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SEEDS AND EMBRYOS OF ARAUCARIA MIRABILIS[1]

RUTH A. STOCKEY
Department of Botany, The Ohio State University, Columbus 43210

ABSTRACT

Seeds and embryos contained within the silicified ovuliferous cone *Araucaria mirabilis* from the Cerro Cuadrado petrified forest are described. One wingless seed, 0.8–1.3 cm in length and 0.2–0.6 cm in width, is embedded in each ovuliferous scale. Seeds show a three-layered integument and a prominent wavy nucellus which is attached basally to the endotesta. The megagametophyte contains cellular inclusions that may represent starch grains. Ovule vascularization is complex and appears most similar to that of *Araucaria bidwillii*. Embryos 2 mm long and 0.25 mm wide appear to be in a telo-stage period of development. Shoot apex, cotyledons, root meristem, and calyptroperiblem are present in the embryos in which vascular tissues and secretory elements were beginning to differentiate at the time of fossilization. Embryo ontogeny is considered in light of stages encountered in extant gynmosperm taxa. The absence of the microgametophyte phase in the Cerro Cuadrado collection is discussed.

SILICIFIED CONIFEROUS remains from the Cerro Cuadrado petrified forest in Patagonia have been scattered to various universities and museums around the world. These fossil cones, seedlings, and wood from the volcanic peaks of Cerro Cuadrado, Cerro Alto, and Cerro Madre e Hija have been sold in Argentina as curios and kept in private collections because of their beauty and excellent preservation (Wieland, 1935). The Cerro Cuadrado petrified forest of southern Argentina was initially described by Windhausen (1924) who first collected silicified wood and cones in 1919 and 1922. The collection was passed on to Professor Gothan (1925, 1950) of Berlin who later described some of these specimens. At about the same time a second large collection was made by members of the Field Museum (Chicago) of Natural History's Patagonian expedition (Riggs, 1926). This collection of wood, seedlings, and cones was later reviewed by Wieland (1929, 1935) and incorporated in the Field Museum collections. A few years later Dr. Franz Mansfeld collected and purchased many specimens while on a fossil vertebrate expedition in 1936 (Gordon, 1936; Calder, 1953).

Calder (1953) has provided the most critical study to date of these fossils from the collections of the British Museum. She reviewed the literature on the Cerro Cuadrado petrified forest and presented revised diagnoses on twigs and branches (*Araucarites santaecrucis* [Speg.] Calder) and cones (*Pararaucaria patagonica* Wieland, *Araucaria mirabilis* [Speg.] Calder).

The silicified ovuliferous cone *Araucaria mirabilis* (Speg.) Calder was first described by Spegazzini (1924) as *Araucarites mirabilis*. Wieland's (1935) review established several species of cones belonging to the genus *Proaraucaria*. Calder (1953) undertook a more detailed study of the cones since Wieland's work was done mainly on the gross morphological level and failed to emphasize anatomical details. She emended the original diagnosis and also included *Proaraucaria mirabilis* (Speg.) Wieland, *P. elongata* Wieland, *P. patagonica* Wieland, *P. mirabilis* var. *minima* Wieland, and *P. mirabilis* var. *elongata* Wieland under the binomial *Araucaria mirabilis*, concluding there were no anatomical differences to separate these taxa from each other. Calder further notes that the generic name *Araucaria* should be used, with *Araucarites* reserved for cones or branches of the araucarian habit which lack preservation of structural details. Since *Araucaria mirabilis* is structurally preserved, the generic name *Araucaria* is used even though *Araucarites* has taxonomic priority. Calder has assigned *Araucaria mirabilis* to the section *Bunya* Wilde and Eames (1952) that contains the extant *Araucaria bidwillii* Hook., native to Queensland, Australia.

A short paper by Darrow (1936) on one cone (*P13854*, Field Museum Collection) illustrated the general features of the embryo of *Araucaria mirabilis*. Darrow principally used polished slabs for her investigation, with few thin sections; the cellular organization of the embryos was not dis-

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cussed. Calder (1953) also illustrated a few embryos in her study; however, little attention was paid to cellular preservation. Detailed structure of cones was not made available by extensive thin sectioning, probably because of difficulty in sectioning and grinding the silicified material. Moreover, an ontogenetic sequence of embryo development has never been attempted for the fossil cone, nor have tissues and meristems of the cellulary preserved embryos been illustrated. It is the intent of this paper to provide a more detailed analysis of the cones with particular emphasis directed at seeds and embryos.

MATERIALS AND METHODS—The present study utilizes 87 cone specimens assignable to the fossil taxon *Araucaria mirabilis* on the basis of external morphology. Fifty-three were selected for sectioning on examination of previous cuts made by Wieland (1935) and Darrow (1936). External features of uncut cones, as well as those showing already exposed embryos, were considered before mounting the specimens for sectioning.

The cones are completely silicified by α-quartz, as determined by x-ray diffraction. Darrow (1936) points out the tendency for different colors of quartz to replace different seed tissues. Embryos and seeds appear to be replaced by chalcedony, but the lumen once occupied by the nucellus is filled with transparent quartz. None of the Field Museum cones are embedded in volcanic ash, as occurs in some of the British Museum specimens (Calder, 1953, Pl. 3, Fig. 26).

Slides were made with 2×3 in. double weight glass. One side of the slide was frosted and TRA-Bond 2114 Water White Transparent Epoxy Adhesive (TRA-CON, Inc., Medford, Massachusetts) was used for mounting specimens to the slide. Sections were cut and ground on a Hillquist Thin Section Machine to a thickness of 20–30 micrometers. Since many tissues of the cones show little contrast, various slides were stained in a 5% aqueous Malachite Green solution (Bartholomew, Matten, and Wheeler, 1970) for one and one-half min. Details of pitting on the tracheids, cells of the megagametophyte, and some integumentary cells of the seeds showed a greater contrast with this technique. Bismark Brown stain was also tried on several sections without satisfactory results.

STRATIGRAPHY—Calder (1953) has pointed out that the geologic age of the petrified forest of Patagonia is still not accurately determined. Middle Triassic (Windhausen, 1924, 1931; Wieland, 1935; Gothan, 1925), Jurassic (Feruglio, 1949, 1951; Calder, 1953; Menendez, 1960; Archangelsky, 1970), Cretaceous (Mansfeld, in Gordon, 1936), and Eocene (Frenguelli, 1933; Darrow, 1936; Fossa-Mancini, 1941) ages have all been suggested. Since there is an unconformity which separates this particular series of volcanics from the sediments known to be Late Cretaceous in age, the forest is at least as old as Middle Cretaceous. Feruglio (1949, 1951) has extensively reviewed the problem of the geologic age of the petrified forest and considers the volcanics in this region to be Middle to Late Jurassic in age. His conclusions were based on correlations between fossil plant compressions (*Hausmannia* Dunker, *Cladophlebis* Brogn., and others) in nearby regions which he correlated with the Cerro Cuadrado remains. An additional bit of evidence for the Jurassic age of these plants is the presence of the branchiopod genus *Cyzicus* Audouin (= *Estheria*) in the series of volcanics. *Cyzicus draperi* (Jones) Audouin is generally accepted as being Late Triassic in age (Feruglio, 1949).

DESCRIPTION—*General features*—Cones are nearly spherical to ellipsoid in shape and range from 2.5–8 cm in length and 2.5–8 cm in diam (Fig. 1, 4, 8). Numerous spirally arranged cone scales surround a central axis. Each cone-scale complex is composed of an ovuliferous scale which overlies a woody winged bract (Fig. 1, 2). In some cones the woody bract apophysis, or outer swollen portion of the bract, is covered by a deciduous laminar tip, which has dropped off in most specimens (Fig. 8). Calder (1953, Pl. 2, Fig. 40) illustrates what she believes to be the abscission zone of the laminar tip. The presence of such a deciduous laminar tip occurs on extant araucarian cones, for example, in *A. angustifolia* (Bertol.) O. Ktze. (= *A. brasiliana* Rich.) and is known to fall off when cones are mature (Pilger, 1926). The ovuliferous scale is free from the bract for about half its length, the free extension of the scale having been referred to as the "ligule" (Fig. 1, 6). The space between the ovuliferous scale and the bract is called the ligular sulcus (Fig. 6). Cone scales range from 0.8–2.3 cm in length and 0.5–1.5 cm in width, including the wing-like extensions of the bract. The ligule is 0.5–0.9 cm long, 0.4–0.5 cm wide at its broadest point, and 0.1–0.2 cm high when exposed almost completely to view.

Some cones show evidence of the mode of burial by exhibiting what appear to be scorched areas, pitting of the bract apophyses and ovuliferous scales, and distortions due to crushing prior to silicification. One specimen (*P13896,* Fig. 4) shows the chalaza of several exposed seeds. Sections of this cone reveal that no tissues of the cone are structurally preserved except the woody bract and ligule tissues and some poorly preserved secondary xylem. Many cones which show pitting of their outer surfaces reveal little structural detail in the first few cuts; however, successive cuts indicate these to be some of the best preserved specimens with respect to seed and

embryo tissues (Fig. 18). Many cones are entire, but most are pieces ranging from ¼ to ¾ of a complete cone.

Pieces of cone peduncle range from 0.8–1.2 cm in diam and 1.1 cm in length in some specimens but do not reveal evidence of leaf scars externally. The pith of the axis is wide (0.4–0.6 cm) and contains numerous sclereids (Fig. 2). The vascular system consists of fused bundles that form a continuous ring in the cone peduncle. At higher levels this ring separates to form discrete bundles that surround the pith. These bundles are surrounded by what Calder (1953) refers to as a "sheath of extra-phloem tissue"; however, individual phloem cells are not usually preserved. The vascular supply of the cone-scale complex is double at its source; a lower and an upper inverted series of vascular bundles pass out into the bract apophysis and laminar tip and the ligule, respectively. These vascular strands are accompanied by resin canals which are lined by an epithelium (Fig. 5, 11). The ligule is strongly vascularized by six bundles of short tracheids which have alternate, multiseriate bordered pits on their radial and tangential walls. The vascular bundles in the bract are each underlain by a resin canal which accompanies the bundle into the deciduous laminar tip. The presence of winged cone scales and a strongly vascularized ligule free from the bract has prompted Calder (1953) to put *A. mirabilis* into the section *Bunya* of the genus *Araucaria.*

In general, the cones of *Araucaria mirabilis* are very sclerotic; sclereids or fibers have been observed in the pith, "ligule," bract, seed integuments (sclerotesta), and scattered in cortical regions of the cone axis. The cones appear to have numerous resin canals in the cortex, ovuliferous scale, bract apophyses, and perhaps in the pith, but never in the secondary xylem of the cone peduncle.

Seeds—Seeds measure 0.8–1.3 cm in length and are 0.2–0.6 cm wide (Fig. 11). One wingless seed is embedded in each ovuliferous scale, with the micropyle directed toward the cone axis. No instances were encountered where there was more than one seed per scale as is the case in some living araucarians (Mitra, 1927; Wilde and Eames, 1955). Many seeds are loosely held in place in some of the more porous fossil specimens and often fall out when the cones are sectioned.

Seed integuments are composed of three tissue layers ranging up to 1.0 mm in thickness. The sarcotesta, or outer coat, is often poorly preserved and rarely found in attachment. When present, this layer is most often peeling or separating from the middle stony layer of the seed (Fig. 14). In places where sarcotestal cells are preserved they appear to be in layers of 3–4 cells, with each of the elongate parenchymatous cells about 15 × 75 micrometers. The sarcotesta is continuous with the ovuliferous scale tissues in which the seed is embedded.

The sclerotesta, or middle layer of the integument, is 355–715 μm thick (Fig. 14). In tangential section the elongate, often branched sclereids show a zig-zag pattern of orientation which is present from the chalaza to the micropylar end of the seed (Fig. 10). These thick-walled cells are often branched and may be 150 μm long. Sclerotestal cells become more elongate toward the micropylar region where the layer is thinnest and are thickest at the seed chalaza. These cells generally form the micropylar lining (Fig. 15). Araucarian seed micropyles have been described as "mouth-shaped" in transverse section (Burlingame, 1915), and this feature is also evident for the seed of *Araucaria mirabilis* (Fig. 15).

The third layer of integument is referred to as the endotesta. This layer averages about 150 μm in thickness when present; however, the cells of this tissue are rarely preserved. This inner layer of integument is composed of parenchymatous cells 65–80 μm in diam (Fig. 14).

The nucellus is free from the enclosing integument except in the chalazal region where it is fused to the inner layer of the seed coat (Fig. 14). It appears stalked as Eames (1913) has shown in *Agathis australis* (Lamb.) Steud. and referred to as "stipitate." The nucellar apex is wavy in outline and appears to be tenuously folded and invaginated (Fig. 11, 12). Darrow (1936) suggested this folding was probably due to crushing or digestion by pollen tubes. In several seeds the nucellus has been found extending into the micropyle and still appears cellular (Fig. 12). In most

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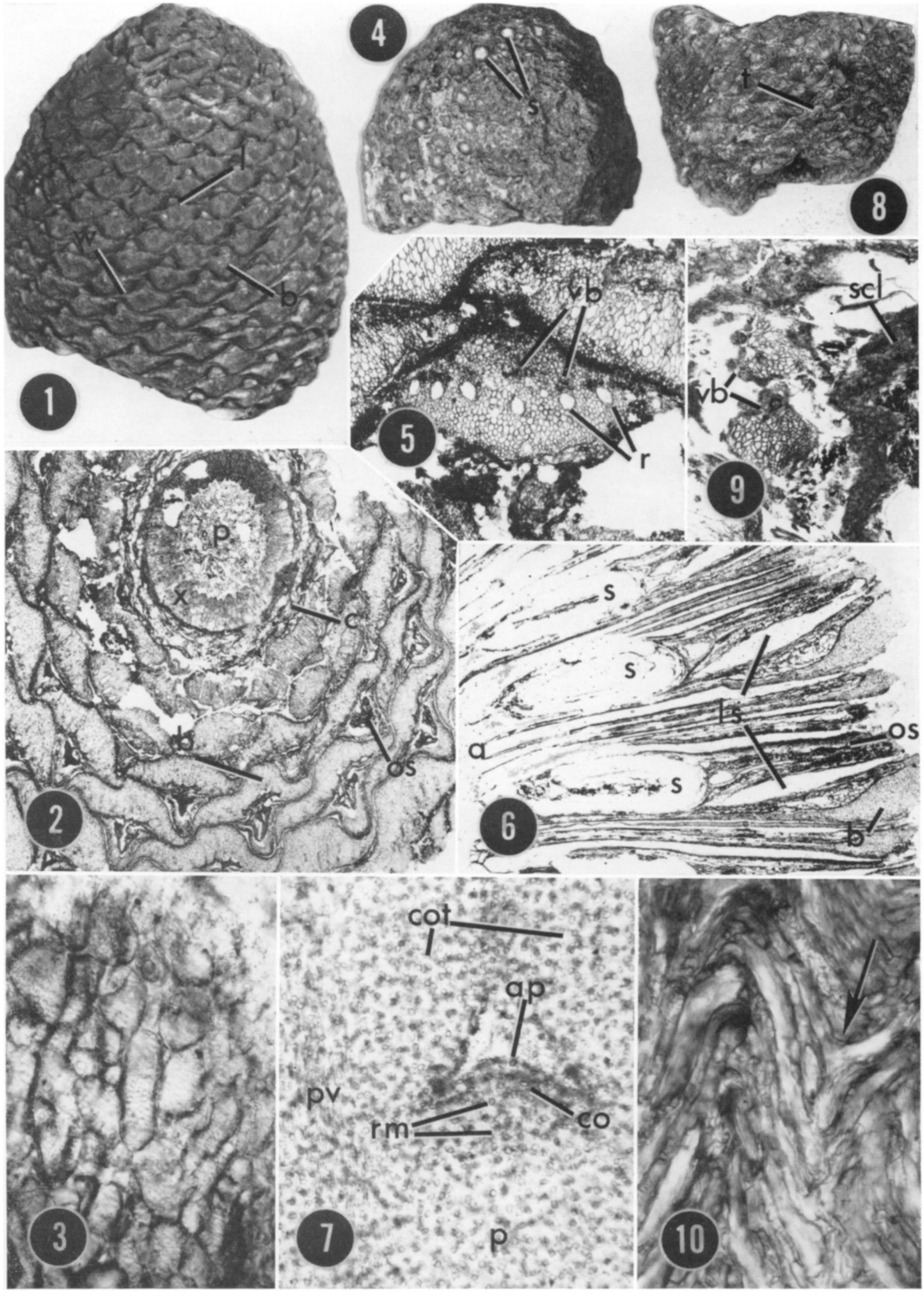
Fig. 1–10. *Araucaria mirabilis.* a, cone axis; ap, apical initials; b, bract; c, cortex; co, corpus or central mother cell group; cot, cotyledon; l, ligule; ls, ligular sulcus; os, ovuliferous scale; p, pith; pv, provascular strand; r, resin canal; rm, rib meristem; s, seed; scl, sclerotesta; t, laminar tip; vb, vascular bundle; w, wing; x, xylem. **1.** External surface of ovulate cone. *P13852.* × 1. **2.** Transverse section of cone in peduncle region. *P13929 #1.* × 4.2. **3.** Tracheids with circular bordered pits in ovuliferous scale vascular bundle. *P13888 #1.* × 170. **4.** External surface of cone showing exposed seeds. *P13896.* × 1. **5.** Transverse section of bract apophysis. *P13929 #1.* × 15.7. **6.** Longitudinal section of cone, showing ovules and ovuliferous scales. *P13820 II A #1.* × 7.4. **7.** Longitudinal section of embryo shoot apex. *P13816 #4.* × 275. **8.** External surface of cone, showing deciduous laminar tip. *P13825.* × 1. **9.** Vascular bundles in region of seed chalaza. *P13888 #1.* × 26.8. **10.** Tangential section of seed integument sclerotestal cells. Arrow indicates branched sclereid. *P13885 #2.* × 162.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seeds, however, the nucellus is thin and appears lignified (Fig. 16, 18).

Traces have been found of what may be the remnants of the megaspore membrane. It is usually crushed and discontinuous. The scanty preservation of the megaspore membrane is probably due to the fact that it was not an intact structure at the time of petrification because of megagametophyte and embryo expansion. Thomson (1905b), Eames (1913), and Burlingame (1915) all comment on the thin and poorly differentiated megaspore membrane of *Araucaria* compared to that, for example, of *Agathis*, which has a cap-like structure formed over the top of the megagametophyte. This cap presumably forces pollen tubes to enter the megagametophyte tissue from the sides, thus preventing the destruction of the upper portion of the gametophyte (Eames, 1913). When extant araucarian seeds are mature, the megaspore membrane is not usually visible (Thomson, 1905b).

Tissue of the megagametophyte is present in most seeds and is fully developed, filling most of the nucellar cavity (Fig. 11, 16, 18). Megagametophyte tissue commonly reaches a length of 0.5 cm and a width of approximately 0.2 cm. The cells are isodiametric and 50–70 μm in diam. In most seeds the delicate cell walls are not preserved but are represented by what appear to have been cellular inclusions or contents. A granular matrix with small spherical bodies is sometimes visible. These structures, that measure 3–5 μm in diam, appear to be slightly angular in outline (Fig. 17). The megagametophyte cells selectively take up the Malachite Green stain more consistently than other tissues of the seed or embryo. Cells in contact with the embryo appear to be flattened slightly; those toward the micropylar end of the seed are more elongate. Although no ovules with a solid megagametophyte tissue have been found in which no embryo is present, wall formation in *A. mirabilis* megagametophyte tissue may have been of the *Pinus* type (Chamberlain, 1935). In this type of megagametophyte formation cell walls are laid down centripetally, the periclinal walls being the last to be laid down. The result is a random orientation of cells and a megagametophyte with a weak central region caused by the line of closure where two cell walls are in contact (Chamberlain, 1935). The other type of wall formation common in conifers is illustrated by *Taxus*, where the cell walls are laid down extending to the middle of the megaspore cavity. The cells appear long and tube-like and have been referred to as "alveoli" (Chamberlain, 1935). Subsequent divisions break up the "alveoli" into smaller cells. The resulting megagametophyte shows a regular arrangement of cells. Since the megagametophyte cells of *A. mirabilis* show no evidence of "alveoli," wall formation may have been similar to that of *Pinus*.

The absence of cell walls in *Araucaria mirabilis* megagametophyte tissue is probably due to their thin and delicate nature. Burlingame (1915), in a study of *A. angustifolia* (= *brasiliensis*), notes the delicate nature of megagametophyte cell walls as well as difficulty in photographing the tissue because of the numerous starch grains.

One megagametophyte (*P13892 #4*) contains what may be two sunken archegonia (Fig. 19). These consist of two elliptical cavities in the upper portion of the megagametophyte tissue on either side of the micropylar canal. No neck cells or any cellular preservation inside the cavities was observed. The nature of jacket or other surrounding cells is unknown. These elliptical cavities not only appear to be in the appropriate position to be araucarian archegonia but, moreover, are of a uniform size and shape. They appear to be similar to those described by Eames (1913) for *Agathis australis*, which are produced superficially, near the surface of the megagametophyte, and by a subsequent proliferation of the megagametophyte tissue become sunken.

Vascularization of the ovule is complex and corresponds to some extent with the vascular system that Wilde and Eames (1948) show for the seed of *Araucaria bidwillii*. Vascular bundles are extensive at the chalaza of the ovule, with 1–4 bundles present in the ovule bases (Fig. 14). A series of six vascular bundles enters the tip of the ovuliferous scale (Fig. 13). Worsdell (1899) reports "concentric bundles" in the ligule of *A. bidwillii*, each with spiral thickenings, although he never notes the actual number of bundles. Vascular bundles, often with the protoxylem elements surrounded by a ring of metaxylem tracheids, are typical of those seen in *A. mirabilis*. In cone transverse sections two large vascular bundles are present in the ovuliferous scale (Fig. 9). Tracheids in this region are nearly as wide as they are long (60–70 μm) and have alternately arranged multiseriate circular bordered pits on their radial and tangential walls (Fig. 3).

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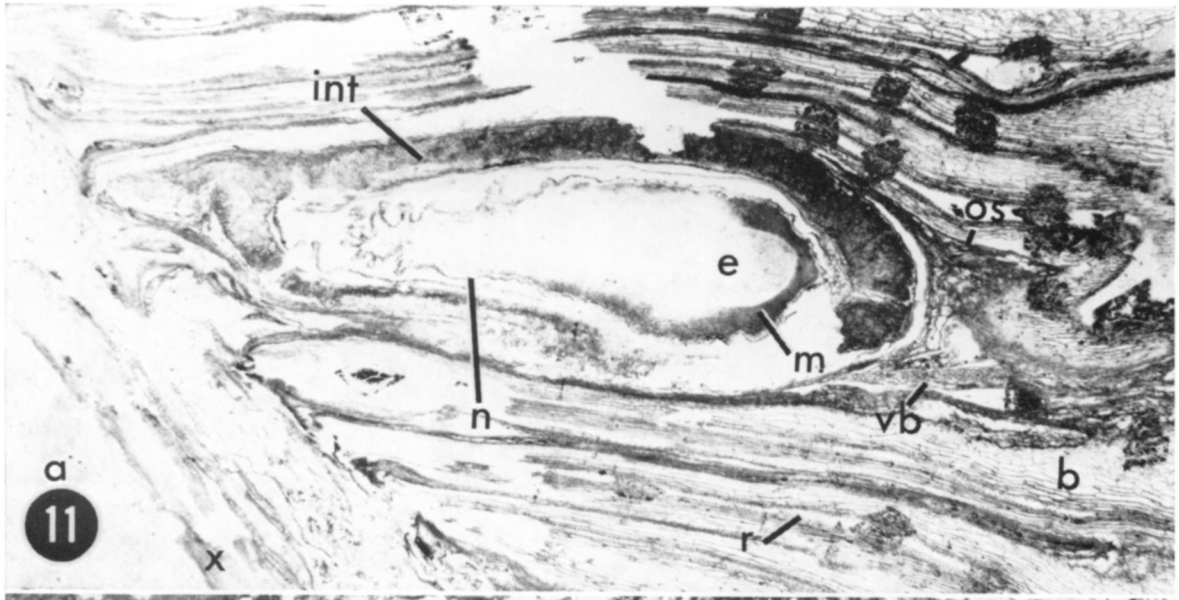
Fig. 11–15. *Araucaria mirabilis*. a, cone axis; b, bract; e, embryo; end, endotesta; int, integument; m, megagametophyte; mi, micropyle; n, nucellus; os, ovuliferous scale; r, resin canal; sar, sarcotesta; scl, sclerotesta; vb, vascular bundle. **11.** Longitudinal section of seed, showing principal features. *P13939 #1*. × 11. **12.** Oblique section of ovule, showing remnants of nucellus. *P13888 #1*. × 18. **13.** Transverse section of bract and ovuliferous scale near region of ligular sulcus. *P13929 #1*. × 29. **14.** Longitudinal section of seed in region of chalaza. *P13939 #1*. × 35.8. **15.** Transverse section of ovule, showing irregularly shaped micropyle. *P13939 #4*. × 24.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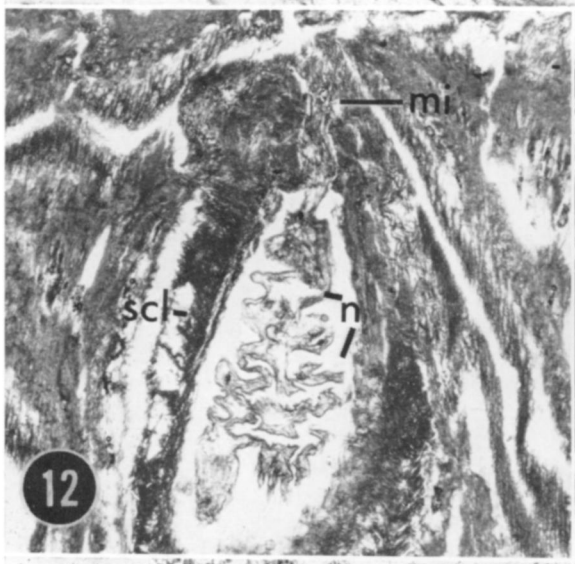
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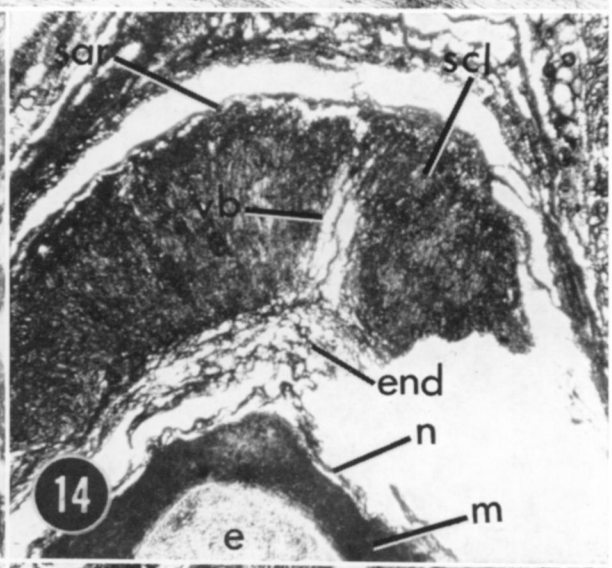
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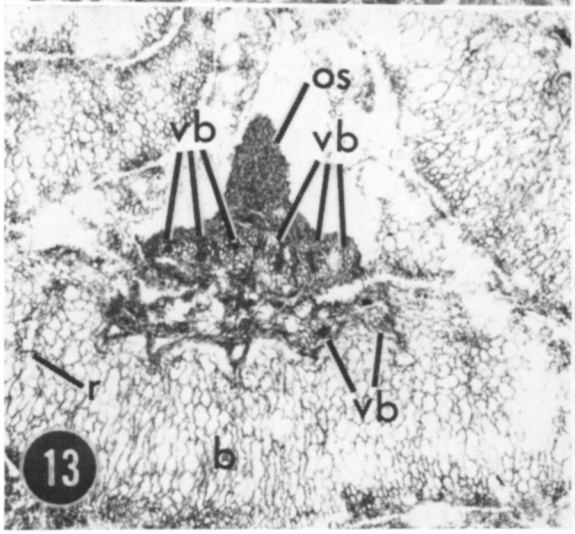
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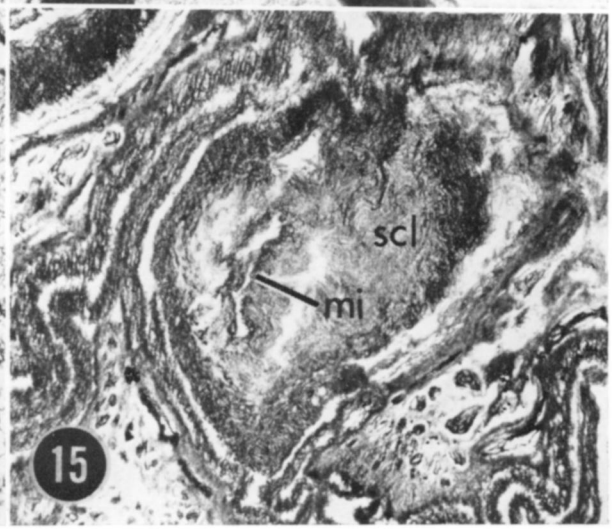
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These two large groups of tracheids may represent the portions of the upper (ovuliferous scale) series in the cone-scale complex which Eames (1913) referred to as "the crowded twisted groups which come together behind the ovule in a horizontal series and pass on into the free ligule as strong bundles." Eames (1913) describes eight bundles that eventually pass into the ligule of *A. bidwillii*.

Embryos—The embryos of *Araucaria mirabilis* appear to be in what Schopf (1943) has referred to as the telo-stage period of development. Embryos are found embedded in the megagametophyte tissue, and as Calder (1953) has pointed out, the orientation of the plane in which the cotyledons lies varies from one almost parallel to one at right angles to the surface of the ovuliferous scale. Longitudinal sections of the embryo show the typical features of a conifer telo-stage embryo: shoot apex, cotyledons, area of permanent initials of the root meristem, columella, and calyptroperiblem (Fig. 7, 16, 18, 20, 23–28). In the Cerro Cuadrado embryos the vascular tissues are just beginning to differentiate. Darrow (1936) has reported that there are only two cotyledons present in the embryo, with each reaching a length of about 2 mm and a width and depth of 0.25 mm (Fig. 16, 18, 26–28). Each cotyledon is supplied by six provascular strands (Fig. 26).

Calder's (1953) figures of *A. mirabilis* embryos show what appears to be cellular preservation although she never makes note of this fact. Most embryos do not show preservation of the delicate cell walls. Each cell is represented in the embryo by a small spherical body 5–6.5 μm in diam (Fig. 7). In embryos where cell walls are preserved, one of these spherical bodies is present in each cell (Fig. 21). They become more elongate in the regions of the provascular strands, where they measure 17 $\times$ 2 μm. These spherical bodies or inclusions in the cells of the embryos provide excellent markers for the tissue zones of the telo-stage embryo. Their possible origins in the living embryo may have been protein granules or globules, starch grains, or some other stored material. They may also represent nuclei, since there is only one per cell, and they are elongated in the provascular regions. Living nuclei in differentiating xylem elements are often larger and more elongate than nuclei in cells where differentiation is not occurring (Scott, 1940).

The shoot apex has a diam of 112–135 μm and appears to have a surface meristem composed of a layer of apical initials. Some apices show what appear to be two surface layers. Griffith (1952) reports a discrete surface layer or tunica in living araucarian shoot apices. In two species (*Araucaria araucana* [Molina] K. Koch [= *A. imbricata* Pavon] and *A. bidwillii*) a second discrete layer is present in 90 % of the shoot apices; sometimes more than two surface layers are found in these species. Embryos of *A. mirabilis* show one and what may be two surface layers of cells. In apices with a so-called tunica-corpus arrangement of the meristem, the numbers of parallel surface layers may vary during the ontogeny of the plant and also with seasonal growth changes (Romberger, 1963; Griffith, 1952). The central mother cell group of most gymnosperms underlies the surface meristem (Romberger, 1963). This region is referred to as the corpus in angiosperms, *Gnetum*, and *Araucaria* (Griffith, 1952; Romberger, 1963). In *A. mirabilis* there is a corpus-like region beneath the surface initials in which there is a more or less random orientation of cells (Fig. 7). No leaf primordia, like those that occur in the dormant stage of some gymnospermous embryos (Buchholz and Old, 1933), have been observed. Beneath this corpus-like area is the rib meristem in which the cells are oriented in longitudinal rows that in living meristems show a gradual maturation away from the apex to form pith cells. The longitudinal rows of cells which make up the central column or hypocotyl region of *A. mirabilis* embryos are very prominent (Fig. 20), and the hypocotyl region extends for a length of 1.2 mm (Fig. 16).

The generative meristem of the root is represented by a group of permanent initials (Fig. 18, 20). Beneath this region of initials the column or columellar region extends for a length of 1.2 mm (Fig. 16, 20). This temporary meristematic region adds cells to the "root cap" by periclinal divisions (Allen, 1947). The "root cap" or calyptroperiblem region (Buchholz and Old, 1933) measures about 1.1 mm $\times$ 0.9 mm and extends

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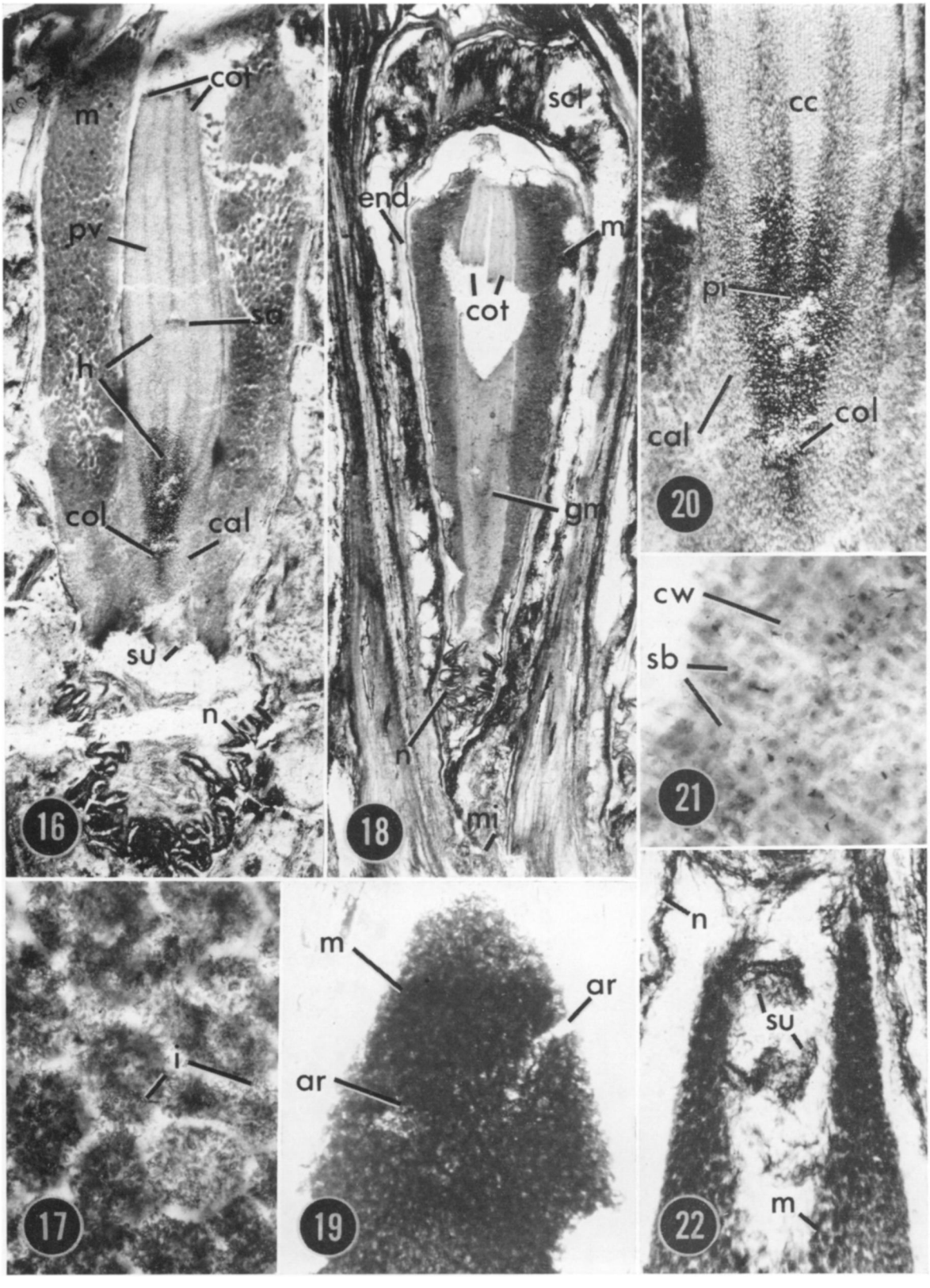
Fig. 16–22. *Araucaria mirabilis*. ar, archegonium; cal, calyptroperiblem; cc, central column; col, columella; cot, cotyledon; cw, cell wall; end, endotesta; gm, generative meristem of the root; h, hypocotyl; i, inclusion; m, megagametophyte; mi, micropyle; n, nucellus; pi, permanent initials; pv, provascular strand; sa, shoot apex; sb, spherical bodies; scl, sclerotesta; su, suspensor remains. **16.** Longitudinal section of telo-stage embryo and surrounding tissues. *P13816 #4*. $\times$ 22.3. **17.** Cells of the megagametophyte, showing inclusions. *P13865 #2*. $\times$ 295.7. **18.** Longitudinal section of embryo contained within seed. *P13865 #3*. $\times$ 14. **19.** Oblique section of megagametophyte tissue, showing possible sites of archegonia. *P13892 #4*. $\times$ 59.5. **20.** Longitudinal section of embryo root meristematic region. *P13816 #3*. $\times$ 42. **21.** Cellular organization of the embryo. *P13816 #3*. $\times$ 269. **22.** Longitudinal section of seed, showing suspensor remains in micropylar region. *P13892 #3*. $\times$ 60.2.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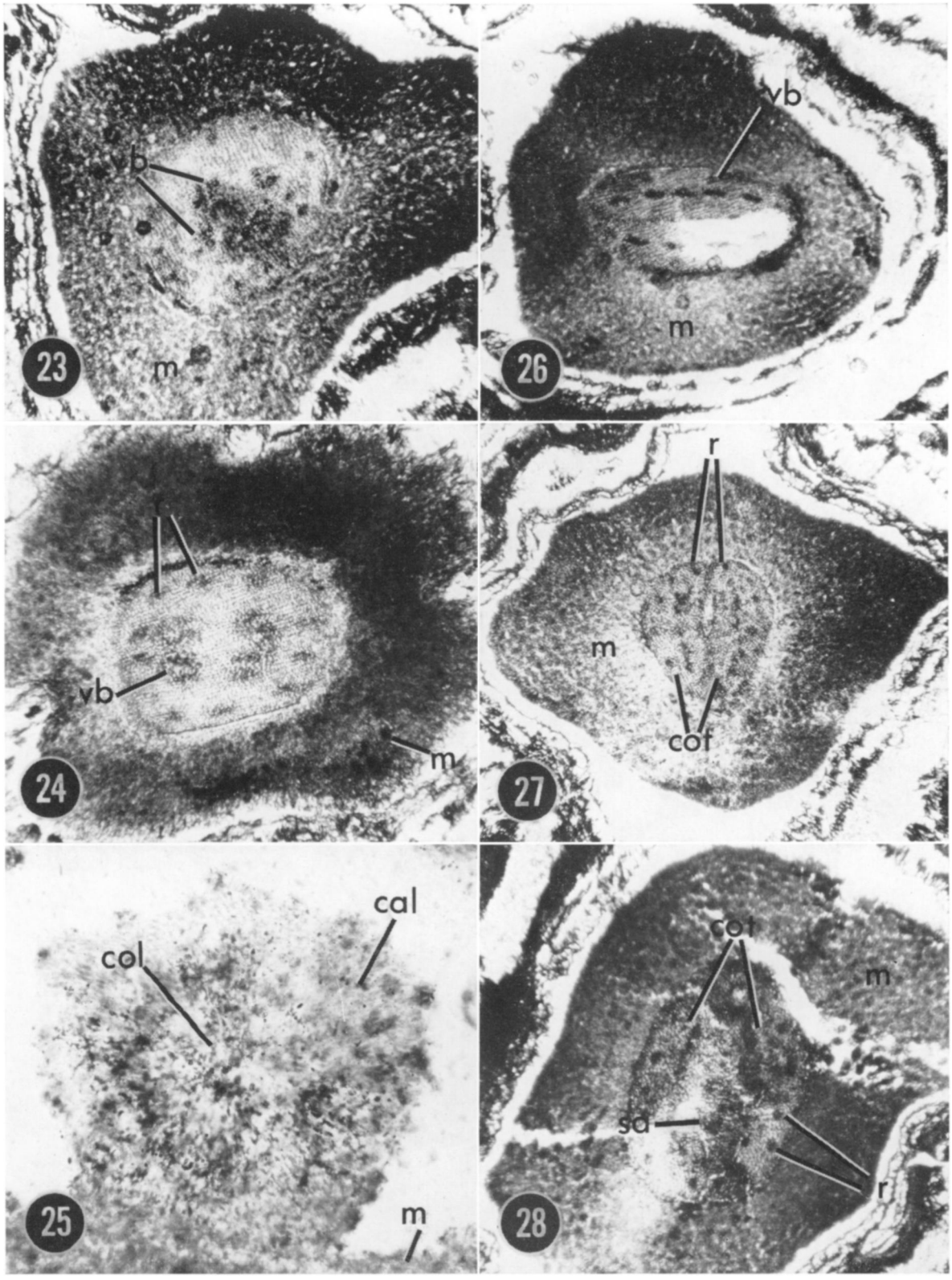

Fig. 23–28. *Araucaria mirabilis* embryo. Successive transverse sections from calyptroperiblem to cotyledons. cal, calyptroperiblem; col, columella; cot, cotyledon; m, megagametophyte; r, resin canal; sa, shoot apex. **23.** Trans-

for about ¼ of the embryo proper (Fig. 16, 18, 20, 25). At the basal portion of the embryo the calyptroperiblem is jagged in outline, probably indicating the place of detachment from the suspensors (Fig. 16).

Beneath the protoderm of the embryo, and in the cortex, is a ring of 12 secretory elements, resin ducts, or canals which also extend into the cotyledons (Fig. 24, 27, 28). Darrow (1936) makes a brief note of their presence in one of her text figure explanations. Individual canals measure 25–40 μm in diam. Burlingame (1915) has reported two such rings of resin ducts in the cortex and pericycle of *A. angustifolia.* Only one ring has been observed in the cortex of the embryos of *A. mirabilis.*

The remains of highly coiled suspensors can be seen in various planes of section (Fig. 22). In only one seed were the suspensors seen to be stretched out and more or less uncoiled or straight. This may be an embryo in an earlier stage of development.

Figures 23–28 are a series of transverse sections taken at various levels of the telo-stage embryo. Figure 25 is a section taken somewhere within the columellar region. Cells converge toward a central point, the column or columellar region, where calyptroperiblem cells originate. A section a little farther up the embryo in the hypocotyl region shows that the vascular cylinder is split into two C-shaped strands (Fig. 24). These two C-shaped strands each divide at a higher level to produce four strands (Fig. 23, 24). At a still higher level each divides to form a total of six strands that enter the cotyledons (Fig. 26, 27, 28). Figure 28 is a section taken at a plane which crosses the shoot apex region and Fig. 27 at a level just above the point of cotyledonary divergence. Cotyledons themselves extend for about ½ of the embryo proper.

DISCUSSION—The fossil ovulate cones of *Araucaria mirabilis* show the greatest resemblance to extant specimens of *A. bidwillii* (section *Bunya*). Calder (1953) reports that the strongly vascularized ligule (free portion of the ovuliferous scale) and the separate origins of bract and ovuliferous scale vascular supplies seem to ally *A. mirabilis* with this species. This pattern of vascularization is only found in the section *Bunya* (Eames, 1913; Aase, 1915; Wilde and Eames, 1948, 1952). Darrow (1936) points out the similarity of the embryo of *A. mirabilis* to that of *A. bidwillii* in the presence of two cotyledons. Cotyledon number in the genus ranges from 2–4 (Butts and Buchholz, 1940). Darrow (1936) also compares the embryo to those of *A. araucana* and *A. angustifolia* in which the hypocotyl constitutes less than ⅙ of the total embryo length. She states that the fossil embryo has a hypocotyl that comprises about half of the embryo proper. The thin section technique used in this study has demonstrated that the region from shoot apex to point of suspensor attachment is about ½ of the embryo proper; the other half is comprised of the cotyledons. The actual hypocotyl constitutes about ¼ of the embryo in *A. mirabilis*, the calyptroperiblem or "root cap" region about one fourth.

The diameter of the shoot apex in araucarians varies from 116 μm in *Araucaria cunninghamii* Ait. ex Lamb. to 168 μm in *A. araucana* (Griffith, 1952). The apex of *A. mirabilis* reaches a diam of 135 μm, thus showing a similarity to the apex of *A. bidwillii*, which reaches 134 μm (Griffith, 1952). There appears to be one discrete layer of apical initials and perhaps two in some apices, as is seen in *A. bidwillii* and *A. araucana* (Griffith, 1952).

The greater depth of the ligular sulcus (space between ovuliferous scale tissue and that of the bract) in *A. mirabilis* as compared to that feature in other araucarian cones prompted Wieland (1935) to institute the genus *Proaraucaria.* Wilde and Eames (1948) point out that at a stage about 3 weeks after pollination the ovuliferous scale is still free for about half its length in *A. bidwillii*; later the length of the fused portion increases. Calder (1953) suggested that the depth of the sulcus alone was not enough to justify a new genus for the fossil cone.

Vishnu-Mittre (1954) described a petrified araucarian cone, *Araucarites bindrabunensis* Vishnu-Mittre, from the Jurassic of India. This cone resembles *A. mirabilis* in several respects and is regarded as an extinct species of the section *Bunya.* One reason for the separation of this cone from *Araucaria mirabilis* is the presence of longer scales and seeds. It should be noted that the range of measurements in seed and scale length in *A. mirabilis*, as reported here, covers the range found for *Araucarites bindrabunensis.* Cells of the pith in most cones of *Araucaria mirabilis* are not sufficiently preserved to determine the presence of resin canals, which are reported in *Araucarites bindrabunensis.* Also, in *Araucaria mirabilis* many bract apophyses in certain sections of

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verse section of embryo in hypocotyl region. *P13925 #7.* × 63. **24.** Transverse section of embryo in hypocotyl region, showing C-shaped strands. *P13853 #4.* × 49. **25.** Transverse section of embryo in columellar region. *P13925 #6.* × 147. **26.** Transverse section of embryo cotyledons, showing 6 vascular bundles. *P13925 #7.* × 38. **27.** Transverse section of embryo just above point of cotyledonary divergence. *P13925 #6.* × 44.3. **28.** Transverse section of embryo at point of cotyledonary divergence with 6 vascular bundles. *P13925 #7.* × 49.

tangential cuts do not readily show the presence of resin canals (Fig. 2, 13). Vishnu-Mittre's drawing of cross sections through cone scales appears much like similar sections of *A. mirabilis*, especially with respect to vascularization.

Wilde and Eames (1955) and Mitra (1927) have suggested that studies of multi-ovulate cone scales may indicate that the one seed per cone-scale complex feature of araucarians is a derived condition from two or three ovulate types. No scales bearing two or three seeds have yet been found among the cones of *A. mirabilis*. The one-seed condition seems to have evolved in the Araucariaceae at least as early as the Jurassic.

The oldest known fossil embryo has been described by Miller and Brown (1973) in a voltzialean cone (*Moyliostrobus texanum* Miller and Brown) from the Permian of Texas. This Paleozoic embryo consists of an elliptical area of parenchyma with three tracheids contained within the megaspore wall. The *Moyliostrobus* embryo seems to be at a later stage of development than that of *Araucaria mirabilis* since tracheids with annular and spiral thickenings are already differentiated. Tracheids have not yet differentiated and laid down secondary thickenings in the araucarian embryo. Unfortunately, no megagametophyte tissue was found in the *Moyliostrobus* seeds. In *Araucaria mirabilis*, however, megagametophyte cells are present and cellular contents suggested by the presence of a granular matrix and roughly angular to spherical cellular inclusions which may be starch grains or protein bodies (Fig. 17).

Most embryos of *A. mirabilis* show the typical telo-stage condition that is common in conifers (Fig. 16). Schopf (1943) has suggested that the monophyletic origin of gymnosperms may be indicated by the apparent similarity of nearly all gymnospermous embryos at the telo-stage period, even though the various groups show differences in vegetative habit as well as in early stages of embryo development. The great similarity of the telo-stage embryo of *Araucaria mirabilis* to that of other conifers indicates that this stage of development was present in conifers as early as the Jurassic.

The presence of no earlier stages in embryo development in *A. mirabilis* may be due to the time and mode of preservation of the forest. If the forest was covered by volcanics while most cones showed telo-stage embryos, and the smaller newly forming cones were just developing ovules, the resultant fossils may be reflected in what is seen in the Cerro Cuadrado remains. Calder (1953) also calls attention to the fossil seedlings in the collection to point out three generations of remains within the petrified forest. Burlingame (1913, 1914) reports that extant ovulate cones of *Araucaria* also take two years to develop; whereas those of *Agathis* (Eames, 1913), as in most conifers, mature in three years.

Conditions of polyembryony have not been observed in *A. mirabilis* since most of the seeds are at relatively the same stage of development. The nature of the embryonic cap of cells characteristic of proembryos of *Araucaria* and *Agathis* has been much speculated upon (Strasburger, 1872; Seward and Ford, 1906; Eames, 1913; Buchholz, 1920). Buchholz (1920) has suggested the cap functioned in preventing cleavage polyembryony. Others including Eames (1913), Seward and Ford (1906), and Strasburger (1872) have suggested that the cap functioned in protection of the embryo. Buchholz (1920) regards the function of the cap for the secretion of enzymes as a remote possibility. The presence of a cap or details of its formation are not available in the embryo of *Araucaria mirabilis* at this time.

Buchholz (1919) has suggested the evolution of dicotyledony from a polycotyledonous condition. He states that the fusion of cotyledonary primordia in *Pinus banksiana* Lamb. and *Cedrus libani* A. Rich. in the early stages of cotyledon elongation or the abortion of cotyledonary primordia may show a manner in which dicotyledony may have arisen. *Araucaria mirabilis* embryos in earlier stages of development have not been found showing only cotyledonary primordia, and consequently their number is unknown. However, the dicotyledonous condition of the araucarian telo-stage embryo is present by the Jurassic period.

The lack of pollen cones has likewise been speculated upon by Calder (1953). She believes their absence is due to the season in which the forest was covered. Perhaps evidence of them will yet be found in the volcanic ash matrix. Menendez (1960) described a pollen cone, *Masculostrobus altoensis* Menendez, from the regions around Cerro Alto. His work dealt with four cone specimens containing spherical pollen grains 100–120 μm in diam. Sufficient details of anatomy were not present to assign the cones to any of the major conifer families. These cones may represent the pollen cones of *Araucaria mirabilis*, *Pararaucaria patagonica*, or perhaps another gymnosperm found in the Cerro Alto forest. As Menendez (1960) and Calder (1953) point out, the cones in the Field Museum collections considered by Wieland (1935) to be "staminate" are actually smaller ovulate cones. No other of the collecting localities has reported pollen cones but the Cerro Alto region.

There is also a lack of pollen tubes or pollen grains on, or embedded in, tissues of the ovulate cone of *A. mirabilis*. Their absence is most puzzling in light of the strong erosive capacity of araucarian pollen tubes in extant species (Eames, 1913). The pollen of *Araucaria* lands on the ex-

tended portion of the ovuliferous scale and grows toward the nucellus (Thomson, 1905a, 1907; Eames, 1913; Burlingame, 1915; Wilde and Eames, 1948). This condition is present only in *Agathis*, *Araucaria*, *Saxegothaea*, and *Tsuga* (Wilde and Eames, 1948; Doyle and O'Leary, 1935a, b). Eames (1913) notes that araucarian pollen tubes may enter all tissues of the cone, e.g., ovuliferous scale, cone axis, including xylem and phloem tissues, as well as the pith and cortex of the cone axis.

Eames (1913) suggests that perhaps parthenogenesis might be found in the Araucariaceae. In *Agathis australis* some ovules that are not pollinated have jacket cells of the archegonia that break down, their contents being freed into the egg cytoplasm. Eames notes that these contents fuse and migrate toward the egg nucleus. He has also observed them indenting the nuclear membrane of the egg (Pl. II, Fig. 15) but expressed some doubt as to the actual fusion with the egg nucleus. No migration of jacket cell contents occurred under normal conditions of fertilization.

The question as to the mode of dispersal of the seeds of *Araucaria mirabilis* is still unanswered. Gothan (1950) notes the absence of free cone scales and suggests that they were retained on the scale. Calder (1953) suggests that perhaps the cone did shed its scales since some of the smaller specimens like those described by Wieland (1935, Pl. 6, E and F) are cone axes that have no cone scales present on their surfaces. The cone figured as F in Wieland's plates has actually been sectioned in this study and shows a pith with surrounding vascular bundles. One cone (*P13836*) of the collection shows a hollow central region where the pith and vascular tissues have been removed before petrifaction. The cone scales themselves have remained together in a compact unit. Seeds of some cones fall out during sectioning, suggesting perhaps that the seeds were actually shed from the scale, as in *A. bidwillii* (Wilde and Eames, 1948, 1955).

The cones of *Araucaria mirabilis* have shown a remarkable state of preservation that has enabled them to be closely compared with extant conifers. The one-seeded condition of araucarian cones is seen to have evolved by the Jurassic period. Detailed structure of the tissues and meristems of the telo-stage embryo have been demonstrated for the Jurassic conifer. Vascularization of the seed and cone-scale complex have appeared similar in many respects to that of the extant *Araucaria bidwillii*, which appears to be its nearest living relative. The rareness of such excellent preservation in silica is appreciated, especially in megagametophyte and embryo tissues where delicate cell walls as well as cellular contents, difficult to examine in living araucarian cones, are apparently preserved.

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