (Rydin *et al*., 2002; Rydin & Korall, 2009), Gnetales sister to Cupressophyta (Raubeson *et al*., 2006), Gnetales sister to all other seed plants (Källersjö *et al*., 1998) or Gnetales sister to all other gymnosperms (Schmidt & Schneider-Poetsch, 2002). Moreover, the various results have often received strong statistical support. In the most recent classification of the land plants (Chase & Reveal, 2009), the gymnosperms are treated as four subclasses (Ginkgoideae, Cycadidae, Pinidae and Gnetidae), but no suggestion was made about the

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interrelationships of these four, reflecting the conflicting results from the different analyses.

There are certainly several reasons for this diversity of results; conflicting information in molecular data is only one. It has, for example, been demonstrated that a slight alteration of species representation in phylogenetic analyses of seed plants can completely change seemingly strongly supported results (Rydin & Källersjö, 2002). Further, the homology of reproductive structures is poorly understood, which is a major problem and limitation when analysing seed plant phylogeny using morphological information. For example, the ancestral angiosperm carpel is of the ascidiate type (not conduplicate) and sealed by secretion (not postgenital fusion) (Doyle & Endress, 2000; Endress & Igersheim, 2000; Endress & Doyle, 2009), but the sister group of angiosperms has not been identified (Mathews, 2009) and the morphological equivalent of the carpel in gymnosperms (if any) is unclear. Several extant and extinct seed plants have extra-ovular protective organs, for example, angiosperms, Gnetales, Erdtmanithecales, Caytoniales, glossopterids and Bennettitales (Friis *et al*., 2007; Doyle, 2008), but are these organs homologous?

Gnetales are constantly in focus in studies of seed plant phylogeny, partly because the phylogenetic position of the clade is unresolved and partly because of their unique and elusive morphology. Extant diversity is restricted to some 70–80 species (Kubitzki, 1990) of three morphologically and ecologically distinct genera (*Ephedra* L., *Gnetum* L., *Welwitschia* Hook. f.). However, recent discoveries of Early Cretaceous fossils have documented a larger diversity in the past. Some fossils have strong affinities to *Welwitschia* (e.g. Crane & Upchurch, 1987; Rydin, Mohr & Friis, 2003; Dilcher *et al*., 2005). Other fossils share most features with *Ephedra* (e.g. Krassilov, 1982; Rydin, Pedersen & Friis, 2004; Yang *et al*., 2005; Rydin *et al*., 2006a; Rydin, Wu & Friis, 2006b), but it has typically not been possible to assign the ephedroids to any particular subclade within *Ephedra*. However, in some cases, it has also not been possible to separate them from the extant genus by any character, and a consequence of the poorly resolved relationships among living and fossil species is that the fossils have been of limited use for further studies; for example, as calibration points in molecular dating analyses. A better understanding of *Ephedra*, ephedroids and basal relationships in Gnetales, might have profound implications for the understanding of the timing of diversification in extant *Ephedra* and could also provide clues to the relationships of Gnetales to other seed plants.

The difficulties in assessing the phylogenetic position of ephedroid fossils in more detail are partly because of restricted knowledge of the phylogeny and morphology of living species. Extant *Ephedra* comprises approximately 45 species of shrubs or climbers distributed in arid environments in subtropical to warm temperate regions. The reproductive units are interpreted as compound, i.e. they are complex and evolutionarily derived from a branching system (e.g. Strasburger, 1872: 238; Land, 1907; Eames, 1952; Bierhorst, 1971: 467; Mundry & Stützel, 2004). Gross morphological variation between extant species of *Ephedra* (i.e. traits used in floras) is limited and has often appeared confusing from an evolutionary perspective. Variable characters (e.g. growth habit, pollen morphology and leaf and cone morphology) show substantial intraspecific variation (Foster, 1972; El-Ghazaly & Rowley, 1997; Ickert-Bond, Skvarla & Chissoe, 2003; Huang, Giannasi & Price, 2005). For example, the number of bracts and seeds per cone are characters that vary between species, but frequently these characters are also variable within species. Species delimitations are sometimes uncertain or under debate and attempts to find morphological support for a subgeneric classification (e.g. Stapf, 1889; Steeves & Barghoorn, 1959; Mussayev, 1978; Freitag & Maier-Stolte, 1994) have proved difficult and have resulted in different hypotheses.

Early attempts to resolve the phylogeny of the genus using molecular data (Ickert-Bond & Wojciechowski, 2004; Rydin *et al*., 2004; Huang *et al*., 2005) tentatively indicated that previous hypotheses of evolutionary relationships were incorrect, but restricted sampling resulted in poorly supported results. A recent study uses an extended sampling of species and molecular markers (Rydin & Korall, 2009) and provides more information, but the earliest divergences in the genus are still uncertain because of few informative characters in investigated loci. *Ephedra foeminea* Forssk. is resolved as sister to all other species, but with poor support (Rydin & Korall, 2009).

With the aim of improving the understanding of the morphology, histology and anatomy, we performed a comparative study of extant *Ephedra* spp. with an evolutionary perspective. Previous studies have provided information regarding reproductive morphology and/or anatomy and histology in *Ephedra* (among others, Tieghem, 1869; Strasburger, 1872; Stapf, 1889; Jaccard, 1894; Thoday & Berridge, 1912; Herzfeld, 1922; Pearson, 1929; Maheshwari, 1935; Mehra, 1950; Eames, 1952; Martens, 1971; Takaso, 1985), generally based only on studies of one or a few species. Variation and patterns of evolution of reproductive characters have never been fully grasped.

Here we focus on the female reproductive unit and present a first assessment of inter- and intraspecific variation. Because of the plasticity of gross morphological features, we have utilized data from several sources (morphology, anatomy, histology, molecular

markers). Our intention is to provide new information on structural diversity and evolutionary patterns, information that is necessary to place fossils more precisely. Are there variable and phylogenetically informative characters within *Ephedra* and, if so, do these characters have potential to be preserved in Early Cretaceous fossils?

MATERIAL AND METHODS

Taxon sampling and scientific approach

We selected 45 species for the present study, largely covering the extant diversity of the genus. Whenever possible, we included several specimens of each species. Anatomy, histology and morphology were studied for a total of 96 specimens, and an additional 29 specimens have been studied for their gross morphology. Specimen vouchers and specification of investigations conducted are given in the Appendix.

To add further support to our conclusions and to address the extreme difficulties with species determinations/delimitations in *Ephedra*, we aimed at producing DNA data [the internal transcribed spacers of the nuclear ribosomal DNA (nrITS): ITS1, 5.8S, ITS2] for at least one of the sectioned vouchers of each species (Appendix). The molecular information was already available for many specimens (produced in previous studies by C.R.: Rydin *et al*., 2002, 2004; Rydin & Korall, 2009), but 30 sequences were newly produced for the present study.

Species identifications based on gross morphology were used as a first hypothesis. We then examined the anatomy and histology of each individual plant, re-examined gross morphology and re-evaluated our hypotheses on species identifications according to the new information. Finally, we tested these hypotheses using molecular data. Any incongruence would have resulted in further studies and re-evaluations of data (but no incongruence was found). The results were consequently used to assess morphological/anatomical/histological variation among and within species and to find synapomorphies of clades at all levels in the phylogenetic hierarchy.

Laboratory procedures and phylogenetic reconstruction

Molecular data

Extraction of DNA, amplification and sequencing were performed as described in Kårehed & Bremer (2007). Primer sequences and methods for alignment are described in Rydin & Korall (2009). Phylogenetic analyses were conducted on a data set comprising 136 *Ephedra* terminals and 8077 characters from the nuclear and plastid DNA (nr18S, nr26S, nrITS, *rbcL, rps4, rpl16, trnS-trnfM*). The backbone of the matrix is formed by the *Ephedra* data from Rydin & Korall (2009), with the addition of new nrITS sequences from vouchers relevant to the present study (for voucher information of specimens, for which no new data have been produced in the present study, see Rydin & Korall, 2009). Bayesian inference of phylogeny was performed as described in detail in Rydin & Korall (2009) (using two data partitions, nuclear data/plastid data, the GTRIG evolutionary model and flat default priors). Ten million generations were run. Trees were rooted on *E. foeminea* (Rydin & Korall, 2009).

Morphological, anatomical and histological data

Female cones with gametophytes at an early stage of development (pollination stage, archegonia developed) were fixed in 70% ethanol or in formalin–acetic acid–ethanol (FAA) and dehydrated under vacuum in an ethanol series (70–80–96–100%). In addition, a few cones at mature developmental stages were chosen for comparison. Herbarium material was softened using bis(2-ethylhexyl)sulphosuccinate sodium salt (following Erbar, 1995), for 7–10 days in room temperature and then placed in 70% ethanol and dehydrated under vacuum as described above.

For serial microtome sections, specimens were embedded in Kulzer's Technovit 7100 (2-hydroethyl methacrylate). We basically followed the procedures outlined in Igersheim & Cichocki (1996), but the seed envelope of *Ephedra* is difficult to infiltrate properly and, to aid in the process, slow and stepwise infiltration is needed. The stepwise infiltration was conducted using the following ratios of 100% ethanol and Technovit solution: (50 : 50, 25 : 75, 0 : 100). The specimens were kept at 6–8 °C for at least 3 days each in 50 : 50 and 25 : 75 and subsequently for several weeks in 100% infiltration solution. Often the material needed the total time of 1 month (or more) to infiltrate properly.

Embedded specimens were sectioned on a Microm HM 355 rotary microtome with a conventional knife D. The sections are 6–7 μm thick and were stained with ruthenium red and toluidine blue for 2 + 2 min and mounted in Histomount. Permanent slides are deposited at the Department of Botany, Stockholm University, Sweden (SUNIV).

Descriptions and terminology

For simplicity, we refer to the female reproductive unit as a cone, but we make no attempts to infer homology with the conifer cone in the present study. The organs that surround the nucellus are called the integument and the seed envelope, reflecting the hypothesis that only the innermost structure is

homologous to the integument of other seed plants (for justification and references, see Discussion).

The sections were investigated and documented using light microscopy. The morphology, anatomy and histology of female cones are described from the outside, in and from the top, downwards, based (mainly) on transverse sections. Median longitudinal sections were produced for a few species. The histology of the cone bracts is reported and compared based on information from the innermost pair or whorl of bracts, at their mid-length. Their thickness is measured as number of cell layers and was observed in the middle between the two vascular bundles. Shape of the seed envelope in transverse section (i.e. the number of angles) is described apically (in the papillate zone) and at mid-length of the ovule. Histology of the seed envelope is described at the distal (upper) half of the ovule, from the papillate zone to mid-length of the ovule. The presence of chemical compounds (lignin, tannins, cutin, etc.) is inferred based on the staining; no microchemical reaction tests have been performed.

Characters observed in all investigated species are reported for the genus. Variable characters are reported for each species (following the phylogenetic results in Rydin & Korall, 2009) and include any observed within-species variation.

RESULTS

Because of variable and overlapping features within/among species, sometimes even within a single specimen, we considered gross morphological examinations alone insufficient for species determinations. The anatomical and histological features investigated here proved to be powerful tools and will usually identify a small subclade within *Ephedra*, to which a specimen belongs, but will not always produce enough data to provide an answer at the species level. Adding DNA data was helpful in these cases because it provided an additional source of information. Phylogenetic results are consistent with results in Rydin & Korall (2009). Deep divergences are often better supported in the present study, whereas some of the higher level resolution found in Rydin & Korall (2009) is collapsed. The data matrix of the present study contains more question marks within *Ephedra* than that of Rydin & Korall (2009) because we only produced one gene region (nrITS) for vouchers new to the present study.

Variation in cellular details (e.g. the size and shape of cells, presence/absence of tannins) is probably of evolutionary significance in some cases, but is obviously also correlated with developmental stage of the plant and whether the material comes from a herbarium specimen or is freshly pickled. We discuss cellular details in cases where we consider them relevant, but they should be interpreted with caution. Further, we found mucilage in the tissues of several specimens; however, except for its presence in the micropylar tube, mucilage appears to be present only in herbarium specimens and is not further discussed in the present study.

Morphology, anatomy and histology of the female cone of *Ephedra*; characters observed in all investigated specimens

The female cone of *Ephedra* (Fig. 1) comprises cone bracts in decussate arrangement or in whorls of three. In the axil of the most distal pair or whorl of bracts there are one to three ovule(s), each surrounded by a seed envelope (Figs 1, 2). At the studied developmental stage (pollination stage) the cones are approximately 0.5–1.0 cm long and the cone bracts are green.

Cone bracts

The number of cone bracts in each cone is variable within and between species and the bracts may be free from each other or fused proximally to various degrees. The cone bracts are differentiated into outer epidermis, mesophyll and inner epidermis (Fig. 3). Free parts of the bracts have a hyaline margin, i.e. the lateral flanks of the bracts consist of only (1–) 2 cell layers (Fig. 3E). The width of the hyaline margin differs substantially between species; most species have a relatively narrow margin (*c.* 5–20 cells wide) but, in some species, the margin is well developed and forms a prominent 'wing' (50–70 cells wide or more) (Fig. 2C).

In addition to the cuticle, epidermis cells also often have a thin cuticle-like layer on the inner tangential side and on the radial sides (Fig. 3). Stomata are present in the outer epidermis and sometimes also in the inner epidermis. In the observed material, they always have the same structure (Fig. 3D); the guard cells are smaller than the normal epidermal cells, ovate in shape and are sunken to the level of the base of the epidermis cell layer. There are no obvious subsidiaries.

There are two parallel longitudinal vascular bundles in a cone bract (Fig. 2A–C) (two exceptions are found, see *Ephedra americana* and *Ephedra major* ssp. *procera*) and no secondary venation. The histology of the mesophyll (Fig. 3) varies between species and will be described for each species.

Bracts of many Old World species become fleshy at seed maturity; in a few species they become dry and papery. Among New World species, dry to semi-dry cone bracts are more common, but fleshy bracts also occur in many species. At the pollination stage of development, the histology of future dry bracts does not differ notably from future fleshy bracts. The width

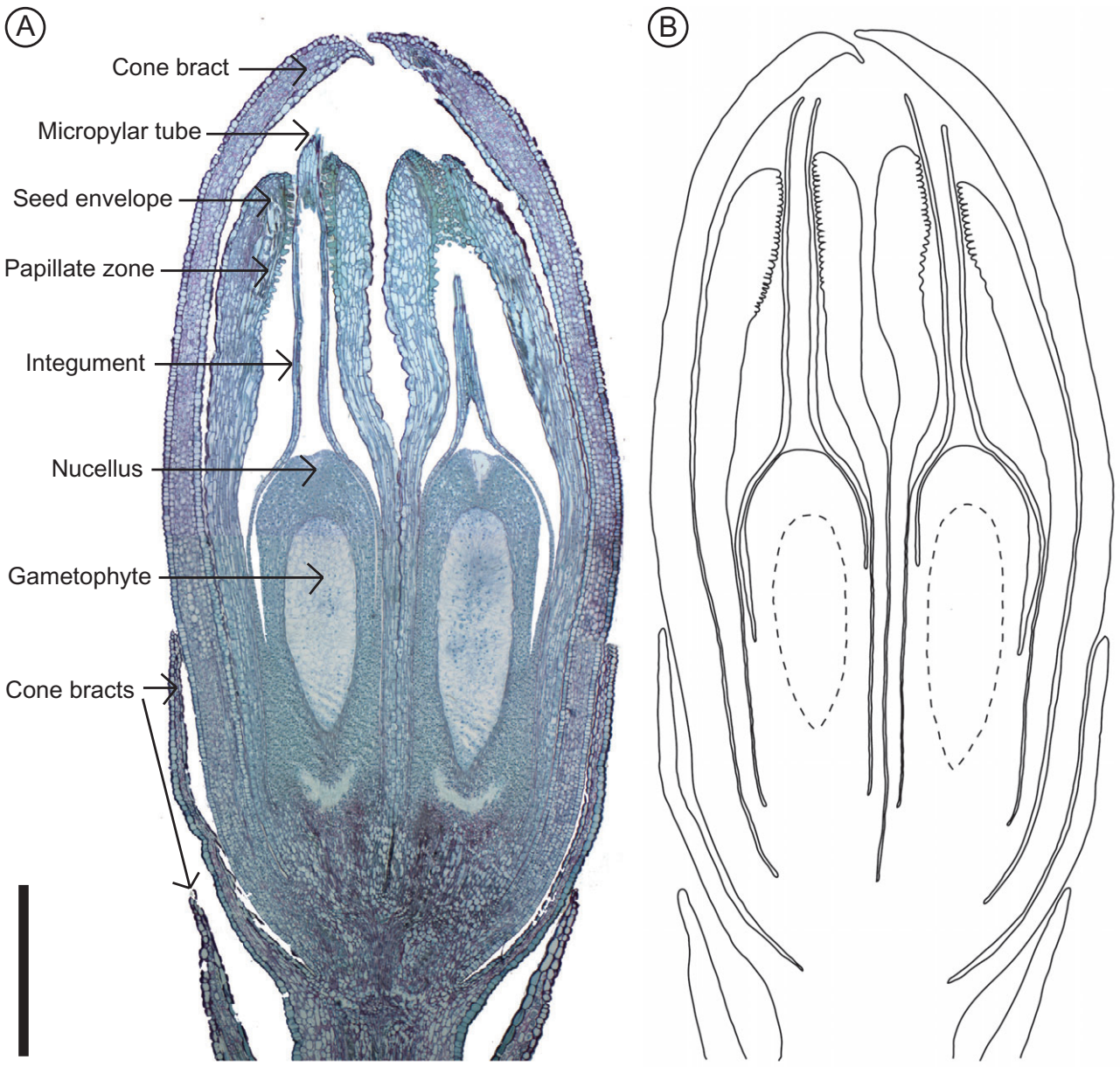


Figure 1. Median longitudinal section of a female cone (*Ephedra foeminea, 159*). A, photograph of microtome section. B, schematic reconstruction of morphology. Uninterrupted lines indicate morphological surfaces; dashed lines indicate the boundary between nucellus and gametophyte. Scale bar, 1 mm.

of the hyaline margin and the amount of mesophyll tissue are the main differences.

Ovules

There are one to three ovules in each cone, positioned in the axil of the distal-most bracts (Figs 1, 2A–C). There is never more than one ovule in the axil of a bract and never more than one ovule in a seed envelope. The ovules are orthotropous and unitegmic (Figs 1, 2). The ovules may be enclosed in the bracts or exserted.

Integument: The integument extends apically (beyond the nucellus apex) into a narrow micropylar tube, which is partly exposed and serves as the pollen-receiving area (Fig. 1). The micropylar tube is hollow throughout (Figs 1B, 4D–E) and more or less straight in investigated material, unless otherwise is stated. In some species, it is slightly bent in the most apical part (reflected by the superficially 'closed' micropylar tub in Fig. 1A). In some species, the micopylar tube is spirally twisted (indicated below). Mucilage was observed inside the micropyle of many investigated specimens.

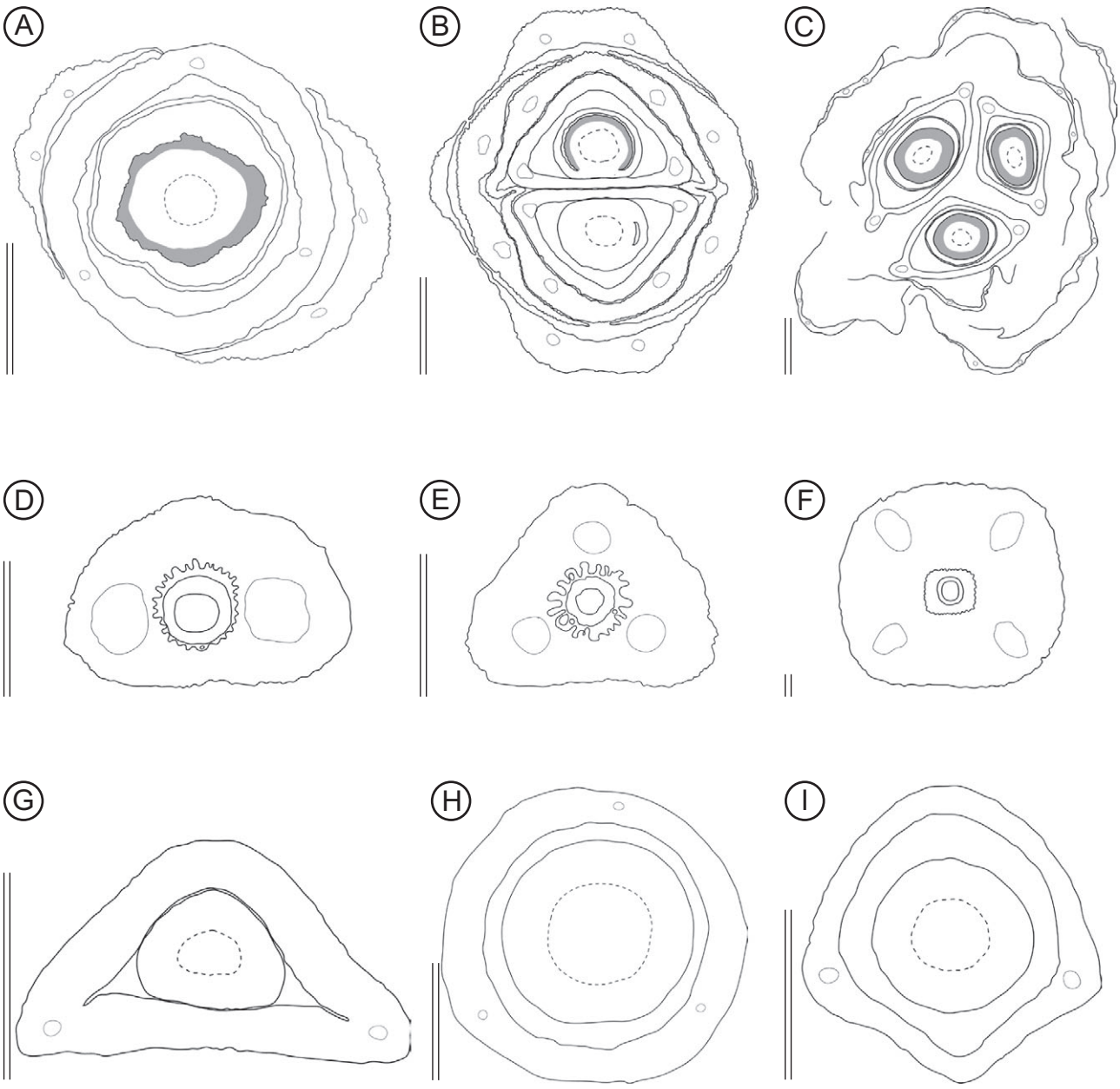


Figure 2. Transverse sections of (A–C) female cones slightly above mid-length of the ovule (integument is free from the nucellus). D–F, ovules with seed envelopes in the distal, papillate zone (strong correlation between the shape of the seed envelope and its anatomy). G–I, ovules with seed envelopes at mid-length of the ovule, below zone of free integument (only limited correlation between the shape of the seed envelope and its anatomy). Uninterrupted lines indicate morphological surfaces; dashed lines indicate the boundary between nucellus and gametophyte. Grey shading indicates secretory tissue. A, cone with one ovule. A single pair of cone bracts (*Ephedra aphylla, 154*). B, cone with two ovules. Four pairs of cone bracts; the innermost pair is fused (*Ephedra intermedia, 06*). C, cone with three ovules. Cone bracts in whorls of three, free from each other and with wide hyaline margins (*Ephedra multiflora, 224*). D, seed envelope with two vascular bundles, ovate in transverse section in the papillate zone (*Ephedra intermedia, 06*). E, seed envelope with three vascular bundles, triangular in transverse section in the papillate zone (*Ephedra foeminea, 130*). F, seed envelope with four vascular bundles, squared in transverse section in the papillate zone (*Ephedra fragilis, 109*). G–I, at mid-length of the ovule, there is only limited correlation between the shape of the seed envelope and anatomy (*Ephedra andina, 25; Ephedra altissima, 132; Ephedra saxatilis, 144*). Scale bars, 1 mm (A–C, G–H); 0.1 mm (D–F).

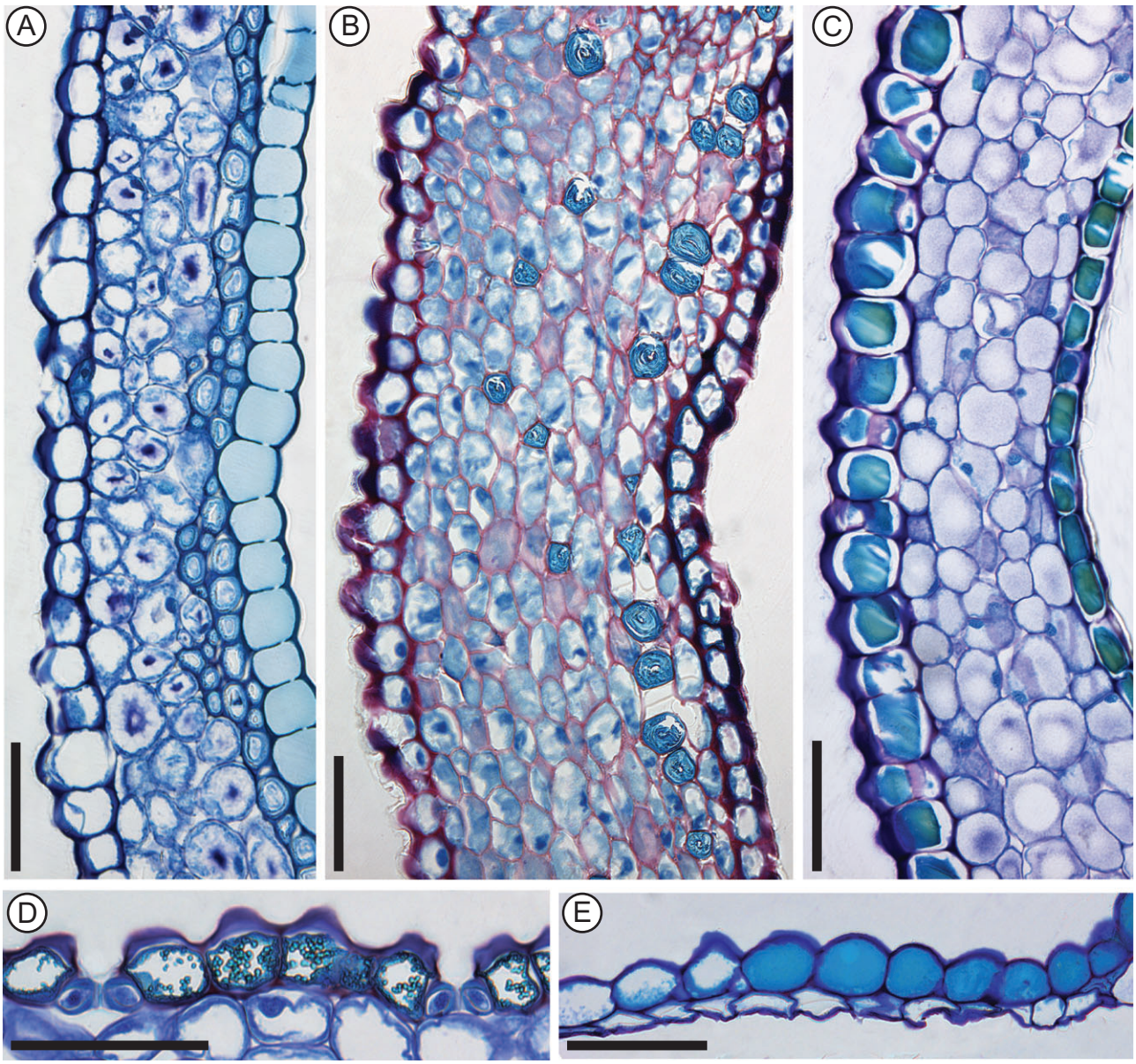


Figure 3. Cone bracts in transverse section. A, mesophyll differentiated into an abaxial zone of parenchymatous tissue and an adaxial zone of fibres (*Ephedra sarcocarpa, 143*). B, mesophyll of parenchymatous tissue and scattered fibres (*Ephedra fragilis, 101*). C, mesophyll of parenchymatous tissue (*Ephedra foeminea, 130*). D, outer epidermis with stomata (*Ephedra minuta, 63*). E, hyaline margin (*Ephedra alata, 128*). Scale bars, 0.1 mm.

There are 3 cell layers in the micropylar tube, but the cells of the outermost two layers become degraded during development of the ovule, leaving only the outer cuticle, which is irregular towards the outside and now superficially covering the inner epidermis (Fig. 4D–E). The cell walls of the inner epidermis are conspicuously thickened, most probably lignified (Fig. 4D–E), in the micropylar tube. In most species, the lignification is only present on the inner tangential side, facing the hollow tube, and on innermost parts of radial sides of the inner epidermis cells (Fig. 4D). In the Mediterranean species complex, the cell walls are often more evenly lignified on all sides of the cells (Fig. 4E) and all cell layers of the micropylar tube may be lignified, not only the inner epidermis.

The number of cell layers in the integument gradually increases downwards, finally to six or seven layers. The integument is completely free from the nucellus, with a space between them, in the distal

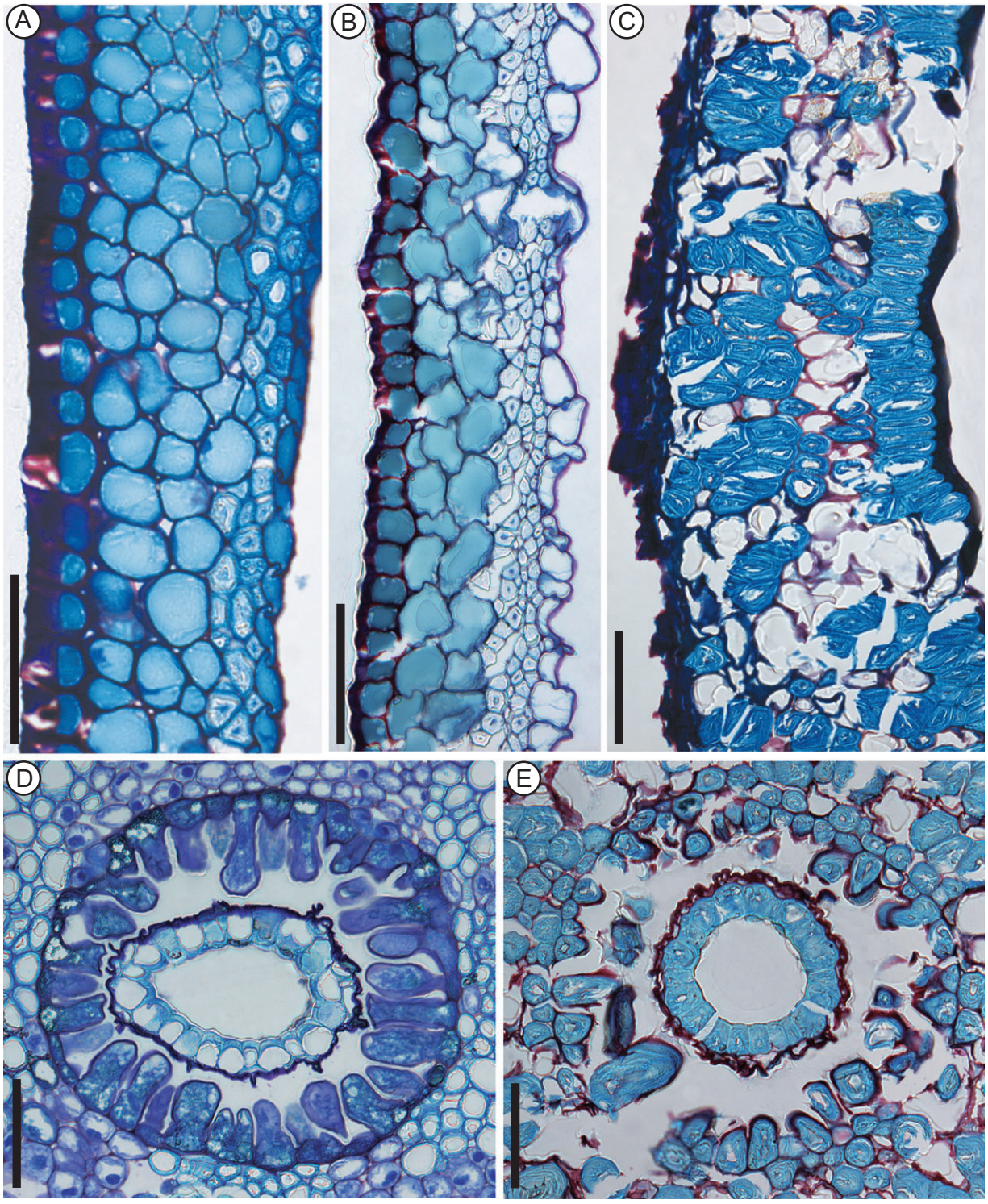


Figure 4. Seed envelope in transverse section A–C, at mid-length. A, mesophyll differentiated into an abaxial zone of rounded parenchymatous cells and an adaxial zone of fibres (*Ephedra transitoria, 111*). B, mesophyll differentiated into an abaxial zone of parenchymatous cells with undulate walls and an adaxial zone of fibres (*Ephedra rupestris, 73*). C, mesophyll of parenchymatous tissue and scattered fibres (*Ephedra fragilis, 109*). D–E, at level of the papillate zone. Papillate epidermis of seed envelope supports the micropylar tube. D, micropylar tube lignified only on the inner tangential side and parts of the radial sides of cells (*Ephedra minuta, 63*). E, papillate epidermis and inner epidermis of the micropylar tube both strongly lignified (*Ephedra altissima, 81*). Scale bars, 0.1 mm.

third of the ovule (Figs 1, 2A, C). Further down, the integument closely surrounds the nucellus, but it is usually distinguishable along approximately the distal half of the ovule (Figs 1, 2B). In the proximal half of the ovule, the integument is congenitally fused with the nucellus (Figs 1, 2G–I). Vascular bundles are never observed.

Nucellus and gametophyte: The nucellus is papillate in its apical part and in this region the tissue has dense cytoplasm, indicating that it is secretory. At the investigated stage of development, the nucellus contains a well-developed gametophyte. A thin cuticle-like layer (the megaspore wall) separates the gametophyte from the sporangial tissue. Usually, two or three archegonia are observed in the gametophytes. Where necessary, additional information on ovule structures is provided in the species descriptions.

Seed envelope

The ovule is surrounded by an outer seed envelope, which is differentiated into outer epidermis, mesophyll and inner epidermis (Fig. 4A–C). As in the cone bracts, the epidermis cells often also have a thin cuticle-like layer on the inner tangential side and on the radial sides. Stomata are usually absent but occur in a few species in the outer epidermis in apical parts of the ovule. When present, the stomata have the same structure as those of the cone bracts (see above).

In the most distal part of the seed envelope (the papillate zone), the inner epidermis is distinctly papillate (Figs 1, 2D–F, 4D–E). The unicellular papillae are contiguous with the micropylar tube, which they support. The papilla cells are rounded to rectangular in transverse section (Fig. 4D), usually tanniferous and sometimes lignified (Fig. 4E). Towards the proximal end of the papillate zone, the papillae become less abundant and less prominent and they are no longer contiguous with the integument (i.e. there is a space between the seed envelope and the integument, Figs 1, 2F, 4E). Below the papillate zone, the cells of the inner epidermis are non-papillate and non-lignified.

There are two, three or four vascular bundles in the seed envelope (Fig. 2D–F). Two vascular bundles are always in a lateral position. In some species, there is in addition an abaxial (dorsal) vascular bundle or distal supportive strand. In a few species, there is a fourth vascular bundle, which is inferred to be in an adaxial position (species with four vascular bundles in the seed envelope always have one-seeded cones). The vascular bundles are usually associated with fibre bundles. In the distal part of the ovule, they contain mainly transfusion tissue.

In all but one species, the seed envelope is partly sclerenchymatous (Fig. 4). The histology of the seed envelope is variable and will be described for each species.

The polyphyly of *Ephedra major* Host

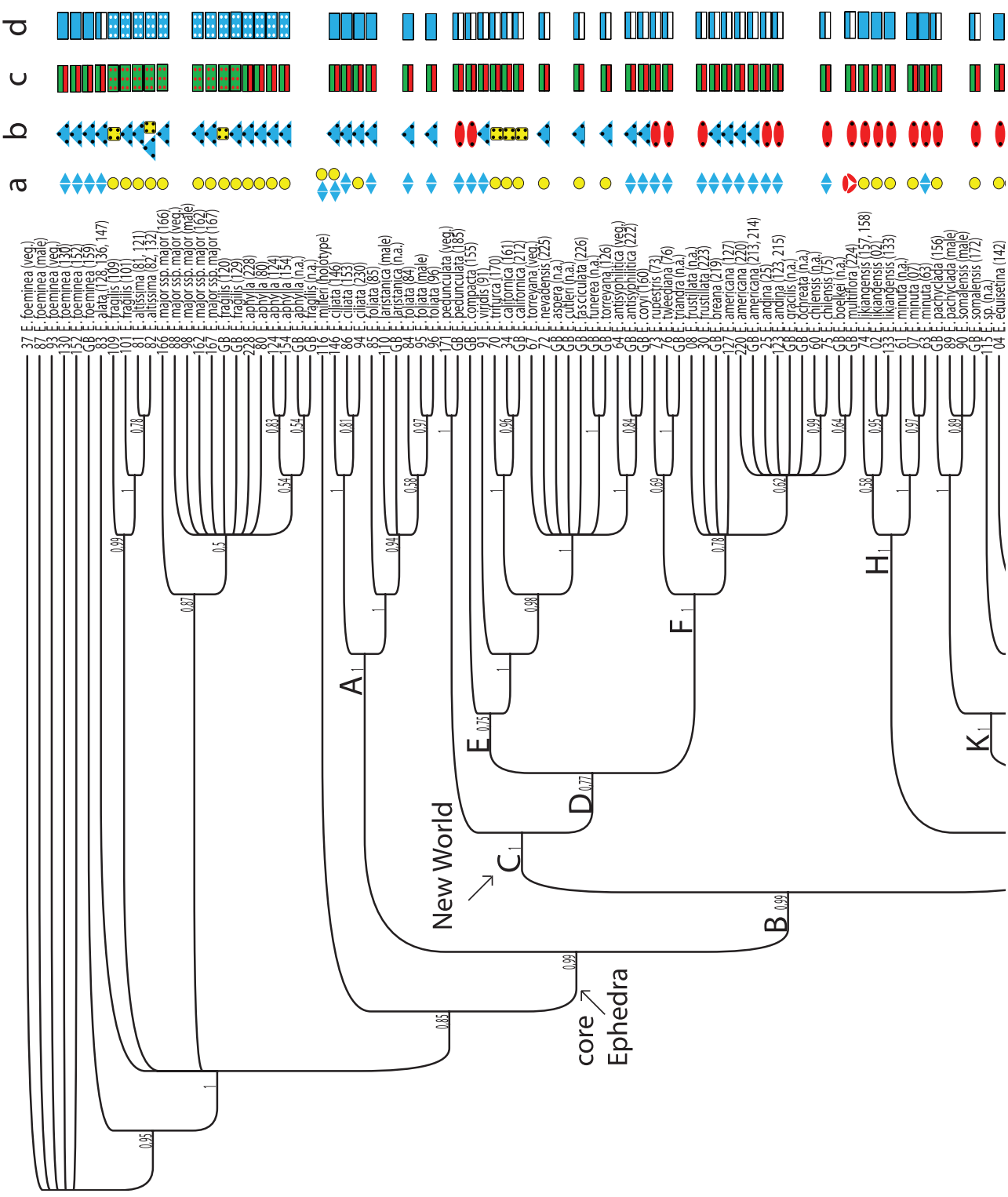
Based on morphology, anatomy, histology and molecular data, it is clear that plants referred to as *E. major* and nomenclatural synonyms, fall into two distinct groups, which are not closely related (Fig. 5). Some specimens belong in the Mediterranean species complex and they are here called *E. major* ssp. *major*. Other specimens belong in the Asian clade and are called *E. major* ssp. *procera*. We have investigated all material determined as *E. major* and the putative synonyms *Ephedra botschantzevii* Pachom., *Ephedra nebrodensis* Tineo, *Ephedra procera* C.A.Mey. and *Ephedra scoparia* Lange, available at Z, S and UPS, and some material from MO. Based on gross morphological examinations, we find that the two groups of *E. major* differ in general habitus as described here. Additional details based on serial sections of selected specimens are described under respective phylogenetic subheading (below).

General features of E. major ssp. major

Ephedra major ssp. *major* is restricted to the western parts of the Mediterranean area according to our data; the investigated material was collected in Algeria and Spain. Branchlets are upright, sometimes greyish or yellowish and relatively thick (2–3 mm). Leaves are usually persistent only on young parts of the plant. Female cones are sessile to subsessile, positioned two to several at nodes. Cone bracts are in three pairs, sometimes up to three additional pairs on a short peduncle, and are connate for 5/6 of their length or more. They are finely ciliate at the margins. There is one ovule in each cone, rounded in transverse section at mid-length.

General features of E. major ssp. procera

Ephedra major ssp. *procera* has a wide distribution ranging from Western Europe and the Western Mediterranean area to Central Asia; the investigated material was collected in Europe (Turkey, Balkan, Iberia, France, Sicily, Sardinia), Africa (Algeria, Morocco) and Asia (Georgia, Transcaucasia, Iran, Turkmenistan, Himalaya). Branchlets are numerous, usually green and thin (*c.* 1 mm). Leaves are connate for most of their length; older leaves split apart and are black–brown on the dorsal side. Female cones are solitary, axillary and often pedunculate (to 1 cm), but may also be (sub)sessile. Cone bracts are in three pairs; the innermost are connate for approximately


a
b
c
d
E. foeminea (veg.)
E. foeminea (male)
E. foeminea (veg.)
E. foeminea (130)
E. foeminea (152)
E. foeminea (159)
E. alata (128, 136, 147)
E. fragilis (109)
E. fragilis (101)
E. altissima (81, 121)
E. altissima (82, 132)
E. major ssp. major (166)
E. major ssp. major (veg.)
E. major ssp. major (male)
E. major ssp. major (162)
E. major ssp. major (167)
E. fragilis (120)
E. fragilis (128)
E. aphylla (228)
E. aphylla (80)
E. aphylla (124)
E. aphylla (154)
E. aphylla (n.a.)
E. fragilis (n.a.)
E. milleri (holotype)
E. ciliata (146)
E. ciliata (153)
E. ciliata (230)
E. foliata (85)
E. laristanica (male)
E. laristanica (n.a.)
E. foliata (84)
E. foliata (male)
E. foliata (96)
E. pedunculata (veg.)
E. pedunculata (185)
E. compacta (155)
E. viridis (91)
E. trifurca (170)
E. californica (161)
E. californica (212)
E. torreyana (veg.)
E. nevadensis (225)
E. aspera (n.a.)
E. cutleri (n.a.)
E. fasciculata (226)
E. funerea (n.a.)
E. torreyana (126)
E. antisyphilitica (veg.)
E. antisyphilitica (222)
E. coryi (160)
E. rupestris (73)
E. tweedyana (76)
E. triandra (n.a.)
E. frustillata (n.a.)
E. frustillata (223)
E. breana (219)
E. americana (127)
E. americana (220)
E. americana (213, 214)
E. andina (25)
E. andina (123, 215)
E. gracilis (n.a.)
E. ochreata (n.a.)
E. chilensis (n.a.)
E. chilensis (75)
E. boelckei (n.a.)
E. multiflora (224)
E. likiangensis (157, 158)
E. likiangensis (02)
E. likiangensis (133)
E. minuta (n.a.)
E. minuta (07)
E. minuta (63)
E. pachyclada (156)
E. pachyclada (male)
E. somalensis (male)
E. somalensis (172)
E. sp. (n.a.)
E. equisetina (142)
37
87
93
130
152
GB
83
109
101
81
82
166
88
98
162
167
GB
GB
228
80
124
154
GB
GB
116
146
86
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08
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127
220
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123
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60
75
GB
GB
74
02
133
61
07
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89
90
GB
115
04
0.78
1
0.99
0.95
0.87
1
0.5
0.83
0.54
0.54
1
0.81
1
A
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0.94
0.58
0.97
1
1
0.96
1
0.988
1
1
0.84
1
0.69
1
0.78
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0.99
0.64
0.58
0.95
1
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1
0.89
1
H
1
K
1
E
0.725
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D
0.977
F
1
C
1
New World
B
0.999
0.99
core
Ephedra
0.85

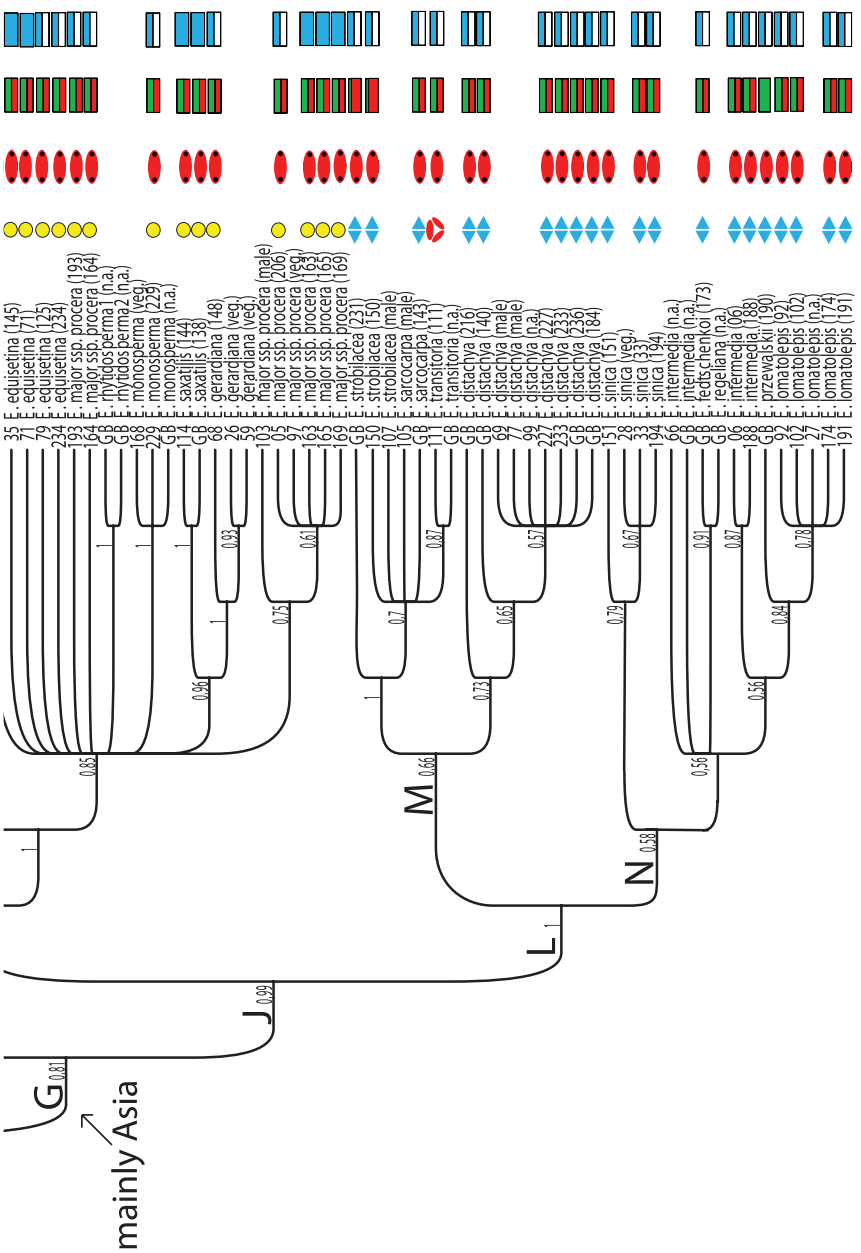

Figure 5. Phylogenetic relationships in *Ephedra* and evolution of four selected characters. Bayesian inference of phylogeny is based on data from seven loci [nuclear ribosomal 18S, 26S, nrITS (nrITS1, 5.8S and nrITS2); plastid *rbcL* and *rps4* genes; *rpl16* intron; and $trnS^{UGA}$-$trnfM^{CAU}$ intergenic spacer]. Posterior probabilities of clades are indicated above branches. Clades A–N are discussed in the text. DNA vouchers are indicated before the taxon name. Morphological vouchers are indicated in brackets after taxon names. Abbreviations: n.a., not available for morphological studies; male, DNA voucher is a male plant; veg., DNA voucher is vegetative). Evolution of selected morphological traits, based on the results in the present study: (a) number of ovules in each cone: ○, 1; ▲, 2; ▼, 3; (b) number of vascular bundles in the seed envelope: ⠿, 4; ▲, 3; ●, 2; (c) histology of the seed envelope (simplified): ▬, mesophyll differentiated into an abaxial zone of parenchymatous tissue and an adaxial zone of fibres, ▬, mesophyll of parenchymatous tissue and scattered fibres, ▬, mesophyll of parenchymatous tissue; (d) histology of the cone bract (simplified): ▭, mesophyll differentiated into an abaxial zone of parenchymatous tissue and an adaxial zone of fibres, ⠿, mesophyll of parenchymatous tissue and scattered fibres, ▬, mesophyll of parenchymatous tissue.

half their length, outer bracts less. There is one, rarely two, ovules in each cone, angled in transverse section at mid-length.

The species in the Mediterranean species complex: *Ephedra foeminea, Ephedra alata, Ephedra altissima, Ephedra aphylla, Ephedra fragilis, Ephedra milleri*

Ephedra foeminea Forssk. (syn. Ephedra campylopoda C.A.Mey.)

Cone bracts: Two or three pairs of bracts enclose the ovules. The innermost bracts are 6.5–7.5 mm long and fused for almost all their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (4–5 cell layers) and inner epidermis (1 cell layer) (Fig. 3C). Hyaline margins are 2–4 cells wide or lacking. The outer epidermis is papillate and tanniferous. The mesophyll consists only of parenchymatous, non-tanniferous tissue at mid-length and in the distal part; fibres are only seen in association with the vascular bundles. The inner epidermis is not papillate; the cells are rectangular (tangential sides longer than radial sides) and tanniferous. Stomata are not observed.

Ovules: There are two ovules in each cone (Fig. 1), approximately 4.5–6.5 mm long.

Integument: The micropylar tube is lignified and lignification of cell walls is present on all sides of the cells, but is often most prominent on the inner tangential side and inner parts of radial sides of cells.

Seed envelope: The seed envelope is distinctly triangular in transverse section in the papillate zone (Fig. 2E) and at mid-length. The cells of the outer epidermis are rectangular (tangential sides shorter than radial sides) in transverse section, some are tanniferous and the cell walls are slightly undulate. The cuticle is thick. The mesophyll consists, in the papillate zone, of parenchymatous tissue with rare fibres just below the inner epidermis. At mid-length of the ovule, the mesophyll is differentiated into two distinct zones: an abaxial zone of rounded parenchymatous cells (3 or 4 cell layers) and an adaxial zone (2 or 3 cell layers) of fibres, which are often triangular or polygonal in shape. There are three vascular bundles. The papilla cells of the inner epidermis are tanniferous. Below the papillate zone, the cells of the inner epidermis are non-tanniferous and the walls slightly undulate. The cuticle is thick and prominent.

Ephedra alata Decne.

Cone bracts: Four to five pairs of bracts enclose the ovules. The innermost bracts are at least 6 mm long and free throughout their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll around the vascular bundles (2 or 3 cell layers) and inner epidermis (1 cell layer). Hyaline margins (2 cell layers, Fig. 3E) are broad, *c.* 30–50 cells at bract mid-length, and form lateral wings. The cells of the outer epidermis are rounded or papillate and tanniferous. The cuticle is thin. Stomata are only present around the vascular bundles. Mesophyll is only present in a small area around and between the two vascular bundles. It consists of parenchymatous tissue in the apical parts. At mid-length of the bracts, it is differentiated into 0–1 (–2) cell layers of parenchymatous tissue and an adaxial zone of fibres (1 cell layer). The inner epidermis has the same structure as the outer epidermis. Stomata are not observed.

Ovules: There are two ovules in each cone, *c.* 7–8 mm long.

Integument: The micropylar tube is evenly lignified; i.e. lignification of the cell walls is present on all sides of the cells. The lignification continues below the micropylar tube and is also present in the most apical 400 μm of the nucellus.

Seed envelope: The seed envelope is distinctly triangular in transverse section in the papillate zone; the sides are concave. At mid-length of the ovule, the seed envelope is triangular with convex sides. The cells of the outer epidermis are rounded and tanniferous. Stomata are frequently present in the papillate zone, but were not found further down. The cuticle is thick. The mesophyll consists mainly of the three vascular bundles (massive transfusion tissue) in the papillate zone; there are only 0–3 cell layers of mesophyll in the areas between the three vascular bundles and small areas of parenchymatous tissue outside of the strands. An adaxial zone of fibres (1–3 cell layers) is present just below the inner epidermis. At mid-length of the ovule, the mesophyll is differentiated into two distinct zones: an abaxial zone of tanniferous parenchymatous cells (1–3 cell layers thick) and an adaxial zone (1–3 cell layers thick) of fibres. The papilla cells of the inner epidermis are tanniferous. Below the papillate zone, the cells of the inner epidermis are tanniferous and slightly irregular in shape in transverse section.

Ephedra altissima Desf.

Cone bracts: Two pairs of bracts are present and the ovules are exserted. The innermost bracts are *c.* 4–5 mm long and fused for almost 9/10 of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (6 or 7 cell layers) and inner epidermis (1 cell layer). The outer epidermis is

papillate, rarely tanniferous. The mesophyll consists of (usually) non-tanniferous parenchymatous tissue and fibres. In the apical parts, the fibres are sparsely scattered in the mesophyll, but become more abundant further down. At mid-length of the ovule, they are regularly positioned in groups of 1–5 cells in one row just below the inner epidermis and scattered in the remaining mesophyll. Further down, the mesophyll gradually becomes more sclerenchymatous. The inner epidermis has the same structure as the outer epidermis, but is not prominently papillate and the cuticle is thinner. Stomata are observed, but only rarely in the most distal part of the cone bract.

Ovules: There is one ovule in each cone, *c*. 4.0–4.5 mm long.

Integument: The micropylar tube is strongly and evenly lignified; lignification of cell walls is present on all sides of the cells (Fig. 4E). The lignification continues below the micropylar tube and is also present in the apical 200 μm of the nucellus (specimen *82*).

Nucellus and gametophyte: Megaspore wall is prominent in this material.

Seed envelope: The seed envelope is squared with convex sides (specimen *132*) or triangular with convex sides (specimens *81, 82, 121, 132*) in transverse section in the papillate zone (variable in specimen *132*). At mid-length, it is circular (Fig. 2H). The outer epidermis is not papillate; the cells are rounded, tanniferous in some specimens (*121, 132*). The cuticle is thick. The mesophyll consists mainly of parenchymatous tissue in the papillate zone. At mid-length, the mesophyll consists of parenchymatous tissue (tanniferous cells) scattered with fibres, often in groups. There is an adaxial zone of fibres (3 cell layers). There are three (*81, 82, 121, 132*) or four (*132*) vascular bundles (specimen *132* has three vascular bundles in the seed envelope of some ovules, four in others). The papilla cells of the inner epidermis are strongly lignified (Fig. 4E). Below the papillate zone, the cells of the inner epidermis are tanniferous.
Note: The distinction between *E. altissima*, *E. fragilis* and *E. major* ssp. *major* is uncertain.

Ephedra fragilis Desf.
Cone bracts: Two or three pairs of bracts are present and ovules are exserted. The innermost bracts are *c*. 6 mm long (3.5 mm in *120, 129*) and fused for 2/3 of their length or more. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (4–5 cell layers in *109, 129*, 6–7 in *101, 120*) and inner epidermis (1 cell layer). In the distal part of the bracts, the hyaline margins are *c*. 10 cells wide or less. The outer epidermis is papillate, tanniferous in some specimens (*109, 120*). The cuticle is prominent and thick. The mesophyll consists of non-tanniferous parenchymatous tissue and fibres. In apical parts, the fibres are sparsely scattered in the mesophyll (Fig. 3B), but become more abundant further down. At mid-length of the ovule or slightly higher, fibres are regularly positioned in groups of 1–5 cells in a zone just below the inner epidermis and scattered in the remaining mesophyll. Further down, there may be a continuous zone of fibres (1–2 cell layers thick) below the inner epidermis, i.e. the mesophyll gradually becomes more sclerenchymatous proximally in the cone. The inner epidermis has the same structure as the outer epidermis, but is thinner and not papillate. Stomata are observed in specimen *129*, but only rarely in the most distal part of the cone bract.

Ovules: There is one ovule in each cone, which is approximately 4–5 mm long (specimens *120, 129*), or 7 mm in specimen *101*.

Integument: The micropylar tube is strongly and evenly lignified; lignification of the cell walls is present on all sides of the cells. In specimen *101*, lignification continues below the micropylar tube and is also present in the apical 300 μm of the nucellus.

Seed envelope: The seed envelope is triangular with convex sides (*101, 120, 129*) or squared (*109,* Fig. 2F) in transverse section in the papillate zone and rounded at mid-length. Specimen *109* is largely squared in transverse section, also at mid-length. The outer epidermis is tanniferous and may be papillate in the apical part of the ovule. Tangential sides of cells are shorter than radial sides and the cuticle is unusually thick. The mesophyll consists mainly of parenchymatous tissue in the papillate zone. At mid-length, the mesophyll consists of parenchymatous tanniferous tissue, which is scattered with fibres, often in groups (Fig. 4C). There is typically an adaxial zone of fibres (1–3 cell layers). There are three (*101, 129*) or four (*109, 120*) vascular bundles. The papilla cells of the inner epidermis are lignified. Below the papillate zone, the cells are rounded to rectangular (tangential sides longer than radial sides) and usually tanniferous.

Ephedra major Host. ssp. major (synonym E. nebrodensis Tineo)
Cone bracts: Three pairs of bracts are present and the ovule is exserted. The innermost bracts are *c*. 5–6 mm long and fused more than 4/5 of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (6 or 7 cell layers), inner epidermis (1 cell layer). In the distal part of the

bracts, the hyaline margins are *c.* 5 cells wide. The outer epidermis is strongly papillate and tanniferous. The cuticle is thick and prominent. The mesophyll consists of non-tanniferous parenchymatous tissue and fibres. In apical parts, the fibres are rarely scattered in the mesophyll, but become more abundant further down. At mid-length, they are regularly positioned in groups of 1–5 cells in an adaxial zone and scattered in the remaining mesophyll. Further down, there may be a continuous zone of fibres (1–2 cell layers thick) below the inner epidermis. The mesophyll gradually becomes more sclerenchymatous, proximally in the cone. The inner epidermis has the same structure as the outer epidermis, but is not papillate. Stomata are not observed.

Ovules: There is one ovule in each cone, *c.* 6 mm long.

Integument: The micropylar tube is strongly and evenly lignified; lignification of the cell walls is present on all sides of the cells.

Seed envelope: The seed envelope is triangular with convex sides in transverse section in the papillate zone and rounded at mid-length. The outer epidermis is not strongly papillate; the cells are rounded to rectangular (tangential sides shorter than radial sides) and tanniferous. The cuticle is thick. The mesophyll consists of parenchymatous tissue in the papillate zone. At mid-length, the mesophyll consists of parenchymatous, tanniferous tissue, which is scattered with fibres, often in groups. There is an adaxial zone of fibres (3 cell layers). There are three vascular bundles. The papilla cells of the inner epidermis are tanniferous and some appear lignified. Below the papillate zone, the cells of the inner epidermis are rounded to rectangular (tangential sides longer than radial sides) and tanniferous.

Ephedra aphylla Forssk.
Cone bracts: Two or three pairs of bracts enclose the ovules. The bracts of the innermost pair are *c.* 4.3 mm long and fused for approximately 3/4 of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (4–5 cell layers) and inner epidermis (1 cell layer). The hyaline margin is approximately 6 cells wide. The outer epidermis is strongly papillate and tanniferous. The mesophyll consists only of parenchymatous tissue in the papillate zone (scattered fibres are rarely present in specimen *228*). Fibres become more abundant proximally in the cone, scattered at first and at mid-length of the bracts or, further down, there may be fibres in an adaxial zone (1 or 2 cell layers). The abaxial zone (3 or 4 cell layers) consists of non-tanniferous parenchymatous tissue, scattered with fibres. The inner epidermis has the same structure as the outer epidermis, but is not prominently papillate. Stomata are observed, but only rarely in the most distal part of the cone bract.

Ovules: There is one ovule in each cone (Fig. 2A), *c.* 3 mm long.

Integument: The micropylar tube is evenly lignified; lignification of the cell walls is present on all sides of the cells.

Seed envelope: The seed envelope is triangular with convex sides in transverse section in the papillate zone and rounded at mid-length (Fig. 2A). The outer epidermis is strongly papillate and tanniferous. The cuticle is thick. The mesophyll consists mainly of parenchymatous, tanniferous tissue in the papillate zone. There are scarce fibres just below the inner epidermis. At mid-length, the mesophyll is differentiated into two distinct zones: an abaxial zone of parenchymatous, tanniferous tissue and an adaxial zone (2 or 3 cell layers) of fibres. There are three vascular bundles. The papilla cells of the inner epidermis are tanniferous and some appear lignified. Below the papillate zone, the cells of the inner epidermis are tanniferous and slightly irregular in shape. The cuticle is thick.

Ephedra milleri Freitag & M.Maier-Stolte
Most cones of *E. milleri* have three pairs of bracts and the ovules are exserted. In one cone, the bracts are in whorls of three. The innermost bracts are *c.* 6 mm long in most cones and usually fused for 1/2–4/5 of their length. In the distal part of the bracts, the hyaline margins are relatively broad, *c.* 0.5–1 mm. There are usually two ovules in each cone, sometimes only one. They are generally *c.* 7 mm long. In two-seeded cones, the (surrounding) seed envelope is triangular in transverse section, in the papillate zone as well as at mid-length. The shape of the seed envelope in the 1-seeded cones is not clear, but probably more roundish.
Note: Ephedra milleri (the holotype) was investigated from a photograph, i.e. the anatomy and histology could not be investigated.

The Clades of core *Ephedra*

Clade A: *Ephedra foliata*

Ephedra foliata Boiss. & C.A.Mey. (synonyms E. ciliata Fisch. & C.A.Mey., E. laristanica Assadi)
Cone bracts: Two pairs of bracts are present and they do not enclose the ovules completely. The innermost bracts are *c.* 8 mm long and fused for approximately 4/5 of their length. At mid-length, they are differen-

tiated into outer epidermis (1 cell layer), mesophyll (2 or 3 cell layers) and inner epidermis (1 cell layer). In the apical parts of the bracts, the hyaline margins are *c*. 15 cells wide. The outer epidermis is prominently papillate and non-tanniferous. The mesophyll consists of parenchymatous tissue. Fibres are sparsely scattered. The inner epidermis is papillate in one specimen (*230*). The cells are narrowly rectangular (tangential sides longer than radial sides) and non-tanniferous. Stomata are observed in specimen *146*, but only rarely in the most distal part of the cone bract.

Ovules: There are usually two ovules in each cone, *c*. 6.0–8.5 mm long. Specimen *146* has two ovules in some cones and one ovule in other cones. Specimen *230* has one ovule in the cone. Both types were investigated.

Seed envelope: The seed envelope is triangular in transverse section in the papillate zone. At mid-length, it is largely circular when there is one ovule in the cone and triangular to almost half-circle shaped when there are two ovules in the cone. The cells of the outer epidermis are rounded and tanniferous. The cuticle is thick. The mesophyll consists of tanniferous parenchymatous cells and scattered fibres in the papillate zone. At mid-length, the mesophyll is differentiated into an abaxial zone of tanniferous, parenchymatous cells and an adaxial zone (2 or 3 cell layers) of fibres. There are three vascular bundles. The papilla cells of the inner epidermis are tanniferous and sometimes lignified. Below the papillate zone, the inner epidermis cells appear slightly irregular in shape. Some are tanniferous.

Clade C: the New World clade

Ephedra pedunculata Engelm. ex S.Watson

Cone bracts: There are three pairs of bracts and the ovules are exserted. The innermost bracts are *c*. 6 mm long and fused for a little more than half of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (6 or 7 cell layers) and inner epidermis (1 cell layer). The hyaline margins are *c*. 10 cells wide. The outer epidermis is prominently papillate and tanniferous. The cuticle is thick. The mesophyll is differentiated into two zones, an abaxial zone of parenchymatous tissue and an adaxial zone (1–3 cell layers) of fibres. The cells of the inner epidermis are similar to those of the outer epidermis, but smaller and sometimes more rectangular (tangential sides longer than radial sides). Stomata are not observed.

Ovules: There are two ovules in each cone, *c*. 7 mm long.

Seed envelope: The seed envelope is triangular in transverse section in the papillate zone and at mid-length. The outer epidermis is strongly papillate and tanniferous and the cuticle is thick. The mesophyll consists, in the distal part, of parenchymatous cells and scattered fibres. At mid-length, the mesophyll is differentiated into an abaxial zone of tanniferous parenchymatous cells (2–4 cell layers) and an adaxial zone (2–4 cells layers) of fibres. These layers fluctuate in thickness. There are two vascular bundles. In the papillate zone, there is a third strand of supportive tissue in one of the ovules. It disappears proximally (without fusing with another bundle). The papilla cells of the inner epidermis are tanniferous and the cuticle is thick. At mid-length, the cells of the inner epidermis are rectangular (tangential sides longer than radial sides) to slightly irregular in shape and tanniferous.

Clade E: the North American clade

Ephedra compacta Rose

Cone bracts: There are three pairs of bracts and the ovules are exserted. The innermost bracts are *c*. 4.5 mm long and fused for approximately 3/4 of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (7 or 8 cell layers) and inner epidermis (1 cell layer). The hyaline margins are *c*. 10–15 cells wide. The cells of the outer epidermis are rounded or papillate and tanniferous. The cuticle is thinner than in most other species. The mesophyll is differentiated into two zones, an abaxial zone of tanniferous, parenchymatous cells (4 or 5 cell layers) and an adaxial zone of fibres (3 or 4 cell layers). The inner epidermis is not papillate. The cells are non-tanniferous and cell walls undulate. Stomata are not observed.

Ovules: There are two ovules in each cone, *c*. 5 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and elliptic to triangular at mid-length. The outer epidermis is tanniferous and cell walls undulate. The cuticle is thick. The mesophyll consists, in the distal part, of tanniferous, parenchymatous cells. At mid-length, the mesophyll is differentiated into two zones, an abaxial zone of tanniferous, parenchymatous cells with undulate walls (2–5 cell layers) and an adaxial zone of fibres (3–8 cell layers). The thickness of these zones fluctuate in a distinct way; where the fibre zone is thin, the parenchymatous zone is thick and vice

versa. There are two vascular bundles. The papilla cells of the inner epidermis are not well preserved but appear tanniferous and lignified. Below the papillate zone, the inner epidermis cells are tanniferous with undulate walls.

Ephedra viridis Coville
Cone bracts: There are three pairs of bracts and the ovules are exserted. The innermost bracts are *c*. 4.5 mm long and free from each other for most of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (7 or 8 cell layers) and inner epidermis (1 cell layer). The hyaline margins are *c*. 10–15 cells wide. The outer epidermis is papillate and tanniferous. The mesophyll is differentiated into two zones; an abaxial parenchymatous zone (2–4 cell layers) and an adaxial zone of fibres (5 or 6 cell layers). The inner epidermis is not papillate; cells are tanniferous with undulate walls. Stomata are not observed.

Ovules: There are two ovules in each cone, *c*. 5 mm long.

Seed envelope: The seed envelope is triangular in transverse section in the papillate zone and at mid-length. The cells of the outer epidermis are large, tanniferous and narrowly rectangular (tangential sides shorter than radial sides), sometimes with undulate walls. The mesophyll consists, in the distal part, of parenchymatous tissue. Some cells are tanniferous. At mid-length, the mesophyll is differentiated into two zones, an abaxial zone of parenchymatous, tanniferous tissue, sometimes with undulate cell walls (2–4 cell layers), and an adaxial zone of fibres (7 or 8 cell layers). There are three vascular bundles. The papilla cells of the inner epidermis are tanniferous and the cuticle is thick. At mid-length, the cells of the inner epidermis are tanniferous with undulate walls.

Ephedra californica S.Watson
Cone bracts: The bracts are in whorls of three and the ovules are exserted. Number of whorls, length and degree of fusion of the cone bracts was not observed; only the distal part of the cones could be sectioned because of problems in the laboratory process. The bracts are differentiated into outer epidermis (1 cell layer), mesophyll (5–7 cell layers) and inner epidermis (1 cell layer). The hyaline margins are approximately 30–50 cells wide. The outer epidermis is papillate; the cells are tanniferous and the cuticle is thick. The mesophyll is differentiated into an abaxial zone of parenchymatous, tanniferous tissue and an adaxial zone of fibres. The cells of the inner epidermis are tanniferous with undulate walls and the cuticle is thin. Stomata are not observed.

Ovules: There is one ovule in each cone, *c*. 6–7 mm long.

Seed envelope: The seed envelope is distinctly squared in transverse section in the papillate zone, rounded at mid-length. The cells of the outer epidermis are tanniferous and rounded to papillate. The cuticle is thick. Stomata are present in the papillate zone but absent further down. The mesophyll consists, in the distal part, of parenchymatous tissue and fibres. At mid-length, the mesophyll is differentiated into several zones, an abaxial zone (3–5 cells layers) of parenchymatous, tanniferous tissue, a middle zone of large, fibre-like cells and scattered parenchymatous cells (3–5 cell layers) and an adaxial zone of fibres (3–7 cell layers). There are four vascular bundles. The papilla cells of the inner epidermis are tanniferous and the cuticle is thick. At mid-length, the cells of the inner epidermis are rectangular (tangential sides shorter than radial sides) to irregular in shape and tanniferous.

Ephedra trifurca Torrey ex S.Watson
Cone bracts: There are three whorls of bracts, three bracts in each whorl. The ovule is exserted. The innermost bracts are *c*. 5 mm long and free from each other for most of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (5–7 cell layers) and inner epidermis (1 cell layer). The hyaline margins are approximately 20–40 cells wide. The outer epidermis is papillate and tanniferous and the cuticle is thick. The mesophyll is differentiated into an abaxial zone of parenchymatous tissue (1 or 2 cell layers) and an adaxial zone of fibres (4 or 5 cell layers). Some of the parenchymatous cells are tanniferous. The inner epidermis is tanniferous when present; the lateral parts of the bracts lack a well-defined inner epidermis. The cells are rounded, sometimes with undulate walls and the cuticle is thin. Stomata are not observed.

Ovules: There is one ovule in each cone, *c*. 7 mm long.

Seed envelope: The seed envelope is squared with concave sides in transverse section in the papillate zone and rounded at mid-length. The cells of the outer epidermis are rounded and tanniferous. The cuticle is thick. The mesophyll consists, in the distal part, of parenchymatous tissue and scares fibres. At mid-length, the mesophyll is differentiated into two distinct zones, an abaxial zone (7–9 cell layers) of parenchymatous, tanniferous cells and an adaxial zone of fibres (*c*. 7 cell layers). There are four vascular

bundles. The papilla cells of the inner epidermis are tanniferous and the cuticle is thick. At mid-length, the cells of the inner epidermis are rectangular (tangential sides shorter than radial sides) to irregular in shape and tanniferous.

Ephedra nevadensis S.Watson
Cone bracts: There are four pairs of bracts and the ovule is exserted. The innermost bracts are *c*. 5.5 mm long and fused for approximately 1/3 of their length. They are differentiated into outer epidermis (1 cell layer), mesophyll (6–8 cell layers) and inner epidermis (1 cell layer). The hyaline margins are *c*. 15–30 cells wide. The outer epidermis is papillate and tanniferous. The cuticle is thick. The mesophyll is differentiated into an abaxial zone of parenchymatous tissue (3 or 4 cell layers) and an adaxial zone of fibres (3 or 4 cell layers). The cells of the inner epidermis are tanniferous and cell walls are undulate. The cuticle is thin. Stomata are not observed.

Ovules: There is one ovule in each cone, *c*. 7 mm long.

Seed envelope: The seed envelope is triangular in transverse section in the papillate zone and at mid-length. The outer epidermis is not papillate; the cells are tanniferous and rounded, sometimes with undulate walls. The cuticle is thick. The mesophyll consists, in the distal part, of parenchymatous tissue and fibres. At mid-length, the mesophyll is differentiated into several zones, an abaxial zone (4 or 5 cells layers) of parenchymatous, tanniferous cells, a middle zone of parenchymatous, non-tanniferous tissue (4 or 5 cell layers) and an adaxial zone of fibres (4–7 cell layers). There are three vascular bundles. The papilla cells of the inner epidermis are tanniferous. At mid-length, the cells of the inner epidermis are tanniferous and rectangular (with longer tangential sides) or irregular in shape.

Ephedra fasciculata var. clokeyi (H.C.Cutler) Clokey
Cone bracts: There are four pairs of bracts and the ovule is exserted. The innermost bracts are *c*. 5.5 mm long and free for most of their length. They are differentiated into outer epidermis (1 cell layer), mesophyll (7–9 cell layers) and inner epidermis (1 cell layer). The hyaline margins are approximately 20 cells wide. The cells of the outer epidermis are rounded and tanniferous. The cuticle is thin. The mesophyll is differentiated into an abaxial zone of parenchymatous tissue (3 or 4 cell layers) and an adaxial zone of fibres (3 or 4 cell layers). The cells of the inner epidermis are rectangular (tangential sides longer than radial sides) and tanniferous. The cuticle is thin. Stomata are not observed.

Ovules: There is one ovule in each cone, *c*. 6 mm long.

Seed envelope: The seed envelope is triangular in transverse section in the papillate zone and at mid-length. The cells of the outer epidermis are tanniferous and rounded to papillate in shape. The cuticle is thick. The mesophyll consists, in the distal part, of parenchymatous tissue and fibres. At mid-length, the mesophyll is differentiated into two zones, an abaxial zone of parenchymatous tissue and an adaxial zone of fibres. Details of the histology are uncertain because of poor results. There are three vascular bundles. The papilla cells are tanniferous and the cuticle is thick. At mid-length, the cells of the inner epidermis are rectangular (tangential sides longer than radial sides) to irregular in shape and tanniferous.

Ephedra torreyana S.Watson
Cone bracts: There are six whorls with three bracts in each, which largely enclose the ovules. The innermost bracts are *c*. 10 mm long and free throughout their length. Distally there are only 2 cell layers in the bracts. At mid-length, they are differentiated into epidermis (1 cell layer), mesophyll [3–5 (–7) cell layers] and inner epidermis (1 cell layer). The hyaline margins are *c*. 30–50 cells wide; *c*. 80 cells or more in distal parts, but the cells are often torn and the width is difficult to estimate. The outer epidermis can be up to 3 cell layers thick. It is weakly papillate. The cells are tanniferous in the proximal parts of the bracts. The cell walls are undulate and the cuticle is thin. In some cells, the outer tangential wall is extended and forms distinct outward projections. Stomata are not observed. When present (in the proximal 3/4 of the length of the bracts), the mesophyll consists of parenchymatous, non-tanniferous tissue and fibres. At mid-length of the bracts, the mesophyll is differentiated into two zones, an abaxial zone of parenchymatous tissue (0–4 cell layers) and an adaxial zone of fibres (1–3 cell layers). The inner epidermis is non-tanniferous. The cell walls are undulate and the cuticle is thin. Stomata are not observed.

Ovules: There is one ovule in each cone, *c*. 7.5 mm long.

Seed envelope: The seed envelope is distinctly triangular, with concave sides in transverse section in the papillate zone and triangular with convex sides at mid-length. The outer epidermis is non-papillate and tanniferous. Cell walls undulate and the cuticle is thick. In some cells, the outer tangential wall is extended and forms distinct outward projections. The inner tangential wall of these cells is in line with that of the other cells of the outer epidermis. The mesophyll does not take part in the formation of the

outward projections. It consists, in the papillate zone, of parenchymatous tissue and fibres and is restricted to the areas around the three vascular bundles. At mid-length, the mesophyll is differentiated into two distinct zones, an abaxial zone of tanniferous, parenchymatous cells with undulate walls (2 or 3 cell layers) and an adaxial zone of fibres (3 or 5 cell layers). The cells of the abaxial zone are indistinguishable from those of the outer epidermis. The papilla cells of the inner epidermis are tanniferous and the cuticle is thin. At mid-length, the cells of the inner epidermis are rectangular (tangential sides longer than radial sides) to irregular in shape and tanniferous.

Ephedra antisyphilitica Berlandier ex C.A.Mey.
Cone bracts: There are three pairs of bracts and the ovules are exserted. The innermost bracts are *c.* 4.5 mm long and fused for approximately half of their length. They are differentiated into outer epidermis (1 cell layer), mesophyll (5–7 cell layers) and inner epidermis (1 cell layer). The hyaline margins are approximately 10–20 cells wide. The outer epidermis is papillate tanniferous and the cuticle is thick. The mesophyll is differentiated into an abaxial zone of parenchymatous tissue (3 or 4 cell layers) and an adaxial zone of fibres (2 or 3 cell layers). The inner epidermis is tanniferous and cell walls undulate. The cuticle is thin. Stomata are not observed.

Ovules: There are two ovules in each cone, *c.* 5.5 mm long.

Seed envelope: The seed envelope is triangular in transverse section in the papillate zone and at mid-length. The cells of the outer epidermis are tanniferous and rounded to papillate in shape. The cuticle is thick but not papillate. The mesophyll consists, in the distal part, of parenchymatous tissue and fibres. At mid-length, the mesophyll is differentiated into two zones, an abaxial zone (3 or 4 cells layers) of parenchymatous, tanniferous tissue and an adaxial zone of fibres (4–7 cell layers). There are three vascular bundles. The papilla cells of the inner epidermis are tanniferous and the cuticle is thick. At mid-length, the cells of the inner epidermis are rectangular (with longer tangential sides) or irregular in shape and tanniferous.

Ephedra coryi E.L.Reed
Cone bracts: Four pairs of bracts enclose the ovules almost completely. The innermost bracts are *c.* 5.5 mm long and free from each other for most of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (5 or 6 cell layers) and inner epidermis (1 cell layer). The hyaline margins are 15–20 cells wide. The outer epidermis is papillate and tanniferous. The cuticle is thinner than in many other species. The mesophyll is differentiated into two zones; an abaxial zone of parenchymatous tissue (3 cell layers) and an adaxial zone of fibres (3 cell layers). The cells of the inner epidermis are large, rectangular (tangential sides shorter than radial sides), slightly papillate and tanniferous. Stomata are present near the vascular bundles.

Ovules: There are two ovules in each cone, *c.* 5.5 mm long.

Seed envelope: The seed envelope is triangular in transverse section in the papillate zone and at mid-length. The cells of the outer epidermis are large, narrowly rectangular (tangential sides shorter than radial sides) and tanniferous. The mesophyll consists, in the distal part, of parenchymatous tissue. At mid-length, the mesophyll is differentiated into two distinct zones, an abaxial zone of tanniferous cells with undulate walls (*c.* 5 cell layers) and an adaxial zone of fibres (7 or 8 cell layers). There are three vascular bundles. The papilla cells of the inner epidermis are tanniferous and the cuticle is thick. At mid-length, the inner epidermis is tanniferous. The cells are much larger than those of the outer epidermis and with undulate walls.

Clade F: the South American clade

Ephedra rupestris Benth.
Cone bracts: Three pairs of bracts enclose the entire ovules or nearly so. The innermost bracts are *c.* 4.5 mm long and connate for approximately 1/3 of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (5 cell layers) and inner epidermis (1 cell layer). The outer epidermis is papillate and tanniferous. The cuticle is thick. Stomata are present, but less frequently than in most species. The mesophyll is differentiated into two zones, an abaxial zone of non-tanniferous parenchymatous tissue (5 cell layers) and an adaxial zone of fibres (1 cell layer). The cells of the inner epidermis are rectangular (tangential sides longer than radial sides) and tanniferous. Stomata occur, more frequently than in the outer epidermis.

Ovules: There are two ovules in each cone, *c.* 5 mm long.

Seed envelope: The seed envelope is elliptic to triangular in transverse section in the papillate zone and triangular at mid-length. The outer epidermis is tanniferous and non-papillate; the cells are squared to rectangular (tangential sides shorter than radial

sides), sometimes with undulate walls. The cuticle is thick. The mesophyll consists, in the distal part, of parenchymatous tissue. At mid-length, the mesophyll is differentiated into two distinct zones, an abaxial zone (2 or 3 cell layers) of tanniferous, undulate cells and an adaxial zone of fibres (3–5 cell layers) (Fig. 4B). The cells of the outer zone are sometimes indistinguishable from those of the epidermis. There are two vascular bundles. The papilla cells of the inner epidermis are tanniferous and the cuticle is thick. At mid-length, the cells of the inner epidermis are tanniferous and have undulate walls.

Ephedra tweediana Fisch. & C.A.Mey.
Cone bracts: There are three pairs of bracts, which do not enclose the ovules entirely. The innermost bracts are *c*. 6 mm long and connate for approximately 1/3 of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (4–6 cell layers) and inner epidermis (1 cell layer). The hyaline margin is approximately 20 cells wide. The outer epidermis is papillate and tanniferous. The cuticle is thin. The mesophyll is differentiated into two zones, an outer zone of non-tanniferous parenchymatous tissue and an inner zone of fibres. The cells of the inner epidermis are tanniferous, rectangular (tangential sides longer than radial sides), sometimes papillate. Stomata occur but are rarer than in the outer epidermis.

Ovules: There are two ovules in each cone, *c*. 6.5 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and largely triangular at mid-length. The outer epidermis is slightly papillate and tanniferous. Tangential sides of cells are shorter than radial sides. The cuticle is thick. The mesophyll consists, in the distal part, of parenchymatous tissue and fibres. At mid-length, the mesophyll is differentiated into two distinct zones, an abaxial zone (4–6 cell layers) of tanniferous cells, sometimes with undulate walls, and an adaxial zone of fibres (2–4 cell layers). The cells of the abaxial zone are sometimes indistinguishable from those of the outer epidermis. There are two vascular bundles. In the distal part of the ovule there is an additional strand of supportive tissue, which disappears proximally (without fusing with another bundle). The papilla cells of the inner epidermis are tanniferous and the cuticle is thick. At mid-length, the cells of the inner epidermis are tanniferous and have undulate walls.

Ephedra frustillata Miers
Cone bracts: There are two pairs of bracts, which enclose the ovules almost entirely. The innermost bracts are *c*. 3.5 mm long and connate for approximately 1/3 of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (3 cell layers) and inner epidermis (1 cell layer). The hyaline margin is approximately 10 cells wide. The outer epidermis is papillate and tanniferous. The cuticle is thick. The mesophyll is differentiated into two zones, an abaxial zone of non-tanniferous parenchymatous tissue (1 or 2 cell layers) and an adaxial zone of fibres (1 or 2 cell layers). The cells of the inner epidermis are larger than those of the outer epidermis, tanniferous and rectangular (tangential sides shorter than radial sides). Stomata are not observed.

Ovules: There are two ovules in each cone, *c*. 3.5 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and largely triangular at mid-length. The outer epidermis is slightly papillate and tanniferous. Tangential sides of cells are shorter than radial sides. The cuticle is thick. The mesophyll consists, in the distal part, of parenchymatous tissue and fibres. At mid-length, the mesophyll is differentiated into two distinct zones, an abaxial zone (2 or 3 cell layers) of tanniferous cells, sometimes with slightly undulate walls, and an adaxial zone of fibres (5 or 6 cell layers). The cells of the abaxial zone are sometimes indistinguishable from those of the epidermis. There are two vascular bundles. The papilla cells of the inner epidermis are tanniferous and the cuticle is thick. At mid-length, the cells of the inner epidermis are rectangular and tanniferous.

Ephedra breana Phil.
Cone bracts: There are three pairs of bracts and the ovules are exserted. The innermost bracts are *c*. 3 mm long and connate for approximately half of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (8 cell layers) and inner epidermis (1 cell layer). The hyaline margin is narrow, *c*. 5–10 cells wide. The cells of the outer epidermis are rounded to papillate and tanniferous. The cuticle is thick. The mesophyll is differentiated into two zones, an abaxial zone of non-tanniferous parenchymatous tissue (3 cell layers) and an adaxial zone of fibres (3–5 cell layers). The cells of the inner epidermis are tanniferous and rectangular (tangential sides longer than radial sides). Stomata are not observed.

Ovules: There are two ovules in each cone, *c*. 3.5 mm long.

Seed envelope: The seed envelope is triangular in transverse section in the papillate zone and largely triangular at mid-length. The outer epidermis is slightly papillate and tanniferous. Tangential sides of cells are shorter than radial sides. The cuticle is thick. The mesophyll consists, in the distal part, of parenchymatous tissue and fibres. At mid-length, the mesophyll is differentiated into two distinct zones, an abaxial zone (1 or 2 cell layers) of tanniferous cells, rounded or with slightly undulate walls, and an adaxial zone of fibres (8–10 cell layers). The cells of the abaxial zone are sometimes indistinguishable from those of the outer epidermis. There are three vascular bundles. The papilla cells of the inner epidermis are tanniferous and the cuticle is thick. At mid-length, the cells of the inner epidermis are rectangular and tanniferous.

Ephedra americana Humb. & Bonpl. ex Willd.
Cone bracts: At least four pairs of bracts enclose the ovules. The innermost bracts are *c*. 4.6 mm long and connate for approximately half of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (5–8 cell layers) and inner epidermis (1 cell layer). The hyaline margin is narrow to absent at mid-length but approximately 20 cells wide distally. The outer epidermis is papillate and tanniferous. The cuticle is thick. The mesophyll is differentiated into two zones, an abaxial zone of non-tanniferous parenchymatous tissue and an adaxial zone (1–4 cell layers) of fibres. In specimen *220*, there is a middle (third) strand of supportive tissue in the distal-most part of one cone bract. It ends freely in the mesophyll in the upper half of the bract. The cells of the inner epidermis are rectangular (tangential sides longer than radial sides), slightly papillate and tanniferous. Stomata are observed, but only rarely in the distal-most part of the cone bract.

Ovules: There are two ovules in each cone, *c*. 3.5–4.0 mm long.

Seed envelope: The seed envelope is triangular in transverse section in the papillate zone and at mid-length. The cells of the outer epidermis are rounded or with undulate walls and tanniferous. The cuticle is thick. The mesophyll is differentiated into two zones, an abaxial zone of parenchymatous, tanniferous cells, rounded or with undulate walls (2–4 cells layers), and an adaxial zone of fibres (3–7 cell layers). The two zones fluctuate in thickness. There are three vascular bundles. The papilla cells of the inner epidermis are tanniferous and the cuticle is thin. At mid-length, the cells of the inner epidermis are rounded, slightly papillate and tanniferous.

Ephedra andina Poepp. & Endl.
Cone bracts: There are three to four pairs of bracts and the ovules are exserted. The innermost bracts are *c*. 4 mm long and connate for approximately 1/3 of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (5–8 cell layers) and inner epidermis (1 cell layer). The hyaline margin is narrow to absent at mid-length but approximately 20 cells wide distally. The outer epidermis is papillate and tanniferous and the cuticle is thick. The mesophyll is differentiated into two zones, an abaxial zone of non-tanniferous parenchymatous tissue and an adaxial zone (1–4 cell layers) of fibres. The inner epidermis is papillate and tanniferous. The tangential sides of cell walls are shorter than radial sides. Stomata are not observed.

Ovules: There are two ovules in each cone, *c*. 5–6 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and largely triangular at mid-length (Fig. 2G). The cells of the outer epidermis are large, rectangular (tangential sides are shorter than radial sides) and tanniferous. The cuticle is thin. The mesophyll consists, in the distal part, of parenchymatous tissue. At mid-length, the mesophyll is differentiated into two zones, an abaxial zone of rounded and tanniferous parenchymatous cells (2–4 cell layers) and an adaxial zone (2–8 cell layers) of fibres. There are two vascular bundles (Fig. 2G). The papilla cells of the inner epidermis are tanniferous and the cuticle is thin. At mid-length, the cells of the inner epidermis are large, rectangular and tanniferous.

Ephedra chilensis C.Presl
Cone bracts: There are four pairs of bracts and the ovules are exserted. The innermost bracts are *c*. 4.5 mm long and connate for approximately half of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (4 cell layers) and inner epidermis (1 cell layer). The hyaline margin is approximately 20 cells wide. The outer epidermis is papillate and tanniferous and the cuticle is thick. The mesophyll is differentiated into two zones, an abaxial zone of non-tanniferous parenchymatous tissue and an adaxial zone of fibres. The cells of the inner epidermis are rectangular (tangential sides longer than radial sides), not papillate and tanniferous. Stomata are observed.

Ovules: There are two ovules in each cone, *c*. 5 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and triangular at mid-length. The outer epidermis is papillate and tanniferous. Tangential sides of cells are shorter than radial sides. The cuticle is thick. The mesophyll consists, in the distal part of the ovule, of parenchymatous tissue and fibres. At mid-length, the mesophyll is differentiated into two distinct zones, an abaxial zone of tanniferous cells with undulate walls (2 or 3 cell layers) and an adaxial zone of fibres (3 to 5 cell layers). The cells of the abaxial zone are sometimes indistinguishable from those of the outer epidermis. There are two vascular bundles. The papilla cells of the inner epidermis are tanniferous and the cuticle is thick. Below the papillate zone, the cells of the inner epidermis have undulate walls and are tanniferous.

Ephedra multiflora Phil. ex Stapf
Cone bracts: There are four whorls of bracts, which enclose the ovules entirely or almost so. The innermost bracts are *c*. 9 mm long and free for most of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (3 or 4 cell layers) and inner epidermis (1 cell layer). The hyaline margin is broad (Fig. 2C), at least 70 cells wide. The cells of the outer epidermis are rounded to papillate and tanniferous. The cuticle is thin. The mesophyll consists (when present) mostly of fibres, but there is usually an abaxial zone of parenchymatous tissue (0–2 cell layers). The cells of the inner epidermis are smaller than those of the outer epidermis and tanniferous. Stomata are not observed.

Ovules: There are three ovules in each cone (Fig. 2C), *c*. 8.5 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and largely squared at mid-length. The cells of the outer epidermis are rounded to rectangular (tangential sides longer than radial sides) and tanniferous. The cuticle is thick. The mesophyll consists, in the distal part, of parenchymatous tissue and fibres. At mid-length, the mesophyll is differentiated into two distinct zones, an abaxial zone (1 or 2 cell layers) of tanniferous cells, rounded and sometimes with slightly undulate walls, and an adaxial zone of fibres (1–3 cell layers). There are two vascular bundles (Fig. 2C). The papilla cells of the inner epidermis are tanniferous. At mid-length, the cells of the inner epidermis are rectangular and tanniferous.

Clade G: the mainly Asian clade
Clade H

Ephedra minuta Florin
Cone bracts: Three or four pairs of bracts are present and the ovules are exserted. The innermost bracts are *c*. 5 mm long and fused for less than half their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (3 cell layers) and inner epidermis (1 cell layer). The hyaline margins are 20–25 cells wide in the distal parts of the bracts and *c*. 5–7 cells wide at the mid-length of the bracts. The outer epidermis is papillate and tanniferous (Fig. 3D). The tannin may be enclosed in small globules (Fig. 3D), but is sometimes present throughout the cells. The cells are rounded or rectangular (tangential sides longer than radial sides). The mesophyll consists of parenchymatous tissue. The inner epidermis has the same structure as the outer epidermis, but is not prominently papillate and the cuticle is thinner. Stomata are present, but less frequently than in the outer epidermis.

Ovules: There are one or two ovules in each cone. They are *c*. 5–6 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone. At mid-length, the seed envelope is narrowly half-moon shaped in transverse section when there are two ovules and more roundedly triangular when there is one ovule. The outer epidermis is strongly papillate and tanniferous. Tannins are sometimes in globules. Tangential sides of cells are shorter than radial sides and the cuticle is thick. The mesophyll consists of parenchymatous tissue in the papillate zone. At mid-length, the mesophyll is differentiated into two distinct zones: an abaxial zone of parenchymatous, tanniferous cells (tannins often in numerous small globules) and an adaxial zone of fibres. There are two vascular bundles. Distally, there is an additional abaxial strand of supportive tissue, which disappears below the papillate zone (without fusing with another bundle). The papilla cells (Fig. 4D) of the inner epidermis are tanniferous. Below the papillate zone, the cells of the inner epidermis are slightly irregular in shape and with tannins in globules. The cuticle is thin.

Ephedra likiangensis Florin
Cone bracts: Three pairs of bracts enclose the ovules. The innermost bracts are *c*. 5 mm long and fused for a little over half of their length. At mid-length, they are differentiated into outer epidermis (1 or 2 cell layers), mesophyll (6–8 cell layers) and inner epidermis (mostly 1 cell layer). The hyaline margins are *c*. 8–12 cells wide in the distal part of the bracts. The

outer epidermis is distinctly papillate, with a warty appearance, and tanniferous. It is uneven in thickness (sometimes there is more than 1 cell layer). The cuticle is thick. The mesophyll consists of parenchymatous tissue. The inner epidermis comprises 1 (rarely 2) cell layers; cells are squared (not papillate), sometimes tanniferous. Stomata are observed.

Ovules: There is one ovule in each cone, *c*. 4.5–5.5 mm long.

Seed envelope: The seed envelope is largely elliptic in transverse section in the papillate zone and rounded at mid-length. The outer epidermis is not papillate; the cells are squared to rectangular (tangential sides shorter than radial sides); some cells have undulate walls and/or are tanniferous. The cuticle is thick. The mesophyll consists of parenchymatous tissue in the papillate zone. At mid-length, the mesophyll is differentiated into two distinct zones, an abaxial zone of parenchymatous, often tanniferous tissue and an adaxial zone of fibres. There are two vascular bundles. The papilla cells of the inner epidermis are tanniferous (tannins in globules). Below the papillate zone, the cells of the inner epidermis are tanniferous and have slightly undulate walls. The cuticle is thin.

Clade K

Ephedra pachyclada Boiss.
Cone bracts: There are four pairs of bracts. The length of the innermost bracts was not calculated because results were unsatisfactory for the lower part of the cone. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (3–5 cell layers, uncertain observation) and inner epidermis (1 cell layer). The hyaline margins are narrow, less than 10 cells wide. The outer epidermis is papillate and tanniferous. The cuticle is thick. The mesophyll consists of parenchymatous tissue and an adaxial zone of fibres (1–3 cell layers thick). The cells of the inner epidermis are large, rounded to rectangular and tanniferous. The cuticle is thin. Stomata are not observed.

Ovules: There is one ovule in each cone, *c*. 5 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and at mid-length. The cells of the outer epidermis are rounded, sometimes with slightly undulate walls, and tanniferous. The cuticle is thick. The mesophyll consists of parenchymatous tissue and an adaxial zone (1 or 2 cell layers) of fibres in the papillate zone. At mid-length, the mesophyll is differentiated into two distinct zones, an abaxial zone (2 or 3 cell layers) of parenchymatous, tanniferous cells with undulate walls and an adaxial zone (1 or 2 cell layers) of fibres. There are two vascular bundles. Below the papillate zone, the inner epidermis consists of narrowly rectangular (tangential sides longer than radial sides) and tanniferous cells in 1 or 2 cell layers. Cell walls sometimes undulate.

Ephedra somalensis Freitag & Maier-St.
Cone bracts: There are four pairs of bracts in the cone. The innermost bracts are 4.2 mm long and fused for a little less than 1/10 of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (5 or 6 cell layers) and inner epidermis (1 cell layer). The hyaline margins are narrow, less than 10 cells wide. The outer epidermis is papillate and usually non-tanniferous. The cuticle is thick. The mesophyll is differentiated into an abaxial zone of parenchymatous tissue and an adaxial zone of fibres (1–3 cell layers). The cells of the inner epidermis are large, rounded to rectangular, sometimes tanniferous. Stomata are observed, but only rarely in the distal-most part of the cone bract.

Ovules: There is one ovule in each cone, *c*. 2.5 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone. At mid-length it is almost squared; there are abaxial and adaxial 'angles' not associated with vascular bundles. The cells of the outer epidermis are narrowly rectangular (tangential sides shorter than radial sides) and tanniferous. The cuticle is thin. At mid-length of the ovule, the epidermis cells are rounded, sometimes with slightly undulate walls. The mesophyll consists of parenchymatous tissue and an adaxial zone (1–4 cell layers) of fibres in the papillate zone. At mid-length, the mesophyll is differentiated into two distinct zones, an abaxial zone (7 or 8 cell layers) of tanniferous, parenchymatous cells, irregular in shape, and an adaxial zone (1–3 cell layers) of fibres. There are two vascular bundles. The papilla cells of the inner epidermis are tanniferous. Below the papillate zone, the inner epidermis consists of narrowly rectangular (tangential sides shorter than radial sides) and tanniferous cells, sometimes with slightly undulate walls.
Note: The investigated material of *E. somalensis* (the holotype) is in an earlier developmental stage than that of the remaining species.

Ephedra equisetina Bunge
Cone bracts: There are three pairs of bracts and the ovules are exserted. The innermost bracts are 3.7 mm (specimens *71*, *142*), 5.2 mm (specimen *125*) and > 6 mm in specimen *234*. They are fused for little over

2/3 of their length in *125, 142, 145, 234* and for approximately half of their length in *71*. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (3–5 cell layers) and inner epidermis (1 cell layer). The hyaline margins are 10–15 cells wide in the apical parts of the bracts. The outer epidermis is strongly papillate. The cells are usually non-tanniferous and the cuticle is thick. The mesophyll is differentiated into an abaxial zone of parenchymatous tissue and an adaxial zone of fibres. The fibres are rare and scattered in some specimens, but more frequent in an adaxial zone in specimens *125* and *234*. The inner epidermis is non-papillate; cells are rectangular, sometimes tanniferous and with slightly undulate walls. The cuticle is thinner than that of the outer epidermis. Stomata are observed in specimen *142*, but only rarely in the most distal part of the cone bract.

Ovules: There is one ovule in each cone, *c*. 4–5 mm long.

Integument: The inner epidermis cells are tanniferous.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and almost rounded, with a weak adaxial 'angle' at mid-length. The outer epidermis is strongly papillate and sometimes tanniferous. Tangential sides of cells are shorter than radial sides and the cuticle is thick. At mid-length of the ovule, the cell walls are undulate. The mesophyll consists of parenchymatous tissue and fibres in the papillate zone. At mid-length, the mesophyll is differentiated into two distinct zones, an abaxial zone (3 or 4 cell layers) of parenchymatous cells, which become tanniferous as the ovule develops, and an adaxial zone (3 or 4 cell layers) of fibres. There are two vascular bundles. The papilla cells of the inner epidermis are lignified and tanniferous. Below the papillate zone, the cells of the inner epidermis are large and non-tanniferous. Cell walls are undulate and the cuticle is thin.
Note: The histology and some morphological features of *E. equisetina* are quite variable and the distinction between *E. equisetina* and *E. major* ssp. *procera* is uncertain.

Ephedra major Host ssp. procera (synonyms E. botschantzevii Pachom., E. nebrodensis Tineo, E. procera C.A.Mey., E. scoparia Lange)
Cone bracts: There are three pairs of bracts and the ovules are exserted. The innermost bracts are 3.0–5.5 mm long and fused for approximately half of their length (more, approximately 2/3, in specimen *193*). At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (3–7 cell layers) and inner epidermis (1 cell layer). The hyaline margins are narrow, < 10 cells wide. The outer epidermis is strongly papillate and tanniferous. The cuticle is thick. The mesophyll consists mainly of parenchymatous tissue, but fibres are present just below the inner epidermis. In specimens *163, 165, 169*, fibres are infrequently to rarely scattered. In specimen *193*, there are fibres in 1 cell layer but with frequent gaps in the layer. In specimens *164* and *206*, there are fibres in an uninterrupted adaxial zone (1 or 2 cell layers). There are four to six vascular bundles in specimen *165*. In all other specimens, there are two vascular bundles. The inner epidermis is non-papillate and tanniferous. The cells are rectangular, sometimes with slightly undulate walls. The cuticle is thinner than that of the outer epidermis. Stomata are observed in specimens *71, 163* and *169*, but only rarely in the most distal part of the cone bract.

Ovules: There is one ovule in each cone, *c*. 4.5–6.5 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone. At mid-length, it is rounded but with abaxial and adaxial 'angles', which give the seed envelope an almost squared impression. The adaxial angle is poorly developed or absent in *193* and in *206* the seed envelope is more ovate (two-angled) also at mid-length. The outer epidermis is tanniferous; Cells are rounded and walls are sometimes undulate. The cuticle is thick. The mesophyll is differentiated into an abaxial zone of parenchymatous tissue and an adaxial zone (3 or 4 cell layers) of fibres in the papillate zone. At mid-length, the abaxial zone is tanniferous and 4–6 cell layers thick. Cells walls are undulate. The adaxial zone of fibres is 1–3 (–5) cell layers thick. There are two vascular bundles. The papilla cells of the inner epidermis are lignified and tanniferous. Further down, the inner epidermis is 1 or 2 cell layers thick and tanniferous. Cell walls are undulate.

Ephedra monosperma J.G.Gmel. ex C.A.Mey.
Cone bracts: Two pairs of cone bracts enclose the ovule. The innermost bracts are *c*. 4.5 mm and fused for approximately half of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (5 or 6 cell layers) and an inner epidermis (1 cell layer). The hyaline margins are approximately 15–20 cells wide. The outer epidermis is papillate and tanniferous. Tangential sides of cells are longer than radial sides. The mesophyll is differentiated into two zones: an abaxial zone of parenchymatous tissue and an adaxial zone (1 or 2 cell layers)

of fibres. The cells of the inner epidermis are similar to those of the outer epidermis. Stomata are not observed.

Ovules: There is one ovule in each cone, *c.* 4.3 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and elliptic to weakly squared at mid-length. The cells of the outer epidermis are rectangular and tanniferous, sometimes with undulate walls. The cuticle is thick. The mesophyll is differentiated into two distinct zones in the papillate zone and downwards. At mid-length, there is an abaxial zone (3–5 cell layers) parenchymatous, tanniferous cells with undulate walls, and an adaxial zone (1 or 2 cell layers) of fibres. There are two vascular bundles. The papilla cells of the inner epidermis are lignified. Further down, the histology of the inner epidermis is unclear in the investigated material.

Ephedra saxatilis (Stapf) Royle ex Florin
Cone bracts: There are three pairs of bracts and the ovules are exserted. The innermost bracts are *c.* 6.5 mm and fused for approximately half their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (7 cell layers) and inner epidermis (1 cell layer). The bracts lack the hyaline margins at the lateral flanks, which are present in most species. The outer epidermis is papillate and non-tanniferous. The mesophyll consists of parenchymatous tissue, sclerids are not observed. The inner epidermis is non-papillate and non-tanniferous. The cuticle is thinner than that of the outer epidermis. Stomata are present.

Ovules: There is one ovule in each cone, *c.* 3.5–7 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and rounded at mid-length, with abaxial and adaxial 'angles', which gives the seed envelope a squared impression (Fig. 2I). The outer epidermis is strongly papillate and tanniferous. Tangential sides of cells are shorter than radial sides in the papillate zone. At mid-length of the ovule, cell walls are undulate. The cuticle is thick. The mesophyll consists of parenchymatous tissue in the papillate zone. At mid-length, the mesophyll is differentiated into two non-distinct zones (probably attributable to early developmental stage), an abaxial zone (3 or 4 cell layers) of large parenchymatous cells, rounded or with undulate walls, and an adaxial zone (3 or 4 cell layers) of fibres. There are two vascular bundles and a zone of tanniferous cells outside of each vascular bundle. The papilla cells of the inner epidermis are tanniferous. Further down, the cells of the inner epidermis are large with undulate walls and non-tanniferous. The cuticle is thin.

Ephedra gerardiana Wall. & Florin
Cone bracts: There are two pairs of bracts and the ovules are exserted. The innermost bracts are *c.* 4.5 mm long and fused for approximately half their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (at least 3 layers) and inner epidermis (1 cell layer). The bracts have narrow hyaline margins (*c.* 5 cells wide). The outer epidermis is papillate and tanniferous. The mesophyll consists of parenchymatous tissue and probably also of fibres (uncertain results). The inner epidermis is non-papillate and tanniferous; cells are rectangular. Stomata are not observed.

Ovules: There is one ovule in each cone, *c.* 5 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and rounded but with 'angles', which give the seed envelope an almost squared impression at mid-length. The outer epidermis is tanniferous and not strongly papillate; cell walls are undulate. The cuticle is thick. The mesophyll is differentiated into two distinct zones, an abaxial zone (3 or 4 cell layers) of large, parenchymatous, tanniferous cells, and an adaxial zone (3 or 4 cell layers) of fibres. There are two vascular bundles. The papilla cells of the inner epidermis are tanniferous and probably lignified. The cuticle is thin. Further down, the inner epidermis is tanniferous (but not lignified). The cuticle is thin.

Clade L

Ephedra strobilacea Bunge
Cone bracts: There are four or five pairs of bracts and the ovules are exserted. The innermost bracts are *c.* 7–10 mm long and free for almost their entire length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (4 or 5 cell layers) and inner epidermis (1 cell layer). The hyaline margins of free parts of the bracts are broad. Distally they constitute most of the bracts. At mid-length of the bracts, they are 30 cells wide or more. The outer epidermis is tanniferous and prominently papillate over the vascular bundles. Cells are otherwise generally rounded, sometimes with slightly undulate walls. The mesophyll is differentiated into two zones: an abaxial zone (2 or 3 cell layers) of parenchymatous tanniferous tissue (cells are rectangular, sometimes with undulate walls), and an adaxial zone (1–3 cell layers) of fibres. The cells of the inner epidermis are similar to

those of the outer epidermis but non-papillate and the cuticle is thinner. Stomata are not observed.

Ovules: There are two ovules in each cone, *c*. 6.5–7 mm long.

Seed envelope: The seed envelope is largely triangular in transverse section in the papillate zone and at mid-length. The outer epidermis is tanniferous and not prominently papillate; cells are rounded, sometimes with slightly undulate walls. The cuticle is thick. The mesophyll consists apically, mainly of fibres. At mid-length, it is differentiated into two zones, an abaxial zone of tanniferous, parenchymatous tissue (1 or 2 cell layers), and a prominent adaxial zone of fibres (2–5 cell layers). There are two vascular bundles. The papilla cells of the inner epidermis are (partly) tanniferous and the cuticle is thin. At mid-length, the cells of the inner epidermis are tanniferous and rectangular, sometimes with undulate walls. The cuticle is thinner than that of the outer epidermis.

Ephedra sarcocarpa Aitch. & Hemsl.
Cone bracts: Three pairs of bracts enclose the ovules almost completely. The innermost bracts are *c*. 4.3 mm long and fused for slightly less than half of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (2 cell layers) and inner epidermis (1 cell layer). The hyaline margins of free parts of the bracts are approximately 25 cells wide. The outer epidermis is papillate and non-tanniferous. The cuticle is thick. The mesophyll is differentiated into two zones: an abaxial zone of parenchymatous tissue and an adaxial zone (1–3 cells layers) of fibres (Fig. 3A). Starch is observed in cells of the mesophyll. The inner epidermis is different from the outer epidermis; it is tanniferous and non-papillate; the cells are larger and some have slightly undulate walls. The cuticle is thinner. Stomata are not observed.

Ovules: There are two ovules in each cone, *c*. 4.8 mm long.

Integument: The integument fuses with the nucellus higher up than usual and never becomes thicker than 2 cell layers.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and triangular at mid-length. The outer epidermis is tanniferous and not prominently papillate. The cells are rectangular (tangential sides shorter than radial sides), sometimes with slightly undulate walls. The mesophyll consists of parenchymatous tissue in the papillate zone. At mid-length, the mesophyll is differentiated into two zones, an abaxial zone of large parenchymatous cells with slightly undulate walls (2–4 cell layers) and an adaxial zone of fibres (3 or 4 cell layers). The cells of the mesophyll (and outer and inner epidermis cells) gradually become more densely tanniferous proximally. There are two vascular bundles. The papilla cells of the inner epidermis are tanniferous and the cuticle is thin. At mid-length, the cells of the inner epidermis are similar to those of the outer epidermis but the cuticle is thinner.

Ephedra transitoria Riedl
Cone bracts: There are three whorls of bracts (three bracts in each whorl); the ovules are exserted. The innermost bracts are *c*. 4 mm long and fused for approximately half of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (5 cell layers) and inner epidermis (1 cell layer). The hyaline margins of free parts of the bracts are approximately 25 cells wide. The outer epidermis is papillate and tanniferous. Some cells have slightly undulate walls. The mesophyll is differentiated into two zones: an abaxial zone of parenchymatous tissue and an adaxial zone (1 or 2 cell layers) of fibres. The cells of the inner epidermis are similar to those of the outer epidermis but smaller and non-papillate. The cuticle is thinner. Stomata are observed, but only rarely in the most distal part of the cone bracts.

Ovules: There are three ovules in each cone, *c*. 5 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and squared at mid-length. The outer epidermis is tanniferous and non-papillate; cells are rounded and the cuticle is thick. The mesophyll is differentiated into two distinct zones in the papillate zone and at mid-length: an abaxial zone of tanniferous parenchymatous tissue (2–5 cell layers) and an adaxial zone of fibres (Fig. 4A). The parenchymatous cells are rounded in the papillate zone and rounded or have undulate walls further down. The inner zone of fibres is 4 cell layers thick in the papillate zone and 1 or 2 cell layers at mid-length. There are two vascular bundles. The papilla cells of the inner epidermis are (partly) tanniferous and the cuticle is thin. At mid-length, the cells of the inner epidermis are tanniferous and rectangular, sometimes with undulate walls. The cuticle is thinner than that of the outer epidermis.

Ephedra distachya L.
Cone bracts: There are three or four pairs of bracts and the ovules are exserted in most specimens (not in *227*). The innermost bracts are *c*. 2.7–3.8 mm long

and fused for approximately half their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (4–7 cell layers) and inner epidermis (1 cell layer). The hyaline margins are approximately 10–15 cells wide distally. The outer epidermis is papillate and tanniferous. The mesophyll is differentiated into two zones: an abaxial zone of parenchymatous tissue and an adaxial zone (1–3 cell layers) of fibres. The inner epidermis cells are rectangular (papillate over the vascular bundles) and tanniferous. They are smaller than those of the outer epidermis and the cuticle is thinner. Stomata are observed in specimens *140* and *236*, but only rarely in the most distal part of the cone bract.

Ovules: There are two ovules in each cone, *c.* 4–5.5 mm long (2.5 mm in specimen *227*).

Nucellus and gametophyte: The gametophyte of specimen *236* contains eight archegonia.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and largely triangular at mid-length. The outer epidermis is tanniferous and not prominently papillate; cells are rectangular (tangential sides shorter than radial sides) and sometimes have slightly undulate walls. The cuticle is thick. The mesophyll is differentiated into two zones, an abaxial zone of tanniferous, parenchymatous cells, rounded and sometimes with undulate walls (3 or 4 cell layers), and an adaxial zone of fibres (2–4 cell layers). There are two vascular bundles. The papilla cells of the inner epidermis are tanniferous, lignified in specimens 184, 233. At mid-length, the cells of the inner epidermis are similar to those of the outer epidermis but smaller and the cuticle is thinner.

Ephedra sinica Stapf
Cone bracts: Three pairs of bracts enclose the ovules. The innermost bracts are 5–6 mm long and fused for approximately 1/2 to 3/4 of their length (less in specimen *194*). At mid-length, the bracts are differentiated into outer epidermis (1 cell layer), mesophyll (3–5 cell layers) and an inner epidermis (1 cell layer). The hyaline margins are 5–10 cells wide distally. The outer epidermis is papillate and tanniferous. The cells sometimes have slightly undulate walls. The mesophyll is differentiated into two zones: an abaxial zone of parenchymatous tissue and an adaxial zone (1 or 2 cell layers) of fibres. The cells of the inner epidermis are similar to those of the outer epidermis, but slightly smaller. Stomata are observed, but only rarely in the most distal part of the cone bract.

Ovules: There are two ovules in each cone, *c.* 5–6 mm long.

Integument: The micropylar tube is spirally twisted or straight. The two types may occur in a single specimen.

Seed envelope: The seed envelope is elliptical in transverse section in the papillate zone and elliptical to triangular at mid-length. The outer epidermis is tanniferous and not prominently papillate. The cell walls are sometimes undulate. The cuticle is thick. The mesophyll is differentiated into two distinct zones: an abaxial zone (2–5 cell layers) of undulate, tanniferous, parenchymatous cells, and an adaxial zone (1–3 cell layers) of fibres. There are two vascular bundles. The papilla cells of the inner epidermis are tanniferous and the cuticle is thin. Further down, the cells of the inner epidermis are tanniferous and rectangular, sometimes with undulate walls. The cuticle is thinner than that of the outer epidermis.

Ephedra intermedia Schrenk & C.A.Mey.
Cone bracts: Three to five pairs of bracts enclose the ovules (Fig. 2B). The innermost bracts are 4–4.5 mm long and fused for approximately 1/3 to more than half of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (3–5 cell layers) and inner epidermis (1 cell layer). The hyaline margins are approximately 10–15 cells wide distally. The outer epidermis is papillate and tanniferous. The mesophyll is differentiated into two zones: an abaxial zone of parenchymatous tissue and an adaxial zone (1 or 2 cell layers) of fibres. Starch was observed in cells of the mesophyll in specimen *06*. The cells of the inner epidermis are similar to those of the outer epidermis. Stomata are not observed.

Ovules: There are two ovules in each cone (Fig. 2B), *c.* 3.3 mm long.

Integument: The micropylar tube is spirally twisted.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone (Fig. 2D) and triangular at mid-length (Fig. 2B). The outer epidermis is tanniferous and not prominently papillate; cells are rectangular (tangential sides shorter than radial sides) in the distal part of the ovule. At mid-length cell walls are undulate. The cuticle is thick. The mesophyll consists of parenchymatous tissue in the papillate zone. At mid-length, the mesophyll is differentiated into an abaxial zone of parenchymatous tissue and an adaxial zone of fibres [1 (or 2) cell layers]. The parenchymatous tissue (and the epider-

mis) gradually becomes more densely tanniferous, starting with the abaxial cell layers and is, at mid-length, strongly tanniferous. Cell walls are slightly undulate. There are two vascular bundles (Fig. 2B, D). The papilla cells of the inner epidermis are tanniferous and the cuticle is thin. At mid-length, the cells of the inner epidermis are rectangular (tangential sides shorter than radial sides) and tanniferous. The cuticle is thinner than that of the outer epidermis.

Ephedra fedtschenkoi Paulsen
Cone bracts: Three pairs of bracts enclose the ovules. The innermost bracts are *c*. 4.7 mm long and fused for approximately 5/6 of their length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (4 or 5 cell layers) and inner epidermis (1 cell layer). The hyaline margins are approximately 10–15 cells wide distally. The outer epidermis is papillate and tanniferous. The mesophyll is differentiated into two zones: an abaxial zone of parenchymatous tissue and an adaxial zone (1 cell layer) of fibres. The inner epidermis is tanniferous and non-papillate; cells are rounded or have undulate walls. The cuticle is thinner than that of the outer epidermis. Stomata are not observed.

Ovules: There are two ovules in each cone, *c*. 7 mm long.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and elliptic to triangular at mid-length. The outer epidermis is not prominently papillate; the cells are tanniferous and have undulate walls. The cuticle is thick. The mesophyll is differentiated into two distinct zones in the papillate zone and at mid-length: an abaxial zone of parenchymatous tissue (2–4 cell layers), in which the cells are tanniferous and have undulate walls, and an adaxial zone (1, rarely 2, cell layers) of fibres. There are two vascular bundles. The papilla cells of the inner epidermis are poorly preserved in the material but appear tanniferous with a thin cuticle. At mid-length, the inner epidermis is tanniferous; cells are rectangular (tangential sides longer than radial sides). The cuticle is thinner than that of the outer epidermis.

Ephedra przewalskii Stapf
Cone bracts: Four (or five) pairs of bracts enclose the ovules completely. The innermost bracts are *c*. 4 mm long and free for almost their entire length. At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (2 or 3 cell layers) and inner epidermis (1 cell layer). The hyaline margins of free parts of the bracts are wide. Distally they constitute the entire bracts and are 40–80 cells wide. At mid-length of the bracts, they are ≥ 30 cells wide. The outer epidermis is non-tanniferous and strongly papillate at mid-length of the bracts. In the distal part of the bracts, the cells are rounded. The cuticle is thick. The mesophyll is differentiated into two zones: an abaxial zone (1 or 2 cell layers) of non-tanniferous, rounded, parenchymatous cells and an adaxial zone (1 cell layer) of fibres. The cells of the inner epidermis are similar to those of the outer epidermis. Stomata are not observed.

Ovules: There are two ovules in each cone, *c*. 2.5–3 mm long.

Seed envelope: The seed envelope is largely triangular in transverse section in the papillate zone and at mid-length. The cells of the outer epidermis are tanniferous, large and rounded or with slightly undulate walls. The cuticle is thick. The mesophyll consists of a single zone of distinctly tanniferous, parenchymatous tissue (3 cell layers); cells are rounded or have undulate walls. There are two vascular bundles. The papilla cells of the inner epidermis are tanniferous and the cuticle is thin. At mid-length, the cells of the inner epidermis are tanniferous and rectangular (tangential sides longer than radial sides), sometimes with undulate walls. The cells are smaller than those of the outer epidermis and the cuticle is thinner.

Ephedra lomatolepis Schrenk
Cone bracts: There are three pairs of bracts and the ovules are exserted. The innermost bracts are *c*. 4.2 mm and can be free for most of their length (specimens *92, 102*) or fused in the proximal third of their length (specimens *191, 174*). At mid-length, they are differentiated into outer epidermis (1 cell layer), mesophyll (3–6 cell layers) and inner epidermis (1 cell layer). The hyaline margins are approximately 5 cells wide at bract mid-length. The outer epidermis is papillate and tanniferous. The mesophyll is differentiated into two zones: an abaxial zone of parenchymatous tissue and an adaxial zone (1 or 2 cell layers) of fibres. Starch is observed in cells of the mesophyll in some specimens. The inner epidermis is tanniferous and non-papillate; cells are rectangular, sometimes with slightly undulate walls. The cuticle is thinner than that of the outer epidermis. Stomata are not observed.

Ovules: There are two ovules in each cone, *c*. 4.5 mm long.

Integument: The micropylar tube is slightly twisted.

Seed envelope: The seed envelope is elliptic in transverse section in the papillate zone and largely triangular at mid-length. The outer epidermis is tanniferous and not prominently papillate. Cells have undulate walls. The cuticle is thick. The mesophyll is differentiated into two distinct zones, an abaxial zone (3 or 4 cell layers) of parenchymatous, tanniferous tissue and an adaxial zone (2 cell layers) of fibres. There are two vascular bundles. The papilla cells of the inner epidermis are tanniferous. Below the papillate zone, the cells of the inner epidermis are rectangular and tanniferous. The cuticle is thinner than that of the outer epidermis.

DISCUSSION

Comparative structural evaluation

Female cone bracts

Morphology: Cone bracts arranged in opposite pairs are inferred to be the ancestral and most common condition in extant *Ephedra*. Cone bracts in whorls (of three) occur in several clades but are rarer and/or variable within species. We found trimerous whorls in *E. milleri* (endemic to Oman) and in the Mediterranean–Asian *E. foliata*, in the North American *E. californica, E. trifurca* and *E. torreyana*, in the South American *E. multiflora* and in the Asian *E. transitoria*. Phyllotaxis is partly correlated with the number of ovules in the cone; for species with whorled bracts, there are sometimes three ovules in the cone (*E. multiflora* and *E. transitoria*) and sometimes only one (the North American species). In *E. milleri* and *E. foliata*, the cone bract arrangement and the number of ovules are variable but we never found two ovules in cones with cone bracts in whorls of three.

Number of cone bracts and degree of fusion: The number of pairs/whorls of cone bracts in extant species of *Ephedra* is quite variable and ranges from two or three to five or six (to nine). The degree of fusion of the bracts in one pair or whorl vary from fused for almost all of their length to completely free from each other, or anything in between. Intraspecific variation of these features is substantial.

Structure: At seed maturity, the gross morphological differences between cone bracts of species adapted for various seed dispersal mechanisms are obvious. A pronounced developmental transformation occurs in species with fleshy cone bracts at seed maturity. The parenchymatous zone expands to become 20–30 cells thick and the cells enlarge. The tissue is frequently penetrated by ascomycete fungi. In species with dry, papery cone bracts at seed maturity, no such clear histological difference between developmental stages is observed.

At the pollination stage, the only pronounced morphological differences between future dry bracts and future fleshy bracts are the amount of mesophyll and the width of the hyaline margin at the lateral flanks. The margin is already broad (30–50 cells or more) in, for example, *E. alata*, *E. multiflora*, *E. strobilacea*, *E. przewalskii* and *E. torreyana,* at the immature stage of development, and mesophyll is often only present around and between the vascular bundles. In contrast, Old World and South American species with future fleshy cone bracts typically have narrow margins (2–15 cells wide). In North American species, seed dispersal mechanisms and the texture of the cone bracts at seed maturity are more variable (Hollander, Vander Wall & Baguley, 2010), as is the width of the hyaline margin. All the above-mentioned morphological characters have been widely used as bases for classification and species delimitation and determination, but their plasticity indicates that this is problematic.

Anatomy: The anatomy of the cone bracts appears largely constant throughout the *Ephedra* lineage. There are generally two unbranched vascular bundles and they are not strictly parallel, converging towards the apex. These findings are in accordance with a report of the venation of vegetative leaves (Foster, 1972).

Rare deviations from the general pattern of two veins can be found in cone bracts and in vegetative leaves. We found four to six vascular bundles in cone bracts of *E. major* ssp. *procera*, *165.* Further, in *E. americana, 220,* there is a third strand in the distal parts of one cone bract, which ends freely in the mesophyll in the upper half of the bract. These features were not observed in other specimens of these species. Similarly, vegetative leaves of *E. chilensis* may have three veins and the middle vein (if present) sometimes ends freely in the mesophyll (proximally) without connecting with other vasculature (Foster, 1972).

Monoyer (1937, 1938) suggested an evolutionary reduction of the vascular system in vegetative leaves of *Ephedra*, from the ancestral three veins (in *E. foeminea*) to a more commonly occurring derived state of two-veined leaves. The hypothesis is interesting because recent phylogenetic studies indicate that *E. foeminea* is sister to all other species, and because we hypothesize a reduction of the vascular system in the seed envelope (see below). However, vegetative leaves in the specimens of *E. foeminea* investigated here have two veins. Variation patterns in vegetative leaves are not investigated here and have to our knowledge never

been documented for the entire genus. From studies of *E. chilensis* (Foster, 1972), however, and our results on cone bracts, it seems likely that three-veined leaves (and cone bracts) are rare exceptions in *Ephedra*, not an ancestral state.

Histology: Based on histology at the pollination stage of development, the evolutionary importance of the much-noted (and clearly homoplastic) difference in cone bract texture seems overestimated. Most species of *Ephedra* (i.e. the New World clade and most Asian clades) have the same general histological pattern: an adaxial zone of fibres and an abaxial parenchymatous zone, regardless of seed dispersal mechanism. However, there are exceptions and detailed arrangements may vary between subclades. In some clades (i.e. in *E. foeminea*, *E. foliata* and the *E. minuta–E. likiangensis* clade), fibres are absent. Thoday & Berridge (1912), who (mainly) investigated *E. fragilis, E. altissima, E. major* and *E. distachya*, suggested that all cone bracts have scattered 'fibrous cells' in the tissue and that fibres are particularly numerous in a layer just under the inner epidermis. Here, with a greatly expanded taxon sampling, we find that only the species in a Mediterranean clade (*E. fragilis, E. altissima, E. major* ssp. *major, E. aphylla*) have fibres scattered throughout an otherwise parenchymatous mesophyll. In these species, the sclerenchymatous cells become more abundant proximally in the cone bracts and, in some specimens, they are arranged in a continuous or almost continuous adaxial zone towards the base of the bracts, similar to the histological pattern of most other species.

The epidermis is papillate in many species, but the shape of the cells and the thickness and shape of the cuticle is usually variable within a specimen. The dry bracts of *E. alata* and *E. strobilacea* have a thin cuticle compared with most species, although they live in extremely arid environments. In *E. przewalskii*, the cuticle is thicker. In all these drought-adapted species, the cells of the outer epidermis are distinctly papillate.

Stomata are present on the outer epidermis. More rarely stomata are also observed on the inner epidermis, but (if present) usually only in the most distal part of the cone bracts. Pant & Mehra (1964) reported that stomata on stems and leaves are surrounded by four or five subsidary cells, which differ from the ordinary epidermal cells in that the sides facing the stomatal pore are slightly thickened and papillate. However, no such differences can be seen in transverse sections of cone bracts. Epidermal cells are papillate, with a thick cuticle in many species, and the shape of cells is variable, but as seen in transverse sections there is no clear distinction between cells adjacent to guard cells and other epidermal cells.

Seed envelope

Morphology: Generally, the shape of an organ is partly determined by intrinsic factors and sometimes partly by pressure from other organs (Endress, 2008). The ephedran seed envelope may be squared, triangular, elliptic or circular in transverse section and the shape at mid-length of the ovule often differs from the apical shape (in the papillate zone). The apical shape in transverse section is, with few exceptions, consistent with anatomy; i.e. the seed envelope is squared apically when there are four vascular bundles, triangular when there are three vascular bundles and elliptic when there are two vascular bundles (Fig. 2D–F). Exceptions are the Asian *E. strobilacea* and the American species *E. pedunculata* and *E. rupestris*, in which the seed envelope is largely triangular apically, although there are only two vascular bundles. In contrast, the transverse shape at mid-length of the ovule is only rarely correlated with the vasculature (Fig. 2G–I) and appears rather to be linked with the number of ovules in the cone. Thus, apically, the transverse shape of the seed envelope is determined by an intrinsic factor (i.e. the vasculature), whereas the shape at mid-length is determined by pressure from other organs (mainly the other ovules).

Anatomy: The ephedran seed envelope is always vascularized (in contrast to the integument, which is never vascularized). The vascular bundle is usually associated (on its inner side) with a thick strand of fibres (see also Thoday & Berridge, 1912). The number of vascular bundles varies between four (in lateral, abaxial and adaxial position), three (lateral and abaxial position), and two (lateral position). Eames (1952) states that there may be up to six vascular bundles but we never found more than four and the feature is surprisingly constant within clades and species. We find only a few cases of intraspecific variation (i.e. *E. fragilis* and *E. altissima*).

In a few species with two vascular bundles (*E. pedunculata, E. tweediana* and *E. minuta*), there may be an extra strand of supportive tissue in the papillate zone, located where the third vascular bundle would have been if present. It disappears further down (without fusing with another bundle). Several features in *Ephedra* seem to serve the function of ensuring an upright position of the micropylar tube and this 'extra strand' of supportive tissue may have been retained as the only evolutionary remainder of an otherwise reduced vascular bundle, in response to the clear adaptive value of an upright micopylar tube. However, we also find an 'extra distal strand' in one cone bract (*E. americana, 220*) and Foster (1972) documented a similar pattern for vegetative leaves (see also above).

The adaptive value of an 'extra distal strand' in cone bracts and leaves is more difficult to explain.

We found only four species (in two unrelated clades) that have four vascular bundles in the seed envelope: the Mediterranean sister taxa *E. altissima* and *E. fragilis,* which have three or four vascular bundles, and the North American sister taxa *E. californica* and *E. trifurca*, which from our results appear to consistently have four vascular bundles. This is largely in line with previous reports of the anatomy of the seed envelope; Thoday & Berridge (1912) found that *E. altissima* and *E. trifurca* have three, sometimes four, vascular bundles, whereas *E. distachya* has two. Herzfeld (1922) reported that *E. campylopoda* (= *E. foeminea*) has three vascular bundles in the seed envelope.

The morphological and evolutionary bases for this variation is, however, not clear. Thoday & Berridge (1912) described the vascularization of the cone in detail for a few species and considered the presence of four vascular bundles to be a result of evolutionary fusion of the vascular systems of seed envelopes from two ovules. Eames (1952) also regarded the ovule of the single-seeded cones in *E. altissima* and *E. trifurca* as 'double' (i.e. derived from fusion of two ovules) and the presence of four vascular bundles in a seed envelope to be a reflection of this fusion. However, *E. altissima* and *E. trifurca* are both members of clades in which all other species have three vascular bundles in the seed envelope, not two. A simple 'doubling' of the vascular systems (one seed envelope originating from two) thus does not fit our observations. Further, differences in the position of the vascular bundles and phylogenetic results from molecular data indicate that four vascular bundles have evolved separately in two extant clades, and there may thus be several, independent explanations for the evolutionary origin of the feature.

Histology: The ephedran seed envelope is always at least partly sclerenchymatous (with the possible exception of *E. przewalskii*). Among early diverging species, the mesophyll either consists of two distinct zones, an abaxial parenchymatous and an adaxial sclerenchymatous (*E. foeminea, E. alata, E. aphylla* and *E. foliata*), or, alternatively, the mesophyll consists of parenchymatous tissue and scattered fibres (*E. major* ssp. *major, E. fragilis* and *E. altissima*). In the New World species and in the Asian clade, the structure is largely the same as in *E. foeminea, E. alata, E. aphylla* and *E. foliata*, i.e. there are two distinct zones. But details may differ; for example, the parenchymatous cells are typically rounded in some species and typically have undulate walls in others (i.e. the cells fit together like pieces of a jigsaw puzzle and intercellular space is sparse or lacking). The occurrence of tannins in the parenchymatous tissue may be diagnostic; for example, in some clades all cells are clearly and evenly tanniferous in a distinctive way (see below).

Features of the epidermis (i.e. size, shape and tannin content of cells) vary in similar patterns, as does the mesophyll. Stomata are usually absent in the seed envelope; however, in a few unrelated species adapted to dry environments (i.e. *E. alata* and *E. californica*) stomata are common, but only in the outer epidermis and in apical parts of the seed envelope. The Chinese *E. rhytidosperma* has a distinctive surface pattern with transverse ridges on the seed envelope (Yang, 2007). A similar pattern is present also in the North American species *E. torreyana*. The ridges are typically 100–500 μm wide in both species and formed by protruding outer tangential walls of up to 40 adjacent epidermis cells (C. Rydin, pers. observ.). The cone bracts of *E. torreyana* have similar protrusions, particularly in proximal parts of the bracts.

The papilla cells formed by the inner epidermis are present in all species of *Ephedra* and have been noticed in a few previous studies (Jaccard, 1894; Thoday & Berridge, 1912; Kahn, 1943; Rydin *et al*., 2004). They do not appear to be secretory; they are tanniferous, the cuticle is often thick and they are strongly lignified in some species. Their function is probably exclusively to support the micropylar tube and possibly also to close the gap between the seed envelope and the integument as suggested by Thoday & Berridge (1912). Lignification of the papilla cells is prominent in *E. altissima* and also present in some other species, but appears to be restricted to three clades (the Mediterranean clade *E. fragilis–E. altissima–E. major* ssp. *major*; the Asian clade K and *E. distachya*). It should be noted, however, that the possibility that lignification of the papilla cells is correlated with developmental stage, as indicated by Thoday & Berridge (1912), has not been tested in the present study.

Homology assessments: The evolutionary origin of the seed envelope and its homology with other organs have been discussed and variously interpreted. Two examples (for a comprehensive summary see Takaso, 1985): some early authors argue for homology with the integument (e.g. Thoday & Berridge, 1912), whereas others argue for homology with bracts (e.g. Jaccard, 1894). Modern developmental studies indicate that the seed envelope is more comparable with vegetative leaves than with the integument (Takaso, 1985). In early development, the seed envelope is horseshoe-shaped, lacking on the adaxial side, but later present on all sides and distinctly thicker on the lateral sides (Takaso, 1985). Thus, two opposite projections on the young seed envelope are in decussate

arrangement with the cone bracts (Jaccard, 1894; Takaso, 1985). The integument is, in contrast, a uniform collar from the beginning (Martens, 1971: 42; Takaso, 1985), as in most other seed plants.

Our data also lend some support for homology with cone bracts. The seed envelope is vascularized, as are bracts and leaves, but the integument is never vascularized. In particular, the seed envelope has, according to our observations, a maximum of four vascular bundles, a number expected if it is homologous to two bracts (leaves). Lower numbers of vascular bundles can be easily interpreted as reductions. Histology may possibly provide additional indications of homology of the cone bracts and the seed envelope (i.e. sclerenchymatous tissue in an adaxial zone, or scattered, in the mesophyll and the presence of stomata in the epidermis of both structures).

Ovule

Integument: In contrast to the seed envelope, the integument is a histologically simple structure, 3 cell layers thick apically, gradually thickened downwards to *c*. 6 or 7 cell layers, and congenitally fused with the nucellus in the proximal half of the ovule. It is never sclerenchymatous (except in the micropylar tube, see below) or vascularized. There are rare reports of vascularization of the integument (Land, 1907; Eames, 1952), but we find no evidence of that in any of our specimens.

The extended micropylar tube presents the pollination drop and serves as the pollen-receiving area. In addition to the support provided by the papilla cells on the seed envelope, the micropylar tube itself is constructed to ensure an upright position through conspicuous thickening, most probably lignification, of the outer walls of the cells of the inner epidermis. In most species, it is mainly the inner tangential side facing the hollow tube and partly also the radial sides that are lignified (Fig. 4D). In the 'basal' species in the Mediterranean species complex, the cell walls are more evenly thickened, on all sides of the cells (Fig. 4E). The partial lignification (Fig. 4D) probably provides some flexibility. When strongly lignified on all sides of the cells, the micropylar tube appears to break more easily than in other species (Fig. 4D–E).

The lignification of the micropylar tube is ancestral; it is present in all species of *Ephedra*, in *Welwitschia* (on the inner tangential side and parts of radial sides similar the example of *Ephedra minuta* in Fig. 4D) and also to a minor extent in *Gnetum gnemon* L. (above the level of the closure tissue there is a thin but even thickening of the inner epidermis cell walls) (C. Rydin, pers. observ.). The lignification has been previously noted, for *Welwitschia* and *Gnetum* by Church (1914) and Thoday (1921), but it is generally referred to as cuticularization (but see Thoday, 1911: 1118). For *Ephedra*, Kahn (1943) mentioned that the cell walls of the innermost layer of the integument become thick and Thoday & Berridge (1912) described the cells as 'cuticularized to a most remarkable extent'. From our investigations, the obvious thickening is not derived from the cuticle, but from the cell wall and the staining indicates lignification.

After pollination the micropylar tube is closed, in *Ephedra* by mucilage, which will become increasingly solidified with ontogenetic development (Thoday & Berridge, 1912). Such mucilage, apparently in different stages of solidification, is found in the micropylar tube of some specimens investigated in the present study and we interpret it as universally present in *Ephedra*, even if it was apparently lacking in many specimens.

Nucellus and gametophyte: The features of the nucellus and gametophyte appear rather constant from the present study, but their histology is often unclear in herbarium material and we cannot cover the full (potential) variation in the genus here. We have, however, made a few noteworthy observations. The apical region of the nucellus is apparently secretory (as indicated in Fig. 2A–C); the cytoplasm is dense and vacuoles are typically rare or lacking. The pollination drop and the mucilage that closes the micropylar tube after pollination are probably secreted from the nucellus, which is in accordance with previous observations in *E. distachya* (Moussel, 1980).

The gametophyte usually contains two or three archegonia, but in one specimen (*E. distachya, 236*) there are eight archegonia. This variation is consistent with previous studies (e.g. Land, 1904; Maheshwari & Sanwal, 1963).

Pollination

The thickening and support for the micropylar tube is conceivably important in Gnetales in order to withstand visits from pollinators. Although pollenkitt is lacking in Gnetales (Hesse, 1984), insect pollination has been demonstrated in *Welwitschia*, where flies, bees and wasps were observed carrying its pollen (Wetschnig & Depisch, 1999). Also in *Gnetum*, there are reports of unspecialized insect pollination; studies of *G. gnemon* and *Gnetum cuspidatum* Blume show that nectar-seeking moths and small flies visit the cones and pollen was attached to their mouth parts and legs (Kato, Inoue & Nagamitsu, 1995). In *Gnetum* and *Welwitschia*, pollination drops are produced by both male and female cones (Endress, 1996); the male cones are morphologically bisexual (but functionally unisexual). The absence of showy

structures seems to be compensated for by strong fragrance (Kato *et al*., 1995).

The pollination biology in *Ephedra* is poorly understood (Endress, 1996). It seems likely that insect pollination occurs, at least in *E. foeminea* (Porsch, 1910; Meeuse *et al*., 1990), which has morphologically bisexual male cones, and perhaps also in *E. aphylla* (Meeuse *et al*., 1990). The reported sugar content in the pollination drops (15%; Meeuse *et al*., 1990), is > 10 times the amount in pines (1.25%; Gifford & Foster, 1989: 470). In remaining *Ephedra*, pollination is probably largely abiotic. For *E. aphylla*, a mix of insect pollination and gravitational pollination was suggested (i.e. male plants growing among females and overtopping the females, thus showering female cones with pollen as a consequence of movement caused by visiting insects and wind; Meeuse *et al*., 1990). Wind tunnel experiments (in *E. nevadensis* and *E. trifurca*) have shown that selective pollen capture might be possible under certain conditions (Niklas & Buchmann, 1987), indicating that wind pollination can also be efficient for sympatric species.

Systematic implications and synapomorphic trends

Deep divergences in Ephedra and evolutionary origin of traits

The deepest divergences in *Ephedra* are somewhat uncertain. Generally, Bayesian analyses indicate that a Mediterranean species complex constitutes a 'basal grade' of species, successive sisters to core *Ephedra*, with *E. foeminea* sister to all other species of *Ephedra* (Fig. 5) (Rydin & Korall, 2009). However, parsimony analyses and Bayesian analyses with a more restricted sampling resolve the Mediterranean species complex as a clade (sister to all other species) (Rydin *et al*., 2004; Ickert-Bond, Rydin & Renner, 2009; Rydin & Korall, 2009). These problems and the repeated parallelisms (Ickert-Bond & Wojciechowski, 2004) and the substantial intraspecific variation (Huang *et al*., 2005; Rydin & Korall, 2009) in characters such as growth habit, leaf morphology and seed dispersal mechanisms have hampered evolutionary interpretations in *Ephedra*; however, interpreting our results it is nevertheless possible to distinguish evolutionary patterns. The morphological, anatomical and histological features discussed here are usually variable in the genus but constant within subclades (Fig. 5). The variation patterns in vascularization of the seed envelope are, for example, fully consistent with phylogenetic results based on DNA data (Fig. 5b). Three vascular bundles are ancestral in the extant clade. This condition is present in the Mediterranean species, North American species and a few South American species. Two vascular bundles are also common and occur in a few North American species, most South American species and the mainly Asian clade.

The number of ovules in a cone and whorled vs. decussate arrangement of the cone bracts are correlated features and can be quite variable, also within species. Nevertheless, it is evident that the number of ovules in the cone is a phylogenetically informative feature. Two ovules are the most common state in some clades of *Ephedra,* whereas one ovule is the prevailing state in other clades (see Fig. 5a and below). As with whorled phyllotaxis, three ovules in a cone have been reported to occur (rarely) in most major clades (Freitag & Maier-Stolte, 1992, 1996; Stevenson, 1993; Hunziker & Novara, 1998; Fu, Yu & Riedl, 1999), but this is usually not a constant character of a species or clade. Here we found only two species with three ovules in the cone: the Asian species *E. transitoria* and the South American *E. multiflora* (Fig. 5a).

Based on current estimates of phylogeny and comparative morphology, it appears likely that an opposite bract arrangement and two ovules in the cone (axillary positioned in the distal-most pair of bracts, Fig. 1) are the ancestral condition in the extant clade. Uniovulate cones apparently evolved several times in *Ephedra* (see Fig. 5). In the present study, we never found more than one ovule within a seed envelope, but Thoday & Berridge (1912) reported that, in *E. altissima*, two ovules may be found within what appears to be a single seed-envelope. The ovules were in all stages of development from fully developed to aborted at an early stage (Thoday & Berridge, 1912). They thus argued that the single, seemingly terminal ovule in the cone of *E. altissima* represents fusion of two ovules (and seed envelopes) over the shoot apex. In *E. foliata*, one of the ovules (and its seed envelope) is occasionally aborted and the remaining ovule occupies a 'false' terminal position (Maheshwari, 1935). Similar observations have been made on triovulate cones of *E. intermedia,* with ovule(s) aborted at different stages of development (Yang, 2004; 2007). An origin of uniovulate cones from abortion of the remaining ovule(s) appears to be the most common scenario. The fusion of ovules described for *E. altissima* is probably an exception. These findings are in accordance with interpretations of the male cones of *Ephedra* and *Welwitschia* (Mundry & Stützel, 2004); developmental studies indicate that each half of the antherophores of *E. distachya* (and *Welwitschia*) represents a reduced axillary cone (Mundry & Stützel, 2004).

Several other features show substantial plasticity; for example, the number of cone bracts in a cone and their degree of fusion. Thoday & Berridge (1912) suggested that *E. alata* and *E. torreyana*, with many free cone bracts, represent the ancestral state in *Ephedra*, but we find no support for this hypothesis.

Fleshy bracts, fused for most of their length, are ancestral in crown group *Ephedra* and are found, for example, in *E. foeminea, E. altissima and E. foliata*. Dry cone bracts, which are free from each other (an adaptation for dispersal by wind or seed-catchers), have evolved independently in most major subclades (i.e. the Asian clade, New World clade and the Mediterranean species complex, Rydin & Korall, 2009) (see also, for example, Ickert-Bond & Wojciechowski, 2004; Hollander *et al*., 2010).

The Mediterranean species complex

Ephedra foeminea, sister to remaining *Ephedra* (Fig. 5) (Rydin & Korall, 2009), differs from the other species in the Mediterranean species complex in three notable ways: the cone bracts do not contain any sclerenchymatous tissue, there are usually two ovules in the cones and the seed envelopes are triangular in transverse section, at mid-length and in the papillate (distal) zone. These characters are also present in *E. foliata,* probably also in *E. milleri*, and in some species in core *Ephedra*, and are probably plesiomorphic in the genus.

Ephedra alata is clearly morphologically distinct from the remaining species in the Mediterranean species complex with its many, free, dry, papery cone bracts with wide hyaline margins. In most other respects, *E. alata* does not differ morphologically, histologically and anatomically from *E. foeminea*, i.e. there are generally two ovules in each cone, the seed envelope is triangular in transverse section at mid-length and in the papillate zone and the mesophyll consists of rounded parenchymatous cells and an adaxial zone of fibres. *Ephedra alata* has, however, a distinct innermost layer of fibres in the mesophyll of the cone bracts, which is lacking in *E. foeminea*.

Ephedra aphylla, *E. altissima, E. fragilis* and *E. major* ssp. *major* constitute a poorly supported clade in previous phylogenetic studies (Rydin & Korall, 2009) (collapsed in the present study, Fig. 5), and the clade is well defined on the basis of morphology, anatomy and histology. Species in the clade have more or less strictly one-seeded cones, rounded seed envelopes at mid-length and scattered fibres in the mesophyll of the seed envelope and the cone bracts. The seed envelopes are slightly angled in the apical (papillate) zone and the three or four angles reflect the number of vascular bundles. The papilla cells of the seed envelope are usually strongly lignified.

Species determination and delimitation within this clade are clearly problematic; *E. altissima*, *E. fragilis* and *E. major* ssp. *major* are often misidentified for each other and confusions with *E. aphylla* also seem to occur frequently (see also Freitag & Maier-Stolte, 1994). DNA data have not been able to clearly separate the individual species in the clade (Rydin & Korall, 2009). Based on anatomy and histology, *E. aphylla* appears more or less distinct (recognizable) and can be separated from the complex of *E. altissima, E. fragilis* and *E. major* ssp. *major* based on the histology of the seed envelope and the cone bract, but we find no apparent and consistent distinctions between investigated specimens of *E. altissima*, *E. fragilis* and *E. major* ssp. *major*.

Ephedra milleri is sister to core *Ephedra* (i.e. *E. foliata* + the New World-Asia clade) in Bayesian analyses, but part of a Mediterranean clade in parsimony analyses (Rydin & Korall, 2009). There are few accessions of *E. milleri* and it appears as if female reproductive structures currently are available only on the holotype. We have therefore only been able to investigate the gross morphology of *E. milleri*. It appears to have most features in common with *E. foeminea* and *E. foliata*, i.e. shared features are probably plesiomorphic.

Core Ephedra

Core *Ephedra* was defined as all species other than those comprising a Mediterranean grade or clade in Rydin & Korall (2009). This clade is relatively well supported (and resolved), but we have not been able to find any clear morphological synapomorphy for the entire clade.

Clade A: the Ephedra foliata–E. ciliata–E. laristanica clade: Ephedra foliata is sister to the remaining species in core *Ephedra* (Rydin & Korall, 2009). Based on DNA data, *E. laristanica* is nested within *E. foliata* and *E. ciliata* is sister to the *E. foliata–E. laristanica* clade (Rydin & Korall, 2009) (Fig. 5). No clear boundaries can be found between *E. foliata* and *E. ciliata* based on reproductive anatomy and histology. We have investigated specimens from Morocco, Somalia, Egypt, Iran and Russia and, in contrast to the substantial gross morphological variation, the anatomy and histology of the female cones appear largely constant in the clade. Fertile female structures of *E. laristanica* were not available to us, but the results from molecular data are well supported and based on several specimens. *Ephedra foliata, E. laristanica* and *E. ciliata* are probably best treated as synonyms of a single grossly morphologically variable (but anatomically and histologically constant) species.

Many features of *E. foliata* are most probably plesiomorphic. The triangular seed envelope with three vascular bundles and the two distinct histological layers in the mesophyll are also frequently present in *E. foeminea* and many other species.

Clade B: New World + Asia: Clade B is characterized by a reduction to two vascular bundles in the seed envelope (but with several independent reversals

back to three and four vascular bundles). Further, most species (but see below on clade H and clade K) have two distinct zones in the cone bracts, an abaxial zone of parenchymatous tissue and an adaxial zone of fibres.

Clade C: the New World clade: The monophyly of New World *Ephedra* is well supported by molecular data, but we find no obvious morphological/anatomical/histological synapomorphies for the clade.

Clade E: the North American clade: Many features are variable within this clade. *Ephedra compacta* and *E. viridis* (successive sisters to the remaining species in the clade) have two ovules in the cone. All others have one ovule in each cone, except for a reversal to two ovules in the sister taxa *E. antisyphilitica* and *E. coryi*. Cone bracts may be in pairs or whorls of three. The number of vascular bundles in the seed envelope is also variable within the clade; there are ancestrally two (*E. compacta*), three in most species and four in *E. californica* and *E. trifurca*. The parenchymatous cells of the seed envelope often have undulate walls, as in the Asian clade, but the significance of this character is uncertain.

Clade F: the South American clade: Most South American species have two ovules in each cone and cone bracts in pairs. However, *E. multiflora* has three ovules in the cone and bracts in whorls of three. Most species have two vascular bundles in the seed envelope, but two species (*E. breana* and *E. americana*) have three. Species delimitation in this clade have partly been under debate; *E. andina* is sometimes treated as a synonym of *E. americana* (Stapf, 1889; Macbride, 1936) or of *E. chilensis* (Marticorena & Rodriguez, 1995). Our data might tentatively support the latter, whereas we find a clear distinction between *E. americana* and *E. andina*, most obviously the number of vascular bundles in the seed envelope, with three in the former and two in the latter.

Clade G: the mainly Asian clade

The mainly Asian clade is clearly defined by the anatomy of the seed envelope; there are always two vascular bundles. Consequently, the seed envelope is, with few exceptions, ovate ('2-angled') in transverse section in the papillate zone (because of the strong correlation between anatomy and apical transverse shape).

Clade H: Ephedra minuta and *E. likiangensis* constitute the sister clade of all remaining species in the mainly Asian clade. There are one or two ovules in the cone and the seed envelope is half-circle shaped at mid-length if there are two ovules, more rounded when there is one ovule. Parenchymatous cells of the seed envelope are rounded with intercellular spaces and the cone bracts lack sclerenchymatous tissue. As in all other species in the Asian clade, the two vascular bundles in the seed envelope are in the lateral position, but in *E. minuta*, there is a third supportive strand in the papillate zone, which disappears further down (without fusing with another bundle). It is in the abaxial (dorsal) position, exactly matching the position of the third vascular bundle in species with three vascular bundles. The same feature is also found in the sister species of the remaining species in the New World clade, *E. pedunculata*.

Clade J, remaining Asia: All species in clade J appear to have parenchymatous cells with distinctively undulate walls in the mesophyll of the seed envelope and sometimes also in the epidermis, but the significance of the feature is uncertain.

Clade K: This clade comprises the species, *E. pachyclada*/*somalensis, E. major* ssp. *procera, E. saxatilis, E. gerardiana, E. rhytidosperma, E. monosperma* and *E. equisetina*. We have investigated all except *E. rhytidosperma*. These species typically have one-seeded cones. In all species but *E. pachyclada*/*somalensis* (sister of the remaining species), the seed envelope is largely squared in transverse section at mid-length of the ovule, attributable to dorsal and ventral 'angles', not associated with vascular bundles (Fig. 2I). The cells of the inner epidermis are often much larger than those of the outer epidermis, a feature not seen in other clades, and the papilla cells are lignified in most species. The histology of the cone bracts is slightly variable in the clade, also within species. Most species have an adaxial zone of fibres in the mesophyll, but scares fibres may also be scattered in an innermost layer of the mesophyll in some specimens (*E. major* ssp. *procera, E. equisetina*) or missing (*E. saxatilis*). However, fibres are never scattered throughout the mesophyll of the cone bracts and seed envelope, as may be the case in the Mediterranean species complex.

Analyses of molecular data (Rydin & Korall, 2009) failed to support the distinction between *E. somalensis*, described as an endemic plant from the Horn of Africa (Freitag & Maier-Stolte, 2003) and *E. pachyclada,* distributed from the Near East to Pakistan. Anatomical and histological data show the same pattern; differences found (e.g. absence to rare presence vs. presence of tannins in the inner epidermis of the cone bracts and the mesophyll of the seed envelope) are indistinct and probably reflect the early developmental stage of the *E. somalensis* type material used here. An affinity between *E. somalensis* and *E. pachyclada* was also considered by Freitag &

Maier-Stolte (2003) and, from the results found here and in Rydin & Korall (2009), *E. somalensis* is probably best treated as a westernmost outpost of *E. pachyclada*, dispersed into the Horn of Africa.

The distinction between the closely related species *E. major* ssp. *procera*, *E. equisetina*, *E. gerardiana* and *E. saxatilis* is well supported by molecular data in Rydin & Korall (2009), although within-species variation of gross morphological features is often more prominent than among-species variation. Some of the names have been considered as synonyms (Freitag & Maier-Stolte, 1994). Based on histology, *E. saxatilis* differs from *E. equisetina, E. major* ssp. *procera* and *E. gerardiana* by the lack of fibres in the mesophyll of the cone bract. *Ephedra equisetina* and *E. major* ssp. *procera* are distinct from *E. gerardiana* in that they have a papillate surface of the seed envelope, which is visible from scanning microscopy but not obvious in transverse sections. *Ephedra equisetina* can possibly be separated from *E. major* ssp. *procera* (and *E. gerardiana*) by the lack of tannins in the outer epidermis of the cone bracts of *E. equisetina*, but the distinctiveness of the feature is uncertain.

Clade L: The species of clade L typically have two-seeded cones and seed envelopes that are triangular at mid-length. Further, all parenchymatous cells in the seed envelope are distinctly tanniferous and fibres are always present as an innermost zone of the mesophyll of the cone bracts. Clade L comprises two subclades, clade M (*E. strobilacea, E. sarcocarpa, E. transitoria* and *E. distachya*) and clade N (*E. sinica, E. intermedia, E. fedtschenkoi, E. regeliana, E. przewalskii* and *E. lomatolepis*).

In clade N the cone bracts are often contiguous with the lateral sides of the ovules and each pair of cone bracts gives a distinctive 'rectangular' appearance in transverse section. The spirally twisted micropylar tube is a prominent feature in *E. intermedia*, but is also present in *E. lomatolepis* and *E. sinica*. *Ephedra przewalskii* and *E. lomatolepis* are sister taxa; they are sympatric and similar (Freitag & Maier-Stolte, 1994), but differ by the completely dry cone bracts of *E. przewalskii* vs. fleshy bracts with dry wings in *E. lomatolepis*. Further, *E. przewalskii* seems to be the only species in *Ephedra* which lacks sclerenchymatous tissue in the seed envelope (except in association with the vascular bundles). The closely related *E. intermedia* (Fig. 5) is more similar to *E. lomatolepis* in anatomical and histological features, but they differ in degree of cone bract fusion (partly fused in *E. intermedia*, largely free in *E. lomatolepis*) and in the seeds being largely enclosed by fleshy cone bracts in *E. intermedia*, but exserted in *E. lomatolepis*.

Clade M (*E. strobilacea, E. sarcocarpa, E. transitoria* and *E. distachya*) is well supported by molecular data, but it is difficult to find uniquely derived features for this clade. Many anatomical and histological features are constant in the clade and often also shared with the species in clade N.

Nomenclatural comments on Ephedra major Host

Previous studies based on molecular data (Rydin *et al*., 2004; Rydin & Korall, 2009) have indicated that specimens assigned to *E. major* fall into two distantly related clades, one in the Mediterranean species complex (here referred to as *E. major* ssp. *major*) and one in the Asian clade K (here called *E. major* ssp. *procera*). These results are also supported by the present study; for example, by the anatomy of the seed envelope (three vs. two vascular bundles) and the histology of cone bracts and seed envelopes (scattered fibres vs. parenchymatous and sclerenchymatous zones). The distribution of *E. major* ssp. *procera* is wide in Asia, Europe and Africa and it appears to be sympatric with *E. major* ssp. *major* in western Africa and western Europe.

The (neo)type material of *E. major* (Riedl, 1993) has unfortunately not been possible to trace, but from a photograph in the publication and investigations of other material from Dalmatia, Croatia (the region of the type locality), it is most probable that the type belongs to the Asian *E. major* ssp. *procera*, whereas the Mediterranean *E. major* ssp. *major* would need a new name. It should be noted, however, that neither of these species is clearly distinct from their respective close relatives; *E. major* ssp. *major* cannot be clearly distinguished from *E. fragilis* and *E. altissima* and it is uncertain if *E. major* ssp. *procera* can be distinguished from *E. equisetina*.

Comparison with the fossil record

Knowledge of the fossil record of Gnetales and extinct relatives has expanded rapidly in recent years and the results presented here have implications for the systematic interpretation of fossils. One of the most striking features of Gnetales is the extended micropylar tube, present in all extant species and beautifully preserved in many Early Cretaceous fossils. Its importance for pollination probably explains the remarkable consistency of traits involved in assuring an upright position of the micropylar tube. For example, the thickening (lignification) of the inner epidermis of the micropylar tube seems to occur widely in the Bennettitales–Erdtmanithecales–Gnetales (BEG) clade *sensu* Friis *et al*. (2007). It is not only present in all extant species of *Ephedra*, in *Gnetum gnemon* and *Welwitschia* (C. Rydin, pers. observ.), but probably also in fossil *Ephedra* (Rydin *et al*., 2006a), in Bennettitales (similar to *Gnetum* as suggested by Crane & Herendeen, 2009: figs 13, 14,

19), in unassigned Early Cretaceous seeds (also similar to *Gnetum,* as suggested, for example, by Friis, Pedersen & Crane, 2009: figs 16, 75) and in an Early Cretaceous compression fossil with uncertain systematic position in Gnetales (Rydin & Friis, 2010).

Several fossils from the Early Cretaceous are probably most closely related to *Ephedra*; for example, compression/impression fossils such as the *Liaoxia* Cao & S.Q.Wu (emend. Rydin, S.Q.Wu & Friis) specimens (Rydin *et al*., 2006b), *Ephedra archaeorhytidosperma* Y.Yang, B.Y.Geng, D.L.Dilcher, Z.D.Chen & T.A.Lott (Yang *et al*., 2005) and coalified mesofossils such as *Ephedra portugallica* Rydin, Pedersen, Crane & Friis and *Ephedra drewriensis* Rydin, Pedersen, Crane & Friis (Rydin *et al*., 2006a). Some Early Cretaceous fossils were clearly anemochorous (e.g. *Liaoxia chenii* Cao & S.Q.Wu (emend. Rydin, S.Q.Wu & Friis)), but at least one species may have had fleshy cone bracts (i.e. *Liaoxia robusta* Rydin, S.Q.Wu & Friis) (Rydin *et al*., 2006b). As in extant *Ephedra*, there were two vascular bundles in each cone bract in most (but not all) species (Rydin *et al*., 2006b). The degree of fusion of cone bracts is difficult to assess in the fossils, but they appear largely free from each other in most species of *Liaoxia*. Ovules are always positioned in the axil of a bract, as in extant species, but some of the extinct ephedroids also had (such as *Welwitschia* and most conifers) ovules in proximal parts of the cone (Rydin *et al*., 2006b).

In spite of an indisputable overall similarity between these fossils and extant *Ephedra*, it is in most cases not possible to assess their phylogenetic position with certainty. A few morphological features may be useful, for example the number of ovules in the cone, but morphology is generally too variable (even within individuals) and too homoplastic to be evolutionarily informative. However, two species from the Early Cretaceous, *E. portugallica* and *E. drewriensis* (Rydin *et al*., 2006a), can be unambiguously assigned to the *Ephedra* clade based on the presence of diagnostic features: *Ephedra* pollen and papilla cells on the seed envelope. But are these fossils nested among living species in the extant clade or do they represent extinct sister lineage(s) to extant *Ephedra*?

The vascularization of the seed envelope is clearly informative in inferring phylogenetic relationships within *Ephedra* and could thus indicate the position of the fossils. The anatomy of the seed envelope has so far never been clearly documented for any *Ephedra* fossil, but the strong correlation in extant species between the apical shape of the seed envelope in transverse section and the anatomy of the seed envelope tentatively indicates that at least the Early Cretaceous *E. portugallica*, and perhaps also *E. drewriensis*, had four vascular bundles in the seed envelopes (which are distinctly squared in the papillate zone). The finding of a xylem strand in the probable median plane of one specimen of *E. drewriensis* (Fig. 3C, H; Rydin *et al*., 2006a) lends weak support for the hypothesis.

The feature (four vascular bundles in the seed envelope) has evolved independently twice in the extant clade, in *E. fragilis–E. altissima* of the Mediterranean species complex and in *E. californica–E. trifurca* in the North American clade, but to assign the fossils to either of these clades is problematic. The fossils seems to share a strong lignification of the micropylar tube and the papilla cells of the seed envelope with the Mediterranean species, but the variable anatomy and the histology (scattered fibres in an otherwise parenchymatous mesophyll) of the Mediterranean species do not fit with observations of the fossils. From anatomy and histology, a relationship of the fossils with the North American species would be more probable, but *E. californica* and *E. trifurca* do not have the strong lignification of the micropylar tube and papilla cells.

Thus, there is no unambiguous anatomical and histological correlation between the fossils and either of these two extant clades. Additional studies of the fossils may be worthwhile, but the most straightforward interpretation based on currently available data is that the fossils constitute extinct sister lineages to the extant clade and that there has been a general pattern of reduction of the number of vascular bundles in the ephedran seed envelope from probably four in the fossils (reflecting an evolutionary origin of the seed envelope from a pair of bracts), to ancestrally three in the living clade, and later to two.

Concluding remarks and the bottleneck effect in ephedran evolution

A long and diverse evolutionary history of Gnetales was inferred based on the pronounced differences between the extant genera in morphology, ecology and distribution patterns long before gnetalean plants were well documented in the fossil record (Arber & Parkin, 1908). Later, pollen studies supported this interpretation; ephedroid pollen rapidly increase in density in Cretaceous strata, reaching a peak around 100 Mya (Crane & Lidgard, 1989). Now, the fossil evidence of a large Cretaceous diversity of gnetalean plants is overwhelming (see Taylor, Taylor & Krings, 2009 for a summary) and include members of extant Gnetales (e.g. Rydin *et al*., 2003) and extinct groups, the relationships of which to living plants are uncertain (e.g. Friis *et al*., 2007).

A few ephedroid fossils could potentially have been nested among extant species in crown group *Ephedra* because they share uniquely derived morphological features with the extant clade (Rydin *et al*., 2004, 2006a). However, the morphological and anatomical data presented here indicate that the fossils are extinct sister lineages to the extant clade, not nested within it. This finding is in accordance with recent results from other data; for example, the *Ephedra* topology (Rydin & Korall, 2009) (Fig. 5) and molecular dating analyses (Ickert-Bond *et al*., 2009), which suggest that current diversity is the result of a radiation in the Palaeogene, i.e. after the Cretaceous–Tertiary (K–T) boundary and after the final separation of the Gondwana continent in the Albian–Cenomanian. Migration and long-distance dispersal, not vicariance, are responsible for current distribution patterns and phylogenetic relationships, which are congruent with a Beringian disjunction, an origin in Eurasia with subsequent dispersal into North America and later to South America (Ickert-Bond *et al*., 2009), not, for example, with a more ancient Gondwana distribution. Extant speciation in the genus is estimated to have occurred during the last 30 Myr, in the Asian and New World clades during the last 5–10 Myr (Ickert-Bond *et al*., 2009) and the repeated parallelisms and intraspecific variation patterns appear to reflect recent and perhaps parallel adaptations at the population level to local ecological and climatic conditions.

In spite of a striking similarity between fossil and living *Ephedra,* in distribution patterns, in reproductive and vegetative morphology, in histological details and pollination mechanisms, including the indisputable presence of shared unique features (Rydin *et al*., 2006a), the fossils appear to be the remains of an earlier radiation in the Early Cretaceous. This diversity must have gone almost entirely extinct towards the end of the Cretaceous, causing a bottleneck effect in *Ephedra*, which is still reflected today by an extraordinarily low amount of genetic and structural diversity.

ACKNOWLEDGEMENTS

We thank Helmut Freitag (University of Kassel), Guy-Georges Guittonneau, Mats Thulin (Uppsala University), Shuangquan Huang (Wuhan University) Peter Litfors (Stockholm University), the Royal Botanic Gardens, Kew, the Botanical Museum and Botanical Garden, Berlin-Dahlem and the curators of the herbaria E, K, KAS, MO, N, O, P, S, UC, UPS, Z, WH, for access to plant material, and Merran Matthews, Julien Bachelier and Yannick Staedler (University of Zurich) for many fruitful discussions and help with laboratory techniques.

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APPENDIX

MATERIAL AND INVESTIGATIONS

	Taxa	Voucher	Collection information	Reproduct. anatomy/ histology	General morphology	nrITS
128	*E. alata* Decne.	Anderberg 481 (S)	Algeria 1980	*	*	–
136	*E. alata* Decne.	Guittonneau 136 (Z)	Tunisia 2008	*	*	–
147	*E. alata* Decne.	Cosson *s.n.* C-311 (S)	Algeria 1858	*	*	–
121	*E. altissima* Desf.	Jordan 23 (Z)	Algeria 1851	*	*	–
081	*E. altissima* Desf.	Samuelsson 6227 C-628 (S)	Algeria 1936	*	*	AY755772§
082	*E. altissima* Desf.	Botan SU 18 C-7688 (S)	Tunisia 1972	*	*	AY755773§
132	*E. altissima* Desf. var. *mauritanica*	Freitag 35.001 (KAS)	Morocco 2008	*	*	–
220	*E. americana* Humb. & Bonpl. ex Willd.	Novara 10622 (S)	Argentina 1994	*	*	GU968567*
213	*E. americana* Humb. & Bonpl. ex Willd.	Hieronymus 1308 (Z)	Argentina 1878	*	*	–
214	*E. americana* Humb. & Bonpl. ex Willd.	Britton & Rusby 9 (Z)	Bolivia 1890	*	*	–
127	*E. americana* Humb. & Bonpl. ex Willd.	Novara 8219 (S)	Argentina 1988	*	*	GU968545*
215	*E. andina* Poepp. ex C.A.Mey.	Geisse *s.n.* (Z)	Chile 1912	*	*	–
180	*E. andina* Poepp. ex C.A.Mey.	19169.000 (K)	Cult.	*	*	–
123	*E. andina* Poepp. ex C.A.Mey.	Gay 400 (Z)	Chile 1933	*	*	GU968543*
025	*E. andina* Poepp. ex C.A.Mey.	Chase 10140, 1967-25610 (K)	Cult.	*	*	AY755744‡
222	*E. antisyphilitica* Berlandier ex C.A.Mey.	Schaffner 279 (S)	Mexico 1879	*	*	–
124	*E. aphylla* Forssk.	Kramer 4727 (Z)	Palestine 1981	*	*	GU968544*
154	*E. aphylla* Forssk.	Amdursky 402 (S)	Israel 1950	*	*	GU968552*
228	*E. aphylla* Forssk.	Bot. Dep. C-7689 (S)	Tunisia 1972	*	*	GU968569*
080	*E. aphylla* Forssk.	Anderberg 853 (S)	Libya 1982	*	*	AY755771§

APPENDIX *Continued*

	Taxa	Voucher	Collection information	Reproduct. anatomy/ histology	General morphology	nrITS
219	*E. breana* Phil.	Hunziker 1912 (S)	Argentina 1947	*	*	–
161	*E. californica* S.Watson	Tidestrom 9692 (S)	California 1919	*	*	–
212	*E. californica* S.Watson	Abrams 3489 (Z)	California 1903	*	*	–
060	*E. chilensis* C.Presl.	Odum 7780 E00130260 (E)	Chile 1980 (cult.)	–	*	AY755754§
075	*E. chilensis* C.Presl.	Forbes 49.0542 (UC)	Chile 1949 (cult.)	*	*	AY755767§
146	*E. ciliata* Fisch. & C.A.Mey.	Balls B2487 C-635 (S)	Morocco 1936	*	*	GU968548*
086	*E. ciliata* Fisch. & C.A.Mey.	Rechinger 16183 (S)	Afghanistan 1962	–	*	AY755776§
153	*E. ciliata* Fisch. & C.A.Mey.	Androssov 3367 (S)	Turkmenistan 1915	*	*	–
230	*E. ciliata* Fisch. & C.A.Mey.	06002312 (WH)	No information	*	–	–
155	*E. compacta* Rose	Purpus *s.n.* (S)	Mexico 1908	*	*	–
160	*E. coryi* E.L.Reed	Corell 32762 (S)	Texas 1966	*	*	–
099	*E. distachya* L.	Pidopliczka *s.n.* 88/58 (UPS)	Ukraine 1925	–	*	FJ958013¶
184	*E. distachya* L.	46126.000 (K)	Cult.	*	*	–
233	*E. distachya* L.	Kolakovsky 3651 (S)	Turkmenistan 1951	*	*	GU968571*
140	*E. distachya* L.	46126.000 (K)	Cult.	*	*	–
236	*E. distachya* L. ssp. *helvetica*	Endress *s.n.* (Z)	Switzerland 1969	*	–	–
216	*E. distachya* L.	Karo 336 (P)	South-eastern Russia 1889	*	*	–
227	*E. distachya* L.	Filarszky & Schilberszky 2288 II (S)	Hungary	*	*	GU968568*
125	*E. equisetina* Bunge	Sintenis 666 (S)	Turkmenistan 1900	*	*	–
142	*E. equisetina* Bunge	Freitag 05.2008 (KAS)	Russian Altai 1972 (cult.)	*	*	–
145	*E. equisetina* Bunge	Lipsky 3087 (S)	Russia 1912	*	*	–
071	*E. equisetina* Bunge	Merello *et al*. 2241 04903487 (MO)	Tbilisi, Georgia 1999 (cult.)	*	*	AY755763§
234	*E. equisetina* Bunge	Lipsky 2610 (S)	Turkmenistan 1912	*	*	GU968572*
226	*E. fasciculata* var. *clokeyi* (H.C.Cutler) Clokey	Clokey 8697 (S)	Nevada 1941	*	*	–
173	*E. fedtschenkoi* Paulsen	Zhu Taiyan 650764 (N)	Xinjiang, China 1965	*	*	–
130	*E. foeminea* Forssk.	Rydin 130 (Z)	Greece (cult.)	*	*	GU968546*
159	*E. foeminea* Forssk.	Freitag 19.807 (KAS)	Greece (cult.)	*	*	–
152	*E. foeminea* Forssk. (det. *E. campylopoda* C.A.Mey.)	Fries C-7619 (S)	Dalmatia 1938	*	*	GU968551*
084	*E. foliata* Boiss. & C.A.Mey.	Täckholm C-7816 (S)	Egypt 1929	*	*	–
085	*E. foliata* Boiss. & C.A.Mey.	Rechinger & Rechinger 3979 (S)	Iran 1948	*	*	AY755775§

APPENDIX *Continued*

	Taxa	Voucher	Collection information	Reproduct. anatomy/ histology	General morphology	nrITS
096	*E. foliata* Boiss. & C.A.Mey.	Thulin 10745 (UPS)	Somalia 2002	*	*	FJ958010¶
120	*E. fragilis* Desf.	Freitag 328-40 (Z)	Hispaniola 1964	*	*	–
101	*E. fragilis* Desf.	Jonsell 5412 V-54673 (UPS)	Morocco 1989	*	*	FJ958014¶
109	*E. fragilis* Desf.	Denk (179-01) (S)	Morocco 2005	*	*	FJ958019¶
129	*E. fragilis* Desf. *var. dissoluta*	Freitag 35.052 (KAS)	Morocco 2008	*	*	–
223	*E. frustillata* Miers	Dusén 5280 (S)	Argentina 1904	*	*	–
148	*E. gerardiana* Wall. ex Florin	Parker 2099 (S)	Almora, India 1923	*	*	–
188	*E. intermedia* Schrenk & C.A.Mey.	Bartholomew 8211 (MO)	Xinjiang, China 2001	*	*	GU968563*
006	*E. intermedia* Schrenk & C.A.Mey.	Rydin 03-925 (S)	Tien-Shan mount. 1971 (cult.)	*	*	AY755741‡
158	*E. likiangensis* Florin	18480.000 (K)	Cult.	*	*	–
157	*E. likiangensis* Florin	1988-844 (K)	Cult.	*	*	–
133	*E. likiangensis* Florin	Rydin 133 (Z)	Cult.	*	*	GU968547*
002	*E. likiangensis* Florin	Rydin 03-926 (S)	Cult.	*	*	AY755739‡
191	*E. lomatolepis* Schrenk	Krasnoborov 192 (MO)	Novosibirsk 1995	*	*	GU968564*
174	*E. lomatolepis* Schrenk	Bosshard *et al*. 803.24 (Z)	Pakistan 1989	*	*	GU968562*
092	*E. lomatolepis* Schrenk	Baitulin *et al*. *s.n.* (UPS)	Kazakhstan 1997	*	*	FJ958006¶
102	*E. lomatolepis* Schrenk	Titow 488 (S)	Soviet 1914	*	*	FJ958015¶
166	*E. major* Host subsp. *major*	Hofmann 013-1971 (Z)	Algeria 1971	*	*	GU968557*
167	*E. major* Host subsp. *major*	Juillet 94 (Z)	Algeria 1896	*	*	GU968558*
162	*E. major* Host subsp. *major*	Ipse 71/677E (Z)	Spain 1971	*	*	GU968553*
88	*E. major* Host subsp. *major*	Debreczy *et al*. 46206 (S)	Spain 1995	–	*	AY755778§
98	*E. major* Host subsp. *major*	Julin *s.n.* (UPS)	Spain 1979	–	*	FJ958012¶
–	*E. major* Host subsp. *major*	Reverchon 94 (Z)	Algeria 1897	–	*	–
–	*E. major* Host subsp. *major*	Reverchon 94 (S)	Algeria 1896	–	*	–
–	*E. major* Host subsp. *major*	Reverchon C-7717 (S)	Algeria 1897	–	*	–
–	*E. major* Host subsp. *major*	Juillet 94 (Z)	Algeria 1897	–	*	–
–	*E. major* Host subsp. *major*	Thellung *s.n.* (Z)	Algeria 1912	–	*	–
169	*E. major* Host subsp. *procera*	Zogg & Gassner 8388 (Z)	France 1984	*	*	GU968559*
05	*E. major* Host subsp. *procera*	Rydin 03-929 (S)	Balkan 1955 (cult.)	–	*	FJ958003¶
97	*E. major* Host subsp. *procera*	Behr *s.n.* (UPS)	Macedonia 1936	–	*	FJ958011¶

APPENDIX *Continued*

	Taxa	Voucher	Collection information	Reproduct. anatomy/ histology	General morphology	nrITS
103	*E. major* Host subsp. *procera*	Uggla C-242 (S)	Algeria 1936	–	*	FJ958016¶
–	*E. major* Host subsp. *procera*	Schlyter C-246 (S)	Dalmatia, Croatia 1880	–	*	–
–	*E. major* Host subsp. *procera*	Rechinger 806 (S)	Iran 1937	–	*	–
–	*E. major* Host subsp. *procera*	Heyde C-269 (S)	Himalaya 1870	–	*	–
–	*E. major* Host subsp. *procera*	Maire C-266 (S)	Algeria 1924	–	*	–
–	*E. major* Host subsp. *procera*	Cosson C-240 (S)	Algeria 1853	–	*	–
193	*E. major* Host subsp. *procera* (det. *E. botschantzevii* Pachom.)	Kurbanov 630 (MO)	Turkmenistan 2001	*	*	GU968565*
163	*E. major* Host subsp. *procera*	Montserrat 319171 (Z)	Spain 1971	*	*	GU968554*
165	*E. major* Host subsp. *procera*	Baenitz *s.n.* (Z)	Herzegovina 1898	*	*	GU968556*
–	*E. major* Host subsp. *procera*	Orphanides 267 (Z)	Greece 1851	–	*	–
–	*E. major* Host subsp. *procera*	Martelli *s.n.* (Z)	Sardinia 1894	–	*	–
–	*E. major* Host subsp. *procera*	Hochreutiner 671 (Z)	Algeria 1901	–	*	–
–	*E. major* Host subsp. *procera*	Jahandiez 838 (Z)	Morocco 1924	–	*	–
–	*E. major* Host subsp. *procera*	Markgraf 11032 (Z)	Turkey 1958	–	*	–
–	*E. major* Host subsp. *procera*	Vicioso 1153 (Z)	Spain 1910	–	*	–
164	*E. major* Host subsp. *procera*	Grossheim *s.n.* (Z)	Transcaucasia 1924	*	*	GU968555*
–	*E. major* Host subsp. *procera*	Merello *et al*. 2459 (MO)	Georgia 1999	–	*	–
–	*E. major* Host subsp. *procera*	Stevens *et al*. 314 (MO)	Georgia 2003	–	*	–
206	*E. major* Host subsp. *procera* (det. *E. scoparia* Lange)	Quer & Gros 103 (Z)	Spain 1924	*	*	–
–	*E. milleri* Freitag & M.Maier-Stolte	Miller 7667 B (E)	Oman 1985	–	†Photo	–
007	*E. minuta* Florin	Rydin 03-930 (S)	Sikang, China 1934 (cult.)	*	*	AY755742‡
063	*E. minuta* Florin	Rydin 04-486 (S)	cult.	*	*	AY755756§
168	*E. monosperma* J.G.Gmel. ex C.A.Mey.	Honegger 92/111 (Z)	Russia 1992	–	*	GU968560*
229F	*E. monosperma* J.G.Gmel. ex C.A.Mey.	Maltzev 06002315 (WH)	USSR 1907	*	*	GU968570*

APPENDIX *Continued*

	Taxa	Voucher	Collection information	Reproduct. anatomy/ histology	General morphology	nrITS
224	*E. multiflora* Phil. ex Stapf	Johnston 6142 (S)	Argentina 1926	*	*	–
225	*E. nevadensis* S.Watson	Epling & Robison C-222 (S)	California 1932	*	*	–
156	*E. pachyclada* Boiss.	Regel s.n. (S)	Turkestan 1884	*	*	–
171	*E. pedunculata* Engelm. ex S.Watson	Taylor Edwards 385 (S)	Mexico 1937	–	*	GU968561*
185	*E. pedunculata* Engelm. ex S.Watson	Parks R3196 (MO)	Texas 1942	*	*	–
190	*E. przewalskii* Stapf	Grant 15.309 (MO)	Baluchistan 1964	*	*	–
073	*E. rupestris* Benth.	Ornduff 9675 87.1368 (UC)	Ecuador 1987 (cult.)	*	*	AY755765§
143	*E. sarcocarpa* Aitch. & Hemsl.	Freitag 13.988 (KAS)	Iran (cult.)	*	*	–
144	*E. saxatilis* (Stapf) Royle ex Florin	Freitag 098-38-74-84 (KAS)	Nepal (cult.)	*	*	–
138	*E. saxatilis* (Stapf) Royle ex Florin	1947-2603 (K)	Cult.	*	*	–
151	*E. sinica* Stapf	Eriksson 05-9020 (S)	Inner Mongolia 1926	*	*	GU968550*
033	*E. sinica* Stapf	Rydin *s.n.* (S)	Hebei, China 2000	*	*	AY755749‡
194	*E. sinica* Stapf (var. *pumila*)	Totzouekokov *s.n.* (MO)	Kazakhstan 1955	*	*	GU968566*
172	*E. somalensis* Freitag & M.Maier-Stolte	Thulin 10986 (UPS)	Somalia 2002	*	*	–
231F	*E. strobilacea* Bunge	06002261 (WH)	No information	*	*	–
150	*E. strobilacea* Bunge	Androssov 1900 (S)	Turkmenistan 1905	*	*	GU968549*
126	*E. torreyana* S.Watson	Porter et Porter 8998 (S)	New Mexico 1962	*	*	–
111	*E. transitoria* Riedl	Collenette 9095 B (E)	Saudi Arabia 1994	*	*	FJ958021¶
170	*E. trifurca* Torr.	Stauffer 5921 (Z)	California 1964	*	*	–
076	*E. tweediana* Fisch. & C.A.Mey.	Forbes 66.0742 (UC)	Argentina (cult.)	*	*	AY755768§
091	*E. viridis* Coville	Holmgren *et al*. 1826 (UPS)	Utah 1965	*	*	FJ958005¶

*Data newly produced for the present study.
†The holotype; investigated from photo.
Previously published DNA data: ‡Rydin *et al*. (2002); §Rydin *et al*. (2004); ¶Rydin & Korall (2009).
Note: for voucher information of specimens, for which no new data has been produced in the present study (and remaining GenBank accessions), see Rydin & Korall (2009).
Cult., specimen cultivated after collection; nrITS, internal transcribed spacers of the nuclear ribosomal DNA.