University of Alberta

Morphology, anatomy, and phylogeny of fossil and extant Saururaceae: insights from the Middle Eocene Princeton Chert

by

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The most exciting phrase to hear in science, the only one that heralds new discoveries, is not "Eureka!" but rather, "Hmm... that's funny...."

— Isaac Asimov

You will have to experiment and try things out for yourself and you will not be sure of what you are doing. That's all right, you are feeling your way into the thing.

—Emily Carr

The universe is wider than our views of it.

—Henry David Thoreau

ABSTRACT

Hundreds of small flowers and fruits, and one partial inflorescence, from the Middle

Eocene Princeton Chert, British Columbia, Canada, were examined to determine their

relationship to extant angiosperms. Numerous minute perianthless flowers are borne in an

indeterminate raceme. Each flower is subtended by a bract to which flowers are fused. The

five stamens are basally adnate to the carpels. Anthers are tetrasporangiate with

longitudinal, latrorse dehiscence, and pollen is frequently found in situ. The gynoecium is

composed of four basally connate, lobed carpels that are broad at the base and taper

apically, with recurved styles. One ovule with marginal placentation is attached near the

base of each carpel. Flower structure and pollen are indicative of Saururaceae (Piperales).

Phylogenetic analyses using morphological characters support the inclusion of these fossils

within Saururaceae. Fossil flowers are most similar to *Saururus* L., and are described here

as a new species. Fruits of extant *Saururus* are described anatomically for the first time. A

new interpretation of the seed coat is presented along with confirmation of a previous

report that endocarp is present. Anatomy of fossil fruits found in association with the

flowers is described and compared to extant *Saururus,* and is shown to be similar. A

developmental sequence shows that the fruits belong to the same taxon as the flowers and

inflorescence. Finally, pollen ultrastructure in Saururaceae is examined for all six extant

species and from the fossil flowers. The first detailed scanning electron and transmission

electron micrographs for all species are presented. Fossil pollen is shown to be similar to

extant monosulcate saururaceous pollen, which is characterized by its small size and

punctate tectum. *Gymnotheca* shows several unique characters relative to other genera in

the family, such as having a striate, not smooth, tectum, and absence of papillae around

puncta. These fossil flowers and fruits from western North America show that Saururaceae were once much more widespread in the Eocene, and that their current distribution is relictual. These are the oldest fossils, as well as the first North American record of Saururaceae, and provide the first evidence for saururaceous pollen.

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Chapter 1

Introduction

The Middle Eocene Princeton Chert locality has been studied for over 35 years. Located in south-central British Columbia, the locality is composed of interbedded layers of chert and coal, with nine interspersed ash layers. The Princeton Chert is a site of exceptional preservation, where many different types and parts of fossil plants are silicified in three dimensions and retain anatomical details (Stockey, 2001). This allows for detailed comparisons of plants with modern and extinct relatives. Fine details of stems, roots, leaves, fungi, spores, pollen, cones, flowers, fruits and seeds are preserved. To date, ten fungi, four ferns, three conifers, 24 angiosperms (including five monocotyledons) have been described from the Princeton Chert (Pigg and Stockey, 1996; Stockey, 2001) (Table 1.1) and there are more plants yet to be described. The data presented in this thesis are one more step towards fully understanding both the taxonomic and paleoenvironmental diversity represented by the Princeton Chert assemblage.

Princeton Chert geology—The Princeton Chert is located in south-central British Columbia, Canada, near the abandoned mining town of Allenby. It crops out approximately 8.4 km south of the town of Princeton, on the east bank of the Similkameen River. The locality has been dated as Middle Eocene based on mammals (Russell, 1935; Gazin, 1953), freshwater fish (Wilson, 1977, 1982), palynology (Rouse and Srivastava, 1970) and K-Ar dating (Hills and Baadsgaard, 1967). Potassium-argon dating of the ash in layer 22 gives an absolute date of 48.7 Ma (Baadsgard, pers. comm., 1999). Thus the Princeton Chert is near the Early/Middle Eocene boundary, which is dated as 48.6 ± 0.2 Ma (Luterbacher et al., 2004).

The outcrop is part of the Allenby Formation, Princeton Group (Boneham, 1968) and is underlain by a sandy shale, and overlain by a fossiliferous black shale (Basinger, 1981). The outcrop consists of 49 major layers of chert interbedded with coal and volcanic ashes that have been systematically sampled (Stockey, 1983). Chert layers split and merge at different points along the outcrop, resulting in over 70 layers that vary in thickness from 1 to 50 cm (pers. obs.). There are nine ash layers, mostly in the upper parts of the outcrop: layers 22, 26-28, 30-31, 33-34, and 35 (M. Matsumoto, pers. comm., 2003).

The chert itself represents a silicified peat. The amount of organic material in the chert varies from 10 - 80% (M. Matsumoto, pers. comm., 2003). Several different chert macrotextures are present (following macrotexture categories of Trewin, 1996): massive, vuggy, brecciated, lenticular and possibly laminated (M. Matsumoto, pers. comm., 2003; pers. obs.). Sometimes the chert layer intergrades with the coal layer above or below it, i.e., not all the contacts are sharp. Trewin (1996) interpreted massive to vuggy cherts as having formed where an area of plant growth was flooded by hot spring waters (as for the early Devonian Rhynie Chert), while lenticular cherts were interpreted as having formed in small pools that were filled with such water. While it is generally accepted that the Rhynie Chert represents a hot spring (Trewin, 1996; Rice et al., 2002), the Princeton Chert probably represents a small lake or pond that experienced an occasional influx of mineral-rich waters (Basinger, 1979; Stockey, 2001). It appears that the Princeton Chert is less diverse in terms of the types of chert and the range of preservation types than the Rhynie Chert. A more detailed examination of the Princeton Chert geology needs to be done before we can clarify some of these questions about specific composition of the

chert, how it was formed, and how it relates to palaeoenvironment and taphonomic processes.

Depositional environment–Basinger (1981) and Cevallo-Ferriz et al. (1991b) have interpreted the Princeton Chert locality as being representative of a shallow or near-shore lacustrine environment. Support for this aquatic habitat comes from both the anatomical characters and affinities of the organisms preserved here. For example, seeds of the waterlily (Nymphaeaceae), *Allenbya collinsonae* Cevallos-Ferriz & Stockey (1989) are known from several layers. Stems and roots of *Decodon allenbyensis* Cevallos-Ferriz & Stockey (1988b; Lythraceae) are similar to the extant swamp willow, *Decodon verticillatus* (Little and Stockey, 2003). This plant grows on the edge of lakes and can bend in (toward the lake centre) and grow out into the water. Its roots produce a delicate, spongy tissue: concentric layers of thin-walled lacunate phellem, a characteristic anatomical feature of submerged aquatic plants (Little and Stockey, 2003).

Another example of anatomical evidence is the presence of aerenchyma, a tissue typically associated with aquatic habitats, as it allows more gas exchange and buoyancy. Aerenchymatous tissues are found in plants such as the ferns *Dennstaedtiopsis aerenchymata* Arnold & Daugherty and *Trawetsia princetonensis* Smith, Stockey, Nishida & Rothwell (2006), the dicot *Eorhiza arnoldii* Robison & Person (1973), and the monocot *Heleophyton helobiaeoides* Erwin & Stockey (1989). In addition to aerenchyma, the structure of vascular bundles in the petiole of *Heleophyton*, with protoxylem lacunae, reduced thin-walled tracheary elements, and relatively large phloem strands, suggests that the plant grew in an aquatic habitat (Erwin and Stockey, 1989).

Faunal remains are also indicative of a shallow, near-shore lacustrine environment. Turtle bones are occasionally found in the chert (Cevallos-Ferriz et al., 1991b) and soft-shelled (trionychid) turtle remains have been found in the shales above the chert (Wilson, 1982). Freshwater fish remains of bowfin (*Amia* sp.), suckers (*Amyzon* sp.) and trout-perches (*Libotonius* sp.) are also found in the shales above the chert (Wilson, 1977, 1980, 1982). Wilson (1980) examined various Eocene lake environments in British Columbia and Washington State, and concluded that these three fish found at the Princeton Chert locality represent a shallow water/near-shore assemblge.

The most likely scenario for the permineralization process is by periodic and repeated flooding by mineral-rich waters (Basinger, 1981). Alkaline waters could have accumulated dissolved minerals, perhaps by percolating through neighbouring silica-rich rocks. Then when they mixed with the neutral pond/lake water, a rapid pH change would cause precipitation of silica and permineralization of the upper layer of peat (Basinger, 1981). As time continued, non-silicified peat became compressed into coal. Some rocks that have been examined show a gradation from cherty coal at the top and bottom to less organic chert in the middle of a block. Occasionally it is possible to find plant specimens that are caught within this gradation, which can be seen compressed in the brown coaly area, that are distinct in the chert matrix below. Sometimes there is a sharp boundary between chert and coal at the top; other times there is a more gradual change. As mentioned below, few plants show evidence of compression, and this silicification must have been relatively rapid. There are areas within certain layers that appear to have undergone more compression. In addition, there are some layers, e.g., Layer #35, where the chert splits into multiple beds separated by coal (pers. obs). This may have occurred because of fluctuations in silica-rich

water penetration of the peat. This aspect of the geology needs further study, which would help us to understand the process of silicification at the locality.

Transport and time-averaging—Some elements of the Princeton Chert assemblage are rare and could represent allochthonous elements. Examples are rare seeds of Vitaceae and Rosaceae, or infrequently found twigs of Magnoliaceae and Rosaceae (Cevallos-Ferriz and Stockey 1990a, 1990b, 1990c, 1991; Cevallos-Ferriz et al., 1991b). However, for the most part, plants preserved by the Princeton Chert seem to be parautochthonous or autochthonous in origin. The occurrence of rooted axes, such as those of *Eorhiza* (Stockey and Pigg, 1994; Stockey, 2001), indicate that certain plants were preserved in growth position. There are many plants that are represented by large numbers of organs. If these plant remains had been transported in, there would be fewer types of organs since there would be more taphonomic filters acting on the material. Examples of whole plants include *Metasequoia milleri*, where roots, leaves, wood, pollen cones and ovulate cones are known (Basinger, 1981, 1984; Basinger and Rothwell, 1977; Rothwell and Basinger, 1979), and the palm, *Uhlia*, known from stems with attached roots, petioles and leaf laminae (Erwin and Stockey, 1991b, 1994). Finally, delicate tissues are sometimes preserved that would not remain intact with transport, such as the phellem of *Decodon* roots (Little and Stockey, 2003). In living plants this tissue is fragile.

Because peat is derived from an accumulation of organic matter, there is a potential for a considerable amount of time averaging in this outcrop. Basinger (1981) concluded that the coal at Princeton represented an accumulation of material over many years up to several centuries. However, it is likely that the individual chert layers themselves represent short intervals of time. There are some developmental sequences preserved, such as

Princetonia allenbyensis Stockey (1987) flowers and fruits (Stockey and Pigg, 1991);

Decodon roots (Little and Stockey, 2003, 2005); lauraceous flowers and fruits (Little,

2006); and saururaceous flowers developing into fruits (Smith and Stockey, 2006; this

thesis). The different stages often co-occur in a chert layer. In extant Saururaceae and

Lauraceae, flowers and fruits develop at the same time on a plant (Thien et al., 1994). In

Lauraceae flowers and fruits can abscise throughout development (Gazit and Degani, 2002;

Little, 2006), and thus different stages could enter the fossil record at the same time.

Other lines of evidence that there is little time averaging include the presence of five

different taxa that are represented by flowers. Such delicate structures do not have a high

preservation potential and are unlikely to be preserved over a long time period of time

without degradation. Some layers (such as lenticular cherts) have areas with a more

compressed look, which may represent more "encapsulated" time, with less anatomical

fossil data preserved. In general, individual chert layers probably represent at most a few

months or a season of time over which plant material accumulated and was then

permineralized.

Preservation—This locality represents a Konservat-Lagerstätte (Seilacher et al., 1985;

Allison, 1988), as preservation of the material in the Princeton Chert is excellent. Delicate

tissues such as lacunate phellem and aerenchyma are found in plants from this locality.

Another example of the excellent preservation of the Princeton Chert is that in one

specimen of *Keratosperma allenbyense* Cevallos-Ferriz and Stockey (1988a) probable

mucilage is seen in the seed micropyle, comparable to that found in the living seeds related

to this taxon (Smith and Stockey, 2003).

Also, some rarely fossilized plants are found here: several permineralized monocots are

known (Cevallos-Ferriz and Stockey, 1988a; Erwin and Stockey, 1989, 1991a, 1991b, 1992,

1994; Smith and Stockey, 2003). Monocots are rarely preserved in the fossil record due to

their herbaceous, frequently aquatic habit (Herendeen and Crane, 1995), but to date five

taxa have been described from the Princeton Chert. The rush/sedge

(Cyperaceae/Juncaceae) *Ethela sargantiana* Erwin & Stockey (1992) and "lily" (Liliales

sensu Cronquist, 1981; with the more recent changes in classification (e.g., APG, 2003),

reinvestigation may show different affinities for this plant) *Soleredera rhizomorpha* Erwin &

Stockey (1991a) are known from stems with attached roots and leaves. A water plantain

(Alismataceae), *Heleophyton helobiaeoides* (Erwin and Stockey, 1989), is known from

petioles, and seeds of *Keratosperma allenbyense* provide the earliest record for Araceae,

subfamily Lasioideae (Cevallos-Ferriz and Stockey, 1988a; Smith and Stockey, 2003). A

small fan palm (Arecaceae), *Uhlia allenbyensis* Erwin & Stockey (1991b, 1994), is known

from stems with attached petioles, roots, midribs and laminae. In addition, there are at least

two distinct monocot vegetative axes whose affinities remain elusive (pers. obs.).

Fungi are abundant in the Princeton Chert. There is evidence for mycorrhizal

associations in the roots of *Pinus* and *Metasequoia milleri* (Basinger, 1981; LePage et al.,

1997; Stockey et al., 2001). Despite the excellent preservation, there is some degradation of

material. Sometimes presumably saprophytic fungi are found in seeds, on leaves or in

rhizomes (LePage et al., 1994). Others, such as the ascomycetous tar spot

Paleoserenomyces Currah, Stockey & LePage found on palm leaves, give insight into plant-

fungus interactions from the past (Currah et al., 1997).

Trewin (1996) looked at preservation effects in the Rhynie Chert and how they relate to the amount of degradation plants underwent before permineralization. In the Princeton Chert, such a range of preservation has not been reported, but continued investigation with this in mind may reveal more preservation types. Many plants are fairly complete, with little degradation. Sometimes fungal hyphae are found in plant tissues, and fragments of plant organs do occur. Many of the undescribed monocot axes tend to be degraded in the middle of the axis and filled with chert, like Trewin's (1996) "straws" (plant axes that are filled with matrix in the centre).

The present work—Given the aquatic nature of the locality and its exceptional preservation, it is not surprising that we may find rare types of fossils in the Princeton Chert. My thesis focuses on one such example. Here I describe the remains of an inflorescence, hundreds of flowers and fruits, and pollen of a fossil saururaceous taxon as distinct chapters presented in paper format. I plan to publish each chapter as a paper, in the same order that they are presented here. While these fossils were previously thought to be alismatid flowers and fruits (Currah and Stockey, 1991; LePage et al., 1994; Stockey 1994, 2001, 2006; Pigg and Stockey 1996; Smith and Stockey 2004, 2005), anatomy and three-dimensional reconstructions have allowed detailed investigations that have shown the correct identity of these fossils as Saururaceae (Smith and Stockey, 2006).

Saururaceae are a small family consisting of four genera and six species: *Anemopsis californica* Hook et Arnott, *Gymnotheca chinensis* Decaisne, *G. involucrata* Pei, *Houttuynia cordata* Thunb., *Saururus cernuus* L., and *S. chinensis* (Lour.) Baill. (Wu and Kubitzki, 1993). The family is likely monophyletic and is sister to Piperaceae in the order Piperales (Tucker and Douglas, 1996; Meng et al., 2002, 2003; Jaramillo et al., 2004;

Neinhuis et al., 2005). As circumscribed by APG II (2003) Piperales also include the

families Aristolochiaceae, Hydnoraceae and Lactoridaceae. The order is sister to

Canellales (=Winterales) in the larger magnoliid clade that also includes Laurales and

Magnoliales (Qiu et al., 1999, 2000, 2005; Graham and Olmstead, 2000; Nickrent et al.,

2002; Zanis et al., 2002; Borsch et al., 2003; Hilu et al., 2003; Soltis and Soltis 2004;

Graham et al., 2006).

Relationships within Saururaceae have been examined using both morphological

(Tucker et al., 1993; Tucker and Douglas, 1996; Meng et al., 2003) and molecular (Meng

et al., 2002, 2003; Jaramillo et al., 2004; Neinhuis et al., 2005) data. Analyses tend to result

in three hypotheses of relationships in Saururaceae: 1) *Saururus* sister to the rest of

Piperaceae and Saururaceae (Saururaceae not monophyletic); 2) *Saururus* sister to the rest

of Saururaceae, and *Gymnotheca* sister to *Anemopsis* + *Houttuynia*; 3) *Saururus* +

Gymnotheca sister to *Anemopsis* + *Houttuynia*. Analyses using molecular data or

combined morphological-molecular data tend to recover the third topology (Meng et al.,

2003; Jaramillo et al., 2004; Neinhuis et al., 2005).

The six species of Saururaceae are found in Asia and North America. The Asian

species, *Saururus chinensis*, *Houttuynia cordata*, *Gymnotheca involucrata* and *G.*

chinensis, are found in wet areas, including forests, streambanks and lakes (Wu and

Kubitzki, 1993; Liang, 1995; Xia and Brach, 1999). *Anemopsis californica* is found in

western North America, and grows in alkaline waters; *Saururus cernuus* grows in the

wetlands of eastern North America (Wu and Kubitzki, 1993; Liang, 1995; Xia and Brach,

1999). There is some horticultural interest in North America in these wetland plants:

lizard's tail (*S. cernuus*), chameleon plant (*Houttuynia*) and yerba mansa (*Anemopsis*) are grown in gardens.

Fossils of Saururaceae are relatively rarely recognized. The only previously known fossils to date for this family are fruits and seeds of *Saururus bilobatus* (Nikitin) Mai from the late Eocene to Pliocene of Europe and Siberia and seeds of *Houttuynia bavarica* Mai from the Miocene of Germany (Mai and Walther, 1978; Friis, 1985; Lesiak, 1994; Mai, 1999). These are known from gross morphology and have not been anatomically examined. No fossil vegetative or pollen remains have been firmly placed in the family Saururaceae.

The plants in Saururaceae all have a rhizomatous, sympodial growth habit. Flowers are borne on racemes (*Saururus, Gymnotheca*) or spikes (*Anemopsis, Houttuynia*) (Liang and Tucker, 1990; Xia and Brach, 1999). Infloresences of *Anemopsis, Gymnotheca involucrata* and *Houttuynia* have large basal bracts that resemble petals, giving the inflorescence the appearance of a single flower, much like Asteraceae inflorescences (Classen-Bockhoff, 1990; Wu and Kubitzki, 1993). All species have flowers that are subtended by a bract and lack perianth. Flowers of *Saururus* and *Gymnotheca* have six stamens and four carpels; those of *Anemopsis*, six stamens and three carpels; and flowers of *Houttuynia* have three stamens and three carpels (Liang and Tucker, 1990; Wu and Kubitzki, 1993; Xia and Brach, 1999). Flowers of the different taxa vary in the degree of connation and adnation of parts. *Saururus cernuus* flowers have free stamens and basally connate carpels; in *S. chinensis* stamens are basally adnate to the basally connate gynoecium (Liang and Tucker, 1990). The flowers of *Gymnotheca chinensis* and *G. involucrata* are similar; both have connate carpels with free styles and stamens adnate for

most of the length of the carpels (Liang and Tucker, 1990). In *Houttuynia*, stamens are adnate to about halfway up the syncarpous ovary (Liang and Tucker, 1990). *Anemopsis* presents a unique floral state in Saururaceae, as flowers are sunken into the inflorescence axis; the three carpels are connate, and stamens are adnate to the ovary for most of its length (Liang and Tucker, 1990). Flowers of *Anemopsis* and *Saururus* are pollinated by wind, insect, or so-called "insect-mediated wind pollination" mechanisms (Tanaka, 1979; Holtzman, 1990; Thien et al., 1994).

In Chapter 2, I describe fossil floral remains of saururaceous affinity. Hundreds of flowers, ca. 1 mm in diameter, are examined morphologically and anatomically. These fossils are described as a new species, *Saururus tuckerae* sp. nov. In addition, phylogenetic analyses using a morphological data set and including the fossil are performed.

Fruits associated with this flower type prompted a survey of anatomy and morphology of extant *Saururus* fruits (Chapter 3), since they have not been studied before. This study allows anatomical comparisons of the fossil fruits with extant fruits of *Saururus*. The fossil fruits are also examined for anatomy and morphology (Chapter 4). Various developmental stages are preserved in the Princeton Chert, and prove these fruits are those of *Saururus tuckerae*. Three-dimensional reconstructions and scanning electron microscopy are used to compare the Princeton fruits to the fossil fruits of *Saururus bilobatus* (Nikitin) Mai from Europe and Siberia. Fruit characters allow for an amplified concept of *Saururus tuckerae* and further distinguish the Princeton fossils from other species of *Saururus*.

Finally, a study on pollen morphology and ultrastructure of Saururaceae is presented in Chapter 5. Since pollen is found in the anthers of the fossil flowers of *Saururus tuckerae*, it provides another line of evidence that the fossils are saururaceous, and additional

evidence that the fossils represent a distinct species. There have been sporadic reports of

pollen characters of Saururaceae over the years, the earliest publications being Erdtman

(1952) and Ikuse (1956). However, there are conflicting reports of some characters (e.g.,

smooth or punctate sculpturing reported for the same species, presence/absence of

endexine). There are also few studies that examine more than one or two species. For

example, *Gymnotheca chinensis* pollen is illustrated once in the literature, by Liang (1992),

and *G. involucrata* pollen twice (Xi, 1980; Liang, 1992). Few studies have used scanning

electron microscopy (SEM) and fewer still, transmission electron microscopy (TEM).

Because of the small size of the grains – less than 20 µm – observations under traditional

light microscopy are very difficult, and electron microscopy is a very necessary tool. Thus,

all species of Saururaceae were examined using both SEM and TEM to confirm the

previously reported characters and to provide a complete study of Saururaceae pollen.

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Table 1.1. Taxa recognized from the Princeton Chert floristic assemblage.

Taxon	References	Affinities	Plant organs
Pteridopsida			
Filicales			
Dennstaedtiopsis aerenchymata	Basinger, 1976a; Basinger and Rothwell, 1977	Dennstaedtiaceae	rhizomes, petioles
Makotopteris princetonensis	Stockey et al., 1999	Athyriaceae	rhizomes, roots, fronds, sori, spores
Dickwhitea allenbyensis	Karafit et al., 2006	Athyriaceae	rhizomes
Trawetsia princetonensis	Basinger 1976a, 1981; Smith et al., 2006	Blechnaceae	roots, rhizomes, petioles
Osmunda	G. W. Rothwell, pers. comm., 2004	Osmundaceae	stipes
Coniferopsida			
Coniferales			
Pinus andersonii	Stockey, 1984	Pinaceae	leaves
Pinus arnoldii	Miller, 1973; Stockey, 1984	Pinaceae	ovulate cones
Pinus princetonensis	Stockey, 1984	Pinaceae	ovulate cones
Pinus similkameenensis	Miller, 1973; Stockey, 1984	Pinaceae	leaves, twigs
Metasequoia milleri	Basinger, 1976, 1981, 1984; Basinger and Rothwell, 1977; Rothwell and Basinger, 1979	Cupressaceae	ovulate & pollen cones, stems, leaves, roots
Angiosperms			
Basal angiosperms			
Magnoliales			
Liriodendroxylon princetonensis	Cevallos-Ferriz and Stockey, 1990b	Magnoliaceae	woody twigs

Table 1.1, continued

Nymphaeales

Allenbya collinsonae	Cevallos-Ferriz and Stockey, 1989	Nymphaeaceae	seeds

Laurales

"Similkameena borealis"	Little, 2006	Lauraceae	inflorescences, flowers, fruits, twigs, leaves

Piperales

"Saururus tuckerae"	Currah and Stockey, 1991; this thesis	Saururaceae	inflorescences, flowers, fruits, pollen

Monocots

Alismatales

Keratosperma allenbyense	Cevallos-Ferriz and Stockey, 1988a Smith and Stockey, 2003	Araceae (Lasioideae)	seeds
Heleophyton helobiaeoides	Erwin and Stockey, 1989	Alismataceae	petiole

"Liliales"

Soleredera rhizomorpha	Erwin and Stockey, 1991a	"Liliales"	stems, attached leaves & roots

Poales

Ethela sargantiana	Erwin and Stockey, 1992	Juncaceae/Cyperaceae	stems, attached leaves & roots

Arecales

Uhlia allenbyensis	Erwin and Stockey, 1991b, 1994	Arecaceae(Coryphoideae)	stems, attached petioles and roots, midribs, laminae
undescribed monocot 1	pers. obs.	?	stems, attached roots
undescribed monocot 2	pers. obs.	?	stems

Table 1.1, continued

Eudicots

Cornales

Diplopanax eydei	Stockey et al., 1997	Cornaceae (Mastixioideae)	fruits

Saxifragales

Ribes sp.	Cevallos-Ferriz, 1995	Grossulariaceae	fruits, seeds

unplaced order

Ampelocissus similkameenensis	Cevallos-Ferriz and Stockey, 1990a	Vitaceae	seeds
Seed-types 1 and 2	Cevallos-Ferriz and Stockey, 1990a	Vitaceae	seeds

Myrtales

Decodon allenbyensis	Cevallos-Ferriz and Stockey, 1988b Little and Stockey, 2003, 2004, 2006	Lythraceae	fruits, seeds, woody axes, roots, leaves
cf. *Lythrum*	Cevallos-Ferriz and Stockey, 1988b	Lythraceae	fruits, seeds
Paleomyrtinaea princetonensis	Pigg et al., 1993	Myrtaceae	fruits, seeds

Rosales

Paleorosa similkameenensis	Basinger, 1976b; Cevallos-Ferriz et al., 1991a	Rosaceae	flowers, pollen
Prunus allenbyensis	Cevallos-Ferriz and Stockey, 1990c	Rosaceae	woody twigs
Prunus sp. (three types)	Cevallos-Ferriz and Stockey, 1991	Rosaceae	fruits

Sapindales

Wehrwolfea striata	Erwin and Stockey, 1990	Sapindaceae	flowers, pollen

Table 1.1, continued

Incertae sedis

Eorhiza arnoldii	Robison and Person, 1973; Stockey and Pigg, 1994	?	stems with attached roots, axillary branches, leaves
Princetonia allenbyensis	Stockey, 1987; Stockey and Pigg, 1991	?	inflorescences, flowers, fruits, seeds, pollen

(modified from Pigg and Stockey, 1996; angiosperm suprageneric names follow APG II (2003) classification).

Chapter 2

Fossil saururaceous flowers from the Middle Eocene Princeton Chert

The Middle Eocene Princeton Chert locality, in south-central British Columbia,

Canada, represents a site of exceptionally well-preserved plant fossils. Plant remains are

silicified in three dimensions in anatomical detail. This preservation allows for detailed

study, sometimes enabling the reconstruction of whole plants, e.g., *Metasequoia milleri*

Rothwell & Basinger (Basinger, 1976a, 1981, 1984; Rothwell and Basinger, 1979). The

Princeton Chert also preserves evidence of plants related to modern families for which

fossils are rarely found, such as various monocot familiess, whose herbaceous and often

aquatic habit typically precludes their having a high preservation potential (Herendeen and

Crane, 1995; Stockey, 2006). To date, numerous fungi, five ferns, a

cupressaceous/taxodiaceaous conifer, at least two pinaceous conifers, and at least 24

angiosperms (including five monocots) have been recognized from the Princeton Chert

assemblage (Pigg and Stockey, 1996; Table 1.1) with more taxa still to be described and

discovered. This site preserves a wetland environment, with many fossil taxa whose

modern-day relatives grow in marginal aquatic areas and others that likely represent the

vegetation bordering a small pond or lake (Cevallos-Ferriz et al., 1991), similar to the

swampy environments in present-day southeastern United States.

Five types of fossil flowers have been recognized from the Princeton Chert: *Paleorosa*

similkameenensis Basinger (1976b) (Rosaceae) (Cevallos-Ferriz et al., 1993), *Wehrwolfea*

striata Erwin and Stockey (1990) (Sapindaceae), *Princetonia allenbyensis* Stockey (1987)

(incertae sedis) (Stockey and Pigg, 1991), a lauraceous flower borne in inflorescences (Sun

and Stockey, 1991; Little and Stockey, 2003; Little, 2006) and a flower thought to have

affinities to Alismatales (Currah and Stockey, 1991; Stockey, 1994, 2001, 2006; Pigg and

Stockey, 1996; Smith and Stockey, 2004, 2005). All of these flowers are known from

anatomically preserved material including pollen. In this chapter I describe this last flower

type. I demonstrate that while this type is similar to certain alismatids, its floral structure,

inflorescence structure, and anther contents suggest that it is related to Saururaceae

(Piperales).

MATERIALS AND METHODS

Fossil material—Fossils were collected from the Princeton Chert outcrop, located on

the east bank of the Similkameen River, 8.4 km south of the town of Princeton, British

Columbia (Boneham, 1968). The outcrop consists of interbedded layers of chert and coal,

with occasional ash layers (Stockey, 1983). There are about 49 major chert layers, but

these split and anastomose to make approximately 70 individual beds ranging in thickness

from 1 to 50 cm (Smith et al., 2006). The Princeton Chert is part of the Princeton Group,

Allenby Formation (Boneham, 1968). A Middle Eocene age has been determined based

on data from freshwater fish (Wilson, 1977, 1982), mammals (Russell, 1935; Gazin, 1953)

and K-Ar dating (Hills and Baadsgaard, 1967). The ash of Layer #22 is currently dated at

48.7 million years (H. Baadsgaard, pers. comm., 1999).

Flowers are commonly found in Layer #43. Other plants co-occuring with the flowers

described here include stems, roots, fruits and seeds of *Decodon allenbyensis* Cevallos-

Ferriz & Stockey (1988b; Little and Stockey, 2003, 2005), seeds of *Keratosperma*

allenbyense Cevallos-Ferriz & Stockey emend. Smith & Stockey (Cevallos-Ferriz and

Stockey, 1988a; Smith and Stockey, 2003), fruits and seeds of *Paleomyrtinaea* Pigg,

Stockey & Maxwell (1993), undescribed monocot vegetative remains, and rare seeds of

Allenbya collinsonae Cevallos-Ferriz & Stockey (1989).

Chert blocks were cut into slabs and studied using the cellulose acetate peel technique

(Joy et al., 1956) modified for concentrated (48%) hydrofluoric acid (Basinger and

Rothwell, 1977; Basinger, 1981). Peels were mounted on microscope slides using Eukitt

(O. Kindler GmbH, Freiburg, Germany) xylene-soluble mounting medium. Images were

captured with a PowerPhase digital scanning camera (Phase One, A/S, Fredriksberg,

Denmark) and a MicroLumina digital scanning camera (Leaf Systems, Bedford,

Massachusetts). Photographs were processed with Adobe Photoshop CS. Three-

dimensional reconstructions were done using photos of serial sections (taken with a Nikon

Coolpix 5400) and the computer visualization software AMIRA 3.1.1 (TGS Software, San

Diego, California, USA).

Electron Microscopy—Scanning Electron Microscopy (SEM) of the fossil pollen

was done using the back side of deeply etched peels. Peel sections were mounted on

double-sided tape on stubs, and covered with 150 Å gold using a Nanotek Semprep II

sputter coater. Samples were observed using a JEOL 6301F (Field Emission Scanning

Electron Microscope). Extant pollen from herbarium sheets was examined in the same

way.

Fossil pollen was prepared for Transmission Electron Microscopy (TEM) by dissolving

the acetate matrix in two changes of acetone, washing with distilled water, then

demineralizing in concentrated (48%) hydrofluoric acid, followed by several washes with

distilled water, and centrifuging after each change. The samples were then placed in 70%

EtOH, rinsed twice with distilled water, placed in 2% OsO_4 for 2 hours, rinsed again in

distilled water and embedded in Spurr's (1969) resin, following an ethanol/propylene oxide

dehydration series. Sections were cut at 80 nm using a diamond knife, collected on 300

mesh formvar coated grids, and stained using uranyl actetate and lead citrate. Sections were

observed using a Philips Morgagni 268 EM.

Pollen from extant *Saururus* was sampled from herbarium material (*S. cernuus*: ALTA

5509, E. H. Moss s. n., 31 July 1914; *S. chinensis*: LSU 72527, Liang Hanxing 8709, 15

June 1987). Pollen was prepared for TEM by an initial fixation in FPA overnight followed

by a wash with distilled water, and was then placed in 2% OsO_4 for 2-3.5 hours. Samples

were then rinsed twice in distilled water and embedded in Spurr's (1969) resin following an

ethanol/acetone dehydration series. Sections were cut at 60 nm using a diamond knife,

collected and observed as above.

Phylogenetics—The data matrices of Tucker et al. (1993), Tucker & Douglas (1996),

and Meng et al. (2003) were modified and the fossil data were added to form a new

morphological data set that was then analysed phylogenetically (Table 2.1, Appendix 2.1).

Molecular data (e.g., Nickrent et al., 2002, Neinhuis et al., 2005) have recognized

Piperaceae/Saururaceae as sister to a clade of Lactoridaceae, Hydnoraceae and

Aristolochiaceae. Analysis of the relationships of the fossil taxon among saururaceous taxa

used *Lactoris* Phil. (Lactoridaceae), *Aristolochia* L. and *Asarum* L. (Aristolochiaceae) as

outgroups. The ingroup consisted of *Piper* L., *Peperomia* Ruiz & Pavon, and *Zippelia*

Blume for Piperaceae, and *Saururus chinensis* (Lour.) Baill., *S. cernuus* L., *Gymnotheca*

Decaisne, *Anemopsis californica* Hook. & Arnott, and *Houttuynia cordata* Thunb. for

Saururaceae. *Piper* and *Peperomia* were coded as generic placeholders using polymorphic

characters. Where inferences about the ancestral states could be made (e.g., stamen and

carpel numbers; see Jaramillo and Manos, 2001), these states were used to represent the genus, rather than also including more derived states, and thus some characters are monomorphic. *Anemopsis* and *Houttuynia* are monotypic, and both species of *Saururus* were used. Although *Gymnotheca* has two species, they do not vary in the floral characters coded in the matrix, and so they were treated as one terminal taxon. The only previously known fossils, fruits and seeds of *Saururus bilobatus* (Nikitin) Mai from the Late Eocene to Pliocene of Europe and Siberia (Mai and Walther, 1978; Friis, 1985; Lesiak, 1994) and seeds of *Houttuynia bavarica* Mai (Mai, 1999), were not included, because of a lack of scorable characters.

Phylogenetic analyses were conducted using PAUP* version 4.0b10 (Swofford, 2002). Heuristic searches were performed using 1000 random addition replicates with tree-bisection-reconnection (TBR) branch swapping and MULTREES on. Characters were unordered and equally weighted. Analyses were done with or without the fossil taxon. Branch support was estimated using bootstrap analyses (Felsenstein, 1985), with 100 bootstrap replicates and the same search criteria as heuristic searches. Character state reconstructions were done using MacClade 4.08 (Maddison and Maddison, 2005).

RESULTS

*Systematics—Order—*Piperales Dumort

*Family—*Saururaceae Martynov

Genus—Saururus L.

Species—Saururus tuckerae Smith sp. nov.

Specific diagnosis— Inflorescence a raceme, at least 2.9 mm long and 1.0 mm diam. Flowers ca. 0.8 mm diam. Bract one, cup-shaped, ovate, subtending flower; flower-bract

stalk and pedicel present. Perianth absent. Stamens five, adnate to carpels, up to 0.8 mm long, tetrasporangiate, with latrorse longitudinal dehiscence. Pollen 6-11 µm, monosulcate, boat-shaped-elliptic; sculpturing punctate. Carpels four, basally connate, tapering at apex, up to 1.2 mm long, 0.4 mm wide. Styles one per carpel, recurved. Seeds one per carpel, attached marginally near base.

Holotype—P1631 Bbot a (Figs. 2.1-2.5).

Paratypes—P1631 Btop a, Btop b, Btop f, Btop h, Bbot c, Cbot e; P5831 Bbot; P5839 A; P5937 Gbot b; P5991 B (Figs. 2.6-2.10, 2.13-2.21, 2,25, 2.26, 2.31, 2.34-2.40).

Etymology—The specific epithet '*tuckerae*' is proposed in honour of Dr. Shirley Tucker, University of California-Santa Barbara, who has done much work on furthering our understanding of floral structure and ontogeny in Saururaceae.

Type locality—Princeton Chert, east bank of the Similkameen River, ca. 8.4 km south of Princeton, British Columbia, Canada. Princeton Map Sheet 92 H/7 (1:50 000) UTM 10U FK 783724).

Stratigraphy and age—Princeton Group, Allenby Fm.; Middle Eocene

Description—*Inflorescence and floral morphology*—A single specimen representing the apical portion of an inflorescence and several hundred isolated flowers have been found in the chert. The inflorescence is a raceme, the preserved portion 2.9 mm long and ca. 1.0 mm in diam (Figs. 2.1, 2.11). Flowers at the apex are very immature, and their bracts are larger than the androecium and gynoecium (Figs. 2.1, 2.3). Those at the base are more mature, showing well-developed anthers and poorly developed carpels (Figs. 2.1, 2.2, 2.4, 2.5). A cup-shaped bract subtends each flower, up to 0.4 mm long, 0.8 mm wide and 48 µm thick.

Flowers are minute, up to 1 mm long and 0.8 mm in diam (Figs. 2.1-2.10, 2.12-2.16). One vascular strand is found in the flower-bract stalk, which divides into multiple strands in the bract, and then into the strands supplying the flower itself (Figs. 2.6-2.10). A very short pedicel, up to 96 μm long, is apparent in some specimens and this separates the carpels and stamens from the bract (Figs. 2.8, 2.15). Distally, the bract becomes less enveloping (Figs. 2.6-2.10). There is no evidence of a perianth in any of the flowers.

Androecium—There are five stamens per flower, although some cross sections show only four (Figs. 2.12-2.14). Five complete flowers have been found and reconstructed, and they all have five stamens. They show that there is one adaxial median stamen and two pairs of lateral stamens. Stamens are adnate to the base of the carpel, and tend to be about the same height as the carpels, at least 0.8 mm long (Figs. 2.15, 2.16). Anthers are tetrasporangiate and most specimens retain pollen inside (Figs. 2.13, 2.14, 2.17). Older dehisced anthers show a longitudinal, latrorse dehiscence pattern (Fig. 2.18). Thickenings in the endothecium are apparent, especially towards the inside of the anther.

Pollen found within the anthers is minute, about 6-11 μm in diam, monosulcate, boat-shaped-elliptic and under light microscopy appears to be psilate (Fig. 2.20). Scanning electron microscopy reveals that the pollen grains have a punctate (or perforate, both sensu Punt et al., 1994) sculpturing and small granula on the aperture membrane (Fig. 2.26, 2.27). Transmission electron microscopy shows that the aperture membrane is thin and the pollen wall is tectate-columellate, up to 380 nm thick (Figs. 2.31, 2.34) (see Text-Fig. 5.1 for diagram of pollen wall stratification). The tectum is ca. 75-130 nm thick, the foot layer ca. 100-175 nm thick (Fig. 2.34). There are perforations of the tectum where the puncta

occur on the surface (Fig. 2.34). Columellae are irregularly spaced in section view and ca. 95-115 nm high (Fig. 2.34).

Pollen of extant Saururaceae—Pollen of *Saururus cernuus* and *S. chinensis* was examined using SEM and TEM. Extant *Saururus* pollen is boat-shaped-elliptic, monosulcate, with granula on the aperture membrane. Grains of *S. cernuus* are ca. 11-13 µm in diam (Figs. 2.21, 2.22), and those of *S. chinensis* ca. 10-12 µm in diam (Figs. 2.23, 2.24). The exine has a pronounced punctate sculpturing and lacks supratectal sculpturing (Figs. 2.21-2.24, 2.25, 2.28). The puncta in extant *Saururus* pollen have raised edges (Figs. 2.25, 2.28, 2.32, 2.33). In both species, the aperture membrane is thin with less developed ectexine, and there are granula (Figs. 2.29, 2.30). In *Saururus cernuus* the ectexine is ca. 450-500 nm thick (Figs. 2.29, 2.32). The tectum is ca. 95-190 nm thick, and the foot layer is ca. 230-270 nm thick. Columellae are irregularly spaced, ca. 95-170 nm tall. The area between the tectum and foot layer is irregular in shape (Fig. 2.32). A dark layer below the foot layer may represent endexine (Fig. 2.32). Ectexine of *S. chinensis* is ca. 360-380 nm thick (Figs. 2.30, 2.33). Columellae are irregularly spaced and very short, ca. 20-95 nm (Fig. 2.33). The tectum is ca. 190-290 nm thick, and the foot layer is ca. 75-170 nm thick (Fig. 2.33). A dark-staining layer below the foot layer may represent endexine (Fig. 2.33).

Gynoecium—Each flower has four carpels, which are basally connate (Fig. 2.35). Carpels are wider at the base (up to 0.4 mm in diam) and taper near the top (ca. 0.1 mm in diam), and are up to 1.2 mm long (Figs. 2.35-2.38). Each carpel has two lateral lobes that are apparent in cross section (Figs. 2.13, 2.35, 2.36). Cells of the carpel wall are generally small (Figs. 2.35-2.39) but the innermost layer is composed of large, thin-walled cells (Figs. 2.35, 2.39). There is one recurved style per carpel (Figs. 2.37, 2.38) and the stigmatic

surface appears to be papillate (Fig. 2.38, arrow). Each carpel is uniloculate with a single

ovule (Fig. 2.35, 2.38, 2.39). Ovule attachment is marginal, towards the base of the carpel,

and helically thickened tracheary elements are preserved in the funiculus of some

specimens (Figs. 2.39, 2.40). There is no evidence of embryos or other internal tissues.

Fungal hyphae are occasionally found inside the ovule or along the cell walls of the carpel.

Spherical fungal structures up to 100 µm in diam also occur in the outermost layers of the

carpels in some specimens. There are no meiospores or conidia found in these structures,

and their exact nature is not clear.

*Phylogenetic analyses—*Phylogenetic analyses of morphological data were done using

only extant taxa, or with the fossil taxon *S. tuckerae* included. Both analyses resulted in a

single most parsimonious tree of 53 steps (extant only; CI=0.679, RI=0.696) or 55 steps

(with fossil taxon; CI=0.673, RI=0.714) (Fig. 2.41). *Lactoris* is sister to (*Aristolochia* +

Asarum). Both Piperaceae and Saururaceae are found to be monophyletic. *Zippelia* is

found to be the sister group of all other Piperaceae. Within Saururaceae, (*Saururus* +

Gymnotheca) is sister to (*Anemopsis* + *Houttuynia*). When the fossil is included in

analyses, it is found sister to extant *Saururus*.

Character evolution was examined in MacClade (Maddison and Maddison, 2005) for

the single most parsimonious tree with the fossil included (Fig. 2.41). Piperaceae are

supported by three synapomorphies: presence of a sessile stigma (character 21), one ovule

per gynoecium (character 23) and basal placentation (character 24). Saururaceae are

supported by having boat-shaped pollen (character 15). A clade of *Anemopsis* +

Houttuynia is supported by two characters showing homoplasy: having sessile flowers

(character 4) and having three carpels (character 18). The presence of a flower-bract stalk

(character 5) and four carpels (character 17; this is homoplasious) support the

Gymnotheca-Saururus clade. The *Saururus* clade is supported by three characters: basally

connate carpels (character 19), one to two ovules per carpel (character 23), and having

marginal placentation (character 24). Only the first is non-homoplasious. Extant *Saururus*

are supported by the presence of trichomes on the bract (character 7).

DISCUSSION

Affinities of the fossil flowers— These fossil flowers have previously been

thought to represent an undescribed alismatid taxon (Currah and Stockey, 1991; Stockey,

1994, 2001, 2006; Pigg and Stockey, 1996; Smith and Stockey, 2004, 2005).

Morphologically, the fossil flowers bear resemblances to some families of Alismatales

(Aponogetonaceae, Juncaginaceae and Potamogetonaceae) and Piperales (Saururaceae), all

of which have minute flowers borne on spikes (or racemes) with few or no perianth parts

and often four carpels. The zygomorphic flowers of Aponogetonaceae typically have two

tepals, six stamens with longitudinal, extrorse dehiscence, and three free carpels, each with

2-12 ovules and a short style, but some species show variability in numbers of parts

(Dahlgren et al., 1985; van Bruggen, 1998). Flowers of Potamogetonaceae are

actinomorphic, usually with four tepals (adnate to the androecium), four stamens with

longitudinal extrorse dehiscence, and four free carpels with a short style and single ovule

(Haynes et al., 1998b). The genus *Maundia* F. Muell. (Juncaginaceae) has four weakly

connate carpels; but *Maundia* flowers have no bract, 2-4 tepals, up to eight stamens, and

the carpels have no style (Haynes et al., 1998a).

Flower structure of the fossils is even more similar to that seen in Saururaceae.

Saururaceae are a small family (four genera and six species) of herbaceous, rhizomatous

plants that usually grow in damp or marshy environments (Wu and Kubitzki, 1993; Xia and Brach, 1999). *Anemopsis* and *Houttuynia* are monotypic, while *Gymnotheca* and *Saururus* each have two extant species. Typical saururaceous flowers are minute, borne on a spike or raceme, with a bract and no perianth, three (*Houttuynia*) or six (*Saururus, Gymnotheca* and *Anemopsis*) stamens and three (*Houttuynia* and *Anemopsis*) or four (*Saururus, Gymnotheca*) carpels (Liang and Tucker, 1990; Wu and Kubitzki, 1993; Igersheim and Endress, 1998). *Saururus* and *Gymnotheca* flowers have a stalk bearing both bract and flower – the "flower-bract stalk" (sensu Liang and Tucker, 1990). Stamens show a strong degree of adnation to carpels, except in *Saururus cernuus,* where stamens are free. In all species of Saururaceae carpels are connate at least at the base, except *S. cernuus,* which is apocarpous (Liang and Tucker, 1990; Igersheim and Endress, 1998). Mature fossil flowers have a flower-bract stalk, a bract, no perianth, and five stamens basally adnate to a four-carpellate, basally connate gynoecium.

While variations on the typical floral plan might allow the inclusion of the fossil in Aponogetonaceae, Potamogetonaceae, Juncaginaceae or Saururaceae, pollen morphology is very more diagnostic. Pollen of Aponogetonaceae is 21-45 µm in diam, ellipsoidal, monosulcate, per-reticulate exine sculpturing, with supratectectal spinules (Erdtman, 1952; Thanikaimoni, 1985; van Bruggen, 1998). In Potamogetonaceae, pollen is inaperturate, ellipsoid to spheroidal, 20-30 µm in diam with homobrochate exine sculpturing (Haynes et al., 1998b; Erdtman, 1952). Pollen grains in *Maundia* (Juncaginaceae) are globose, inaperturate, 27-30 µm in diam (Erdtman, 1952; Hope, G: Australian National University Pollen Database, 2006). Thus, pollen grains in Alismatales are at least twice as large as the fossil pollen grains, with reticulate (rather than punctate in the fossil pollen) sculpturing.

Pollen of Potamogetonaceae and Juncaginaceae is inaperturate, whereas the fossil pollen is monosulcate.

The pollen of the fossil taxon is a key feature for placing it within Saururaceae. Saururaceous pollen is characterized as minute (mostly <15μm) in size, boat-shaped-elliptic to globose, monosulcate with granula in the aperture membrane, and an otherwise punctate sculpturing (Erdtman, 1952; Walker, 1976; Xi, 1980; Takahashi, 1986; Grayum, 1992; Liang, 1992; Pontieri and Sage, 1999; Sampson, 2000). The term 'foveolate' has been used to describe pollen of Saururaceae by authors who define foveolate as pitted exine sculpturing (Walker and Doyle, 1975). Under the definition of Punt et al. (1994) foveolate refers to sculpturing with holes larger than 1 μm in diam, whereas saururaceous pollen is punctate, perforate, or scrobiculate, having tectal holes less than 1 μm in diam (Punt et al., 1994). The fossil pollen shows these same features as pollen of Saururaceae. The fossil grains were originally thought to be fungal spores (Currah and Stockey, 1991; LePage et al., 1994), but I now recognize that size, shape and structure of the fossil pollen are clearly characteristic of Saururaceae.

Within Saururaceae, flowers of *Saururus,* in particular, show similarities to the fossil flowers. *Saururus* flowers are developed on a racemose inflorescence. Flowers of *Saururus* have a flower-bract stalk, which is longer in *S. chinensis* than in *S. cernuus* (Liang and Tucker, 1990) and diverges at a low angle from the inflorescence axis, and flowers are pedicellate. Flowers of *Saururus chinensis* have basally fused carpels (Raju, 1961; Tucker, 1976; Liang and Tucker, 1990), like those in the fossil taxon, but *S. cernuus* is apocarpous. In *Saururus* flowers, there are six stamens initiated in pairs (Tucker, 1975). In *S. cernuus* the stamens have long filaments, are distinct from the gynoecium, and overtop the carpels

at maturity (Raju, 1961; Liang and Tucker, 1990). In *S. chinensis*, stamens are fused

partway up the gynoecium, and filaments are shorter and thicker than in *S. cernuus* (Raju,

1961; Liang and Tucker, 1990). Stamen features of the fossil flowers are most similar to

those of *S. chinensis*. The flowers of *Saururus* are reported to be protogynous, with the

stigma being receptive prior to anthesis, as are many magnoliids (Thien et al., 1994, 2000).

It is difficult to determine exactly if the fossil is protogynous or protandrous (with the

stamens maturing before carpels are receptive). However, the fossil flowers are similar to

extant *Saururus* in having pre-anthesis stage anthers overtopping the carpels and, after

dehiscence, carpels are somewhat larger than the stamens. *Saururus* is self-incompatible

(Pontieri and Sage, 1999), so it is possible that the stigmas are receptive before the stamens

dehisce, without reducing the chances of outcrossing.

There are several differences between the fossil flowers and those of extant *Saururus*.

In size, the fossil flowers are smaller, being ca. 0.8 mm in diam compared to 1.4 mm diam

in *S. chinensis* and 1.7 mm diam in *S. cernuus* (Liang and Tucker, 1990). Flowers of extant

Saururus have trichomes on the bracts and inflorescence axis, but no trichomes are seen in

the fossil material. Bracts are more ovate and cup-shaped in the fossil than in extant

Saururus. Stamens are five in the fossil, and typically six in extant *Saururus*. The fossil

taxon also differs from extant *Saururus* in certain features of the pollen grain: grains of *S.

cernuus* and *S. chinensis* are typically larger than those of the fossil, have fewer and smaller

puncta than the fossil, and the puncta have raised edges not seen in the fossil. In addition,

TEM shows that the pollen wall of the fossil specimens has a more open and regularly

spaced columellate layer than in extant *Saururus*. *Saururus cernuus* has a thicker ectexine

than in the fossil grains. Thus, these fossil flowers clearly fit in Saururaceae, and are most

similar to *Saururus*. However, the differences in size, presence/absence of trichomes,

number of stamens, and pollen features between the fossil specimens and extant *Saururus*

species warrant the description of a new species, *Saururus tuckerae* sp. nov.

Phylogenetics—Piperales have sometimes been considered as a potential sister group

of the monocots, and Burger (1977) details many of the similarities between

Piperaceae/Saururaceae and the alismatid families Aponogetonaceae, Potamogetonaceae

and Araceae. Some cladistic analyses support this idea (Doyle and Endress, 2000;

Barkman et al., 2004). However, in recent molecular analyses, the Piperales are usually

found to be sister to Canellales (=Winterales), in a clade with Laurales and Magnoliales: the

magnoliid clade (Qiu et al., 1999, 2000, 2005; Graham and Olmstead, 2000; Nickrent et

al., 2002; Zanis et al., 2002; APG, 2003; Borsch et al., 2003; Hilu et al., 2003; Soltis and

Soltis 2004; Graham et al., 2006). Soltis et al. (2000a, 2000b) found Piperales as sister to

the other magnoliids. Barkman et al. (2004), using *mat*R data, found Piperales sister to

monocots, as did the combined *rbc*L, 18S, *atp*B and morphological data set of Doyle and

Endress (2000). The relationship of the magnoliid clade to other angiosperms has not yet

been resolved. In the studies mentioned above a variety of topologies involving magnoliids,

Chloranthales, monocots, eudicots and Ceratophyllaceae are found. Further work is

needed to elucidate the relationship between magnoliids and other angiosperms.

Based on molecular data, Saururaceae are consistently accepted as a monophyletic

group within the Piperales, which as circumscribed by APG (2003) consists of

Aristolochiaceae, Hydnoraceae, Lactoridaceae, Piperaceae and Saururaceae. Although

relationships within the order are still somewhat uncertain (APG, 2003), Saururaceae and

Piperaceae are always found as sister groups. Nuclear and mitochondrial data support

inclusion of Hydnoraceae in this order, but delineating the relationships between Hydnoraceae, Lactoridaceae and Aristolochiaceae requires further study (Gonzalez and Rudall, 2001; Nickrent et al., 2002; APG, 2003). In most studies, a clade of (Piperaceae + Saururaceae) is found to be sister to (Aristolochiaceae + Lactoridaceae + Hydnoraceae) (when the latter family is included) (Qiu et al., 1999, 2000, 2005; Doyle and Endress, 2000; Mathews and Donoghue, 2000; Nickrent et al., 2002; Zanis et al., 2002; Borsch et al., 2003; Neinhuis et al., 2005). Occasionally a different topology, such as Asaroideae sister to the (Piperaceae + Saururaceae) clade (Hilu et al., 2003), is found. Chloroplast, mitochondrial and nuclear DNA often support the monotypic Lactoridaceae as sister to Aristolochioideae, within Aristolochiaceae (Qiu et al., 1999, 2005; Doyle and Endress, 2000; Soltis et al., 2000a, 2000b; Zanis et al., 2002; Borsch et al., 2003; Neinhuis et al., 2005) but sometimes alternate hypotheses, such as *Lactoris* sister to Saururaceae (with Piperaceae not included) are found (Graham and Olmstead, 2000).

Several previous studies have examined relationships within Saururaceae. Morphological data were used by Tucker et al. (1993), who analysed the data in different ways. Their results found three recurrent hypotheses of relationships in Saururaceae: 1) *Saururus* sister to the rest of Saururaceae and Piperaceae (Saururaceae not monophyletic); 2) *Saururus* sister to the rest of Saururaceae, and *Gymnotheca* sister to *Anemopsis* + *Houttuynia*; 3) *Saururus* + *Gymnotheca* sister to *Anemopsis* + *Houttuynia*. The second topology is supported by morphological analyses (Tucker and Douglas, 1996) and *atp*B or 18S data (Jaramillo et al., 2004). A fourth topology has resulted from analyses using nuclear genes, with *Anemopsis* basal (Meng et al., 2001, 2003). Meng et al. (2003) further resolved the tree to *Saururus* + *Gymnotheca* sister to *Houttuynia*. This topology was not seen in

morphological analyses or other analyses with molecular data. In his study on karyomorphology, Okada (1986) also showed that *Saururus* is basal, although *Gymnotheca* was not included; thus the second or third topologies are equally likely.

However, there is growing support for the third topology [(*Saururus* + *Gymnotheca*), (*Anemopsis* + *Houttuynia*)]. Molecular analyses, using data from the plastid *rbc*L, *atp*B, *trn*L-*trn*F regions and mitochondrial *matR* and nuclear 18S genes, tend to support this topology (Meng et al., 2002, 2003; Jaramillo et al., 2004; Neinhuis et al., 2005), although (as one might expect) relationships vary when one genus is excluded (e.g., Nickrent et al., 2002; Qiu et al., 2005). The morphological and combined molecular-morphological analyses by Meng et al. (2003) also resulted in a single tree with this topology. Furthermore, the analyses using morphological data from my study, both with and without the fossil taxon included, resulted in a single most parsimonious tree with this topology. The fossil taxon, *S. tuckerae*, is always found in a clade with extant *Saururus*, supporting the placement of the fossil with this genus. In order to resolve relationships between saururaceous taxa confidently, future studies should include *G. involucrata* and *G. chinensis* in addition to the more commonly used taxa *Anemopsis*, *Houttuynia* and *Saururus*.

Variability of flower structure— Although Saururaceae are often described as having three or six stamens and three or four carpels, flowers with other numbers of parts have been documented. In their investigation of *Gymnotheca*, Liang and Tucker (1989) noted the presence of abnormal flowers; some had five or seven stamens rather than six, or three carpels instead of the usual four. Further investigations by Liang (1994) found that *Gymnotheca* flowers had anywhere from 4-8 stamens on three- or four-carpellate flowers.

Liang (1994) examined 381 flowers and found only 79% had the "typical state" of four

carpels and six stamens; nearly 11% had four carpels and seven stamens, while 6% had four

carpels and five stamens, as seen in the fossil flowers. In *Anemopsis,* flowers usually have

six stamens and three carpels. Abnormal *Anemopsis* flowers include those with five

stamens, two carpels, or unisexual staminate flowers (Tucker, 1985). In *Houttuynia,*

flowers at the apex of the inflorescence are unisexual and reduced (Raju, 1961; Tucker,

1981). *Saururus* flowers are also reported to have variable numbers of parts, from five to

eight stamens and three or four carpels (Raju, 1961; Tucker, 1975). Tucker (1975) noted

that these flowers tend to be those found closest to the apex of the inflorescence. Thus,

while the fossil taxon at first seems to have an unusual stamen number compared to other

Saururaceae, as determined from the five complete flowers studied, five stamens are

known from abnormal extant saururaceous flowers.

Variability in the number of floral parts in Saururaceae, as discussed above, and in

Piperaceae, has been noted by several workers (Tucker, 1975, 1981, 1982, 1985; Liang

and Tucker, 1989; Liang, 1994; Jaramillo and Manos, 2001; Remizowa et al., 2005).

Jaramillo and Manos (2001) noted that a variable number of parts in flowers of *Piper*

seems to be correlated with the packaging of flowers in the inflorescence. Loosely spaced

flowers are less constrained in carpel number and tend to have more stamens than tightly

packed ones (Jaramillo and Manos, 2001).

Ontogenetic sequences of stamen development in Piperaceae and Saururaceae are well

understood; these are summarized in Jaramillo et al. (2004), and for Saururaceae in Liang

(1994) and Hufford (1997). All Piperaceae as well as *Anemopsis* and *Houttuynia* start with

the initiation of two lateral stamen primordia. In contrast, the first-initiated primordia in

Saururus are two median stamen primordia (Tucker, 1975; Liang, 1994; Jaramillo et al., 2004). *Gymnotheca* initially has one adaxial median stamen primordium (Liang and Tucker, 1989; Liang, 1994; Jaramillo et al., 2004). However, the development of stamen primordia in *Gymnotheca* then becomes similar to that of *Anemopsis*, *Houttuynia*, *Piper* and *Zippelia* (Jaramillo et al., 2004).

Heterochronic processes have been suggested as a mechanism for evolutionary change (loss or gain) of number or order of initiation of floral organ primordia in Saururaceae (Tucker et al., 1993; Hufford, 1997; Jaramillo et al., 2004). Jaramillo et al. (2004) suggested that the ancestral condition for stamen development was that of *Houttuynia*, with three stamen primordia. This condition is seen in some species of *Piper*, *Houttuynia*, and as an intermediate stage in *Zippelia*, *Anemopsis*, and *Gymnotheca* (the latter three all develop six stamens in total) (Jaramillo et al., 2004). *Peperomia* and some species of *Piper* have only two stamens, which could have resulted from the loss of a primordium. The most parsimonious interpretation of the evolution of androecial states has *Saururus* with a novel change in stamen ontogeny, so that it does not show the primitive floral characters of the family, as previously suggested (Raju, 1961; Liang and Tucker, 1990; Liang, 1994, 1995). The fossil flower with its five stamens and clear affinities to *Saururus*, supports the interpretation of the *Saururus* clade with novel character states, and as Jaramillo et al. (2004) suggested, the six-staminate condition is homoplasious in Saururaceae when ontogeny is taken into account. Although I cannot determine the exact androecial developmental sequence in the fossil flower, the differences seen in the androecium developmental pathways of other saururaceous taxa would support derivation from a simple, three-staminate flower.

Pseudanthia— Raju (1961) first proposed that individual flowers of Saururaceae are pseudanthia, that is, they are actually a reduced inflorescence taking the appearance of a flower. This scenario has been suggested for many taxa, including those in the monocot families Triuridaceae, Aponogetonaceae, Potamogetonaceae, Scheuchzeriaceae and Juncaginaceae (Burger, 1977; Posluszny et al., 1986; Rudall, 2003). Raju's (1961) evidence for pseudanthial saururaceous flowers, such as spirally arranged floral organs and lack of stamens on the last formed carpel, has been refuted by the work of Tucker (1976), Omori (1982) and Liang and Tucker (1990). Tucker (1976) also pointed out that no unisexual flowers have been found in *Saururus*, and that *Saururus* flowers are bilateral, not radial, as are most inflorescences. In addition, no branched inflorescences have been observed in Saururaceae (Tucker, 1976); although floral parts show plasticity, the inflorescence structure does not.

Conclusions—Aquatic and semi-aquatic plants are known to have highly convergent morphological characters (Barrett and Graham, 1997), such as lacunate phellem (Little and Stockey, 2005) and aerenchyma (Cevallos-Ferriz et al., 1991). This may also explain parallelisms in the floral structure of some Piperales and Alismatales, orders that both have wetland-adapted members, such as spicate inflorescences and small flowers with a reduced perianth. Developing an understanding of potential ancestral magnoliid characters through globally parsimonious character reconstructions across magnoliid orders may be useful for direct comparisons with basal monocots (such as Acorales and Alismatales – especially Araceae and Tofieldiaceae) and Chloranthaceae. Also, better understanding of which structures are homologous and which are homoplasious will help to elucidate relationships between magnoliids and other angiosperms. For example, Piperaceae and Saururaceae are

commonly called the "perianthless Piperales" because they lack a perianth, although each flower does have a subtending bract. These flowers are frequently compared to those of alismatid groups such as Potamogetonaceae, Juncaginaceae and Aponogetonaceae (e.g., Burger, 1977), and the fossils of *S. tuckerae* were initially thought to represent one of these families (Currah and Stockey, 1991; Stockey, 1994, 2001; Pigg and Stockey, 1996; Smith and Stockey, 2004, 2005). Alismatids, other than *Scheuchzeria* and Alismataceae, all lack bracts, but have a reduced perianth whorl that can consist of a single tepal.

Recently, advancements have been made in understanding the genetic basis of floral development, e.g., through the ability to test for gene expression in situ (e.g., see Buzgo et al., 2004). Such techniques may be useful to test which floral structures are homologous and which are homoplasious. Particularly relevant for the groups mentioned in this paper might be the question of the nature of 'petaloid' (and bracteate) organs. Our knowledge of what defines a petal, and of what the homologous structures are among "non-typical" flowers, is lacking (Kramer and Jaramillo, 2005). Ontogenetic data have already proven useful for interpreting relationships among taxa (Tucker et al., 1993; Tucker & Douglas, 1996; Hufford, 1997; Jaramillo et al., 2004) and gene expression may provide further ontogenetic characters for phylogenetic inference of extant taxa.

Saururaceae have an interesting distribution, with four species native to eastern Asia (*Gymnotheca chinensis, G. involucrata, Houttuynia cordata,* and *Saururus chinensis*), one to western North America (*Anemopsis californica*) and one to eastern North America (*Saururus cernuus*) (Fig. 2.42). These plants prefer moist or wetland habitats (Wu and Kubitzki, 1993; Xia and Brach, 1999), a characteristic likely shared by the fossil plant described here. This provides yet another piece of evidence that the Princeton Chert

represents a wetland environment. *Saururus tuckerae* is always found with *Decodon allenbyensis*. Layer #43 likely represented a marginal area of a small lake or pond, with *Decodon* and *S. tuckerae* growing along the edge of, and out into the water. Today, *Decodon* and *Saururus* do occasionally co-occur in swampy areas of the southeastern United States (Bennett, 2001; J. Richard Abbott, pers. comm., 2006), and these environments might represent a close modern analog to the Middle Eocene floral assemblage of the Princeton Chert.

The fossil record of Saururaceae is sparse. There is no palynological record (see Muller, 1981), and Song et al. (2004) suggest piperaceous pollen is neglected because it is geologically insignificant and the grains are minute in size. The same could be said for saururaceous pollen. If more fossil pollen records like the one presented here were found for these two groups, we could better understand the past geographic distribution and stratigraphic occurrence of this magnoliid group. The only previously known fossil Saururaceae species are seeds of *Houttuynia bavarica* Mai, from the Lower Miocene of Germany (Mai, 1999) and fruits and seeds of *Saururus bilobatus* (Nikitin ex Dorofeev) Mai, from the Upper Eocene to Pliocene of Europe and Siberia (Mai and Walther, 1978; Friis, 1985; Lesiak, 1994). Thus, the inflorescence and flowers of *S. tuckerae* represent the oldest macrofossils and first North American record for Saururaceae.

Early Cretaceous fruits and associated pollen described as *Appomattoxia ancistrophora* Friis, Pedersen & Crane (1995) have been suggested to have affinities to Piperaceae, Saururaceae, Chloranthaceae or *Circaeaster* (Circaeasteraceae, Ranunculales). Although it is similar in having a thick nexine and sculptured sulcus, *Appomattoxia* pollen is quite distinct from that of Saururaceae in having a continuous (not perforate) tectum, a granular

to columellate infratectum, and verrucate to finely echinate tectal sculpturing (rather than smooth) (Friis et al., 1995). Until we better understand relationships among extant magnoliids, or find floral and vegetative material of *Appomattoxia*, relationships of this fossil will remain elusive (Friis et al., 1995).

Another fossil that has been compared closely to Saururaceae, as well as Piperaceae, Chloranthaceae, Disocoreaceae and Smilacaceae comes from the Aptian of Australia (Taylor and Hickey, 1990). It is known from leaves with attached lateral pistillate inflorescences (Taylor and Hickey, 1990). While the fossil resembles Saururaceae (among other families) in leaf characters and in having small apetalate flowers subtended by a bract, arranged in a spike, the presence of bracteoles (like Chloranthaceae) and single-carpeled flowers with truncate stigmas, is very dissimilar (Taylor and Hickey, 1990).

The occurrence of fossil *Saururus* in western North America, in combination with the fossil fruit record of Europe, shows that ancestral *Saururus* (and perhaps all Saururaceae) was once widespread. The cooling climate of the middle Paleogene may explain why Saururaceae became extinct from Europe and other areas, while it survived in southeast Asia and eastern North America where the climate remained relatively humid and subtropical (Liang, 1995). Developing better search patterns and, especially, looking for minute pollen grains with the characteristic structure of Saururaceae, will help provide unequivocal evidence of the former distribution and evolutionary history for Saururaceae. The Princeton fossils show that Saururaceae were well developed by the Middle Eocene. *Saururus tuckerae* sp. nov. represents the first fossil saururaceous flower, the first fossil pollen record and the first North American fossil species for the family.

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Table 2.1. Morphological data matrix used for phylogenetic analyses.

Character Taxon	1	10	20
Aristolochia	011000?00	02?02001?2	002010
Asarum	001000?05	12?0200112	002010
Lactoris	000000000	0000000000	000000
Piper	110102(01)1(23)	0001(12)01022	11(02)221
Peperomia	110102(01)11	0001201320	01(02)121
Zippelia	110001010	0001?01212	011221
Saururus cernuus	110011110	0011011210	001101
Saururus chinensis	110011110	0101011211	001101
Anemopsis	110101010	1201011022	001011
Gymnotheca	110011010	1211111212	001011
Houttuynia	110101012	0101111022	001011
Fossil *Saururus*	110010014	0101011211	00?101

Sources: Raju, 1961; Tucker, 1975, 1976, 1979, 1980, 1981, 1982, 1985; Xi, 1980; Omori,

1982; Liang, 1992; Kubitzki, 1993; Huber, 1993; Tebbs, 1993; Tucker et al., 1993; Wu

and Kubitzki, 1993; Liang and Tucker, 1995; Tucker and Douglas, 1996; Buddell and

Thieret, 1997; Bernardello et al., 1999; Lei and Lang, 1999; Tseng et al., 1999; Xia and

Brach, 1999; Sampson, 2000; Gonzalez and Rudall, 2001; Jaramillo and Manos, 2001;

Kelly, 2001; Mulder, 2003; Jaramillo et al., 2004.

Appendix 2.1. Characters used in morphological analysis

1. Inflorescence type

    0-solitary flower

    1-raceme or spike

2. Floral symmetry

    0-radial

    1-dorsiventral or zygomorphic

Flowers in Saururaceae have been described as radial, but developmental studies have shown these flowers are zygomorphic (see Liang and Tucker, 1990).

3. Flowers

    0-minute, inconspicuous

    1-showy

4. Presence of pedicel/peduncle

    0- pedicellate or pedunculate

    1- sessile

Solitary flowers will have a peduncle. Note that flowers in *Piper* are coded as sessile, but can be pedicellate in subsection *Arctottonia* (Jaramillo and Manos, 2001).

5. Flower-bract stalk

    0-floral-bract stalk absent

    1-floral-bract stalk present

In *Saururus* and *Gymnotheca* an elongated stalk bears the flower and bract (see Liang and Tucker, 1990).

6. Floral bract shape

    0-ovate

    1-linear/lanceolate, spatulate

    2-peltate

7. Trichomes on bract

    0-absent

    1-present

Appendix 2.1, continued

8. Perianth

    0-present

    1-absent

9. number of stamens

    0-six

    1-two

    2-three

    3-four

    4-five

    5-twelve

Stamens may develop in whorls or in pairs in different taxa, but for the purpose of this analysis the total number of stamens was used regardless of how they are borne. Note that here *Piper* is coded as having three or four stamens, but there are sections with two or six stamens (Jaramillo and Manos, 2001).

10. Stamen connation:

    0-stamens free

    1- stamens connate

11. Stamen adnation to carpels:

    0-free

    1-fused at base of carpel

    2-fused more than half carpel height

12. Length of filaments

    0-less than or equal to gynoecium height

    1-taller than gynoecium

13. Anther dehiscence:

    0-extrorse

    1-latrorse

14. Pollen aperture type

    0-monosulcate

    1-inaperturate

Appendix 2.1, continued

Note that the monosulcate type pollen can include occasionally trichotomosulcate grains, for example, *Houttuynia* (Liang, 1992)

15. Pollen shape

>0-globose

>1-boat-shaped-elliptic

Pollen of Saururaceae is coded here as being boat-shaped-elliptic, as it is not as globose as the pollen of Piperaceae. However, in a larger context (e.g., Doyle, 2005) both Saururaceae and Piperaceae may be interpreted as having globose pollen since that of Saururaceae is somewhat intermediate in shape.

16. Average pollen size

>0-greater than 20 μm

>1-less than 20 μm

17. Carpel number

>0- three

>1- six

>2-four

>3-one

Most species of *Piper* have a tricarpellate gynoecium, but in section *Ottonia* flowers have four carpels (Jaramillo and Manos, 2001).

18. Median sagittal carpels

>0-one carpel (abaxial) in median sagittal plane

>1-adaxial and abaxial carpels present in median sagittal plane

>2-one carpel (adaxial) in median sagittal plane

19. Carpel fusion

>0-apocarpous

>1-syncarpous only at base

>2-syncarpous most of carpel length

20. Styles and stigmas

>0-style and stigma numbers equal to carpel number

>1-style and stigma numbers less than carpel number

Appendix 2.1, continued

21. Style presence

> 0- style present (zonal growth between ovary and stigma)

> 1- sessile stigma (no zonal growth)

22. Stigma shape

> 0-capitate or tufted

> 1-stigmatic stylar cleft

> 2-divided stigma

23. Ovule number

> 0-three or more per carpel

> 1-one or two per carpel

> 2-one ovule per gynoecium

In Piperaceae carpels are fused and a single ovule is produced per gynoecium (Igersheim and Endress, 1998).

24. Placentation

> 0-marginal

> 1-parietal

> 2-basal

25. Ovule orientation

> 0-anatropous

> 1-orthotropous

Figures 2.1-2.5. Inflorescence (Holotype P1631 Bbot a). **2.1.** Longitudinal section through centre of apical part of inflorescence. P1631 Bbot #67a; scale bar = 500 µm. **2.2.** Tangential section through inflorescence. P1631 Bbot #58a; scale bar = 250 µm. **2.3.** Enlarged view of apical part of inflorescence, showing bracts with one pair of developing stamens. P1631 Bbot #67a; scale bar = 100 µm. **2.4.** Longitudinal section through one flower near basal part of inflorescence, with cup-shaped bract, large stamens, and small carpels. P1631 Bbot #68a; scale bar = 100 µm. **2.5.** Longitudinal section through flower showing stalk, gynoecial area, stamens and bract (arrow). P1631 Bbot #58a; scale bar = 100 µm. Abbreviations: B, bract; G, gynoecium; S, stamen.

Figures 2.1—2.5
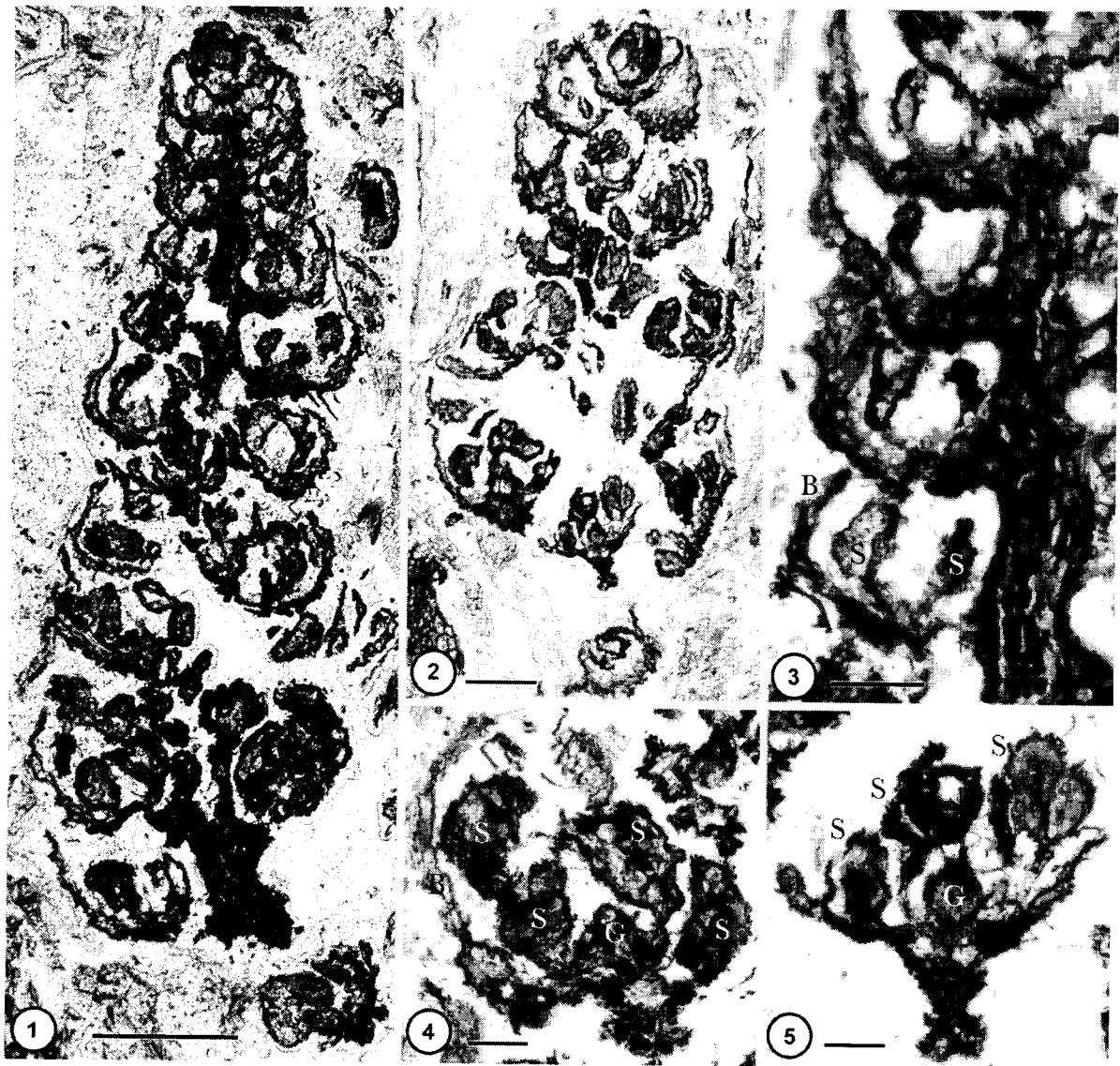
Figures 2.6-2.10. Series of oblique cross sections through one flower (Paratype

P1631 Bbot c). **2.6.** Section showing bract with one thick vascular bundle entering (v), and

small vascular bundles in tips of bract (arrows). P1631 Bbot #32c; scale bar = 100 μm. **2.7.**

Similar view to that in figure 2.6, but part of flower is visible in cup-shaped bract. Arrows

indicate vascular bundles in bracts. P1631 Bbot#34c; scale bar = 100 μm. **2.8.** Distal

section showing carpels, pedicel (arrow), and thin bract surrounding flower on all sides.

P1631 Bbot #36c; scale bar = 100 μm. **2.9.** Bract (arrowhead) separate from stalk, and

carpels are more distinct. P1631 Bbot #38c; scale bar = 100 μm. **2.10.** Four basally fused

carpels, with subtending bract (arrowhead). P1631 Bbot #40c; scale bar = 100 μm.

Figures 2.6—2.10
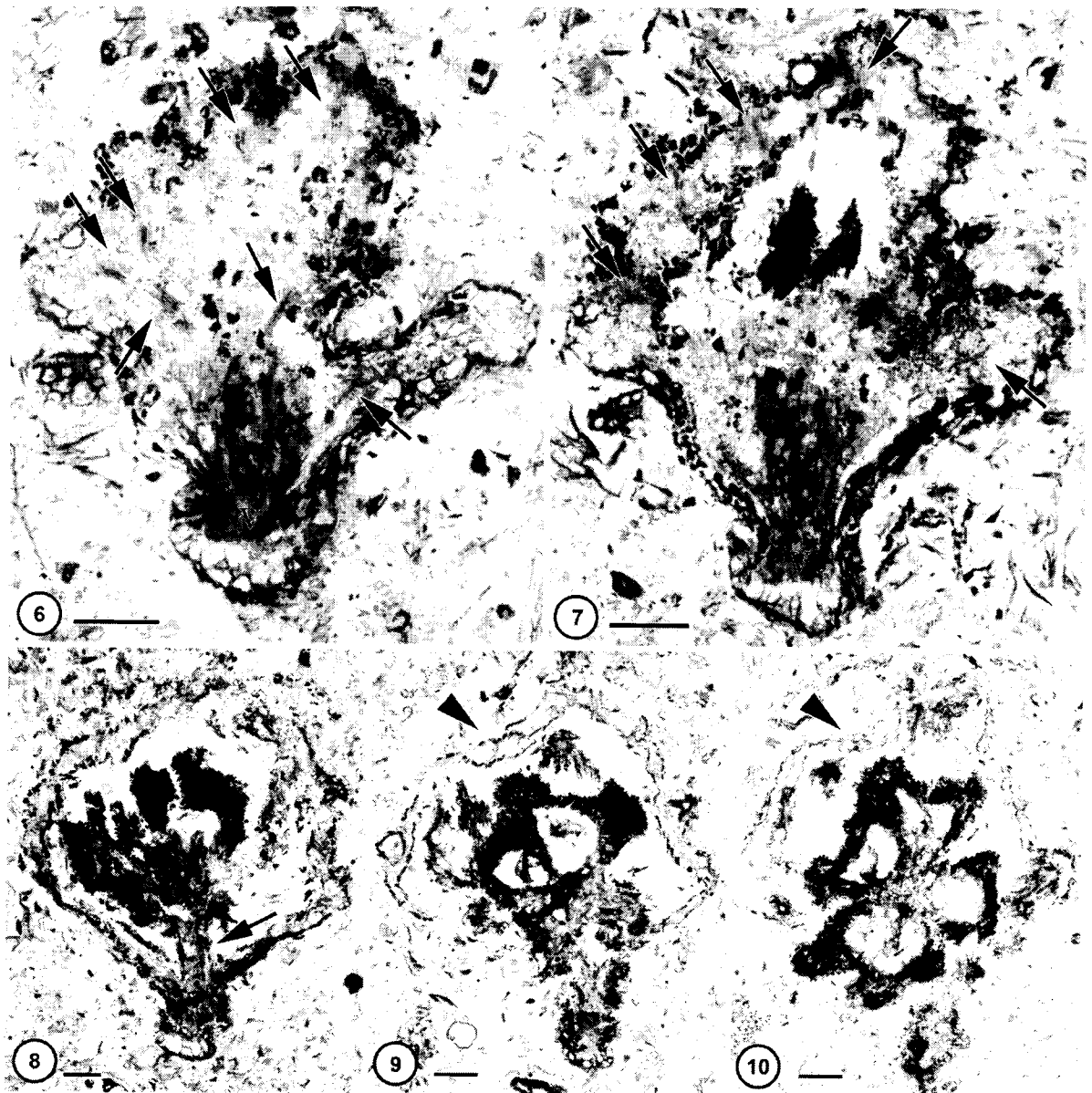
Figures 2.11-2.12. Computer reconstructions based on serial sections through specimens. **2.11.** Inflorescence showing bracts (white); androecium and gynoecium (green) and inflorescence axis (brown). Reconstructed from Holotype P1631 Bbot a. **2.12.** Flower, showing cup-shaped bract (white), five stamens (yellow) about the same height as gynoecium, four carpels (green) with styles reflexing outwards, and inflorescence axis (brown). Reconstructed from Paratype P5937 Gbot b.

Figures 2.11—2.12
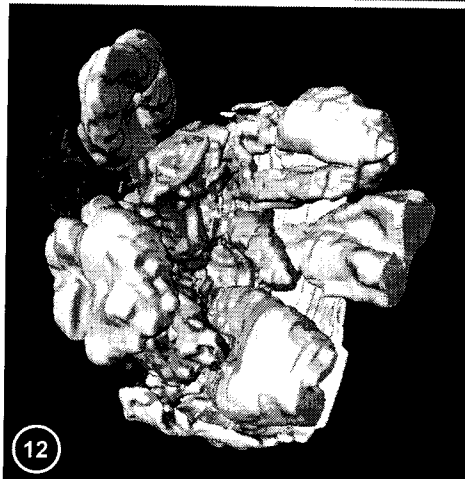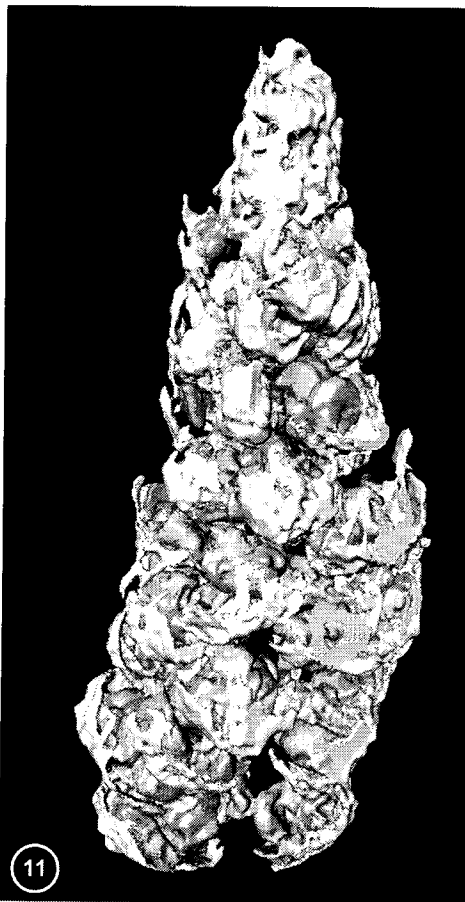
Figures 2.13-2.20. General flower structure and stamens. **2.13.** Cross section through flower at distal level, showing subtending bract, four stamens and four carpels. Paratype P5839 A #0; scale bar = 100 μm. **2.14.** Cross section through flower showing five stamens, and four carpels. Paratype P5937 Gbot #41b; scale bar = 100 μm. **2.15.** Oblique longitudinal section through flower showing bract, pedicel (at arrow), three (of four) carpels, and portions of two stamens, one of which is clearly attached to carpel base. Note thick filament. Paratype P1631 Btop #27h; scale bar = 100 μm. **2.16.** Longitudinal section showing two carpels, each with connate stamen. Paratype P1631 Cbot #69e; scale bar = 100 μm. **2.17.** Cross section through tetrathecal anther showing central vascular strand and enclosed pollen grains. Paratype P5937 Gbot #18b; scale bar = 100 μm. **2.18.** Two stamens showing latrorse dehiscence by longitudinal slits. Paratype P5831 Bbot #3; scale bar = 100 μm. **2.19.** Anther wall, showing endothecium with well-developed secondary thickenings, and small pollen grains. Paratype P1631 Btop #19a; scale bar = 10 μm. **2.20.** Boat-shaped-elliptic pollen grains as viewed using light microscopy. Paratype P5937 Gbot #18b; scale bar = 2 μm. Abbreviations: B, bract; C, carpel; S, stamen.

Figures 2.13—2.20
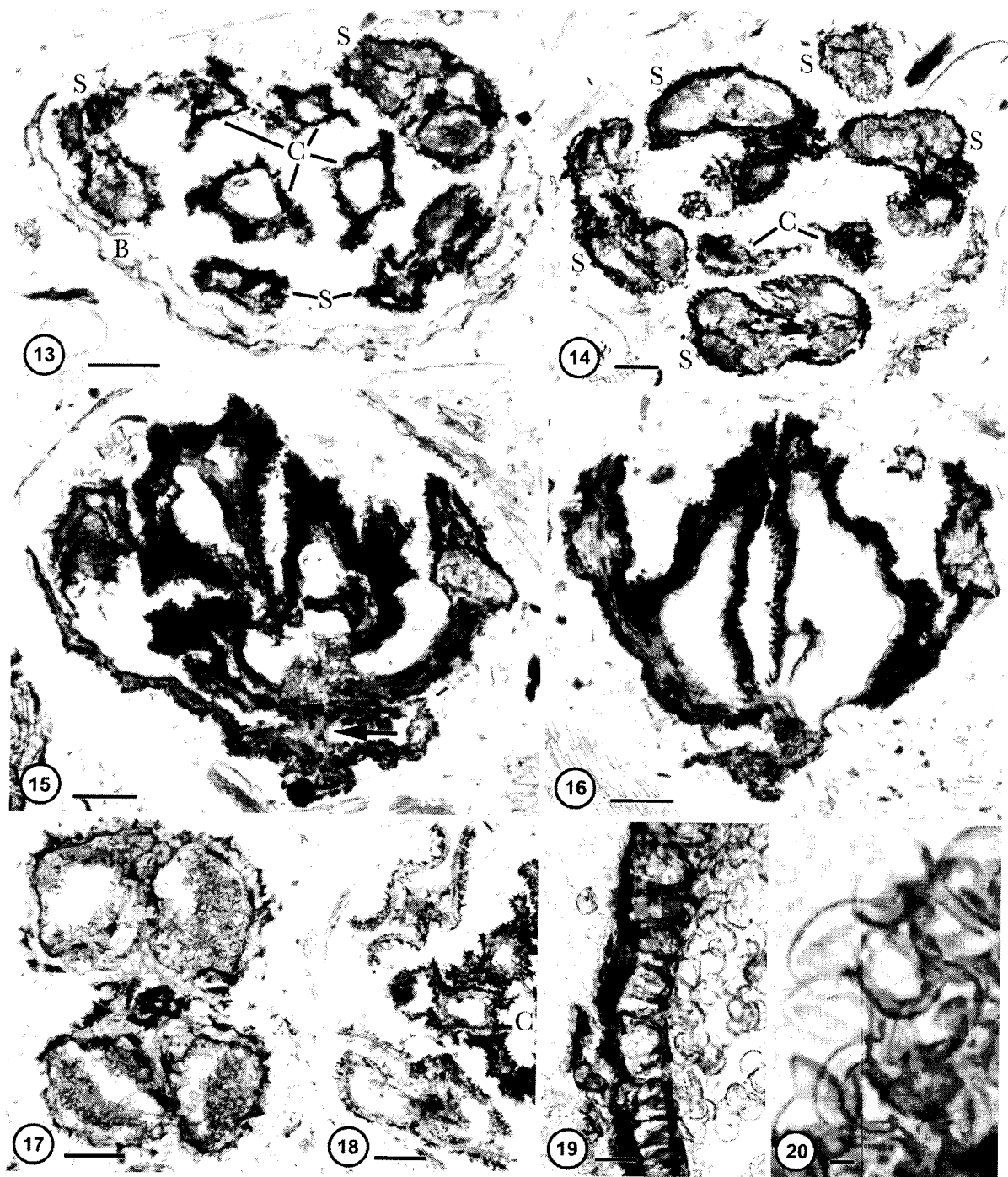
Figures 2.21-2.28. Scanning electron microscopy of *Saururus* pollen. **2.21.** *Saururus cernuus*, boat-shaped-elliptic pollen grains. ALTA 5509, stub A. **2.22.** *Saururus cernuus*, sulcus region with well-developed granula. ALTA 5509, stub A. **2.23.** *Saururus chinensis*, boat-shaped pollen grain viewed from side. LSU 72527, stub D. **2.24.** Pollen grain of *Saururus chinensis*, view of granula in sulcus region. LSU 72527, stub D. **2.25.** *Saururus cernuus* pollen, showing small punctae with raised edges. ALTA 5509, stub A. **2.26.** *Saururus tuckerae* sp. nov. pollen grains. Note small size, granula in sulcus region. Paratype P5991 B. **2.27.** *Saururus tuckerae* sp. nov. pollen showing many punctae. Paratype P5991 B. **2.28.** *Saururus chinensis* pollen, showing punctae with raised edges. LSU 72527, stub D. All scale bars = 1 μm.
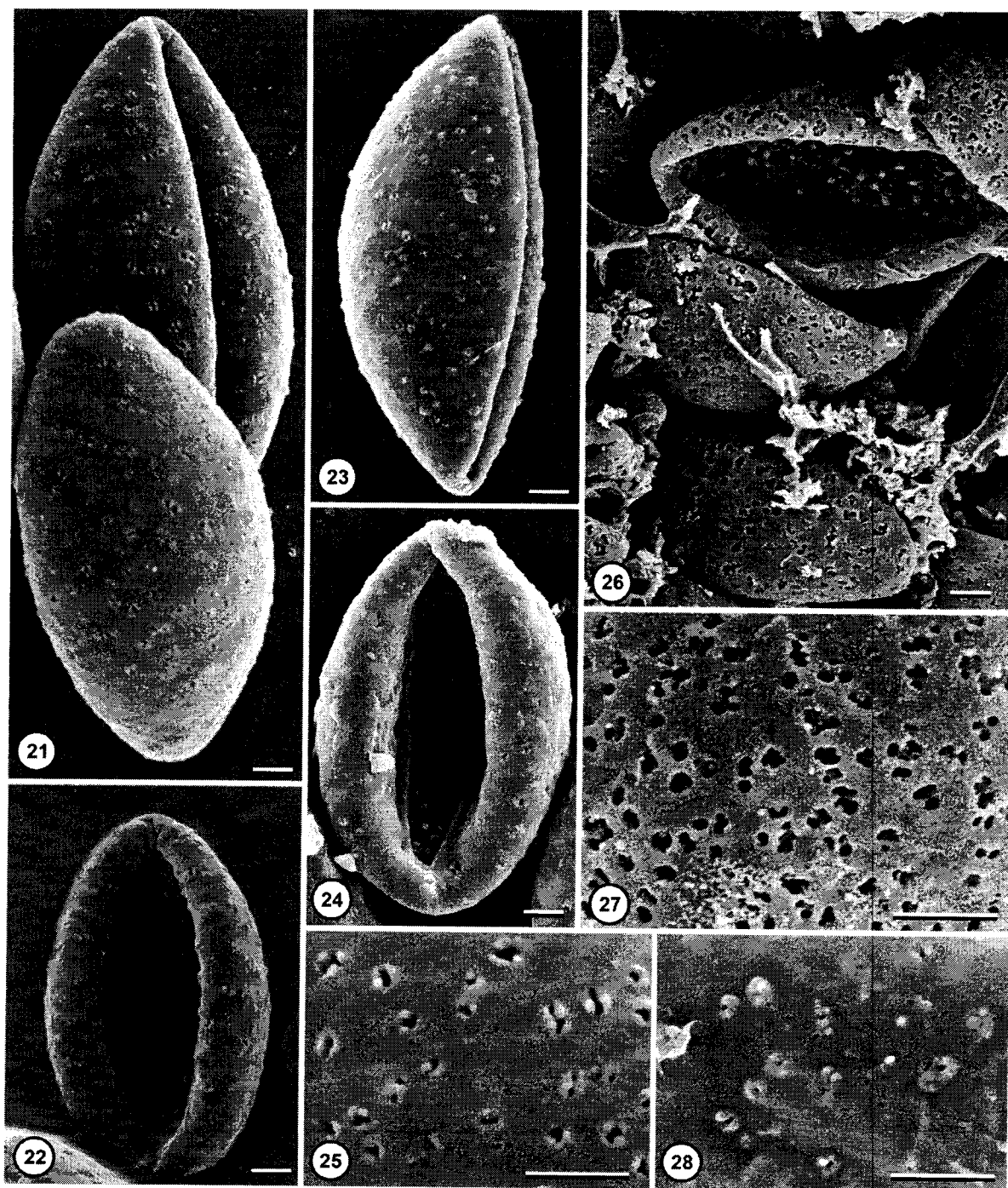
Figures   2.21—2.28

Figures 2.29-2.34. Transmission electron microscopy of *Saururus* pollen. **2.29.** Entire

grain of *S. cernuus*, showing aperture membrane with granula (top right). ALTA 5509.

2.30. Entire grain of *S. chinensis* showing sunken granulate aperture membrane. LSU

72527. **2.31.** Entire grain of *S. tuckerae* sp. nov. with thin, sunken aperture membrane.

P5991 B. **2.32.** Pollen wall of *S. cernuus* with punctate tectum (T), irregular columellar

layer (C), and thick foot layer (F). ALTA 5509. **2.33.** Pollen wall of *S. chinensis* with

punctate tectum (T; note raised edge of puncta), irregular columellar layer, and foot layer.

LSU 72527. **2.34.** Pollen wall of *S. tuckerae* sp. nov. with punctate tectum (T), well-

formed columellar layer (C), and thick foot layer (F). P5991 B. Scale bars: 2.29, 2.30, 2.31

= 1 μm; 2.32, 2.33, 2.34 = 200 nm.

Figures   2.29—2.34
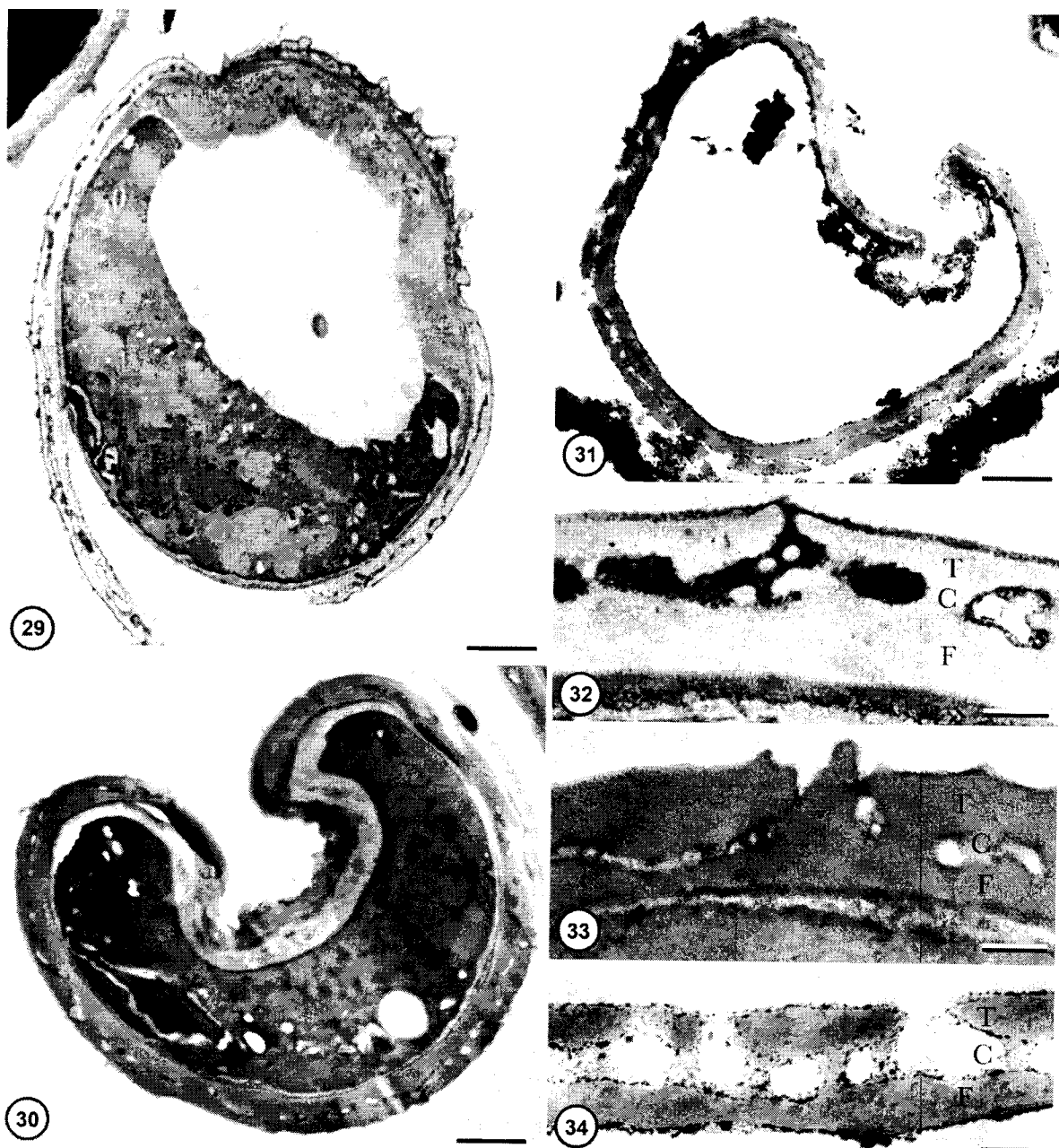
Figures 2.35-2.40. Gynoecium structure. **2.35.** Basal section showing four connate carpels. Note lobes on carpels (arrows) and ovule in each locule. Paratype P1631 Btop #27b; scale bar = 100 µm. **2.36.** More distal section, showing free carpels. Lobes are more prominent. Paratype P1631 Btop #38b; scale bar = 100 µm. **2.37.** Distalmost section showing two styles, recurved outwards. Paratype P1631 Btop #44b; scale bar = 100 µm. **2.38.** Longitudinal section through two basally fused carpels, with recurved styles; papillae indicated by arrow. Paratype P1631 Btop #104f; scale bar = 100 µm. **2.39.** Basal cross section through four carpels; single ovule visible in carpel at right. Paratype P1631 Bbot #43c; scale bar = 100 µm. **2.40.** Enlarged view of funiculus showing vascular tissue (arrow). Paratype P1631 Bbot #42c; scale bar = 50 µm. Abbreviations: C, carpel; E, endocarp; F, funiculus; OV, ovule.

Figures 2.35—2.40

Figure 2.41. The single most parsimonious tree found with the fossil taxon included, resulting from heuristic search including 11 extant taxa and fossil species of *Saururus* (length=55, CI=0.673, RI=0.714). Analysis of only extant taxa results in a single most parsimonious tree of the same topology (length=53, CI=0.679, RI=0.696). Numbers in bold above branches represent bootstrap values. Branch lengths calculated with ACCTRAN optimization (shown in parentheses above branches). Unambiguous character changes are shown below branches with character number followed in parentheses by states (from:to) (see Appendix 2.1 for character descriptions). Bars in grey indicate homoplasious state changes, while those in black indicate non-homoplasious changes.

Figure 2.41
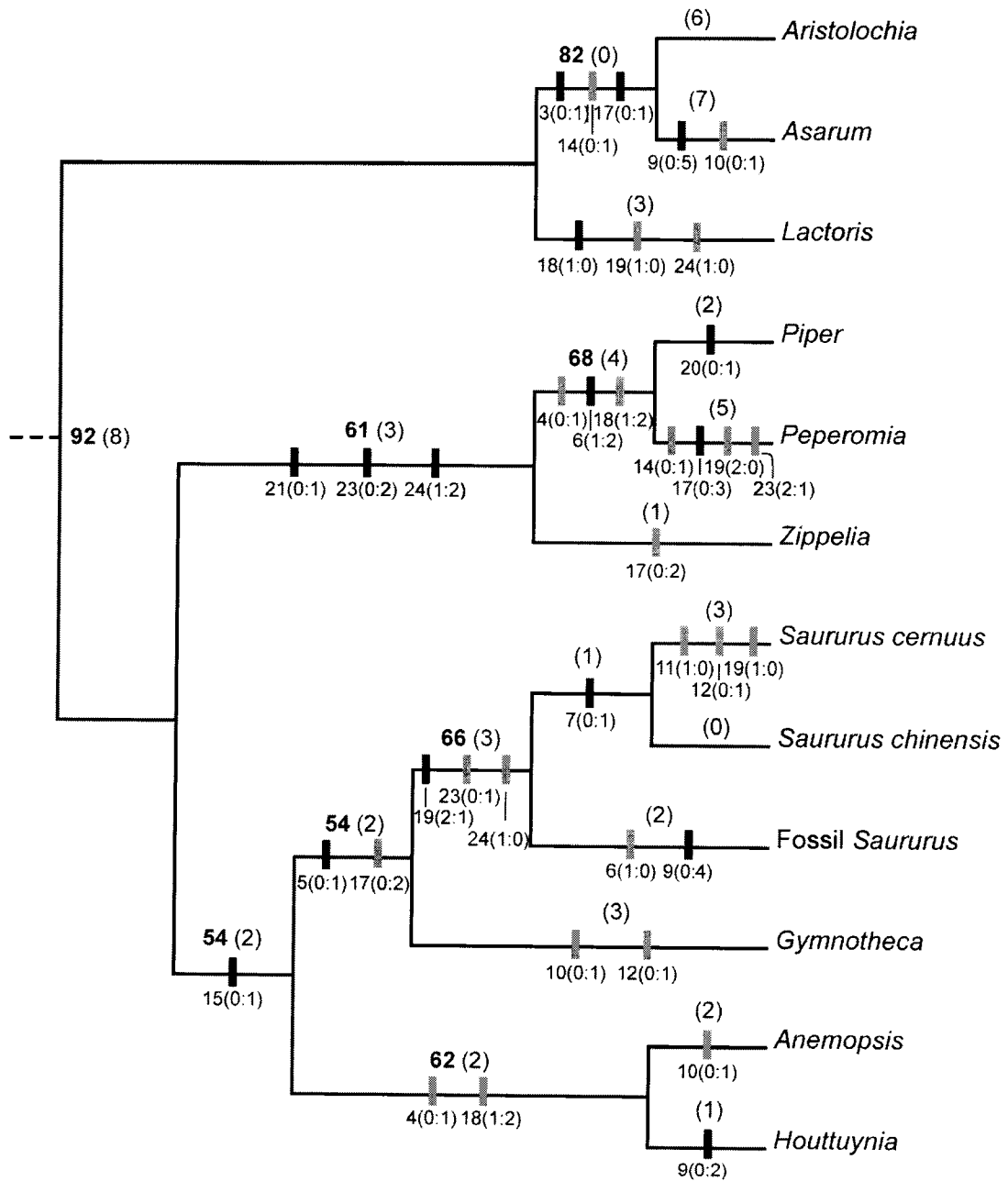
Figure 2.42. Distribution of extant and fossil Saururaceae (modified from Liang, 1995).

Note disjunct distribution of *Saururus* in Asia and eastern North America. Fossils fruits, *S. bilobatus,* indicated by asterisk; Princeton *Saururus tuckerae* sp. nov. indicated by triangle.

Figure   2.42
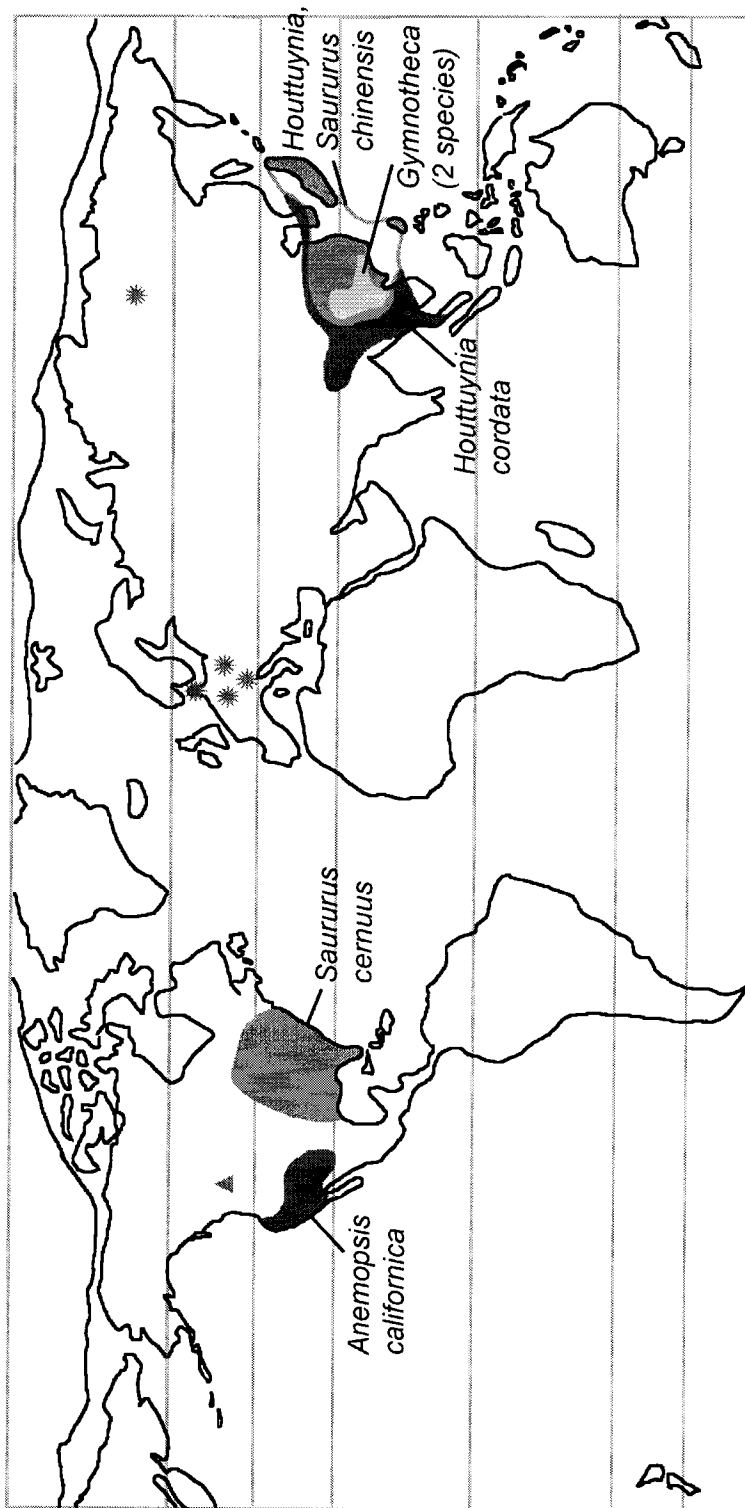
Chapter 3

Fruit anatomy of *Saururus cernuus* and *Saururus chinensis*

Saururaceae are a small family (six extant species) of rhizomatous, herbaceous plants

that grow in wetland or moist forest areas, found in Asia and North America: *Anemopsis*

californica Hook. & Arn., *Gymnotheca chinensis* Decne., *G. involucrata* Pei, *Houttuynia*

cordata Thunb., *Saururus cernuus* L. and *S. chinensis* (Lour.) Baill. These plants can

quickly colonize areas via clonal reproduction (Thien et al., 1994). Studies have shown that

Saururus cernuus and *Anemopsis* are self-incompatible (Thien et al., 1994; Pontieri and

Sage, 1997, 1999) and *Houttuynia* is apomictic and male-sterile (Takahashi, 1986; Pontieri

and Sage, 1997). A few fossil taxa have been recognized: seeds of *Houttuynia bavarica* Mai

and fruits and seeds of *Saururus bilobatus* (Nikitin) Mai, from the Late Eocene to Pliocene

of Europe and Siberia (Mai and Walther, 1978; Friis, 1985; Lesiak, 1994; Mai, 1999), and

more recently flowers and an inflorescence of *Saururus tuckerae* from the Middle Eocene

of North America (Chapter 2).

There are few reports concerning the pollination biology of these plants. Flowers are

borne in a raceme (*Saururus, Gymnotheca*) or spike (*Anemopsis, Houttuynia*) and all

except *Saururus* have larger, white showy bracts at the base of the inflorescence (Liang and

Tucker, 1990). *Saururus cernuus* flowers are borne on long racemes and produce a sweet

odor (Wood, 1971; Thien et al., 1994). Three different pollination modes have been

documented for *S. cernuus*: directly by insects, wind, or insect-mediated wind pollination.

Small flies (Diptera) feed on the pollen and, secondarily, stigmatic secretions; small beetles

(Coleoptera) eat pollen, and various sizes of bees and wasps (Hymenoptera) collect pollen

or look for larvae, respectively (Thien et al., 1994). In addition, the many small pollen

grains produced by the flowers can travel several metres on the wind (Thien et al., 1994).

Sometimes this wind pollination occurs with the help of large insects, such as dragonflies

landing on nodding inflorescences and releasing a pollen cloud (Thien et al., 1994). Leaves

just basal to the inflorescences in *Saururus chinensis* become white or white-green

variegated, which has been suggested to help attract pollinators (Wood, 1971; Liang, 1995).

Saururus chinensis produces more open racemes than *S. cernuus*, and flowers do not smell

as strong as in *S. cernuus* (Wood, 1971; Tanaka, 1979). Tanaka (1979) concluded that *S.

chinensis* was insect-pollinated rather than wind- or self-pollinated.

The inflorescences of *Saururus* are racemes that can be up to 30 cm long (Thien et al.,

1994). Flowers typically have a subtending bract, no perianth, six stamens and four carpels,

which are apocarpous in *S. cernuus* and basally connate in *S. chinensis* (Raju, 1961; Liang

and Tucker, 1990; Wu and Kubitzki, 1993). Carpels mature into wrinkled and warty, dry,

indehiscent fruits (Raju, 1961; Wood, 1971; Tucker, 1976; Wu and Kubitzki, 1993). The

fruit has been described as a berry (Raju, 1961; Tucker, 1976; Plisko, 1988; Wu and

Kubitzki, 1993). However, carpels are fused at the base and split at maturity (Wood, 1971;

pers. obs.), and thus the fruit is more appropriately called a schizocarp, with each carpel

representing an indehiscent fruitlet. Plants of *Saururus* generally grow in wet areas and the

fruits are reported to float in water, persisting in water for over three months (Thien et al.,

1994) (although Wood (1971) reports 95% of fruits sink within three hours). Wood ducks

may be a dispersal mechanism for *S. cernuus* in eastern North America, as some ducks

have been found with thousands of fruits in their stomach (Lesiak, 1994).

Fruits and seeds of *Saururus* have received little attention in the past. In the process of

describing the fossil fruits of *S. bilobatus*, Friis (1985) illustrated *S. cernuus* exterior

surfaces of mature fruitlets, seed integumentary layers, and pitted cells of the fruit wall using scanning electron microscopy. Other reports of fossil *Saururus* have shown the external morphology of *S. cernuus* fruits (Mai and Walther, 1978; Lesiak, 1994; Mai, 1999, 2001). Some illustrations have been published that show extant seed anatomy (Johnson, 1900; Raju, 1961; Wood, 1971; Plisko, 1988). While Plisko (1988) illustrated some details of fruits, fruit anatomy has generally not been shown in great detail. This paper aims to describe fruit and seed anatomy for *Saururus cernuus* and *S. chinensis* in several developmental stages, with the hope of providing both useful information for the understanding of living taxa, and criteria for better recognition of fossil *Saururus* fruits.

MATERIALS AND METHODS

Fruit material of *Saururus cernuus* was provided by the University of Florida Herbarium (FLAS) [USA: Florida, Columbia Co., Falling Creek Falls near Lake City, 28 July 1966, *W. G. D'Arcy 919* (FLAS 94853); Florida, Hillsborough Co., 27 June 1984, *D. White 623* (FLAS 174913)] and was rehydrated in distilled water prior to sectioning. Fresh floral material was also obtained from the Department of Biological Sciences greenhouses at the University of Alberta and a voucher deposited in the University of Alberta herbarium (ALTA 115823). Material of *Saururus chinensis* preserved in alcohol was provided by Harufumi Nishida, Chuo University, Tokyo, Japan and was collected by Hideo Takimoto, in Ibaraki Prefecture, Japan; this material is housed in the spirit collection of the University of Alberta Paleobotanical Collections (UAPC).

All material (except some flowers of *S. cernuus*) that was studied was dried, not fresh, and most carpels were broken apart and solitary. Three size classes of carpels were recognizable and form the basis for distinguishing between developmental stages. Small

carpels either in flower or of similar size to carpels in flower were called immature; the large, well-developed fruitlets were called mature; and a third category of intermediate-sized carpels was called intermediate-stage carpels.

Fruits and flowers were embedded and sectioned using standard paraffin wax technique (Johansen, 1940). Sections, 10 μm thick, were stained with safranin/fast green (Johansen. 1940) and mounted with DPX mounting medium (Electron Microscopy Sciences, Ft. Washington, Pennsylvania, USA). Measurements of extant fruits are given as coronal diameter (lateral width) x height x sagittal diameter (ventral-dorsal width) (Appendix 3.1; see Text-fig. 3.1 for diagram of planes of section). A two-tailed T-test performed in Microsoft Excel for Mac OS X was used to determine the significance of differences between species and between diameter/height measurements within a species (Appendix 3.2). Images were captured with a PowerPhase digital scanning camera (Phase One, A/S, Fredriksberg, Denmark), and photographs were processed with Adobe Photoshop CS.

For scanning electron microscopy (SEM), samples were mounted on stubs using silver paint or gum tragacanth, coated with 150 Å gold using a Nanotek Semprep II sputter coater, and observed using a JEOL 6301F (Field Emission Scanning Electron Microscope). All specimens of *S. cernuus* and some of *S. chinensis* were removed from stubs after SEM and subsequently embedded and sectioned after rehydration in 70% ethanol.

RESULTS

Saururus cernuus—*Fruit morphology*— Flowers of *S. cernuus* typically have six stamens with long filaments and four basally connate carpels (Chapter 2). Fruits are schizocarpic, formed from four mature carpels (Fig. 3.2) that split apart into fruitlets (mericarps). Sometimes fewer than four carpels will mature into fruit (Fig. 3.3). Carpels are

attached to an elongate receptacle or gynophore that is persistent once fruitlets have broken off (Fig. 3.4, arrow). Stamens have long thin filaments that are free from the carpels (Figs. 3.1, 3.3, 3.4).

In the dried material available for study, three size classes of carpels were distinguishable, and form the basis for recognition of immature, intermediate and mature developmental stages. The mean size of an immature carpel is 0.38 mm coronal diameter x 0.78 mm high x 0.37 mm sagittal diameter. Intermediate carpels retain similar proportions with a mean size of 0.75 mm coronal diameter x 1.1 mm high x 0.72 mm sagittal diameter. Mature carpels have a different ratio, showing a mean size of 1.6 mm coronal diameter x1.6 mm high x 1.0 mm sagittal diameter, differing from previous stages in having similar values for height and coronal diameter, rather than having carpels taller than wide as in immature and intermediate stages.

Fruitlets show a wrinkled or pleated morphology that persists throughout development from the immature to mature stages (Figs. 3.1-3.12). Lateral surfaces become more expanded and smooth externally as the fruitlets mature (Figs. 3.4, 3.6, 3.8, 3.9, 3.11, 3.12). Invaginations of the whole fruit wall become less pronounced as the fruitlets mature (Figs. 3.4-3.12). A ventral ridge is formed by the carpel margins (Figs. 3.11, 3.12). Often this ridge is broken open towards the base of the fruitlet where it detaches from the other mericarps (Figs. 3.8, 3.11). Styles are situated on the ventral side of the fruitlet apex and are often straight at the base, ending in a recurved papillate stigma (Figs. 3.1, 3.4, 3.9, 3.12). In dried material, however, they are easily broken off and not always persistent on the fruitlets (e.g., Figs. 3.6-3.8)

Fruit anatomy—Anatomical sections emphasize the warty or pleated nature of the fruit wall. Immature stages have less prominent ridges in section view (Figs. 3.13, 3.14) than do intermediate and mature stages (Figs. 3.15, 3.16, 3.18-3.23). Oil cells are rare in pericarp tissue, distinguishable only in immature stages (Fig. 3.14), and not in later stages of development. Carpels are ovoid in cross section (Figs. 3.13, 3.15, 3.18-3.20). The ventral side of the carpel is very thin (Fig. 3.13, 3.15, 3.17-3.19, 3.22) and in transverse sections the area where the carpel margins meet is visible (Figs. 3.15, 3.17). This thin ventral area is more obvious at the apex and mid-section rather than at the base of the carpel (Figs. 3.18-3.20). Ridges of the fruit wall are longer at the base than at the apex (Figs. 3.18, 3.20) and sometimes fold over so that there appears to be a space in the fruit wall in cross or longitudinal section (Figs. 3.16, 3.20, arrow). Longitudinal sections may look similar to some cross sections (compare Figs. 3.18 (x.s.), 3.21 (l.s.)). In coronal longitudinal section, fruits are less circular and do not show the thin ventral area (Fig. 3.21). A sagittal longitudinal section shows well-developed dorsal fruit wall, thin ventral wall, and recurved style (Fig. 3.22). Fruitlets are found in typically four-parted fruits, attached to the gynophore (Fig. 3.23, arrow) from which they later split.

The single-celled exocarp/epidermis is cutinized (Fig. 3.26, 3.27). Mesocarp in the immature carpels is composed of homogeneous small, densely packed cells with dark contents (Fig. 3.24, 3.25). These cells are enlarged in fruitlets at an intermediate stage of maturity, and have a somewhat disorganized arrangement with some intercellular spaces (Fig. 3.26). In mature fruitlets, cells of the mesocarp are large isodiametric cells with many intercellular spaces (Figs. 3.27, 3.28) giving it a spongy appearance. Cells of the mesocarp are seen to be strongly pitted under the light microscope (Fig. 3.28) and SEM (Figs. 3.39,

3.40). The inner part of the mesocarp becomes cutinized in mature fruitlets (Fig. 3.27, arrow).

The endocarp is formed of large, thin-walled cuboidal to slightly rectangular cells that stain blue. These cells, in very immature carpels, are seen to be closely associated with the mesocarp, but distinguishable by their larger size (Fig. 3.24). In later stages, these cells separate from the mesocarp and become appressed to the seed integument (Figs. 3.27, 3.29, 3.33, 3.34, 3.37, 3.41).

Seed anatomy— A single ovule occurs in each fruitlet (Figs. 3.13, 3.18-3.23); rarely two ovules in a carpel may be found (3.30, 3.31). Ovules are attached marginally at the base of the carpel (Fig. 3.13, 3.19, 3.29). At the micropylar end, integuments form a beak-like projection (Fig. 3.32). Ovules are bitegmic (Fig. 3.33, 3.41), and perisperm is prominent in the seed (Figs. 3.30, 3.37). The composition of the integument is very difficult to interpret. Because integumentary layers are thin and difficult to distinguish, colour plates are provided. Staining with safranin/fast green allowed for consistent interpretation and in the location of tissues and cuticle in these fruits.

The outer integumentary layer is formed of an outer, single-cell thick layer of small, thin-walled rectangular cells (red staining) (Fig. 3.31-3.34) and an inner layer that is thick and structureless (staining pale pink) (Figs. 3.31-3.33, 3.41). The inner integument has an outer amorphous, cuticle-like layer and an inner cellular layer, where the cells have irregular outlines (both stain red) (Figs. 3.29, 3.31, 3.32, 3.33, 3.35, 3.36). The inner cellular layer is continuous with a proliferation of cells in the chalaza (Figs. 3.37, arrow; 3.38). This red-staining layer is closely associated with the perisperm and sometimes interdigitates with perisperm and endosperm cells (Figs. 3.35, 3.38).

***Saururus chinensis*—** *Fruit morphology*— Flowers of *S. chinensis* have four basally connate carpels with recurved styles and six adnate stamens that are shorter than the height of the carpels (Figs. 3.42, 3.43). Immature carpels have a mean size of 0.53 mm coronal diameter x 1.0 mm high x 0.58 mm sagittal diameter. In intermediate stages of development carpels have a mean size of 1.1 mm coronal diameter x 1.4 mm high x 0.85 mm sagittal diameter. Mature fruitlets have a mean size of 1.8 mm coronal diameter x 1.8 mm high x 1.2 mm sagittal diameter. Carpels at all stages of maturity have a wrinkled or pleated surface (Figs. 3.42-3.52). Stamens are almost always persistent on carpels (Figs. 3.44, 3.45, 3.47, 3.50, 3.51, 3.52). Styles are more persistent than those of *S. cernuus*, and are tightly curved up against the fruitlet (Figs. 3.42-3.52). Styles are located towards the ventral side of the fruitlet apex and are a continuation of the ventral ridge that runs up the fruit (Figs. 3.45, 3.48, 3.51). In dried fruits, the ventral ridge is sometimes broken open at the basal point of attachment to other carpels (Figs. 3.45, 3.48, 3.51). Unlike *S. cernuus, S. chinensis* lacks a gynophore. The lateral faces of the fruitlet are smooth and flattened from being appressed to neighbouring carpels (Figs. 3.45, 3.46, 3.48, 3.49, 3.51, 3.52). As they mature, carpels increase more in sagittal and coronal width than in height (Figs. 3.45, 3.46, 3.48, 3.49, 3.51, 3.52).

Fruit anatomy— In anatomical section, fruitlets appear to have less pronounced ridges than in *S. cernuus* (Figs. 3.53-3.57, 3.59-3.62). Transverse sections are roughly bilaterally symmetrical (Figs. 3.53, 3.55, 3.57, 3.59, 3.60). Carpels have a thin ventral wall (Figs. 3.53, 3.54, 3.55, 3.57, 3.59, 3.60, 3.62). In basal sections the ventral ridge may have a widened gap or hole where fruitlets detached from each other (Figs. 3.59, 3.60). In cross section, the ventral fruit wall is smoother in basal sections than in apical sections (Figs. 3.57, 3.59, 3.60).

Coronal longitudinal sections have circular outlines and appear similar to transverse

sections; however, the thin ventral region is not visible in coronal longitudinal sections (Fig.

3.61). Sagittal longitudinal sections show the thickened dorsal fruit wall and the thin, basally

opened ventral wall (Fig. 3.62). Sagittal longitudinal sections show recurved styles (Figs.

3.62), and coronal longitudinal sections show that the style has a central groove, giving it a

V-shaped outline (Fig. 3.56, arrow).

The exocarp/epidermis is visible as a thickly cutinized, single cell layer (Figs. 3.63,

3.64). The mesocarp shows the most change throughout fruitlet development. In immature

carpels, the cells of the mesocarp are small in diam and cell outlines are irregular in shape

(Fig. 3.63). Mesocarp cells in intermediate developmental stages of fruitlets examined here

do not show much difference from those in mature fruitlets; however, cells are smaller in

size and intercellular spaces are not prominent (Fig. 3.64). Mature mesocarp is composed

of large, circular cells in transverse section with prominent intercellular spaces (Figs. 3.65,

3.66). These cells have shallowly pitted secondary walls that are visible under the light

microscope (Fig. 3.66) and SEM (Figs. 3.75, 3.76). The inner wall of the mesocarp

becomes cutinized in mature fruitlets (Figs. 3.65; 3.73; 3.74, arrowhead).

A single cell layer thick endocarp is seen in all stages of fruit maturity. Endocarp is

formed from large, thin-walled cuboidal to slightly rectangular cells (stain blue) (Figs. 3.63,

3.64, 3.65, 3.69, 3.70). In immature stages, these cells are closely associated with the

mesocarp (Figs. 3.53, 3.54, 3.63), and remain so in intermediate stages (Figs. 3.55, 3.56,

3.64). In mature fruits, however, endocarp cells tear away from the mesocarp and become

appressed to the seed integument (Figs. 3.57, 3.61. 3.62, 3.65, 3.69, 3.70, 3.71, 3.73, 3.74,

3.77, 3.78).

Seed anatomy—There is a single ovule per carpel (Figs. 3.53, 3.54, 3.57, 3.60, 3.71). Each ovule has a small beak-like integumentary projection at the micropylar end (Fig. 3.67). Perisperm, with large starch grains, fills most of the seed but endosperm is seen at the micropylar end (Fig. 3.71). Endosperm is distinguishable because it stains less intensely than perisperm and lacks the large starch grains found in the persiperm; there is also an inner, central gap between endosperm and perisperm.

As in *S. cernuus*, the integumentary layers in *S. chinensis* are difficult to interpret without the use of safranin/fast green staining. Therefore, colour illustrations have been provided. The integument is four-layered (Figs. 3.69, 3.70, 3.73, 3.74). The outermost integumentary layer is composed of small, rectangular cells (red staining) with thick walls (Figs. 3.67, 3.68, 3.69, 3.70, 3.72-3.74, 3.78). The inner layer of the outer integument is a solid, amorphous layer (pink staining) (Fig. 3.69. 3.70, 3.73, 3.74, 3.77, 3.78). The inner integument is composed of two dark-staining layers, an outer cuticular layer and an inner layer composed of solid, irregular cells (Figs. 3.67,3.69, 3.70, 3.73, 3.74). At the chalazal end the inner integument proliferates (Figs. 3.71, 3.72). The innermost integumentary layer is closely associated with the perisperm, and where it meets the endosperm, the endosperm and inner integumentary layer become interdigitated (Fig. 3.73, 3.74).

DISCUSSION

The fruits and seeds of *Saururus* have not been given much attention in past studies, and this chapter represents the first examination of detailed anatomy in several fruit developmental stages. Johnson (1900), Raju (1961), Wood (1971), and Igersheim and Endress (1998) have all examined ovule development in *S. cernuus*, but there is little mention of the surrounding carpel tissue, and no studies have included *S. chinensis*.

Although the fruitlets of *S. cernuus* and *S. chinensis* look similar and are similar in dimensions (see Appendix 3.2), there are a few fruit characters that distinguish these two species. The styles on *S. cernuus* are straighter and break off more easily in dried material, than those of *S. chinensis*, which are more tightly coiled against the fruit wall. At all stages of development, I find that stamens of *S. chinensis* have shorter filaments, and thus the filaments and anthers are more persistent and visible on the dried fruits than in *S. cernuus*. *Saururus cernuus* stamens have long filaments that are more readily detached from the fruit, as they are longer than and free from the carpel. Fruitlets of *S. cernuus* are attached to a short gynophore, while fruits of *S. chinensis* lack a gynophore. The ventral ridge is more prominent on fruitlets of *S. chinensis* than on those of *S. cernuus*. The fruitlet surface is more sculptured and wrinkled in *S. cernuus* than in those of *S. chinensis*, and this is seen both in external morphology and in anatomical sections. However, seed structure is virtually identical in both taxa, and there is no significant difference in fruitlet size (less than 2 mm) between the two species (Appendix 3.2).

In both species, I found the fruitlet surface to be pleated or wrinkled, as previously described for *S. cernuus* (Johnson, 1900; Wood, 1971; Friis, 1985; Plisko, 1988). Ventral fruit wall is much thinner than the rest of the fruit wall, and basally may be broken open. There is a ventral ridge formed from postgenital fusion of carpel margins (Igersheim and Endress, 1998). The mesocarp is spongy with air spaces between the cells, a feature mentioned by Johnson (1900) and hypothesized by him to function for floatation and transportation in water.

Contrary to previous studies that have recognized only pericarp (Johnson, 1900; Raju, 1961; Wood, 1971) I observe that, in addition to mesocarp, there is a distinct endocarp.

Mesocarp cells have pitted secondary walls (Friis, 1985; Plisko, 1988; this study) while the endocarp is formed of a single layer of large, thin-walled cells. Plisko (1988) also interpreted the pericarp as having multiple layers, including this layer of thin-walled cells, as well as the layer of elongate cells I interpret as part of the seed integument. In mature fruitlets, however, the endocarp detaches from the mesocarp and becomes appressed to the ovule. It is the endocarp layer that has previously been described as the outermost integumentary layer (Johnson, 1900; Raju, 1961; Wood, 1971; Friis, 1985) except by Plisko (1988).

There is a single ovule in each fruitlet, though rarely two ovules are found in one carpel. It is not uncommon for indehiscent fruits, such as those of *Saururus*, to have much reduced or undifferentiated seed coats (Corner, 1976; Werker, 1997). Young ovules in Saururaceae have been shown to be bitegmic with a two-layered outer integument and three-layered inner integument (Johnson, 1900; Raju, 1961; Igersheim and Endress, 1998). The integument forms a beak-like projection at the micropylar end. The outer integument has been described as consisting of a pale, filmy outer layer of thin-walled, equiaxial, quadrangular cells and an inner layer of elongate, thin walled cells in longitudinal rows (Johnson, 1900; Wood, 1971; Corner, 1976; Friis, 1985; Lesiak, 1994; Mai, 1999). The inner integument at maturity was described as consisting of hard outer and inner epidermal layers, or "sclerotic tegmen," with the cell walls becoming thick enough to obscure cell lumens (Johnson, 1900; Wood, 1971; Corner, 1976; Friis, 1985; Lesiak, 1994; Mai, 1999). Johnson (1900) previously described the presence of many thickened cells that complete the integument at the chalazal end of the ovule; this study has confirmed that observation. These cells can not be called a hypostase, since that implies that they have a nucellar origin

(Werker, 1997); following previous interpretations of integument structure, this layer is integumentary rather than nucellar. The nucellus forms abundant perisperm in seeds of Saururaceae (Johnson, 1900; Raju, 1961; Wood, 1971; Corner 1976; Wu and Kubitzki, 1993; Igersheim and Endress, 1998).

The current investigation leads to a different interpretation of the integument in the mature seed. The large thin-walled cells that were described as the outermost seed coat are in fact an endocarp layer (as interpreted by Plisko, 1988) that becomes appressed to the seed integument. This interpretation is supported by the fact that this layer of large cells is well-developed and attached to mesocarp tissue even in immature carpels, while the ovules themselves at such stages are minute. Also, in mature fruits, sections through the seed attachment show that this filmy layer does not attach at the top of the funiculus, as would be expected if it was an integumentary layer, but rather is found close to the inner fruit wall. A fruit containing two mature ovules (Fig. 2.31) also shows that this layer is restricted to the area adjacent to the inner carpel wall, and is not found in the area between the two seeds; therefore it cannot be integument.

Thus, excluding the endocarp, there are four layers to the seed integument: a layer of elongate, thin-walled but suberized or lignified rectangular cells, a structureless, pink-staining layer; and two deeply red-staining layers, the outer layer being cuticular in appearance, and the inner layer more cellular and continuous with cells of similar appearance in the chalaza. The outermost layer of thin-walled, red-staining elongate cells was interpreted by Plisko (1988) as part of the pericarp. However, this layer is not visible in young carpel stages, even though the endocarp of large, thin-walled cells is well-developed. In addition, when examining the specimen with two seeds, this layer can be seen to occur,

compressed, in between the two seeds. The innermost integument has a smooth contact with perisperm but cells interdigitate with those in the outer layer of endosperm. Detailed studies following integument maturation need to be done in order to confirm the above interpretation of the integument structure.

Saururaceae are accepted as part of the Piperales and are sister to Piperaceae (APG, 2003; Jaramillo et al., 2004; Neinhuis et al., 2005). Aristolochiaceae, Lactoridaceae and Hydnoraceae are also in Piperales (Nickrent et al., 2002; APG, 2003). Seeds of Piperaceae are interpreted in a similar manner as are those of Saururaceae. In both families ovules are orthotropous (Corner, 1976; Nikiticheva, 1988a, 1988b; Plisko, 1988; Ingersheim and Endress, 1998). The inner integument forming a significant part of the seed coat in Piperaceae, and the inner part of the integument becomes crenulated (Corner, 1976; Nikiticheva, 1988a, 1988b; Ingersheim and Endress, 1998; Lei et al., 2002), much like what is observed in *Saururus*. The integuments are described as becoming very tanniniferous and hard (Corner, 1976; Lei et al., 2002), which is similar to descriptions of a hard seed coat in Saururaceae (Wood, 1971; Corner, 1976, Plisko, 1988). Lactoridaceae and Aristolochiaceae have similar ovule structure as Saururaceae, with a two-celled outer integument and three-celled inner integument, but ovules in Lactoridaceae and Aristolochiaceae are anatropous (Igersheim and Endress, 1998). Seed coats differ in structure from Saururaceae, with seeds of Aristolochiaceae having crystalline layers present and some tanniniferous layers of the integument (Gonzalez and Rudall, 2003) and those of Lactoridaceae not having sclerotic layers (Pemrova, 1988). Tobe et al. (1993) found for *Lactoris* that the inner integument accumulates much tannin-like material and is directly associated with the embryo sac. Further studies on the exact nature of the seed coat,

include examination through a series of developmental stages, in *Saururus* and other

piperalean taxa should reveal additional characters to use for comparisons between taxa.

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Appendix 3.1. Measurements of extant *Saururus* carpels. c = diameter in coronal plane; h = height; s = diameter in sagittal plane.

	S. cernuus immature carpels			*S. chinensis* immature carpels		
	c	h	s	c	h	s
	0.4	0.8	0.5	0.5	1.2	0.6
	0.3	0.8	0.3	0.5	1.0	0.6
	0.4	0.8	0.5	0.5	1.2	0.5
	0.3	0.9	(n/a)	0.5	0.8	0.4
	0.3	0.9	0.3	0.6	1.1	0.8
	0.3	0.7	0.3	0.5	1.0	0.4
	0.5	0.9	0.3	0.6	1.2	0.6
	0.2	0.8	0.5	0.4	1.0	0.6
	0.5	0.8	0.4	0.6	0.9	0.5
	0.5	0.8	0.4	0.5	1.0	0.8
	0.6	0.8	0.4	0.6	1.0	0.7
	0.3	0.6	0.3	0.5	1.0	0.6
	0.4	0.7	0.4	0.4	0.7	0.5
	0.4	0.7	0.2	0.4	0.8	0.4
	0.3	0.7	0.5	0.7	1.2	0.7
	0.3	0.7	0.2	0.6	1.0	0.6
				0.5	0.9	0.4
				0.5	1.0	0.6
				0.5	1.1	0.7
				0.6	1.0	0.6
				0.6	1.2	0.6
				0.5	0.8	0.4
				0.6	0.9	0.7
				0.4	1.0	0.5
				0.6	1.0	0.5
				0.6	1.2	0.7
mean	0.4	0.8	0.4	0.5	1.0	0.6
mode	0.3	0.8	0.3	0.5	1.0	0.6

Appendix 3.1, continued

	S. cernuus intermediate carpels				S. chinensis intermediate carpels		
	c	h	s		c	h	s
	0.6	0.8	0.5		0.8	1.4	0.7
	0.4	1.0	0.7		1.2	1.7	0.9
	0.6	1.2	0.8		1.0	1.7	1.0
	1.0	0.9	0.8		1.2	1.7	1.0
	0.7	1.0	0.6		1.3	1.3	0.8
	0.8	1.5	0.8		0.8	1.4	0.8
	0.5	0.9	0.5		1.2	1.7	1.0
	0.5	0.9	0.5		1.4	1.6	0.9
	1.0	1.1	0.9		1.0	1.3	0.9
	0.5	1.0	0.5		1.2	1.6	1.0
	1.1	1.2	1.0		1.1	1.7	0.8
	1.1	1.3	1.0		1.1	1.4	0.9
	0.6	1.0	0.7		0.9	1.2	0.8
	1.1	1.4	0.9		1.2	1.4	0.8
	0.7	1.1	0.6		0.8	1.3	0.8
					1.1	1.7	1.0
					1.0	1.4	0.8
					1.1	1.3	0.8
					1.0	1.4	0.9
					0.8	1.1	0.7
					0.7	1.2	0.7
					0.6	1.1	0.8
					1.1	1.2	0.8
					1.0	1.5	0.8
					0.8	1.6	0.8
					1.1	1.4	0.9
mean	0.7	1.1	0.7		1.0	1.4	0.9
mode	0.6	1.0	0.5		1.1	1.4	0.8

Appendix 3.1, continued

	S. cernuus mature fruitlets			S. chinensis mature fruitlets		
	c	h	s	c	h	s
	1.4	1.6	1	1.7	1.8	1.3
	1.8	1.8	1	1.9	1.6	1.2
	1.6	1.7	1.2	1.7	1.7	1.2
	1.8	1.7	1.4	1.7	1.7	1.2
	1.7	1.6	1	1.8	1.9	1.3
	1.6	1.5	0.9	1.8	1.7	1.3
	1.8	1.6	1.1	1.8	1.7	1.1
	1.4	1.7	1.2	1.9	1.9	1.1
	1.7	1.8	1.2	1.9	1.8	1.4
	1.7	1.6	1	1.8	1.9	1.2
	1.8	1.8	1	1.9	2.2	1.2
	1.8	1.6	1	1.6	1.7	1.1
	1.6	1.6	1.1	1.9	2.3	1.3
	1.3	1.4	0.9	1.6	1.6	1.2
	1.4	1.5	1	2	2.2	1.5
	1.4	1.4	0.8	1.6	1.7	1.1
	1.6	1.6	1	1.8	2	1.4
	1.7	1.7	1.3	1.9	1.9	1.1
	1.2	1.4	1.1	1.8	2	1.3
	1.3	1.5	1.1	1.9	1.7	1.3
	2	1.6	1.1	1.8	1.8	1.2
	1.6	1.6	1	1.6	1.6	1.2
	1.6	1.6	1.1	1.7	1.6	1.2
	1.2	1.4	0.8	1.9	1.7	1.3
	1.4	1.4	0.8	1.6	1.6	1.1
	1.6	1.8	1.1	2	1.8	1.4
mean	1.6	1.6	1.1	1.8	1.8	1.2
mode	1.6	1.6	1	1.9	1.7	1.2

Appendix 3.2. P-values resulting from two-tailed t-tests between fruitlet dimensions, stages and species. A Type 1 (paired) test was used for samples from same fruits (i.e., comparing different dimensions within the same stage and species) while a Type 2 (unmatched pairs) test was used for between-species or -stage comparisons. c = diameter in coronal plane; h = height; imm = immature; int = intermediate; mat = mature; n = no; s = diameter in sagittal plane; y = yes.

Sample set 1	Sample set 2	P-value	Significant at p ≤ 0.05
S. cernuus imm c	*S. cernuus* imm s	0.7	y
S. cernuus imm c	*S. cernuus* imm h	1.2×10^{9}	n
S. cernuus imm h	*S. cernuus* imm s	3.5×10^{9}	n
S. cernuus int c	*S. cernuus* int s	0.5	y
S. cernuus int c	*S. cernuus* int h	2.9×10^{5}	n
S. cernuus int h	*S. cernuus* int s	1.8×10^{7}	n
S. cernuus mat c	*S. cernuus* mat s	6.2×10^{13}	n
S. cernuus mat c	*S. cernuus* mat h	0.5	y
S. cernuus mat h	*S. cernuus* mat s	1.9×10^{18}	n
S. chinensis imm c	*S. chinensis* imm s	0.04	n
S. chinensis imm c	*S. chinensis* imm h	1.1×10^{16}	n
S. chinensis imm h	*S. chinensis* imm s	2.9×10^{15}	n
S. chinensis int c	*S. chinensis* int s	1.6×10^{5}	n
S. chinensis int c	*S. chinensis* int h	3.1×10^{11}	n
S. chinensis int h	*S. chinensis* int s	6.6×10^{17}	n
S. chinensis mat c	*S. chinensis* mat s	4.1×10^{19}	n
S. chinensis mat c	*S. chinensis* mat h	0.6	y
S. chinensis mat h	*S. chinensis* mat s	8.8×10^{15}	n
S. cernuus imm c	*S. chinensis* imm c	2.9×10^{6}	n
S. cernuus imm h	*S. chinensis* imm h	5.9×10^{7}	n
S. cernuus imm s	*S. chinensis* imm s	1.8×10^{6}	n
S. cernuus int c	*S. chinensis* int c	4.0×10^{4}	n
S. cernuus int h	*S. chinensis* int h	3.4×10^{6}	n
S. cernuus int s	*S. chinensis* int s	4.5×10^{3}	n
S. cernuus mat c	*S. chinensis* mat c	4.0×10^{5}	n
S. cernuus mat h	*S. chinensis* mat h	2.5×10^{5}	n
S. cernuus mat s	*S. chinensis* mat s	1.9×10^{6}	n

Text-Figure 3.1. Diagram of *Saururus* fruitlet in cross-section, illustrating sagittal and coronal longitudinal planes of section (dashed lines); ventral and dorsal sides of fruitlet labeled for reference.

Text-Figure 3.1

Ventral

sagittal

coronal

Dorsal
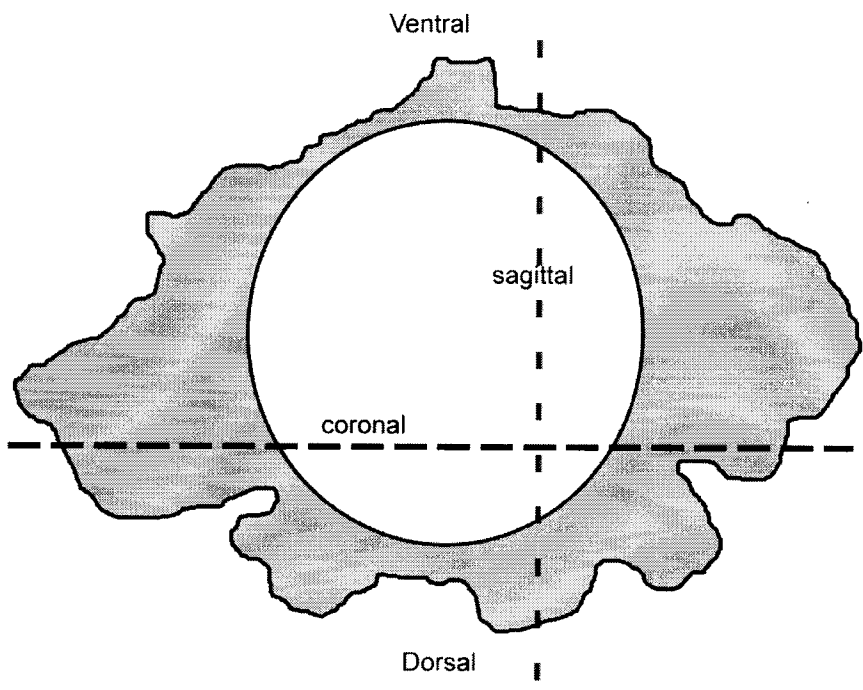
Figures 3.1-3.12. External morphology of carpels of *Saururus cernuus* L. **3.1.** Flower of

S. cernuus, showing stamens on long filaments (arrow) and four carpels with recurved

styles. FLAS 174913. **3.2.** Fruit comprised of four mature fruitlets (arrows). FLAS 94853.

3.3. Fruit with two enlarged, matured carpels (on back side) and two immature carpels

(arrows), showing fruitlets mature at different rates. Stamen filament remnants (S) and bract

(B) also visible. FLAS 174913. **3.4.** Flower with two of four carpels removed, showing

central gynophore (arrow) to which carpels are attached. Note half-moon shape of carpel in

side view, with smooth lateral surface (L) and style situated towards ventral side of carpel.

FLAS 174913. **3.5.** Side view of fruit with four immature carpels, showing finely pleated

carpel surface. FLAS 174913. **3.6.** Ventral view of immature carpel, showing smooth

lateral sides and ridge where carpel margins meet (arrow). Style has broken off. FLAS

174913. **3.7.** Dorsal view of intermediate stage of carpel development, with style broken

off. Pleated mesocarp well defined; carpel much taller than wide. FLAS 174913. **3.8.**

Ventral view, intermediate stage carpel development, showing relatively smooth lateral

surface, and hole in ventral wall where it connected to other carpels in flower. FLAS

174913. **3.9.** Intermediate stage carpel, side view, showing pleated surface and recurved

style situated close to ventral side. FLAS 174913. **3.10.** Dorsal view of mature carpel

(fruitlet), with dorsal ridges. Note that width of fruitlet is approximately equal to height.

FLAS 174913. **3.11.** Ventral view of mature fruitlet showing smooth lateral sides (L),

ventral ridge formed by carpel margins, hole in ventral surface near base of carpel, and

pleated surface. FLAS 174913. **3.12.** Mature fruitlet in side view showing pronounced

crescent shape, smooth sides and small pleats towards ventral side of fruitlet. Style long,

persistent, and situated towards ventral side of fruitlet. All scale bars = 250 µm.
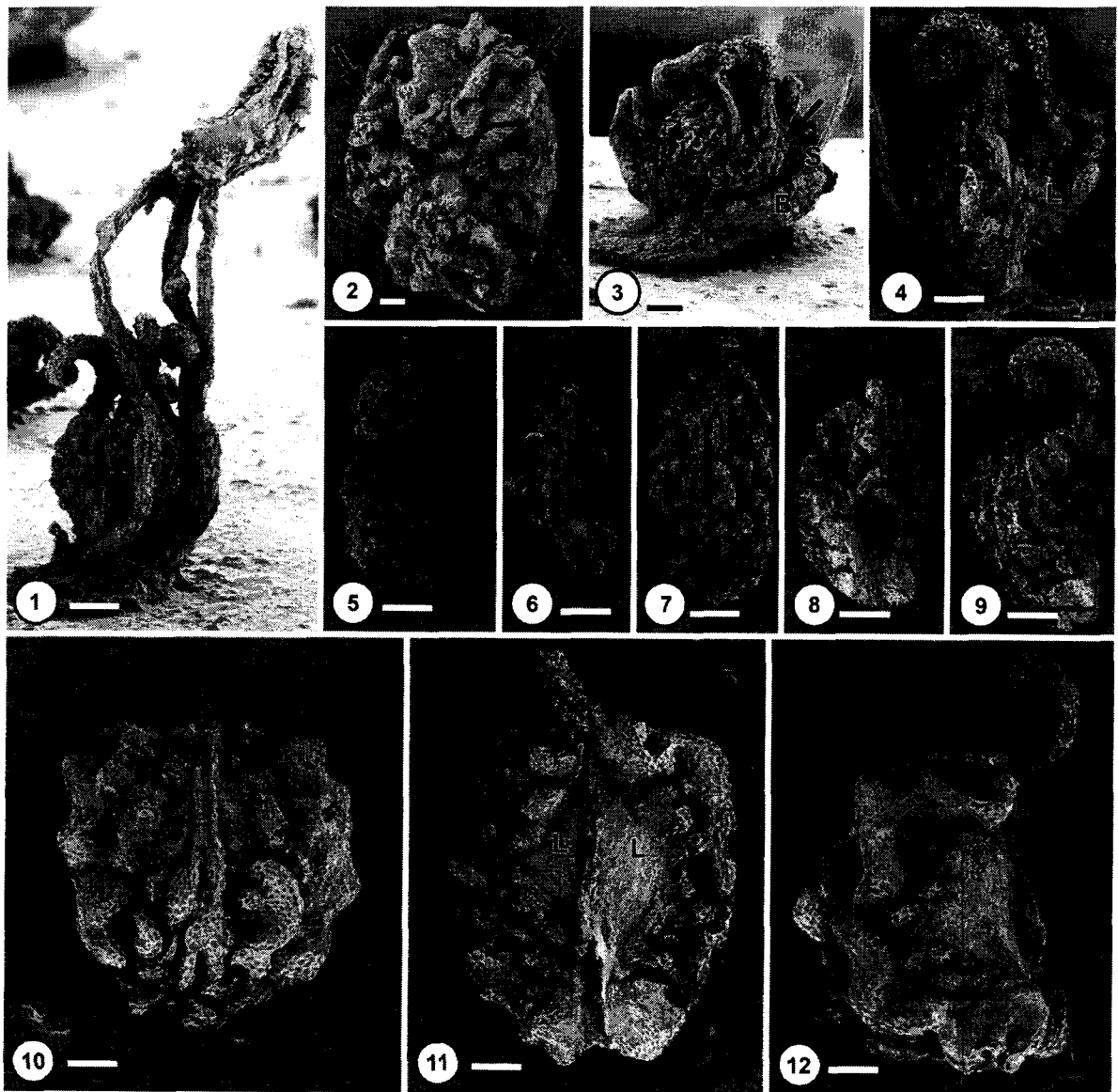
Figures 3.1—3.12

Figures 3.13-3.23. Anatomy of *Saururus cernuus* L. carpels. **3.13.** Immature carpel in cross section showing pleated mesocarp, endocarp (E) of large, thin-walled cells, and single ovule (arrow). SL 14480, scale = 100 μm. **3.14.** Longitudinal section of two carpels showing basal fusion, oil cell idioblasts (arrow), and recurved styles with papillate stigmas. SL 14481, scale = 200 μm. **3.15.** Cross section of intermediate stage carpel, showing more pronounced ridges and better-developed cells in mesocarp (M), and endocarp (E) of large, thin-walled cells. SL 14482, scale = 100 μm. **3.16.** Longitudinal section of intermediate stage carpel, with irregular ridged outline; note space in mesocarp (arrow). SL 14483, scale = 100 μm. **3.17.** Enlargement of ventral area of intermediate stage carpel in cross section, showing ridge formed from carpel margins and thin-walled endocarp (E). SL 14482, scale = 25 μm. **3.18.** Apical cross section of fruitlet showing ovate shape and thin ventral fruit wall (arrow). SL 14484, scale = 250 μm. **3.19.** Basal cross section showing thin ventral tissue, strongly pronounced ridges, and single marginally attached ovule. SL 14485, scale = 100 μm. **3.20.** Cross section at base of fruitlet; note spaces in fruit wall (arrow). SL 14484, scale = 250 μm. **3.21.** Coronal longitudinal section of fruitlet showing ridged outline. SL 14484, scale = 250 μm. **3.22.** Sagittal longitudinal section of fruitlet showing thin ventral wall to left and ridged dorsal wall to right, with recurved style at apex. SL 14484, scale = 250 μm. **3.23.** Basal cross section of fruit showing four fruitlets and central gynophore (arrow). SL 14486, scale = 250 μm.

Figures 3.13—3.23
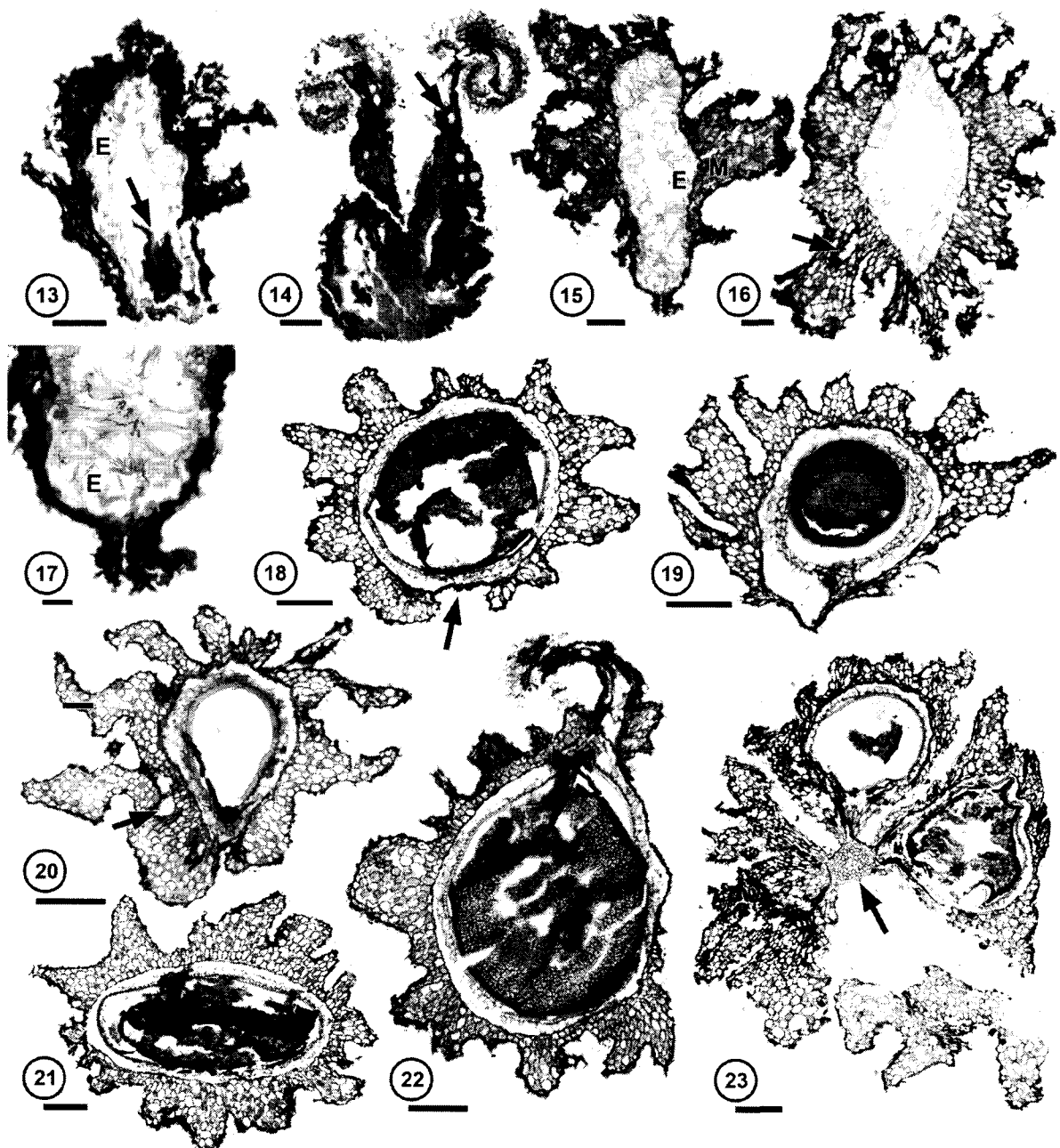
Figures 3.24-3.32. Anatomy of fruit wall and seeds of *Saururus cernuus* L. stained with safranin-fast green. **3.24.** Immature carpel cross section showing mesocarp (M) with small cells with dark contents, central vascular bundle (V), and endocarp layer (E) of thin-walled, cuboidal cells. SL 14487, scale = 25 μm. **3.25.** Mesocarp at an immature stage, with small cells with dark contents. SL 14481, scale = 50 μm. **3.26.** Intermediate stage carpel wall with enlarged mesocarp cells (M) with irregularly shaped walls, and endocarp (E) of large thin-walled cells. SL 14483, scale = 50 μm. **3.27.** Mature mesocarp (M) with large, ovoid cells, intercellular spaces, and cutinized inner wall (arrow); endocarp (E) associated with seed coat (SC). SL 14484, scale = 50 μm. **3.28.** Pitted mesocarp cells. SL 14484, scale = 25 μm. **3.29.** Section showing single ovule, attached to fruit wall, with endocarp tissue (E) attached near base of funiculus and integuments arising from top of funiculus. SL 14485, scale = 100 μm. **3.30.** Cross section showing two seeds in fruitlet. SL 14488, scale = 250 μm. **3.31.** Two seeds in fruitlet showing mesocarp (M) and endocarp (E) outside of seed tissue distinct from surrounding integument (O, *). SL 14488, scale = 50 μm. **3.32.** Beak-like integument at micropylar end of ovule. SL 14484, scale = 50 μm.

Figures 3.24—3.32
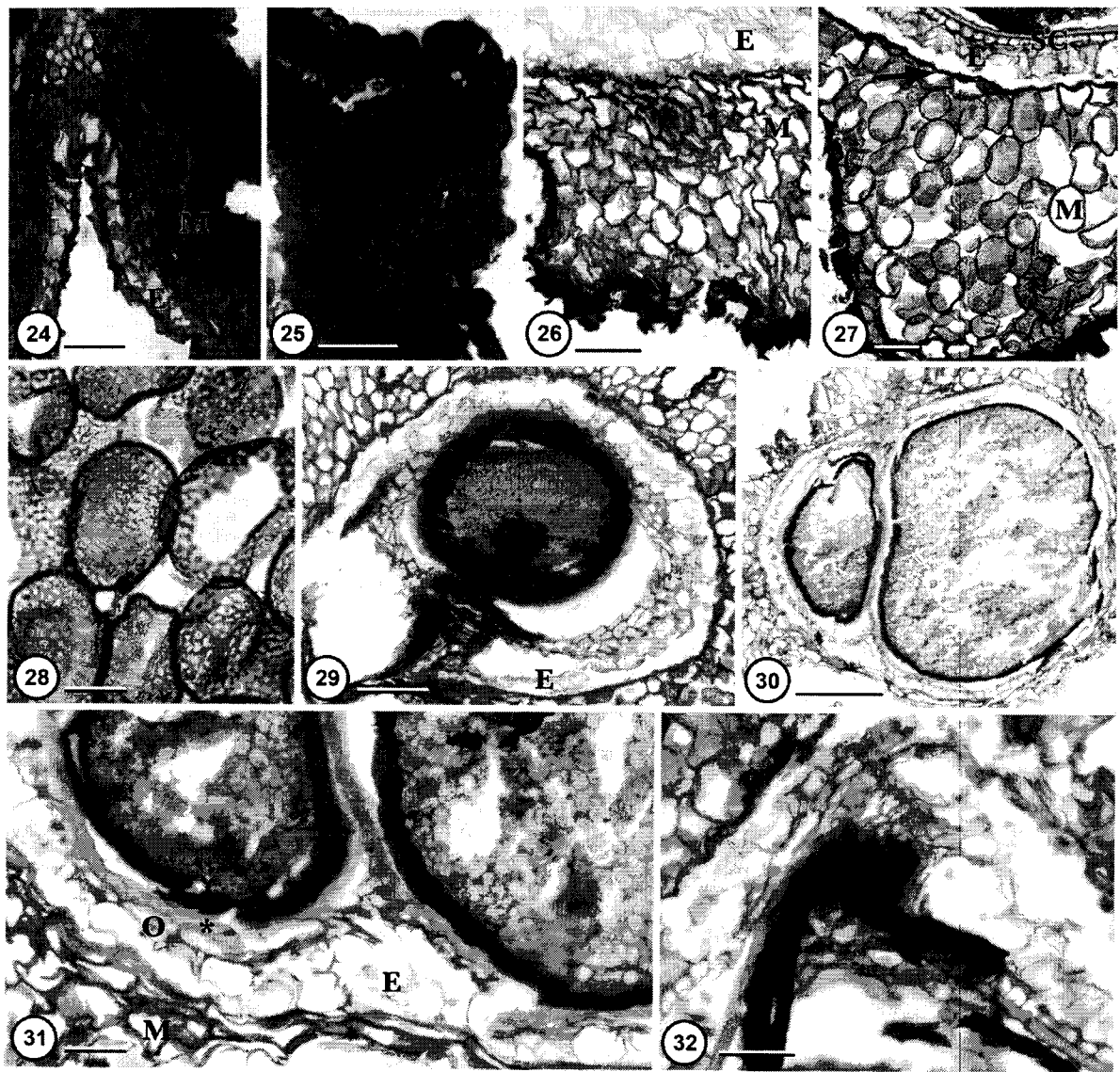
Figures 3.33-3.38. Seed anatomy of *Saururus cernuus* L., stained with safranin-fast

green **3.33.** Longitudinal section of ovule showing perisperm, three of the four integument

layers (O, *, arrow), and thin-walled endocarp (E) detached from mesocarp (M). SL 14489,

scale = 25 μm. **3.34.** Oblique section of seed showing large, circular cells of endocarp (E)

and narrow, rectangular cells of outermost integument (O). SL 14489, scale = 50 μm. **3.35.**

Integumentary layers (red) and perisperm (blue). Cells of inner integument and perisperm

interdigitate. SL 14484, scale = 25 μm. **3.36.** Oblique section of seed showing perisperm in

centre (P), cells with irregular outlines and dark contents (red), cuticular layer (red) and

cuticular layer (pink;*); endocarp (E) and mesocarp (M) to outside. SL 14484, scale = 50

μm. **3.37.** Ovule showing proliferated cells in chalaza (arrow), continuous with innermost

integument. SL 14489, scale = 100 μm. **3.38.** Proliferated cells in chalaza. SL 14489, scale

= 50 μm.
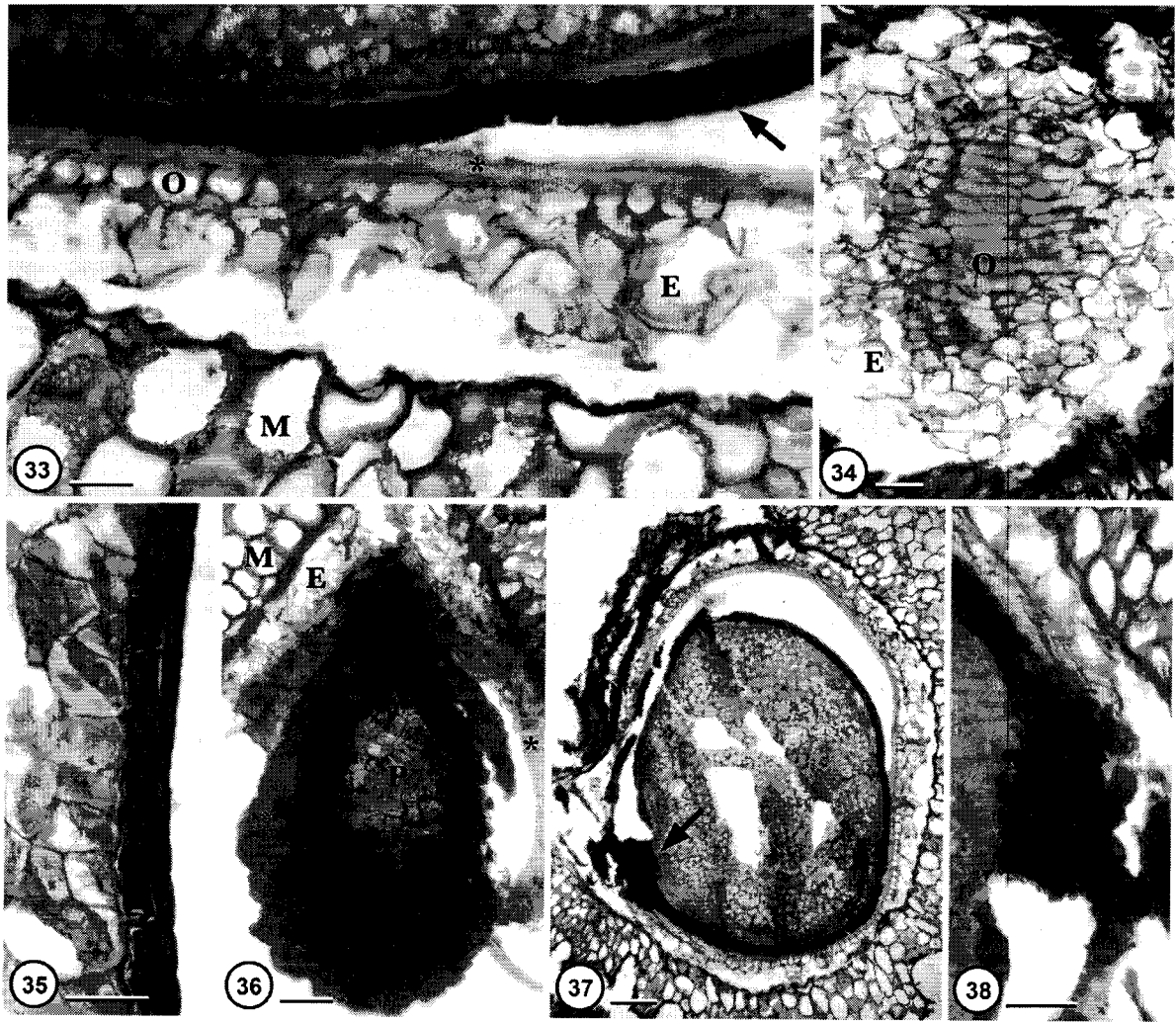
Figures 3.33–3.38

Figures 3.39-3.41. SEM of fruit wall and seed of *Saururus cernuus* L. **3.39.** Mesocarp,

showing cells with strong pitting and presence of air spaces between cells. FLAS 174913,

scale = 20 μm. **3.40.** Single mesocarp cell showing strongly pitted inner wall and smooth

outer walls. FLAS 174913, scale = 10 μm. **3.41.** View of ventral fruit wall (FW) enclosing

ovule, with large cells of endocarp (E), outermost integument (O), cuticular layer (*),

innermost integument (arrows) and persiperm (P). FLAS 174913, scale = 20 μm.

Figures   3.39—3.41
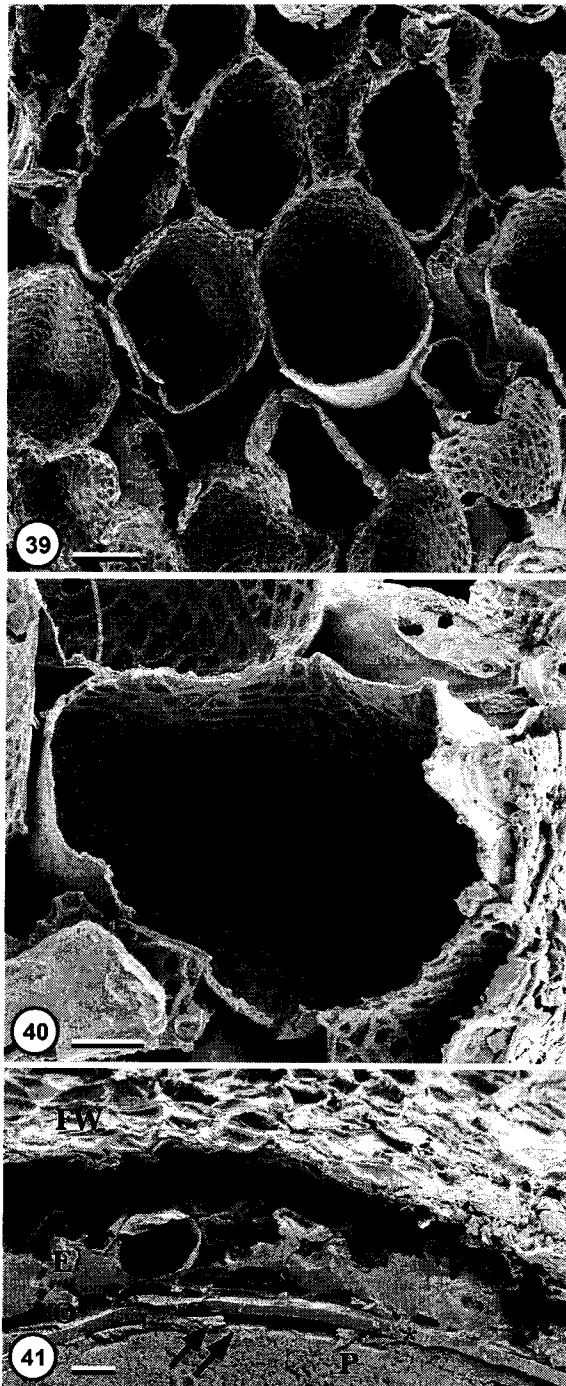
Figures 3.42-3.52. External morphology of carpels of *Saururus chinensis* (Lour.) Baill.

3.42. Side view of flower of *S. chinensis* showing four basally connate carpels with recurved styles, and short stamens (S) adnate to carpels. **3.43.** Top view of flower, showing six stamens (S) and four wrinkled carpels. **3.44.** Dorsal view of immature stage, pleated carpel, with two connate stamens and recurved style. **3.45.** Ventral view of immature carpel, showing adnate stamen and ventral ridge (arrow) formed by carpel margins. **3.46.** Side view of immature carpel showing recurved style and flat lateral side. **3.47.** Dorsal view of intermediate stage carpel, with two adnate stamens (S) and pleated fruitlet surface. **3.48.** Intermediate stage carpel showing ventral ridge formed by carpel margins, and hole (at base) where it connected to other carpels in flower. **3.49.** Side view of intermediate stage carpel showing curled style, ridges and attached stamen (S) with thick filament. **3.50.** Dorsal side of mature fruitlet with style at apex, very pleated mesocarp and attached stamen (S) at right. **3.51.** Ventral view of mature fruitlet, with smooth lateral sides, ventral ridges from carpel margins and hole from detachment from other carpels. **3.52.** Side view of mature fruitlet and attached stamen (lower left). Note size of fruit relative to stamen. All scale bars = 250 μm.

Figures   3.42—3.52
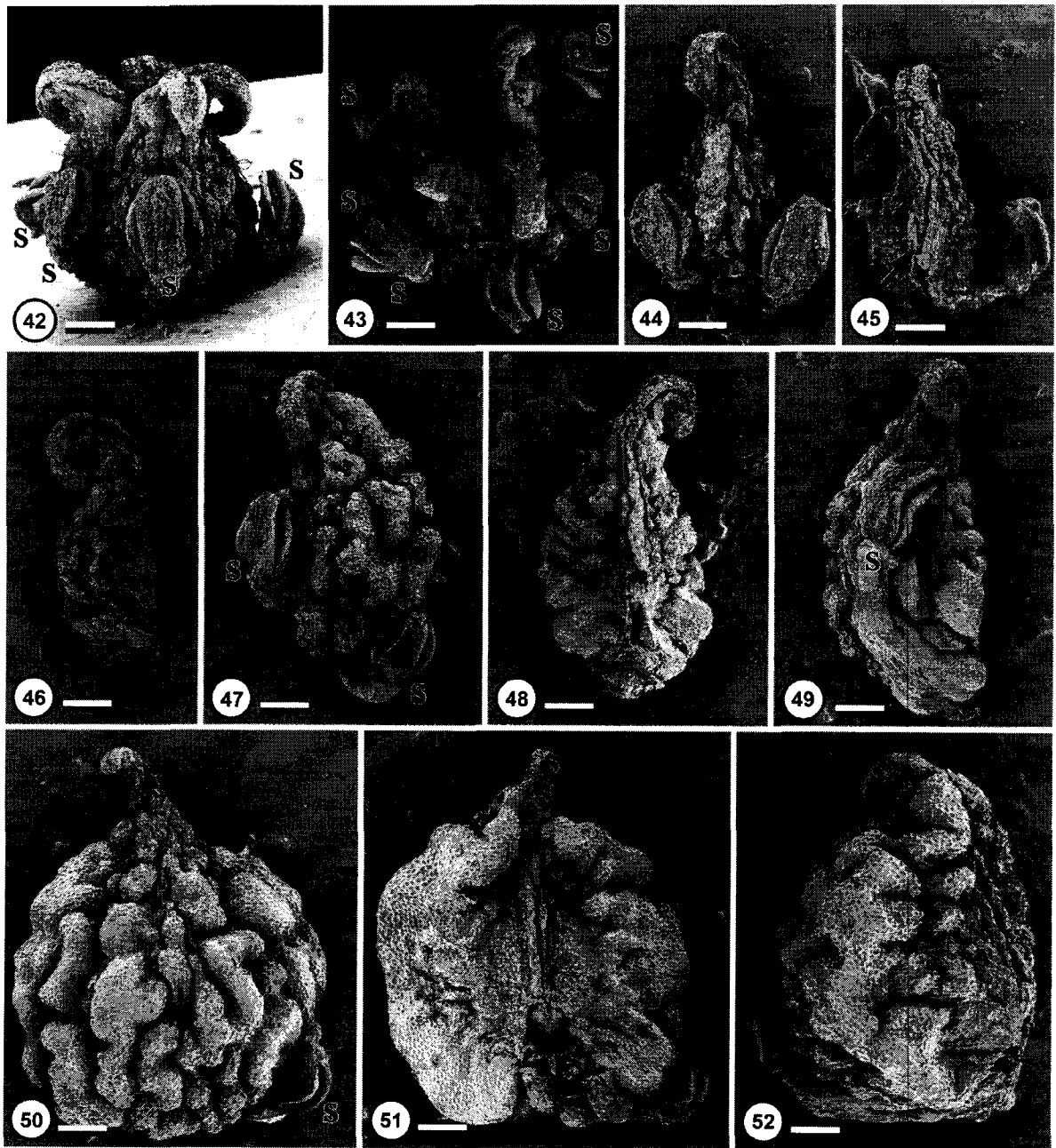
Figures 3.53-3.62. Anatomy of *Saururus chinensis* (Lour.) Baill. carpels. **3.53.**

Immature carpel in cross section showing ridged outline, endocarp (E) of large, thin-walled

cells, and single ovule. SL 14490, scale = 100 μm. **3.54.** Longitudinal section of immature

carpel showing dorsal ridges, thin ventral wall, and recurved style with papillate stigma. SL

14491, scale = 250 μm. **3.55.** Cross section of intermediate stage carpel, showing

pronounced ridges and triangular outline. SL 14492, scale = 250 μm. **3.56.** Coronal

longitudinal section of intermediate fruitlet, showing irregular ridged outline and V-shaped

stigma (arrow). SL 14493, scale = 250 μm. **3.57.** Mid-apical cross section of fruitlet with

ovate shape and bilateral symmetry with two major lobes; dorsal fruit wall well developed.

SL 14494, scale = 250 μm. **3.58.** Ventral wall of fruitlet in cross section, showing fused

carpel margins. SL 14494, scale = 100 μm. **3.59.** Mid-basal cross section through fruitlet

showing thinner ventral wall. SL 14495, scale = 250 μm. **3.60.** Basal cross section through

fruitlet showing more triangular shape, open ventral wall and developed dorsal and lateral

wall. SL 14496, scale = 250 μm. **3.61.** Coronal longitudinal section showing ridged outline.

SL 14497, scale = 250 μm. **3.62.** Sagittal longitudinal section of fruitlet with apical style,

thin ventral wall with basal area broken (left) and ridged dorsal wall (right). Recurved style

at apex. SL 14498, scale = 250 μm.

Figures 3.53—3.62
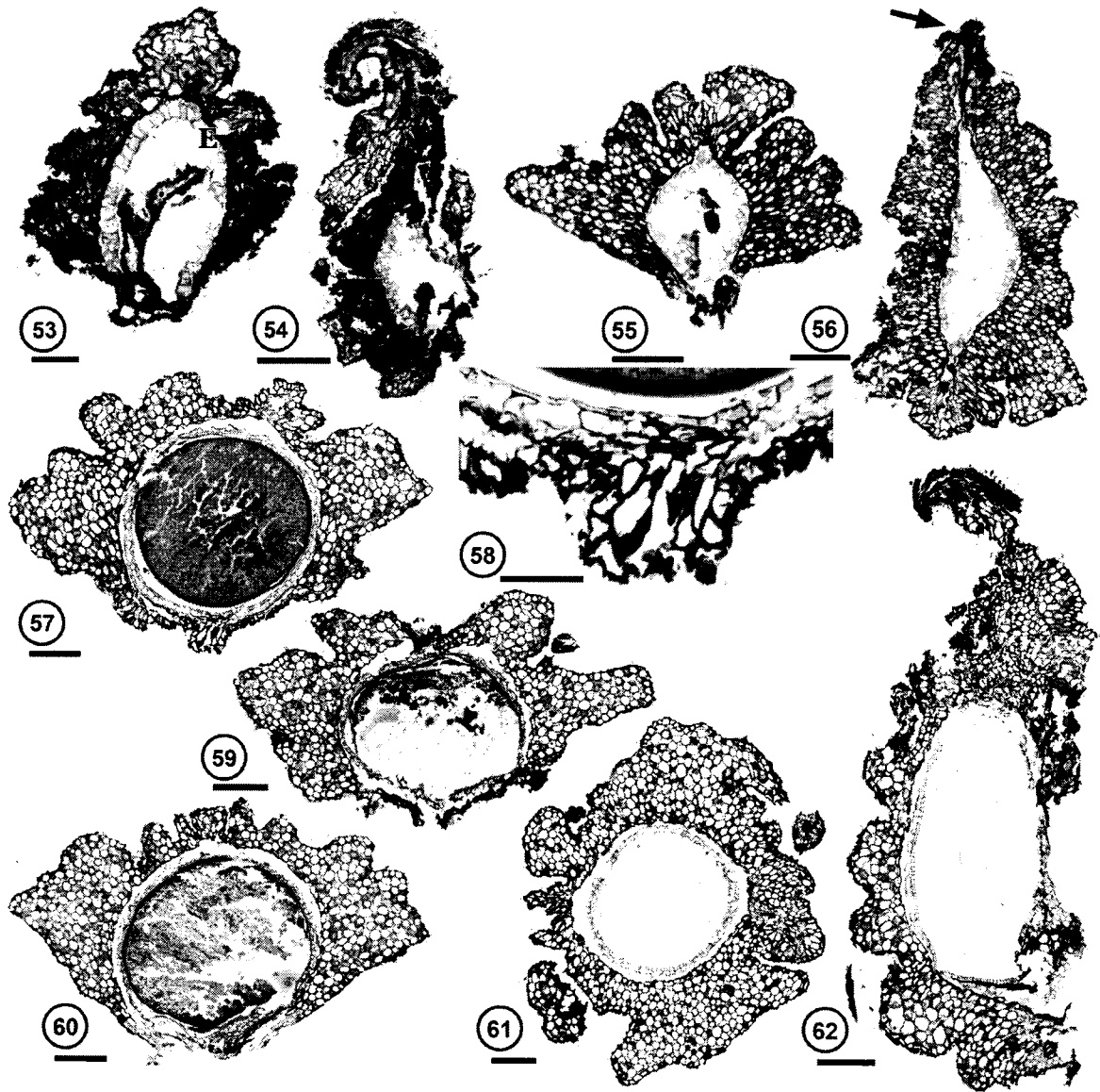
Figures 3.63-3.70. Anatomy of fruit wall and seeds of *Saururus chinensis* (Lour.) Baill. **3.63.** Immature carpel wall, with small-diameter mesocarp cells lacking intercellular spaces and large, thin-walled cells of endocarp (E). SL 14491, scale = 50 μm. **3.64.** Intermediate stage carpel wall, with large mesocarp cells showing some pitting, but few intercellular spaces, and thin-walled endocarp (E). SL 14492, scale = 50 μm. **3.65.** Mature mesocarp with large round cells and prominent intercellular spaces; endocarp (E) separated from mesocarp and now appressed to seed coat (SC). SL 14494, scale = 100 μm. **3.66.** Pitting on mesocarp cells. SL 14494, scale = 25 μm. **3.67.** Integumentary beak-like projection at micropylar end of ovule. SL 14498, scale = 50 μm. **3.68.** Oblique section through integument, showing thin-walled cells of endocarp (E), narrow, rectangular cells of outer integument (O), and inner amorphous layer of the outer integument (pink;*). SL 14498, scale = 50 μm. **3.69.** Integument and fruit wall, showing mesocarp (M) separate from thin-walled endocarp (E), outermost integument of lignified rectangular cells (O), cuticular layer (pink;*), and two layers of inner integument (red; arrows). SL 14499, scale = 25 μm. **3.70.** Seed with layers of inner integument (red) appearing as single layer (arrow), solid cuticular layer (pink;*), and cells of outermost integument (O) adjacent to endocarp (E); mesocarp (M) is separate. SL 14498, scale = 25 μm.

Figures 3.63—3.70
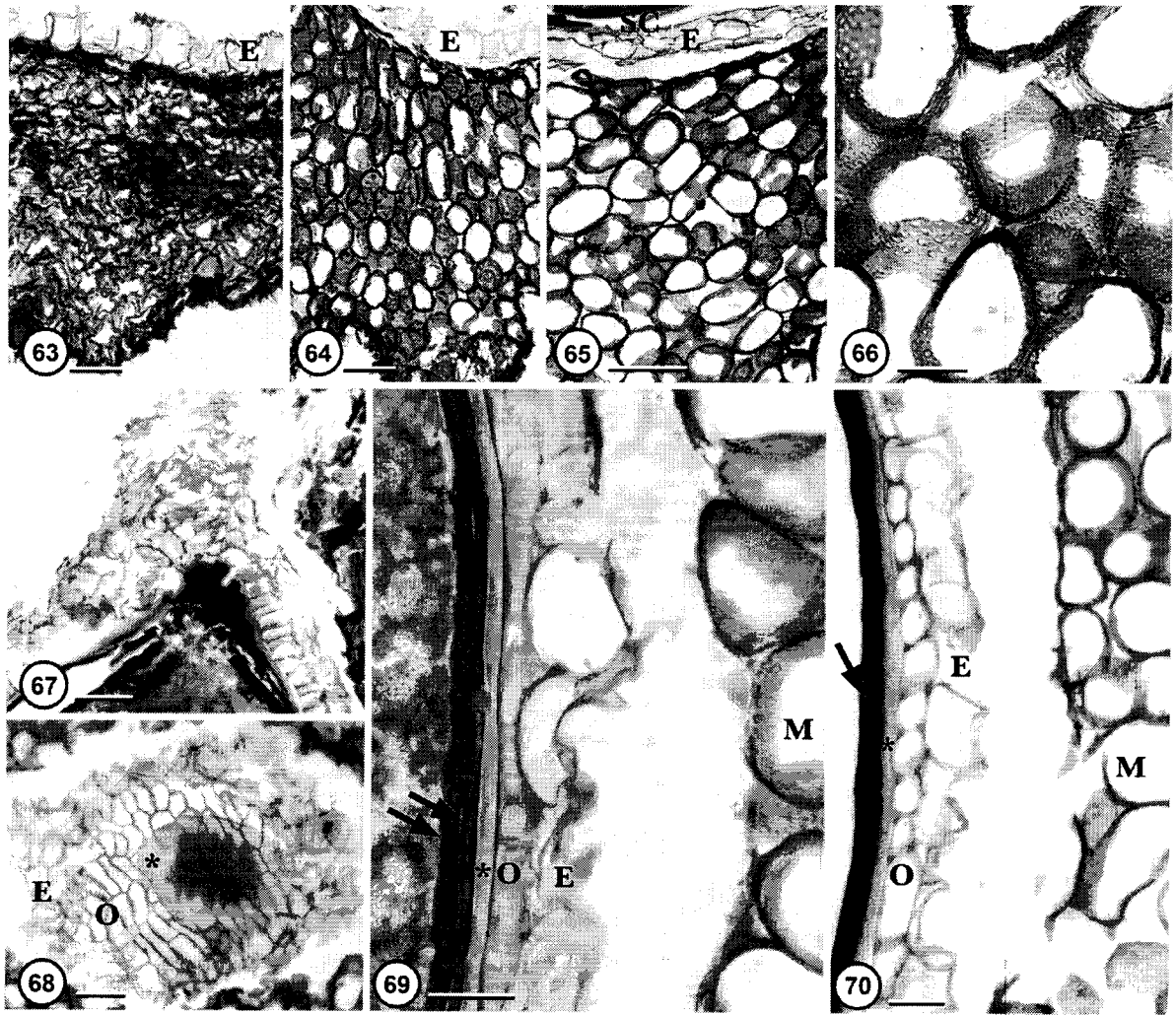
Figures 3.71-3.74. Anatomy of fruit wall and seeds of *Saururus chinensis* (Lour.) Baill.

3.71. Longitudinal section through ovule showing proliferated cells in chalaza, continuous with innermost integument. SL 14498, scale = 250 µm. **3.72.** Chalazal end of seed, showing cells with irregular shape (red), continuous with innermost integument (arrow); outer integument visible (O, *). SL 14498, scale = 25 µm. **3.73.** Longitudinal section of seed showing smooth innermost integument (arrow) to outside of persisperm (P), but interdigitating with endosperm (EN) cells. SL 14500, scale = 25 µm. **3.74.** Inner integument interdigitating with endosperm (blue, at bottom) at micropylar end of seed. Inner integument separated into two layers (arrow); two layers of outer integument (O, *) and cutinized inner wall of mesocarp (arrowhead, M) also visible. SL 14500, scale = 25 µm.
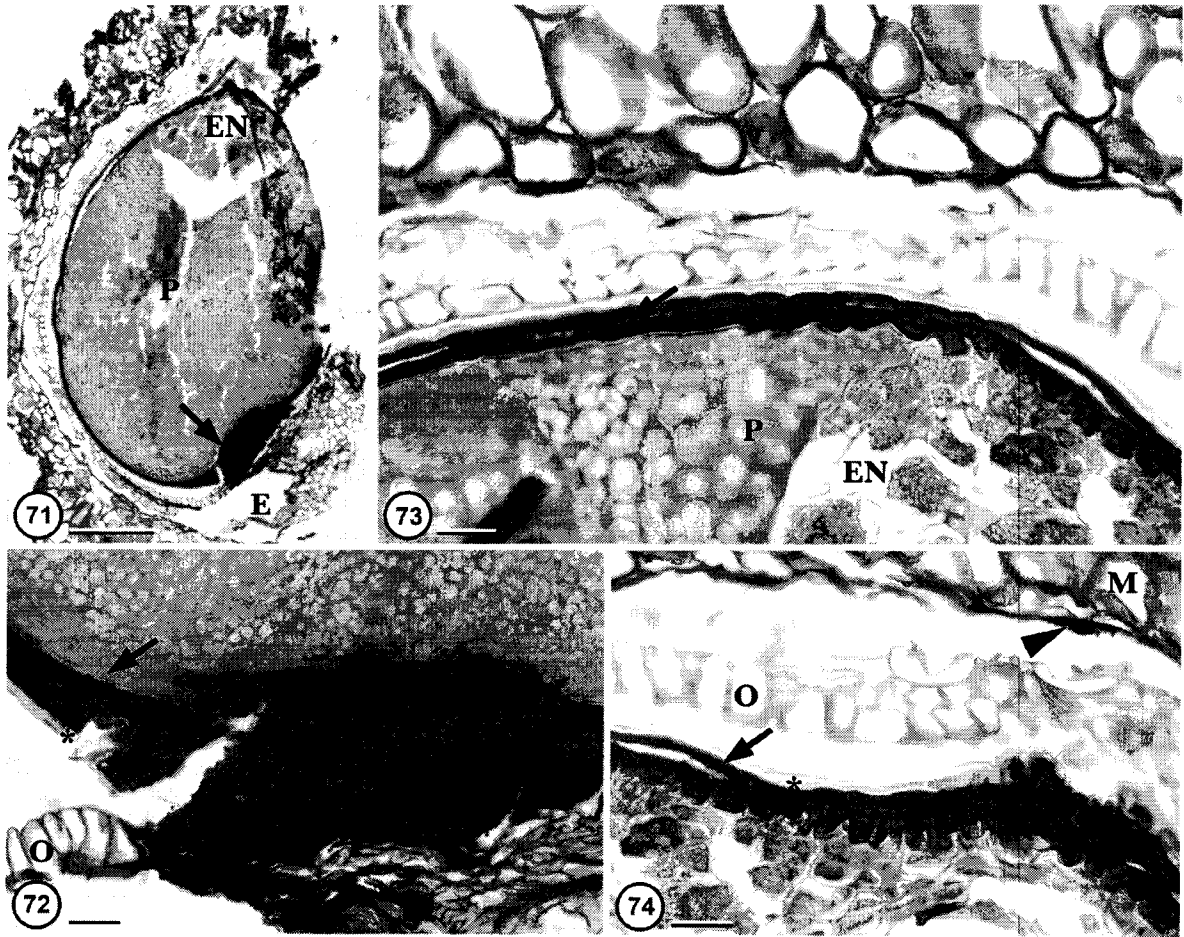
Figures   3.71—3.74

Figures 3.75-3.78. SEM of fruit wall and seeds of *Saururus chinensis* (Lour.) Baill.

3.75. Mesocarp showing cells with moderate pitting and intercellular air spaces. Scale = 20 μm. **3.76.** Single mesocarp cell with moderately pitted inner wall and smooth outer walls. Scale = 10 μm. **3.77.** Seed layers, showing large endocarp cells (E), outer integument (*) and inner integument (arrow) with perisperm (P) inside seed (left) and mesocarp (M) to the outside (right). Scale = 20 μm. **3.78.** Detail of integument showing large thin-walled cells of endocarp (E), outermost integument (O) and thick integumentary layer (*). Scale = 10 μm.

Figures   3.75—3.78
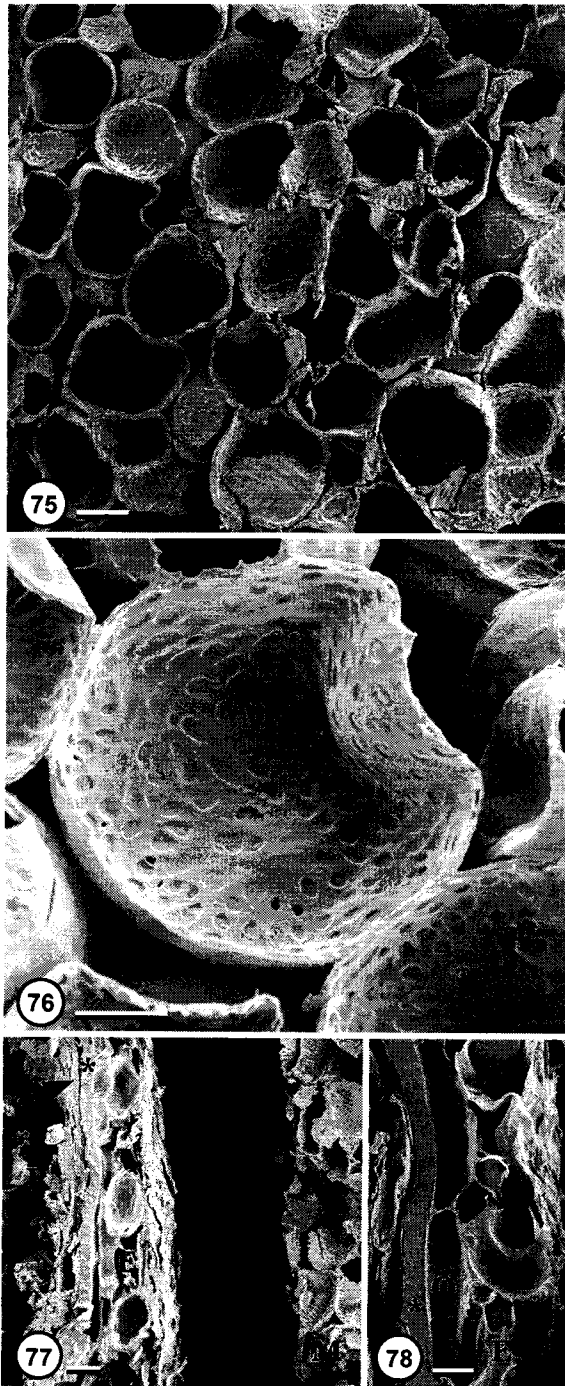
Chapter 4

Anatomy and development of *Saururus*-like fruits from the

Middle Eocene Princeton Chert

There are six extant species of Saururaceae, found in North America and Asia. In western North America, *Anemopsis californica* Hook. & Arn. grows in alkaline waters, while *Saururus cernuus* L. is found in wetlands of eastern North America (Wu and Kubitzki, 1993; Liang, 1995; Xia and Brach, 1999). Wet areas, including forests, streambanks and lakes in Asia, are populated by *Saururus chinensis* (Lour.) Baill., *Houttuynia cordata* Thunb., *Gymnotheca involucrata* Pei and *G. chinensis* Decne. (Wu and Kubitzki, 1993; Liang, 1995; Xia and Brach, 1999). Some species are grown horticulturally in North America as semi-aquatic wetland plants, namely lizard's tail (*S. cernuus*), chameleon plant (*Houttuynia*) and yerba mansa (*Anemopsis*).

Saururus produces long, racemose inflorescences with flowers that have a subtending bract, lack a perianth, and have six stamens and four basally connate carpels (Raju, 1961; Liang and Tucker, 1990; Wu and Kubitzki, 1993; Thien et al., 1994). Carpels mature into wrinkled or pleated indehiscent fruits with a dry-fleshy pericarp (Raju, 1961; Wood, 1971; Tucker, 1976; Wu and Kubitzki, 1993; Thien et al., 1994). Although the fruit has sometimes been called a berry (Raju, 1961; Tucker, 1976; Wu and Kubitzki, 1993), it is in fact a schizocarp as the carpels are fused at the base and split at maturity into separate fruitlets (Wood, 1971; pers. obs.)

Saururaceae fossils are rarely recognized. The only known fossils to date are fruits and seeds of *Saururus bilobatus* (Nikitin) Mai from the Upper Eocene to Pliocene of Europe and Siberia, seeds of *Houttuynia bavarica* Mai from the Miocene of Germany (Mai and

Walther, 1978; Friis, 1985; Lesiak, 1994; Mai, 1999, 2001), and flowers of *S. tuckerae* from the Middle Eocene Princeton Chert (Chapter 2). No fossil vegetative remains are currently known.

The Middle Eocene Princeton Chert has been well-studied for several decades and preserves a variety of plants from an ancient wetland environment (Cevallos-Ferriz et al., 1991; Pigg and Stockey, 1996; Stockey, 2001). Previous work recognized a fruit type putatively assigned to Rosaceae (Cevallos-Ferriz, 1987). In this chapter, I show that this fruit type belongs to *Saururus tuckerae* (Saururaceae), previously known only from minute fossil flowers with in situ pollen, arranged in a racemose inflorescence (Chapter 2). The fruits of *S. tuckerae* are anatomically preserved and the large number of specimens show a developmental series from flowers to mature, isolated fruits. These fossil fruits are compared to fruits of extant *Saururus* (Chapter 3).

MATERIALS AND METHODS

The fossils fruits come from the Princeton Chert locality, located on the east bank of the Similkameen River, 8.4 km south of the town of Princeton, British Columbia (Boneham, 1968). The outcrop consists of interbedded layers of chert and coal with occasional ash layers (Stockey, 1983). There are about 49 major chert layers, but these split and anastomose resulting in 70 individual beds ranging in thickness from 1 to 50 cm (Smith et al., 2006). The chert is part of the Princeton Group, Allenby Formation (Boneham, 1968). Freshwater fish (Wilson, 1977, 1982), mammals (Russell, 1935; Gazin, 1953) and K-Ar dating (Hills and Baadsgaard, 1967) indicate the locality is Middle Eocene in age. The ash of Layer #22 is currently dated at 48.7 million years (H. Baadsgaard, pers. comm., 1999).

Fruits and fruitlets, as well as floral material of *S. tuckerae* are commonly found in

Layer #43; fruits are present also in layer #8. Co-occurring plants in both layers typically

include fruits, seeds, stems and roots of *Decodon allenbyensis* Cevallos-Ferriz & Stockey

(1988b; Little and Stockey, 2003, 2005), seeds of *Keratosperma allenbyense* Cevallos-

Ferriz & Stockey emend. Smith & Stockey (Cevallos-Ferriz and Stockey, 1988a; Smith and

Stockey, 2003), fruits and seeds of *Paleomyrtinaea* Pigg, Stockey & Maxwell (1993),

undescribed monocot vegetative remains, and rare seeds of *Allenbya collinsonae* Cevallos-

Ferriz & Stockey (1989).

Chert blocks were cut into slabs and studied using the cellulose acetate peel technique

(Joy et al., 1956) modified for concentrated (48%) hydrofluoric acid (Basinger and

Rothwell, 1977; Basinger, 1981). Peels were mounted on microscope slides using Eukitt

(O. Kindler GmbH, Freiburg, Germany) xylene-soluble mounting medium. Images were

captured with a PowerPhase digital scanning camera (Phase One, A/S, Fredriksberg,

Denmark) and a Nikon Coolpix 5400. Photographs were processed with Adobe

Photoshop CS. Three-dimensional reconstructions were made using photos of serial

sections and the computer visualization software, AMIRA 3.1.1 (TGS Software, San Diego,

California, USA). Confocal Laser Scanning Microscopy (CLSM) was employed to examine

the fossils, using TRITC profile (543 nm excitation; 560-620 nm emission detection), on a

Leica TCS-SP2 Multiphoton Confocal Laser Scanning Microscope (TCS-MP).

For scanning electron microscopy (SEM) chert samples with fruits were mounted on

stubs using silver paint, after the surface was etched with hydrofluoric acid to dissolve the

siliceous matrix. Samples were coated with 150 Å gold on a Nanotek Semprep II sputter

coater, and observed using a JEOL 6301F (Field Emission Scanning Electron Microscope).

RESULTS

Systematics—Order—Piperales Dumort

Family—Saururaceae Martynov

Genus—Saururus L.

Species—Saururus tuckerae

Amplified specific diagnosis— Inflorescence a raceme, at least 2.9 mm long and 1.0 mm diam. Flowers ca. 0.8 mm diam. Bract one, cup-shaped, ovate, subtending flower; flower-bract stalk and pedicel present. Perianth absent. Stamens five, adnate to carpels, up to 0.8 mm long, tetrasporangiate, with latrorse longitudinal dehiscence. Pollen 6-11 μm, monosulcate, boat-shaped-elliptic; sculpturing punctate. Carpels four, basally connate, tapering at apex, up to 1.2 mm long, 0.4 mm wide. Styles one per carpel, recurved. Seeds one per carpel, attached marginally near base. Seeds one (rarely two) per carpel, attached marginally near base. Fruits schizocarpic, indehiscent, fruitlets up to 1.5 X 0.9 mm diameter and 1.8 mm high, bilobed, smooth to moderately ridged, spongy fruit wall.

*Holotype—*P1631 Bbot a (Chapter 2).

*Specimens examined in this paper—*P1631 (all faces); P3490 Ctop; P4592 B,top; P4947 A; P5102 Btop; P5108 D; P5141 Dtop; P5144 Cbot; P5836 B,top, M,bot; P5937 G; P5964 Dtop; P5993 A; P6025 G.

All specimens are housed in the University of Alberta Paleobotanical Collection (UAPC-ALTA).

Description—*Flowers and immature carpels—*Hundreds of immature carpels of *Saururus tuckerae* have been found in the chert. Often carpels are found singly in the chert matrix, but groups of two to four attached carpels are also found. Floral remains (see

Chapter 2) preserve the earliest stages of carpel development. The four basally connate

carpels are wider at the base (Fig. 4.1) and taper towards the apex (Fig. 4.2). The carpels

measure ca. 0.5 mm in coronal diameter X 0.5 mm high X 0.4 mm in sagittal diameter and

the carpel wall is ca. 11 cells thick. Two lobes of tissue are apparent in cross section,

situated on the dorsi-lateral side of the carpel (Figs. 4.1, 4.2, 4.4). Cells of the carpel wall

are small (ca. 6-12 μm) and indistinct, with dark contents (Fig. 4.5). Immature carpels have

incomplete closure at the base (Figs. 4.1, 4.4). The inner layer of the carpel wall is

composed of large, thin-walled cells, 15-33 μm in diameter, that are often difficult to see in

peels (Figs. 4.2, 4.5). No autofluorescence in any tissue occurs at this stage (Figs. 4.2, 4.3).

A single small orthotropous ovule with lateral placentation is present in each carpel (Fig.

4.1).

Intermediate stages—Some intermediate stages of fruit development are found in the

chert, recognizable by their larger size (ca. 0.7 X 0.5 mm in diameter) relative to immature

carpels, but having the same basic ovoid shape, with two lateral lobes in cross section (Figs.

4.6-4.11), as seen in immature carpels. One specimen (Fig. 4.11) shows an intermediate

carpel still attached to three other carpels, bearing similarities to the preserved floral stages

and providing evidence for the connection of the fruits to the floral material. Cells of the

carpel wall (mesocarp) are more individually distinguishable, and have increased slightly in

size to ca. 12-24 μm. Cell number does not appear to increase. These changes are most

apparent in the two carpel lobes. The endocarp is distinct, a single cell layer thick (Fig. 4.9,

4.10). The large, thin-walled cells of the endocarp do not show any size difference from

immature stages. A central cuticle-like layer in the ovule is more prominent at this stage

(Figs. 4.6-4.8, 4.10, 4.11) and autofluorescent when excited using CLSM (green light) (Figs. 4.7, 4.8).

Mature fruits—Over 3000 mature fruits are known from the Princeton Chert. Fruits are schizocarpic and have four fruitlets (mericarps) (Figs. 4.1, 4.2, 4.11, 4.26). The mature fruitlets of *S. tuckerae* are up to 1.5 mm in coronal diameter X 1.8 mm high X 0.9 in mm sagittal diameter. In cross section fruitlets are narrower at the apex (Fig. 4.12) than the base (Fig. 4.14), and bilobed (Figs. 4.12-4.16). In transverse section, fruitlets have a smooth external surface (Fig. 4.16). Occasionally, transverse and longitudinal sections show an external surface that is ridged (Fig. 4.13, 4.15, 4.18, 4.20, 4.23). A ridge formed from the carpel margins is seen on the ventral surface of fruitlets (Figs. 4.16, 4.17). The ventral surface is thin and is broken open at the base (Figs. 4.14, 4.24, 4.25, 4.38) where fruitlets were attached to other carpels. In longitudinal section fruitlets vary in shape; the point of attachment to receptacle is sometimes seen at the basal end of the fruitlet (Figs. 4.18, 4.19). At the fruitlet apex the two mesocarp lobes are distinct (Fig. 4.19).

Longitudinal sections of mature fruitlets show that three vascular bundles are present in the fruit wall, with one more dorsally located (Fig. 4.20, arrow), leading into the style (Fig. 4.23, arrow) and two lateral bundles (Fig. 4.21, arrows). Tracheary elements have helical thickenings (Fig. 4.22). Style (Fig. 4.23, 4.24) and/or stamen (Fig. 4.25) remnants are occasionally persistent. Fruitlets are sometimes also found attached to stamens and the floral receptacle (Figs. 4.26, 4.27).

Cells of the exocarp/epicarp sometimes have contents preserved (Fig. 4.29). The thickest area of the mature fruitlets is formed by the lateral lobes, which are each up to 15 cells thick. Cells of the mesocarp are 24-36 μm in diameter, and the many intercellular

spaces give the fruit wall a spongy appearance (Figs. 4.28, 4.29, 31). Under SEM, mesocarp

cells are fairly smooth on the inside, with some faint circular depressions that may

represent sculpturing (Figs. 4.31, 4.32). There is no sign of pitting under the light

microscope (Figs. 4.28, 4.29). Endocarp in the mature fruitlets is present as a single layer

with thin-walled, cuboidal to slightly rectangular cells that measure 21-36 µm in diameter

(Figs. 4.21- 4.24, 4.33, 4.39-4.42). At this stage it is more closely associated with the seed

(Figs. 4.21- 4.24, 4.33, 4.39-4.42) than the mesocarp, as in earlier stages of development

(Figs. 4.6, 4.8, 4.10, 4.30).

Three-dimensional reconstructions show the smooth surface of the fruitlets (Figs.

4.34, 4.35, 4.36). Dorsally, there is a groove at the fruit apex, and the lateral lobes are

visible (Fig. 4.34). Ventrally, a groove is formed where the carpel margins meet (Fig. 4.35).

Basally, the ventral region of the fruitlet is open, where it broke apart from other carpels in

the flower. Fruitlets have an expanded ventral surface, and the style is on the ventral side

(Fig. 4.36).

Seeds—Each fruitlet has one seed (Figs. 4.1, 4.6, 4.7, 4.8, 4.10, 4.11, 4.13-4.19, 4.21,

4.23-4.25, 4.27, 4.30, 4.38), although one specimen contains two seeds in a single fruitlet

(Fig. 4.37). Seeds are ovoid, nearly fill the locule, and the integument forms a small beak-

like projection at the micropylar end (Figs. 4.38). The inner walls of the endocarp cells

have an autofluorescent, cuticle-like layer (Figs. 4.40, 4.43, 4.44, 4.47). Small, rectangular,

thin-walled cells arranged in rows can be seen in oblique sections (Figs. 4.41, 4.42) and

represent the outer integument. A thick, crystallized layer that is striated under SEM (Figs.

4.33) is seen between the outer integument and inner integument. The innermost layer of

the integument is a thin, dark layer that is much thickened at the chalazal end of the ovule

(Figs. 4.23, 4.39, 4.40, 4.41, 4.46, 4.47). This layer does not autofluoresce under CLSM

(Figs. 4.43, 4.44). The inner integument shows clearly distinguishable, rectangular cells with

irregular outlines (Fig. 4.45). Cells of perisperm or embryos are not present in any

specimens from Princeton.

DISCUSSION

Mature fossil fruitlets of *Saururus tuckerae* are found dispersed singly and, rarely,

attached in groups of four to the floral receptacle and stamens, indicating that these fruits

were schizocarpic. The open ventral wall at the base is additional evidence that the fruits

split apart from the other basally connate carpels. Mature fruits have a spongy fruit wall and

single seed per fruitlet. The developmental sequence preserved shows that fruitlets

underwent a threefold increase in diameter, and this is seen more as an increase in height

and coronal width than in sagittal width. Immature fruits are ca. 0.5 mm coronal diameter x

0.5 mm high x 0.4 mm sagittal diameter, while mature fruits measure up to 1.5 coronal

diameter x 1.8 mm high x 0.9 mm sagittal diameter. Carpel shape is consistent throughout

development, being broader at the base than the apex and two-lobed, with a fairly smooth

surface. Fruits do not show a marked increase in the number of cells of the mesocarp, but

those cells underwent a sixfold increase in size, from 6 μm in immature carpels to over 36

μm in mature fruitlets. The mesocarp is fleshy with air spaces. Endocarp cells did not

increase in size during fruit maturation.

The fossil fruits share many features with extant fruits of *Saururus* (described in

Chapter 3). They are schizocarpic and occur in groups of four fruitlets. Fossil fruitlets were

probably easily detached from one another, since they are rarely found attached. The

mesocarp of extant *Saururus* is composed of spongy parenchyma, like the fossil. The

presence of air spaces has been hypothesized to help the fruits float in water (Johnson, 1900; Plisko, 1988). The fruitlets are of a similar size across all three species with bilateral symmetry. Fruitlets of *S. chinensis, S. cernuus* and *S. tuckerae* all show a ridge where the carpel margins meet. Many fossil specimens have a basally open ventral surface, as is also observed on the living fruits, representing the area of separation from neighbouring fruitlets (Chapter 3). One, or rarely two, seeds per carpel are found both in extant *Saururus* and in the fossil carpels.

In the fossil, *S. tuckerae,* I observed a similar seed anatomy to that seen in extant *Saururus* (Chapter 3). Both have an outer integument formed of thick-walled rectangular cells. In the fossil, a crystalline layer seems to replace the two thick, amorphous layers seen in extant *Saururus* seeds. In both the fossil and extant *Saururus* there is an innermost layer of thick-walled cells that proliferate at the chalaza.

However, there are some differences between *Saururus tuckerae* fruits and those of the two extant *Saururus* species. The surfaces of the fossil fruitlets are much smoother than the very wrinkled surfaces observed in extant fruits. Cells of the mesocarp in *S. cernuus* are strongly pitted (Friis, 1985; Plisko, 1988; Chapter 3), whereas in *S. chinensis* walls are shallowly dimpled (Chapter 3). In contrast, fruits of *S. tuckerae* have mesocarp cells with weakly pitted to smooth cell walls.

The only fossil record for the family Saururaceae, other than the Princeton Chert material, is that of fossil fruits and seeds of *Saururus bilobatus* (Nikitin) Mai and *Houttuynia bavarica* Mai from the late Eocene to Pliocene of Europe and Siberia (Mai and Walther, 1978; Friis, 1985; Lesiak, 1994; Mai, 1999). The fossil fruitlets of *Saururus bilobatus* have an apical cleft, not seen in extant species, and fruitlets are somewhat

wrinkled, but not to the extent seen in the living species (Mai and Walther, 1978; Friis, 1985; Lesiak, 1994). Fruitlets of *S. bilobatus* have a mean length of 1.22 mm and a mean diameter of 1.08 mm (Friis, 1985), values within the size range of *S. tuckerae* fruitlets. Thus, the fruitlets of *S. tuckerae* are similar to those of *S. bilobatus* in having an apical cleft and smooth sculpturing. However, the fruitlets of *S. tuckerae* differ from those of *S. bilobatus* in the degree of pitting in cells of the fruit wall. *Saururus bilobatus* has strongly pitted mesocarp cells, like *S. cernuus* (Friis, 1985) while those of *S. tuckerae* show weak to no pitting and are more similar to the weakly pitted mesocarp cells of *S. chinensis*. Floral stages are known for *S. tuckerae* (Chapter 2), but unfortunately not for *S. bilobatus*. Thus, more detailed comparisons are not possible between *S. tuckerae* and *S. bilobatus* at this time. As more fossil Saururaceae are found in Europe, the exact relationship between *S. tuckerae* and *S. bilobatus* may be refined.

The discovery here of saururaceous fossils from western North America has contributed valuable data for examining the history of the distribution of Saururaceae, and the genus *Saururus*. Both Saururaceae and *Saururus* show a classic Paleogene disjunct distribution, present in eastern Asia and eastern North America (Tiffney, 1985). *Saururus bilobatus* and *S. tuckerae* clearly show that *Saururus* was once widespread in the northern hemisphere than found at present. For Saururaceae, it would be helpful if more fossils, whether macro- or microfossils, were found to provide more data points that could help define hypotheses on the origin, distribution, and migration of saururaceous taxa. It is almost certain that there is (at least) a pollen record for Saururaceae that has not been recognized (Chapters 2, 5).

This study has shown that the fossil fruits from the Princeton Chert share many similarities with fruits of extant *Saururus*. The developmental series that is preserved allows the fruits to be confidently linked to the floral remains described in Chapter 2 as *Saururus tuckerae*. Together, the referral of the fossils to the genus *Saururus* is reinforced by the detailed knowledge of both flowers and fruits. *Saururus tuckerae* is the most complete fossil saururaceous taxon known. There is a need to look for more fossil Saururaceae elsewhere in the world that will help to clarify the exact nature of relationships between extant and fossil taxa as well as the biogeographic history of the family. However, the recognition of this Princeton fossil gives us hope that other such fossils are out there, awaiting discovery or recognition.

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Figures 4.1 - 4.11. Immature and intermediate developmental stages of carpels of the fossil *Saururus tuckerae*. **4.1.** Basal cross section through flower showing four bilobed, connate carpels. P5108 D #71h, scale = 100 μm. **4.2.** Cross section, viewed using transmitted light microscopy, through flower showing four carpels, each with one ovule and large endocarp cells. P1631 Btop #28, scale = 150 μm. **4.3.** Same as Fig. 4.2 but viewed under CLSM; note absence of any autofluorescent structures. P1631 Btop #28, scale = 150 μm. **4.4.** Cross section of two isolated, immature carpels, showing triangular, bilobed nature, thick dorsal wall and thin, incompletely closed ventral wall (arrow). P1631 Btop #13b, scale = 100 μm. **4.5.** Enlarged view of immature carpel wall, showing mesocarp of small cells with dark contents and endocarp of large, thin-walled cells (E). P1631 Btop #17b, scale = 50 μm. **4.6.** Two isolated, intermediate stage carpels, showing large, rectangular cells of endocarp (E) and single ovule (OV) per locule. P6025 G #85e, scale = 100 μm. **4.7.** Cross section of intermediate stage carpel, viewed under light microscopy, showing bilobed nature of carpel and features of the ovule. P1631 Btop #9a, scale = 80 μm. **4.8.** Same as Fig. 4.7, but viewed under CLSM; note strongly autofluorescent cuticular layer and weakly autofluorescent large rectangular cells of endocarp. P1631 Btop #9a, scale = 80 μm. **4.9.** Enlarged view of cells in carpel wall of an intermediate-stage carpel. Note compact nature of small-diametered mesocarp cells; endocarp cells (E) large and thin-walled. P1631 Cbot #109g, scale = 50 μm. **4.10.** Cross section of isolated intermediate stage carpel, showing two thick dorsi-lateral lobes, thin ventral wall, endocarp (E), and single ovule (OV) in locule. P1631 Cbot #109g , scale = 100 μm. **4.11.** Cross section of flower with two enlarged carpels of an intermediate stage each with an ovule (OV), and subtending bract (arrow). P4592 Bitop #3, scale = 250 μm.
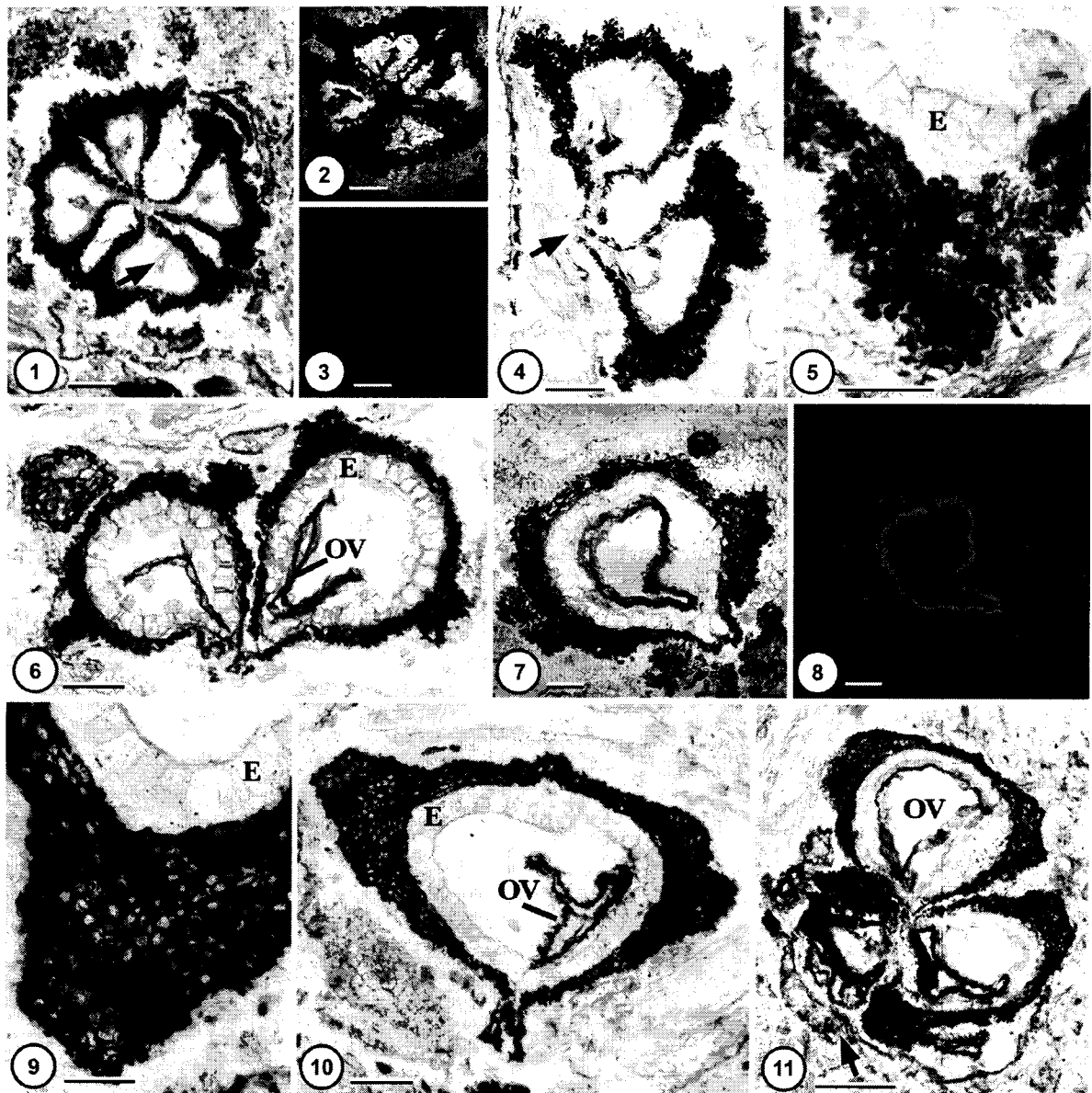
Figures 4.1—4.11

Figures 4.12 - 4.23. Mature fruits of *Saururus tuckerae*. **4.12.** Cross section near apex

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4.20. Longitudinal section through dorsal part of fruit. Note single vascular trace in fruit

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fruitlet showing stylar remnants at apex, vascular tissue (V), and single ovule with beak-like

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Ctop #8, scale = 250 μm.

Figures 4.12—4.23
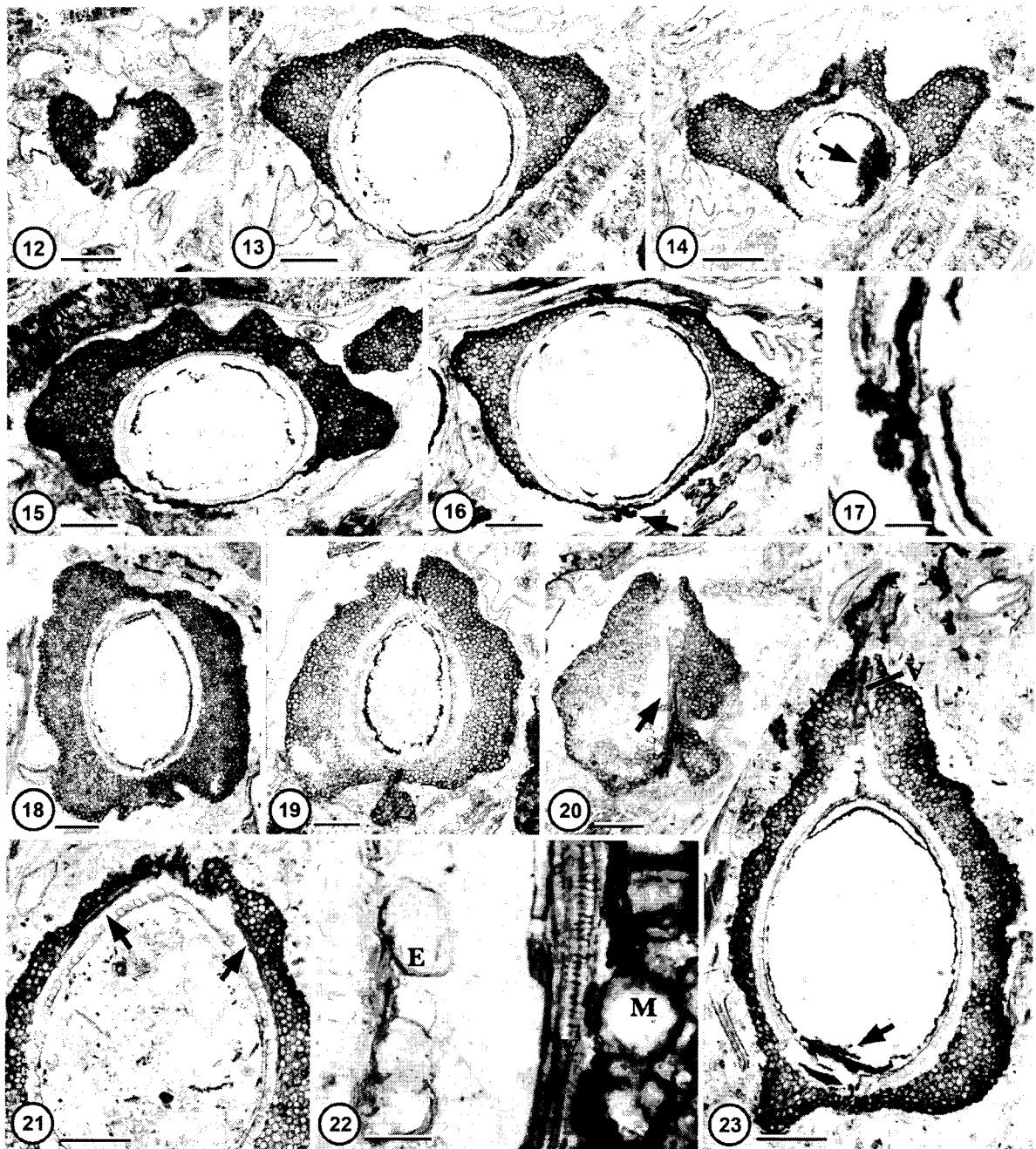
Figures 4.24 – 4.30. Details of mature fruits of *Saururus tuckerae*. **4.24.** Longitudinal section through fruitlet showing recurved style at apex, near ventral side of fruit; thin ventral tissue, missing at base of fruitlet (arrow); single ovule in locule; and slightly undulating dorsal fruit wall. P6025 G #53d, scale = 250 μm. **4.25.** Cross section near base of fruitlet showing attached stamen (S). P6025 G #99g, scale = 250 μm. **4.26.** Oblique cross section through whole fruit with subtending bract (arrow) showing four connate fruitlets. P5144 Cbot #0, scale = 250 μm. **4.27.** Longitudinal section showing two fruitlets, two stamens (S), and attachment to floral receptacle with bract (arrow). P5964 Dtop #8, scale = 250 μm. **4.28.** Detail of mature fruit wall. Note abundance of air spaces between cells. P1631 A #6, scale = 50 μm. **4.29.** Detail of mature fruit wall, showing cells with dark contents in exocarp (arrow). P1631 A #6, scale = 50 μm. **4.30.** Immature carpel showing young endocarp tissue (E) closely associated with mesocarp (M), and single ovule (arrow). P5108 D #71h, scale = 50 μm.

Figures 4.24—4.30
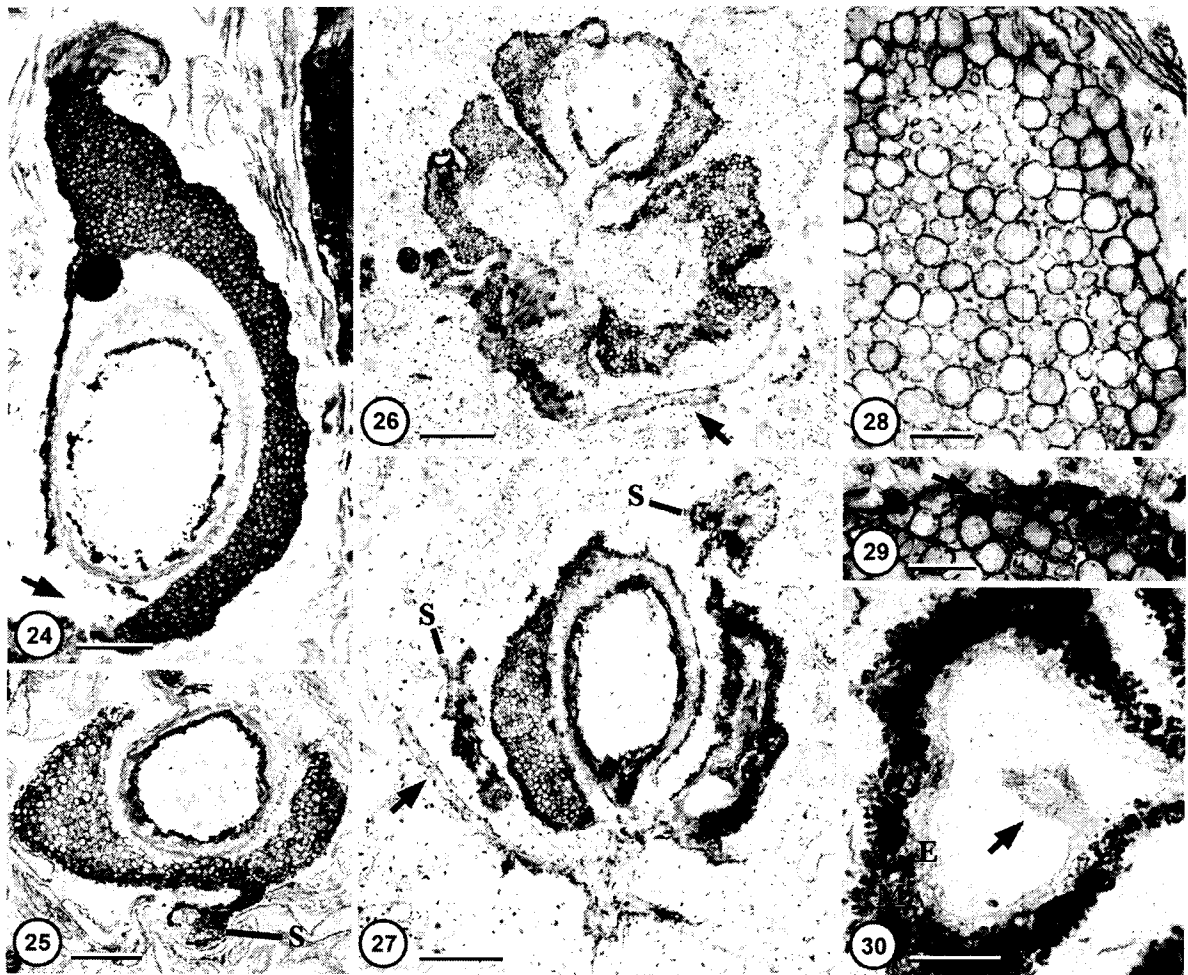
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Figures 4.31—4.36
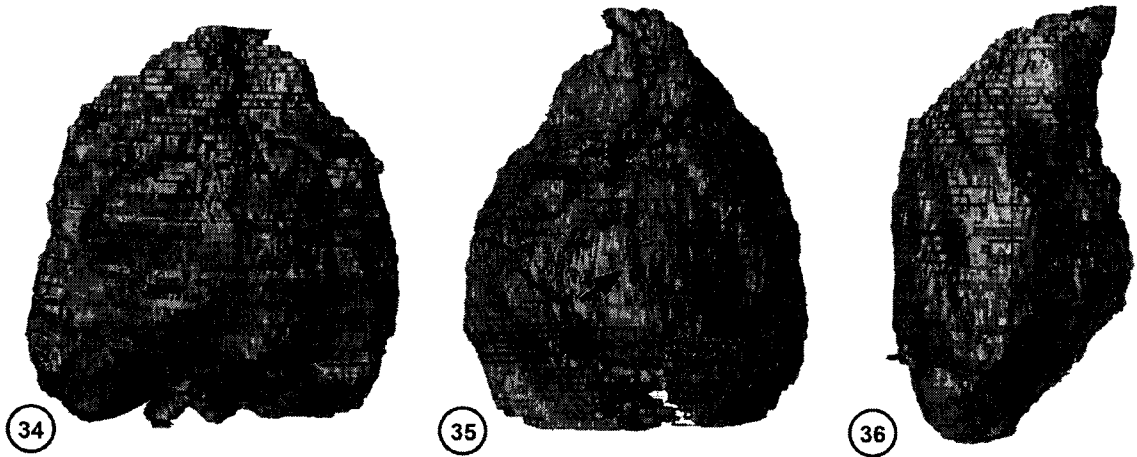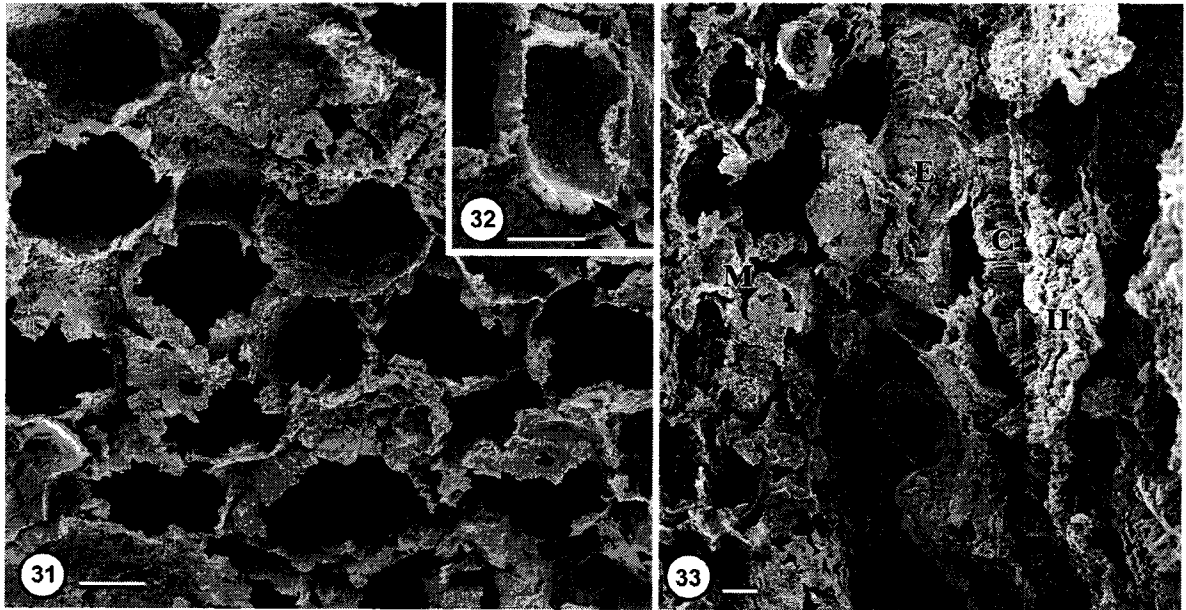
Figures 4.37 - 4.47. Seeds of *Saururus tuckerae*. **4.37.** Section through fruitlet

showing atypical presence of two ovules. P1631 Ctop #80a, scale = 250 μm. **4.38.**

Longitudinal section of fruitlet showing beak (arrow) at ovule apex. P1631 Cbot #14c, scale

= 250 μm. **4.39.** Details of seed coat, showing endocarp (E) separated from mesocarp (M),

outermost integumentary layer of thin-walled rectangular cells (O) and innermost

integument (II). P1631 A #13, scale = 50 μm. **4.40.** Seed coat cross section showing

endocarp (E) with inner cuticle-like layer, crystalline layer (C) replacing integument, and

dark layer of innermost integument (II). P5108 D #86d, scale = 50 μm. **4.41.** Beak-like

integumentary projection at micropylar end of ovule. P1631 Btop #39j, scale = 50 μm.

4.42. Oblique section of seed integument showing large, thin-walled endocarp cells (E)

and, centre, rectangular cells of outer integument (O). P5836 M₁bot #1, scale = 50 μm.

4.43. Section, viewed under light microscopy, showing two distinct layers of integument

(arrow, *). P1631 Btop #33a, scale = 100 μm. **4.44.** Same section as Fig. 4.43, but viewed

under CLSM, showing autofluorescent cuticular outer integument (arrow); inner

integument not autofluorescent. P1631 Btop #33a, scale = 100 μm. **4.45.** Innermost

integument composed of rectangular cells with wavy outlines. P5836 B₁top #31b, scale = 50

μm. **4.46.** Longitudinal section of fruitlet showing innermost integumentary layer

continuous with proliferated cells in chalaza. P6025 G #73c, scale = 150 μm. **4.47.** Detail

of chalazal end of ovule, showing integuments attached to chalazal proliferation of cells.

P5108 D #39d, scale = 50 μm.

Figures 4.37—4.47
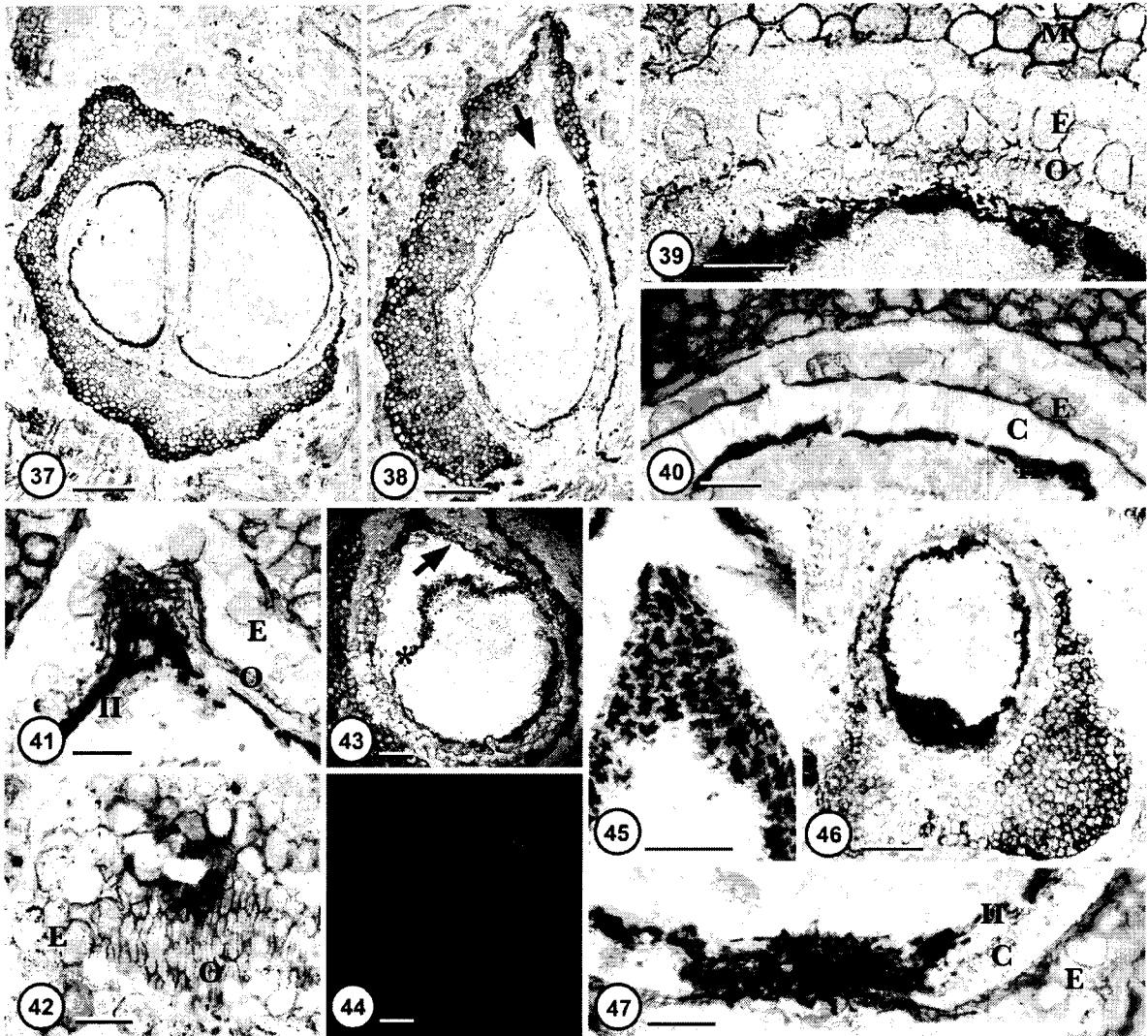
Chapter 5

Pollen morphology and ultrastructure of Saururaceae

Because of its variability in size, structure and ornamentation across taxa, pollen is a good source of many useful systematic characters. Pollen has been used as an important source of phylogenetic characters for elucidating angiosperm relationships and examining character evolution in the angiosperms (Walker and Doyle, 1975; Doyle, 2005). In the case of early angiosperms, pollen is particularly useful as it is often the only evidence for helping to elucidate the relationships of these ancient plants (e.g., Zavada and Benson, 1987; Doyle and Hotton, 1991; Friis et al., 2000, 2004; Doyle, 2005). However, the utility of pollen is limited by the small number of characters available, and by how well we understand the characters of a group. Characters are sometimes based on one or a few exemplar taxa and do not represent the whole range of variation in the family, or there may be uncertainty in some characters. Therefore, studies that examine many taxa and clearly show the pollen features of a group are very valuable.

Detailed studies have not been done on the pollen characters of Saururaceae, and there is no fossil pollen record for the family (Muller, 1981; Song et al., 2004). The four genera and six species of extant Saururaceae are distributed in North America and eastern Asia (Wu and Kubitzki, 1993; Xia and Brach, 1999). *Anemopsis californica* Hook. & Arn. grows in wet areas (including alkaline, saline, and coastal marsh areas) of western North America while *Saururus cernuus* L. inhabits the swamps and other wet or moist forest areas of eastern North America (Wu and Kubitzki, 1993; Buddell and Theiret, 1997). *Saururus chinensis* (Lour.) Baill. is distributed in Asia from Korea to Taiwan, Himalayas to Japan (Wu and Kubitzki, 1993; Xia and Brach, 1999). *Houttuynia cordata* Thunb. is the most

widespread Asian taxon, occurring from Korea south into Indonesia, and from western

India to Japan (Wu and Kubitzki, 1993; Xia and Brach, 1999). *Gymnotheca involucrata*

Pei and *G. chinensis* Decne. have a restricted distribution. *Gymnotheca chinensis* occurs in

central and southwestern China and northern Vietnam, while *G. involucrata* is endemic to

the province of Sichuan in China (Xia and Brach, 1999).

The currently accepted phylogenetic position of Saururaceae is sister to Piperaceae

within Piperales (APG, 2003; Jaramillo et al., 2004; Neinhuis et al., 2005). Piperales have

been placed in various phylogenetic positions within the angiosperms, but seem well

supported as part of the magnoliid clade, which also includes Canellales, Magnoliales, and

Laurales (Graham and Olmstead, 2000; Nickrent et al., 2002; Zanis et al., 2002; APG,

2003; Borsch et al., 2003; Hilu et al., 2003; Soltis and Soltis 2004; Qiu et al., 2005,

Graham et al., 2006).

There are some reports on pollen characters as generalized for the family (e.g.,

Walker, 1976a, 1976b; Grayum, 1992; Doyle, 2005) or for individual species (e.g., Xi,

1980; Takahashi, 1986; Doyle and Hotton, 1991; Pontieri and Sage, 1999). Liang (1992)

published the only study that examined and illustrated features of all six species, using

scanning electron microscopy. Of the scattered accounts focusing on one or a few species,

however, there are sometimes conflicting or inconsistent character reports.

In this chapter, I aim to confirm the characters of saururaceous pollen and contribute

data on wall structure from transmission electron microscopy, which has not been done for

the most of the family. I investigate pollen morphology and ultrastructure of the six extant

taxa and single fossil species of Saururaceae from the Princeton Chert. In addition to

clarifying the features present in saururaceous pollen, such data will provide useful

information for examining pollen character evolution in angiosperm taxa and for the
identification of fossil saururaceous pollen.

MATERIAL AND METHODS

Pollen of the six extant taxa (*Saururus cernuus, S. chinensis, Anemopsis californica, Houttuynia cordata, Gymnotheca chinensis* and *G. involucrata*) and single fossil (*S. tuckerae*) species were examined (Table 5.1). All extant material was obtained dried from herbarium sheets (LSU and ALTA), or for *S. cernuus* was obtained from the Department of Biological Sciences, University of Alberta greenhouse, dried and then used (voucher deposited in ALTA) (see Table 5.1 for list of specimens examined). The fossil pollen is found in situ in siliceous permineralizations of saururaceous flowers from the Middle Ecocene (Allenby Fm.) Princeton Chert, British Columbia, Canada (Chapter 2).

Pollen terminology follows that of Punt et al. (1994). Typical angiosperm pollen wall is stratified into a tectum, columellar (infratectal) layer, foot layer, endexine and intine; the terms sexine and nexine are also sometimes used (Text-Fig. 5.1). Faegri (1956), Walker and Doyle (1975) and Moore et al. (1991) all provide detailed explanations and history on the use of these terms. As a quick reference, I will define here some of the terms used in this chapter (following Punt et al., 1994):

foveola: circular depressions or lumina in the tectum that are more than 1 μm diameter; alternatively used as a synonym of punctum (e.g., Walker and Doyle, 1975; Moore et al., 1991: 74).

granulum: small rounded element less than 1 μm in all dimensions.

perforate: having holes, less than 1 μm in diameter, in the tectum.

psilate: smooth surface.

punctum: perforation of the tectum less than 1 μm in length or diameter; may be round or

　　elongate in shape.

spinule: small spine or tapering, pointed element.

striate: having elongate, parallel elements separated by grooves.

　　Scanning Electron Microscopy (SEM) of the fossil pollen was done using the back side

of deeply etched peels. Peel sections were mounted on double-sided tape on stubs and

covered with 150 Å gold using a Nanotek Semprep II sputter coater. Samples were

observed using a JEOL 6301F (Field Emission Scanning Electron Microscope). Extant

pollen (non-acetolysed) was examined in the same way, with 150 Å gold coating;

occasionally specimens were reexamined after a second coat of 150 Å gold to reduce

charging.

　　Transmission electron microscopy (TEM) was done on non-acetolysed and acetolysed

pollen. Acetolysis was done on anthers following standard procedures (Erdtman, 1960),

with the samples spending four minutes in a hot water bath. Non-acetolysed pollen from

dried specimens was fixed in FPA overnight. Both acetolysed and non-acetolysed samples

were subsequently rinsed with distilled water and placed in 2% OsO_4 for 2 hrs. Samples

were then run through an ethanol dehydration series and embedded in Spurr's (1969) resin

(acetone intermediate). Sections were made at 60 nm, stained using uranyl actetate and

lead citrate, and observed using a Philips Morgagni 268 EM. Samples of fossil pollen were

prepared as in Chapter 2.

　　An effort was made to keep similar images of different taxa to the same scale. However,

many images of *Houttuynia* had to be reduced in scale in order to fit on the plate (Figs.

5.28, 5.29, 5.30, 5.33, 5.34), and the TEM photos of the apertural region of *G. chinensis* (Fig. 5.27) and *S. chinensis* (Fig. 5.55) were enlarged relative to the other images.

Measurements were calculated from SEM and TEM micrographs of non-acetolysed pollen (Appendix 5.1). Except for *Houttuynia* and the fossil *Saururus tuckerae*, for which there was a limited number of samples, at least 25 pollen grains were measured for lengths of the polar and longer equatorial (parallel to sulcus) axes. For other features at least 25 measurements were made, often with multiple measurements taken from one pollen grain or section. The mean measurement for each sample was calculated using Microsoft Excel for Mac OS X (Appendix 5.1).

RESULTS

Anemopsis californica *Hook.* & *Arn.* *(Figs. 5.1-5.9, 5.64)*—Pollen of *Anemopsis* is boat-shaped-elliptic (Figs. 5.1-5.3, 5.6), averaging 5.5 μm in the polar axis and 12.7 μm in the longer equatorial axis. Total exine thickness averages 470 nm. Tectum is psilate and punctate, ca. 180 nm thick (Figs. 5.4, 5.7, 5.8). Puncta average 0.19 μm in diam and are bordered by small papillae, up to six per punctum (Fig. 5.4). The infratectal layer, with a mean thickness of 97 nm, is columellate, but columellae are short, irregularly shaped and, in section view, are separated by undulating spaces (Figs. 5.7, 5.8). The infratectum is similar in structure to that of extant *Saururus*. Nexine comprises more than 1/3 the total exine thickness (Figs. 5.6-5.8). The foot layer averages 163 nm thick. A thin (mean 42 nm) layer situated directly below the foot layer represents endexine (Figs. 5.7, 5.8, 5.64).

Anemopsis pollen is monosulcate. Sculpturing of the aperture is granulate, with the granula often intergrading and forming ridges up to 2 μm long (Fig. 5.5). Granula are

uniform in size (Figs. 5.2, 5.5). Using TEM, the apertural region is seen to be composed of thick intine and laminated endexine, with granula (Fig. 5.9).

Gymnotheca chinensis Decne. *(Figs. 5.10-5.14, 5.20-5.23, 5.65)*—Pollen of *Gymnotheca chinensis* is boat-shaped-elliptic to globose (Figs. 5.10-5.12, 5.20), averaging 5.0 µm in the polar axis and 9.4 µm in the longer equatorial axis. Exine thickness is an average of 550 nm (Figs. 5.20-5.22). Tectum averages 227 nm in thickness and is punctate with weakly striate microsculpturing and very small, sparse spinules (Figs. 5.10-5.13). The mean size of puncta is 0.15 µm in diam, with smooth margins (Fig. 5.13). Infratectum is columellate, averaging 150 nm thick (Figs. 5.21, 5.22). The well-defined columellae in *G. chinensis* are similar to the infratectal layer in pollen of *G. involucrata* and the fossil *Saururus tuckerae*. Nexine is less than 1/3 the total exine thickness. The foot layer measures an average of 134 nm and is thinner than the tectal layer. There is a dark-staining layer, averaging 44 nm thick, below the foot layer that represents endexine in non-acetolysed pollen (Figs. 5.21, 5.22). In acetolysed pollen endexine is visible as a pale-staining layer below the foot layer (Fig. 5.65).

Gymnotheca chinensis pollen is monosulcate. The aperture has granulate sculpturing (Figs. 5.11, 5.14, 5.20. 5.23). Granula average 0.25 µm in diam, separate from each other, and larger in the centre of the sulcus (Figs. 5.11, 5.14). Using TEM, the apertural region is seen to be composed of intine, laminated endexine, and granula (Fig. 5.23).

Gymnotheca involucrata Pei. *(Figs. 5.15-5.19, 5.24-5.27)*—Pollen of *Gymnotheca involucrata* is boat-shaped-elliptic to somewhat globose (Figs. 5.15-5.17, 5.24), averaging 5.8 µm in the polar axis and 10.4 µm in the longer equatorial axis. Exine has a mean thickness of 535 nm (Figs. 5.24-5.26). Tectum averages 226 nm in thickness, and is

punctate, with weakly striate microsculpturing and very small, sparse spinules (Figs. 5.15-

5.18). In this respect *G. involucrata* and *G. chinensis* are similar to each other and show

different features from the other saururaceous taxa, which lack striate microsculpturing and

have papillae around the puncta. Puncta in *G. involucrata* are average 0.18 μm in diam,

with smooth margins (Fig. 5.18). The infratectum is columellate, with an average thickness

of 170 nm (Figs. 5.24-5.26), similar to the infratectum observed in *G. chinensis* and the

fossil *Saururus tuckerae*. The nexine is less than 1/3 total exine thickness. The foot layer

measures an average of 115 nm thick and is thinner than the tectal layer. There is a dark-

staining layer, with a mean thickness of 38 nm, below the foot layer that represents

endexine (Figs. 5.25, 5.26).

Pollen of *G. involucrata* is monosulcate. The aperture has granulate sculpturing (Figs.

5.16, 5.19, 5.24, 5.27). Granula have a mean diam of 0.29 μm and are separate from each

other (Figs. 5.11, 5.14). Using TEM, the apertural region is seen to be composed of intine,

laminated endexine, and granula (Fig. 5.27).

Houttuynia cordata Thunb. (Figs. 5.28-5.36, 5.66)—Pollen of *Houttuynia* is

boat-shaped and globose, averaging 8.5 μm in the polar axis and 13.8 μm in the longer

equatorial axis. This is the largest pollen in Saururaceae. Exine averages 626 nm in total

thickness (Fig. 5.34). Tectum has a mean thickness of 150 nm and is punctate, with smooth

sculpturing (Figs. 5.28-5.31). Puncta average 0.25 μm in diam, bordered by three to five

papillae (Fig. 5.31). The infratectum is columellate, with a mean thickness of 273 nm (Figs.

5.34-5.35). Columellae are broad and the spaces between columellae in section view are

irregular in shape (Fig. 5.34). The infratectum of *Houttuynia* is unlike the other taxa in

Saururaceae. The nexine is less than 1/3 the total exine thickness, with the foot layer

averaging 141 nm thick. Endexine, with a mean thickness of 59 nm, is seen below the foot

layer as a dark-staining layer in non-acetolysed pollen (Figs. 5.34, 5.35) and as a lighter-

staining layer in acetolysed pollen (Fig. 5.66). In contrast to other extant pollen observed,

pollen of *Houttuynia* is empty of cellular contents (Figs. 5.33-5.36).

The aperture of *Houttuynia* is difficult to observe, and is not as prominent as in the

other species. Here I observed only monosulcate grains, although it should be noted that

Liang (1992) clearly showed *Houttuynia* has both monosulcate and trichotomosulcate

pollen. The aperture sculpturing is granulate (Figs. 5.29, 5.32, 5.33, 5.36). Granula are ca.

0.31 μm in diam, and are separate from each other (Figs. 5.29, 5.32). They are less

prominent than in other Saururaceae pollen. Using TEM, the apertural region is seen to be

composed of intine, laminated endexine, and granula (Fig. 5.36).

Saururus cernuus *L.* **(Figs. 5.37-5.42, 5.48-5.51)**—Pollen of *Saururus cernuus*

is boat-shaped-elliptic (Figs. 5.37-5.39, 5.48), averaging 5.5 μm in the polar axis and 11.9

μm in the longer equatorial axis. The average total exine thickness is 488 nm. Tectum is

smooth (although a residue, likely tapetal in origin, sometimes gives it the appearance of

being more rugulate) and punctate, with a mean thickness of 142 nm (Figs. 5.37-5.41, 5.48-

5.50). Puncta average 0.18 μm in diam and are bordered by small papillae, two to four per

pore (Fig. 5.40). Pollen observed from undehisced anthers shows prominent papillae, with

very little observable perforation in the tectum (Fig. 5.41). The infratectal layer, 144 nm, is

columellate. Columellae are short, wide and in section view are separated by undulating

and irregularly shaped spaces (Figs. 5.49, 5.50). The nexine is more than 1/3 the total exine

thickness. The foot layer averages 154 nm thick (Figs. 5.49, 5.50). A dark-staining layer,

with a mean thickness of 42 nm, situated directly below the foot layer represents endexine (Figs. 5.49, 5.50).

Pollen of *S. cernuus* is monosulcate. The sculpturing of the aperture is granulate, with the granula separate from each other. Granula average 0.30 μm in diam (Figs. 5.38, 5.42). Using TEM, the apertural region is seen to be composed of thick intine and laminated endexine, with granula (Fig. 5.51).

Saururus chinensis *(Lour.) Baill.* *(Figs. 5.43-5.47, 5.52-5.55,*

5.67)—Pollen of *Saururus chinensis* is boat-shaped-elliptic, sometimes appearing more globose (Figs. 5.43-5.45), and averages 4.8 μm in the polar axis and 11.0 μm in the longer equatorial axis. Total exine thickness averages 474 nm. The tectum is smooth and punctate, with a mean thickness of 170 nm (Figs. 5.43-5.46, 5.52-5.54). Puncta average 0.13 μm in diam, often more slit-like than circular in shape, and are bordered by three to four small papillae (Fig. 5.46). The infratectal layer averages 67 nm in thickness, is columellate, with short, wide columellae that in section view are separated by elongate spaces (Figs. 5.53, 5.54). Nexine comprises more than 1/3 of the total exine thickness. The foot layer averages 198 nm in thickness (Figs. 5.53, 5.54). A dark-staining layer, with an average thickness of 44 nm, is situated directly below the foot layer and represents endexine in non-acetolysed grains (Figs. 5.53, 5.54); the endexine is lighter staining in acetolysed pollen (Fig. 5.67).

Pollen of *S. chinensis* is monosulcate. Sculpturing of the aperture is granulate, with the granula distinct from each other (Figs. 5.44, 5.47). Granula average 0.30 μm in diam (Figs. 5.44, 5.47). Using TEM, the apertural region is seen to be composed of thick intine and laminated endexine, with granula (Figs. 5.52, 5.55).

Saururus tuckerae *(Figs. 5.56-5.63)*—Pollen is boat-shaped-elliptic, sometimes appearing more globose in polar view (Figs. 5.56-5.58, 5.61). It has an average size of 3.9 μm in the polar axis and 7.4 μm in the longer equatorial axis. Total exine thickness averages 371 nm (Fig. 5.62). The tectum averages 130 nm in thickness, and is punctate with smooth sculpturing (Figs. 5.59, 5.62), as in *Anemopsis, Houttuynia* and extant *Saururus*. Puncta average 0.16 μm in diam and have small papillae at the edges, often in groups of three or four (Fig. 5.59). These papillae are less pronounced than those in *Houttuynia* and more similar to *Anemopsis* and extant *Saururus*. The infratectal layer is columellate, with a mean thickness of 88 nm (Fig. 5.62). The nexine is slightly more than 1/3 of the exine thickness. The foot layer averages 121 nm in thickness. In many specimens a dark-staining layer, with a mean thickness of 32 nm, below the foot layer is visible, which represents endexine (Fig. 5.63).

Pollen is monosulcate (Figs. 5.57, 5.61). The aperture has granulate sculpturing (Figs. 5.57, 5.60). Granula are distinct from each other and measure ca. 0.19 μm in diam.

DISCUSSION

Pollen of *Anemopsis* (Table 5.2) was previously examined by Erdtman (1952; LM), Agababian (1969; LM), Mitroiu (1970), Xi (1980; LM, SEM), Grayum (1992; SEM), and Liang (1992; SEM). No one has previously examined *Anemopsis* pollen using TEM. This investigation confirms the previous reports that *Anemopsis* pollen is monosulcate, with a sculptured aperture; it is boat-shaped and ellipsoidal (Agababian, 1969; Mitroiu, 1970; Xi, 1980; Liang, 1992). Saururaceae tectal sculpturing has been called smooth (Agababian, 1969; Xi, 1980), foveolate (equivalent to punctate as used here) (Liang, 1992) and warty (Mitroiu, 1970). The smooth sculpturing and presence of puncta have been confirmed in

this investigation. In addition, I show here that the puncta have bordering papillae. The

dimensions of pollen grains and tectum are smaller (by 1-2 μm for equatorial axes, with

more discrepancy in polar axis measurements) in the current work than has been reported

before (Erdtman, 1952; Agababian, 1969; Mitroiu, 1970; Xi, 1980; Liang, 1992). As pollen

shape and size changes with hydration (Moore et al., 1991), the smaller sizes observed here

(and for all taxa examined in this chapter) are likely a result of dessication and collapse of

the grains along the sulcus.

Pollen of *Gymnotheca chinensis* (Table 5.2) has been examined by Liang (1992; SEM).

The present work confirms that *G. chinensis* pollen is monosulcate and that the aperture

has granulate sculpturing, as shown by Liang (1992). I also confirm the "foveolate"

(punctate) nature of the pollen reported by Liang (1992). More details of the tectum

sculpturing are reported here, including the microstriate tectal surface (previously noted by

Walker, 1976b) and presence of puncta lacking bordering papillae. In addition, the TEM

work here represents the first done for the species, and shows that the grains have distinct

columellae.

Pollen of *Gymnotheca involucrata* (Table 5.2) has previously been examined by Xi

(1980; LM, SEM) and Liang (1992; SEM). Xi (1980) reported that pollen of *G. involucrata*

was monosulcate to trichotomosulcate, but here and in Liang (1992) only monosulcate

pollen is observed. The aperture is confirmed to be granulate, as shown by Liang (1992).

The striate microsculpturing on the tectum and puncta lacking bordering papillae are

clearly shown here for the first time. This supports Walker's (1976b) description of

Gymnotheca having striate microsculpturing and Liang's (1992) observations that the

pollen is rugate-foveolate, but refutes Xi's (1980) description of smooth pollen in *G.*

involucrata, which was probably due to the low resolution of light microscopy. The current study presents the first TEM sections of the pollen wall for *G. involucrata*. Xi (1980) reported the exine thickness to be 0.8 μm, a measurement differing from the mean exine thickness of 0.5 μm measured here. This could be a result of using different methods: Xi (1980) used light microscopy, where such accuracy is impossible, while I used TEM to observe exine thickness more precisely (especially since the small pollen of Saururaceae is difficult to observe using only light microscopy).

Houttuynia cordata pollen (Table 5.2) was previously studied by Erdtman (1952; LM), Ikuse (1956), Huang (1966; LM), Agababian (1969; LM), Mitroiu (1970), Shimakura (1973; LM), Xi (1980; LM, SEM), Miyoshi and Kato (1982; SEM), Takahashi (1986, TEM) and Liang (1992; SEM). Liang (1992) and Xi (1980) report that pollen of *Houttuynia* is monosulcate to trichotomosulcate [this is clearly illustrated in Liang (1992)], while the others report only monosulcate grains. I confirm that the aperture sculpturing is granular, as was noted by Ikuse (1956), Agababian (1969), Mitroiu (1970) and Miyoshi and Kato (1982). The pollen shape of *Houttuynia* is variable (Miyoshi and Kato, 1982; Takahashi, 1986). The species is reported to be male-sterile and parthenogenic (Shibata and Miyake, 1908; Okabe, 1930; Takahashi, 1986), which may be a reason for finding more variable pollen shape and no cell contents (Takahashi, 1986). The current work confirms previous reports of the tectum being punctate (Miyoshi and Kato, 1982; Takahashi, 1986; Liang, 1992) and shows that the puncta are bordered by papillae. In the morphological data matrix of Meng et al. (2003), their character 36 (absence or presence of papillae around puncta) was coded as present for *Houttuynia*. Takahashi (1986) shows TEM sections of *Houttuynia* pollen that are similar in appearance to those shown in my

study. There is a small discrepancy in the mean measurement of exine thickness. I

calculated a mean thickness of 0.6 µm while Takahashi (1986) reported the exine is 0.4-0.5

µm thick, a measurement that falls within the range I measured. Xi (1980) reported an

exine thickness of 0.8-1.0 µm in *Houttuynia*, using light microscopy, which is a more

imprecise method for these small grains.

Saururus cernuus pollen (Table 5.2) has been previously examined by Erdtman (1952;

LM), Ikuse (1956), Mitroiu (1970), Doyle and Hotton (1991; TEM), Grayum (1992;

SEM), Liang (1992; SEM), Pontieri and Sage (1999; SEM, TEM) and in Chapter 2 (SEM,

TEM). There is no question that the grains are monosulcate with a granulate aperture, as

described by Erdtman (1952), Ikuse (1956), Mitroiu (1970), Liang (1992) and Pontieri and

Sage (1999). The sculpturing of *S. cernuus* pollen has been variously described as obscure

(Erdtman, 1952), a fine reticulum (Ikuse, 1956), warty (Mitroiu, 1970), or foveolate

(Grayum, 1992; Liang, 1992). This chapter shows that the pollen is clearly punctate

(synonymous with foveolate), and that the puncta are bordered by papillae. In their

morphological data matrix used for examining relationships within Saururaceae, Meng et

al. (2003) code *Saururus cernuus* as having papillae around the punctae. Pollen wall

structure of *S. cernuus*, as seen with TEM, has been previously illustrated by Doyle and

Hotton (1991) and Pontieri and Sage (1999). Results from the current study are consistent

with those of previous two studies. Pollen is columellate, has a thick nexine, and, like

Doyle and Hotton (1991), I interpret the grains as having endexine.

Pollen of *Saururus chinensis* (Table 5.2) has been described in varying detail by Ikuse

(1956), Huang (1966; LM), Agababian (1969; LM), Shimakura (1973; LM), Xi (1980, LM,

SEM); Miyoshi and Kato (1982; SEM), Liang (1992; SEM) and in Chapter 2 (SEM, TEM).

My work confirms the previous reports that pollen of *S. chinensis* is monosulcate, with granulate aperture sculpturing (Ikuse, 1956; Agababian, 1969; Shimakura, 1973; Xi, 1980; Miyoshi and Kato, 1982; Liang, 1992). Miyoshi and Kato (1982) and Liang (1992) have previously shown that the tectum is punctate; Miyoshi and Kato (1982) and Meng et al. (2003) state that there are three to five papillae per punctum, a finding that is confirmed by the present study.

A few more details on the pollen of *Saururus tuckerae* (Chapter 2; Table 5.2), the only fossil saururaceous pollen recognized to date, are presented here. It is clearly monosulcate, boat-shaped-elliptic, and the aperture has granulate sculpturing, most similar to that of extant *Saururus* and *Gymnotheca*. The tectum is punctate and smooth between puncta, like *Anemopsis*, *Houttuynia* and *Saururus*. Puncta are surrounded by papillae. The narrow to circular punctum shape is most like that in *Saururus*. *Saururus* and *Anemopsis* are similar to the fossil in having shorter papillae than those of *Houttuynia*. *Saururus tuckerae* has the smallest pollen described within Saururaceae. The infratectum is strongly columellate, and there is a dark-staining layer below the foot layer that is endexine.

Based on the current study and previous data, pollen of Saururaceae shows a unique suite of characters within angiosperms (Table. 5.2). Saururaceous pollen is boat-shaped-elliptic to globose (Walker, 1976a; Grayum, 1992; Liang, 1992; Doyle, 2005). *Houttuynia* has the largest pollen within the family, but is still less than 20 μm in size. All grains are monosulcate, although *Houttuynia* is sometimes trichotomosulcate (Walker, 1976a; Grayum, 1992; Liang, 1992; Doyle, 2005), with granulate apertures (Liang, 1992). Although previously called psilate, smooth, or foveolate, pollen in the family is actually punctate (sensu Punt et al., 1994) in all taxa, with an otherwise smooth (*Anemopsis*,

Houttuynia, Saururus) or microstriate (*Gymnotheca*) tectum. Puncta in *Anemopsis*, *Houttuynia* and *Saururus* are bordered by papillae, as Miyoshi and Kato (1982) reported for *S. chinensis*; however, puncta in *Gymnotheca* are smooth and have no papillae.

Many of the fine details of pollen morphology and ultrastructure in Saururaceae have remained relatively obscure, probably because of the small size of the grains and the limits of using light microscopy to examine such pollen. Thus there has been uncertainty in some characters. While my results are generally consistent with previous reports on pollen features in Saururaceae, I have been able to provide more details on the sculpturing and ultrastructure in pollen of saururaceous taxa. For example, *Gymnotheca* had not been previously illustrated in detail. Liang (1992) provided good overall micrographs, but no detailed illustrations of the tectum sculpturing. I showed here that *Gymnotheca* pollen has a microstriate (rather than smooth) tectum, which is punctate, with no papillae bordering the puncta. I show that the papillae described by Miyoshi and Kato (1982) for *S. chinensis* are in fact present in all saururaceous genera except *Gymnotheca*.

Pollen wall ultrastructure has been illustrated for *Houttuynia* (Takahashi, 1986) and *S. cernuus* (Doyle and Hotton, 1991; Pontieri and Sage, 1999). Here I present complementary data for the other species within Saururaceae, and again for *Houttuynia* and *S. cernuus*. All have a columellar infratectum. *Gymnotheca* and the fossil, *S. tuckerae*, have a more clearly defined columellar infratectal layer than extant *Saururus* or *Anemopsis*, where the columellae are wide and irregular in section, and the infratectal layer is shorter. *Houttuynia* is somewhat intermediate, having a thick infratectal layer, but spaces between columellae are irregular and narrow in section view. The nexine in *Anemopsis* and *Saururus* comprises ca. 1/3 or more of the total exine thickness, but in *Gymnotheca*

and *Houttuynia* this is not the case. In the latter two taxa, nexine appears to be slightly less than 1/3 of the total exine thickness. *Gymnotheca* differs from *Anemopsis, Houttuynia,* and *Saururus* in both general morphology and wall structure.

Previously, Takahashi (1986) stated there is no endexine in *Houttuynia* (though his figures show the same layer seen in my preparations), Sampson (2000) stated that the family lacks endexine, and Doyle (2005) coded endexine as absent in Saururaceae. In contrast, Walker (1976b) stated that endexine is present in Saururaceae, Doyle and Hotton (1991) interpreted endexine as present in *S. cernuus,* and Doyle and Endress (2000) coded endexine as present in Saururuaceae. My work here confirms that endexine is indeed present in the Saururaceae. In all taxa, endexine is seen as a thin (30-60 nm) layer below the foot layer that is dark in non-acetolysed pollen and light-staining in acetolysed pollen.

Saururaceae are accepted as being sister to Piperaceae within Piperales (APG, 2003; Jaramillo et al., 2004; Neinhuis et al., 2005). Piperaceous pollen is globose with variable sculpturing including psilate, scabrate, verrucate and echinate grains (Sampson, 2000), compared to Saururaceae, which has boat-shaped-elliptic to globose pollen with punctate sculpturing. *Zippelia* Blume, a genus previously placed in Saururaceae but now accepted as sister to the rest of Piperaceae (Liang and Tucker, 1995; Jaramillo et al., 2004), also has punctate pollen (Lei and Liang, 1998). Meng et al. (2003), in conducting a phylogenetic analysis on Saururaceae using both morphological and molecular data, code *Zippelia* (along with *Saururus* and *Houttuynia,* but not *Anemopsis*) as having papillae surrounding the puncta. Thus *Zippelia* shows a feature that is similar with most saururaceous pollen, but not the rest of Piperaceae that have been examined to date. Walker (1976b) stated that endexine is present in Saururaceae (as shown in this chapter) but absent in Piperaceae.

Doyle (2005) also accepts that endexine is absent within Piperaceae. Thus, the presence/absence of endexine represents another character to distinguish between the pollen of Piperaceae and Saururaceae.

Recognition of this type of pollen in the fossil record is important to help us understand the evolutionary history of Piperales, and patterns of change in pollen structure within angiosperms. The fossil pollen from the Middle Eocene Princeton Chert shown here is clearly saururaceous because of its small size, monosulcate nature, and sculpturing that matches that of other saururaceous pollen. Pollen of *S. tuckerae* has been found both in situ (Chapter 2) and in bulk macerations (R. Zetter, pers. comm., 2005) of the Princeton Chert, and thus it must be possible to find it in sediments elsewhere. It represents the first fossil pollen record for the family. Because fossil fruits of *Saururus bilobatus* are known from throughout the Late Eocene to Pliocene of Europe and Siberia (Mai and Walther, 1978; Friis, 1985; Lesiak, 1994), there is a possibility that fossil saururaceous pollen is also present in the European fossil record. The reasons for not previously recognizing fossil saururaceous pollen are likely two-fold: 1) the previous lack of data clearly illustrating and characterising pollen in Saururaceae, and 2) small pollen size and the inability to examine (or desire to keep) such small material when so many other, larger, better known pollen types exist and sieving samples with 10 or 20 μm sieves are used that result in the loss of the small grains. I hope that the present study will provide enough data for recognition of disperesed fossil pollen of Saururaceae and new motivation for their discovery.

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Table 5.1. Specimens of Saururaceae examined in this study.

Taxon	Specimen used
Anemopsis californica Hook. & Arn.	USA. California: near San Onofre, 14 July 1935, *W. C. McCalla s.n.* (ALTA 52277).
Gymnotheca chinensis Decne.	CHINA. Guangxi: Nanning, 5 May 1987, *Liang Hanxing 8703* (LSU 72529).
Gymnotheca involucrata Pei	CHINA. Sichuan: near Jiajiang, 29 April 1987, *Liang Hanxing 8701* (LSU 124235).
Houttuynia cordata Thunb.	CHINA. Yunnan: near Xuanwei, 15 June 1987, *Liang Hanxing 8709* (LSU 124234).
Saururus cernuus L.	CANADA. Ontario: Middlesex Co. along Thames River, 31 July 1914, *E.H. Moss s.n.* (ALTA 5509). Alberta: Edmonton, cultivated, University of Alberta Biological Sciences Greenhouse, 18 May 2006 *S.Y. Smith 59* (ALTA 115823)
Saururus chinensis (Lour.) Baill.	CHINA. Sichuan: Omei, 21 May 1987, *Liang Hanxing 8705* (LSU 72527).
Saururus tuckerae	Princeton Chert (Middle Eocene, Allenby Fm), British Columbia, Canada; P1631 Cbot #27; P5108 D #2, 41, 44; P5937 Gbot #11, 12; P5991 B #8, 11 (University of Alberta Paleobotanical Collections (UAPC-ALTA))

Table 5.2. A summary of pollen features in Saururaceae.

	Anemopsis	G. chinensis	G. involucrata	Houttuynia	S. cernuus	S. chinensis	S. tuckerae
Mean pollen size (polar axis x longer equatorial axis; μm)	5.5x12.7	5.0x9.4	5.8x10.4	8.5x13.8	5.5x11.9	4.8x11.0	3.9x7.4
Aperture type	M	M	M	M (-TR)	M	M	M
Aperture sculpturing	granulate, ridges	granulate	granulate	small granula	granulate	granulate	granulate
Tectum sculpturing	P, sm	P, st	P, st	P, sm	P, sm	P, sm	P, sm
Papillae surrounding punctum	up to 6	no	no	3-5	2-4	3-4	3-4
Mean exine thickness (nm)	469	550	535	626	488	474	371
Mean tectum thickness (nm)	179	227	226	150	142	170	130
Infratectum type	wide columellae	columellar	columellar	columellar	wide columellae	wide columellae	columellar
Mean infratectum thickness (nm)	97	150	170	273	144	67	88
Mean nexine thickness (nm)	205	177	153	200	197	241	153
Nexine thickness (relative to exine)	> 1/3	< 1/3	< 1/3	< 1/3	> 1/3	> 1/3	>1/3

Abbreviations: M, monosulcate; TR, trichotomosulcate; P, punctate; sm, tectum smooth in between puncta; st, tectum microstriate in between puncta.

Appendix 5.1. Measurements of Saururaceous pollen.

Anemopsis	Polar (μm)	Equatorial (μm)	Punctum (μm)	Granulum (μm)	Exine (nm)	Tectum (nm)	Infratectum (nm)	Foot layer (nm)	Endexine (nm)
	5.75	11.8	0.26	0.36	428.0	184.5	94.0	190.0	46.0
	5.5	12.3	0.19	0.32	441.0	203	131.0	162.0	25.8
	6.0	12.8	0.26	0.24	450.2	230.6	81.2	184.5	22.1
	7.0	12.5	0.26	0.28	400.4	140.2	99.6	134.7	31.4
	5.0	14.5	0.26	0.35	361.9	141.4	72.0	101.3	51.2
	5.76	13.0	0.19	0.57	454.3	163.7	114.7	141.4	43.4
	6.67	11.8	0.19	0.23	392.9	122.3	103.6	119.6	17.9
	5.67	13.0	0.30	0.20	400.9	154.5	107.1	150.0	15.2
	5.2	13.9	0.33	0.16	376.8	180.4	125.9	165.2	27.8
	5.2	13.1	0.22	0.19	397.6	154.8	137.5	147.0	24.5
	4.9	18.4	0.22	0.60	377.5	147.0	86.9	149.2	40.0
	5.2	10.5	0.19	0.37	500.0	200.0	76.8	220.0	40.0
	6.3	13.3	0.19	0.27	600.0	140.0	100.0	300.0	40.0
	5.6	11.9	0.11	0.44	500.0	180.0	100.0	140.0	40.0
	5.5	12.4	0.14	0.44	480.0	200.0	100.0	140.0	20.0
	5.2	11.9	0.22	0.29	520.0	200.0	100.0	180.0	46.9
	6.3	15.2	0.22	1.07	484.4	156.3	60.0	250.0	54.7
	5.3	12.5	0.25	1.90	453.1	250	39.1	93.8	46.9
	5.8	13.0	0.22	0.80	500.0	140.6	62.5	187.5	46.9
	5.8	12.1	0.22	0.60	453.1	171.9	109.4	171.9	73.8
	5.4	11.9	0.30	0.70	473.8	178.5	62.5	110.8	55.4
	5.4	12.9	0.19	1.40	516.9	209.2	169.2	147.7	49.2
	5.9	12.5	0.19	0.97	535.4	203.1	110.8	101.5	35.7
	5.2	10.2	0.11	0.60	547.6	297.6	116.9	119.0	47.6
	4.4	12.4	0.11	1.58	571.4	190.5	119.0	166.7	59.5
	5.9	13.7	0.13	1.18	500.0	107.1	3.3	261.9	47.6
	4.9	11.1	0.16	1.26	506.0	142.9	166.7	214.3	60.4
	5.3	13.7	0.18	1.05	475.5	143.4	95.2	75.5	52.8
	5.8	13.2	0.13	1.58	505.7	150.9	101.9	211.3	45.3
	5.3	11.1	0.18	0.53	452.8	279.2	90.6	150.9	
	4.8	12.1	0.13				79.2		
	5.5	13.2	0.16						
	5.9	12.6	0.16						
	4.8	10.2	0.18						
	5.5	12.9	0.29						
	6.2	11.2	0.09						
	4.8	12.9	0.09						
	5.5	12.6	0.2						
	5.5	13.3	0.2						
	6.2	13.3	0.29						
		13.1	0.13						
		11.7	0.11						
		12.4							
mean	**5.5**	**12.7**	**0.2**	**0.7**	**468.6**	**178.8**	**97.3**	**162.9**	**41.7**

Appendix 5.1, continued

G. chinensis	Polar (μm)	Equatorial (μm)	Punctum (μm)	Granulum (μm)	Exine (nm)	Tectum (nm)	Infratectum (nm)	Foot layer (nm)	Endexine (nm)
	4.8	9.6	0.16	0.33	606.1	272.7	136.4	121.2	30.3
	5.9	9.1	0.18	0.28	606.1	272.7	163.6	133.3	30.3
	4.9	9.5	0.14	0.23	557.6	345.5	109.1	109.1	30.3
	4.3	9.2	0.20	0.28	820.0	420.0	100.0	280.0	40.0
	4.2	9.2	0.26	0.19	740.0	290.0	240.0	190.0	30.0
	5.0	9.1	0.18	0.28	720.0	270.0	190.0	240.0	40.0
	4.4	9.1	0.08	0.19	492.7	146.3	151.2	146.3	48.8
	5.3	9.1	0.20	0.19	575.6	185.4	146.3	190.2	58.5
	4.4	12.9	0.22	0.23	619.5	297.6	141.5	141.5	43.9
	4.4	8.3	0.24	0.28	434.1	165.9	126.8	92.7	34.1
	4.1	8.4	0.14	0.42	453.7	243.9	122.0	92.7	39.0
	5.9	8.0	0.08	0.19	361.0	126.8	107.3	48.8	34.1
	4.7	8.8	0.16	0.19	395.1	170.7	170.7	107.3	29.3
	5.3	8.8	0.10	0.28	686.8	294.3	245.3	94.3	52.8
	4.7	8.8	0.18	0.14	765.6	250.0	265.6	203.1	62.5
	5.0	8.8	0.08	0.28	487.8	190.2	136.6	126.8	39.0
	5.6	9.3	0.16	0.28	487.8	165.9	180.5	97.6	43.9
	5.6	8.9	0.12	0.23	468.3	209.8	141.5	92.7	24.4
	5.0	11.1	0.14	0.14	492.3	184.6	153.8	73.2	46.2
	5.0	10.0	0.10	0.19	430.8	184.6	92.3	126.2	27.7
	4.8	10.0	0.08	0.19	455.4	203.1	129.2	73.8	49.2
	4.1	9.4	0.08	0.19	492.3	169.2	123.1	138.5	61.5
	5.6	9.7	0.12	0.37	553.8	261.5	76.9	153.8	61.5
	4.4	9.6	0.18	0.29	507.7	153.8	138.5	184.6	61.5
	5.9	9.3	0.26	0.24	553.8	200.0	169.2	123.1	61.5
	5.2	10.7	0.22	0.22	523.1	230.8	153.8	92.3	61.5
	5.9	8.9	0.13	0.34					
	4.4		0.08	0.51					
	5.2		0.08	0.24					
	5.9		0.08	0.22					
	5.2		0.06	0.24					
			0.18	0.24					
			0.20	0.15					
			0.22	0.24					
			0.20	0.27					
			0.13	0.24					
			0.13	0.24					
			0.13	0.29					
			0.11	0.30					
			0.13	0.29					
			0.20	0.26					
			0.16	0.26					
mean	5.0	9.4	0.1	0.3	549.5	227.1	150.4	133.6	43.9

Appendix 5.1, continued

G. *involucrata*	Polar (μm)	Equatorial (μm)	Punctum (μm)	Granulum (μm)	Exine (nm)	Tectum (nm)	Infratectum (nm)	Foot layer (nm)	Endexine (nm)
	6.7	10.3	0.26	0.32	765.9	258.5	356.1	107.3	43.9
	5.6	10.0	0.24	0.24	702.4	282.9	258.5	117.1	43.9
	5.6	10.7	0.21	0.24	515.2	218.2	163.6	109.1	30.3
	5.6	11.5	0.21	0.29	490.9	230.3	103.0	133.3	24.2
	6.7	10.3	0.21	0.26	515.2	242.4	139.4	103.0	36.3
	7.6	9.3	0.29	0.29	548.2	210.8	168.7	132.5	48.2
	4.9	10.0	0.21	0.21	497.6	216.9	138.6	132.5	60.2
	4.9	10.3	0.24	0.21	522.0	323.8	209.8	102.4	20.0
	6.9	9.8	0.24	0.26	488.1	212.2	146.3	143.9	20.0
	5.3	9.8	0.12	0.24	440.5	226.2	107.1	119.0	35.7
	7.1	9.3	0.12	0.24	457.8	160.7	148.8	107.1	23.8
	4.9	10.2	0.12	0.29	457.8	192.8	144.6	96.4	48.2
	5.6	11.1	0.21	0.24	530.1	180.7	156.6	72.3	36.1
	7.4	10.7	0.18	0.18	481.9	216.9	168.7	96.4	36.1
	7.0	9.3	0.12	0.35	530.1	241.0	132.5	84.3	48.2
	6.0	9.8	0.15	0.26	507.7	241.0	96.4	144.6	30.8
	5.6	10.2	0.09	0.41	538.5	230.8	123.1	123.1	46.2
	5.2	11.6	0.12	0.29	569.2	207.7	184.6	176.9	30.8
	4.4	10.0	0.18	0.41	569.2	215.4	169.2	153.8	61.5
	5.2	10.4	0.29	0.41	569.2	230.8	200	76.9	30.8
	6.3	8.3	0.26	0.18	476.2	200.0	269.2	84.6	35.7
	5.2	11.5	0.25	0.50	476.2	196.4	131.0	125.0	35.7
	4.8	8.9	0.18	0.26	452.4	220.2	119.0	113.1	23.8
	4.7	11.2	0.15	0.35	734.4	232.1	89.3	107.1	39.1
	5.6	11.8	0.24	0.41	548.2	257.8	328.1	125.0	48.2
	5.9	9.4	0.15	0.24					
	5.9	11.2	0.13	0.18					
	4.7	11.2	0.27	0.18					
	5.3	10.6	0.18	0.18					
	5.9	9.4	0.22	0.21					
	7.1	11.2	0.11	0.43					
	6.5	10.6	0.24	0.33					
	5.3	10.6	0.22	0.43					
	5.9	10.6	0.13	0.28					
	5.3	8.8	0.09	0.30					
	4.7	11.2	0.20	0.34					
	5.9	12.4	0.10	0.30					
	5.3	10.0	0.24	0.45					
	5.3	10.6	0.13	0.38					
	8.8		0.10	0.23					
			0.16	0.25					
			0.16	0.28					
mean	5.8	10.4	0.2	0.3	535.4	225.9	170.1	115.5	37.5

Appendix 5.1, continued

Houttuynia	Polar (μm)	Equatorial (μm)	Punctum (μm)	Granulum (μm)	Exine (nm)	Tectum (nm)	Infratectum (nm)	Foot layer (nm)	Endexine (nm)
	9.7	16.5	0.47	0.48	486.3	141.2	172.5	125.5	54.9
	9.5	15.9	0.32	0.32	952.4	261.9	345.2	345.2	59.5
	6.5	15.9	0.41	0.56	857.1	202.4	392.9	202.4	59.5
	10.0	13.8	0.35	0.40	773.8	142.9	345.2	226.2	59.5
	8.1	12.7	0.25	0.48	622.2	133.3	246.9	192.6	54.3
	6.0	9.3	0.38	0.40	479.0	163.0	182.7	108.6	29.6
	9.3	14.2	0.35	0.73	409.9	123.5	128.4	111.1	51.9
	8.0	13.3	0.18	0.55	641.5	113.2	347.2	120.8	60.4
	9.0	15.0	0.32	0.82	634.0	196.2	286.8	90.6	75.5
		13.3	0.29	0.27	680.0	120.0	280.0	100.0	60.0
		13.0	0.21	0.21	620.0	180.0	280.0	100.0	60.0
		18.5	0.29	0.12	640.0	120.0	340.0	100.0	80.0
		14.1	0.21	0.20	640.0	160.0	280.0	140.0	60.0
		9.4	0.21	0.22	419.5	117.1	165.9	68.3	58.5
		11.9	0.26	0.27	536.6	78.0	302.4	87.8	58.5
		14.3	0.29	0.20					
		16.8	0.29	0.27					
		15.0	0.21	0.18					
		13.5	0.44	0.24					
		14.0	0.15	0.16					
		12.0	0.24	0.22					
		11.0	0.14	0.20					
		9.0	0.21	0.11					
		16.0	0.17	0.16					
		16.0	0.21	0.16					
		15.0	0.17	0.11					
			0.28						
			0.21						
			0.21						
			0.24						
			0.24						
			0.17						
			0.21						
			0.17						
			0.17						
			0.10						
			0.24						
			0.16						
			0.17						
			0.21						
mean	8.5	13.8	0.2	0.3	626.2	150.2	273.1	141.3	58.8

Appendix 5.1, continued

| S. cernuus	Polar (µm)	Equatorial (µm)	Punctum (µm)	Granulum (µm)	Exine (nm)	Tectum (nm)	Infratectum (nm)	Foot layer (nm)	Endexine (nm)
	5.4	10.7	0.20	0.29	523.8	83.0	178.6	214.3	47.6
	5.4	11.2	0.19	0.32	559.5	59.5	178.6	273.8	47.6
	5.4	12.4	0.14	0.22	535.7	136.9	131.0	220.2	47.6
	5.1	11.2	0.12	0.32	500.0	161.3	161.3	129.0	40.3
	5.8	12.9	0.15	0.22	435.5	169.4	112.9	129.0	24.2
	5.1	11.2	0.15	0.2	467.7	177.4	193.5	88.7	32.2
	5.8	11.5	0.14	0.27	549.0	141.2	180.4	211.8	23.5
	5.8	12.5	0.17	0.32	588.2	137.3	239.2	196.1	23.5
	5.8	11.9	0.15	0.34	476.2	133.3	133.3	171.4	38.1
	5.0	11.7	0.15	0.32	444.4	95.2	193.7	158.7	38.1
	5.0	11.1	0.24	0.22	451.6	177.4	129.0	72.6	56.3
	5.6	12.5	0.2	0.29	451.6	129.0	112.9	161.3	32.3
	5.8	12.2	0.10	0.24	483.9	121.0	185.5	145.2	32.3
	5.3	13.1	0.10	0.39	483.9	145.2	161.3	129.0	48.4
	5.0	11.4	0.17	0.20	476.2	113.1	142.9	220.2	47.6
	6.1	13.3	0.12	0.17	517.9	83.3	125.0	261.9	47.6
	5.8	12.2	0.20	0.28	511.9	142.9	154.8	166.7	47.6
	6.7	11.7	0.20	0.28	483.9	177.4	161.3	80.6	64.5
	6.1	9.0	0.20	0.29	459.7	129.0	64.5	21.0	48.4
	5.3	11.7	0.25	0.25	467.7	169.4	104.8	129.0	64.5
	6.0	11.9	0.18	0.20	467.7	161.3	129.0	88.7	72.6
	5.3	12.4	0.23	0.28	478.4	149.0	133.3	152.9	47.1
	5.5	12.2	0.23	0.28	470.6	196.1	78.4	168.6	27.5
	5.3	12.8	0.28	0.20	454.9	180.4	102.0	133.3	39.2
	5.0	12.8	0.18	0.38	470.6	188.2	117.6	133.3	31.4
	4.5	10.0	0.13	0.38					
	6.0	8.8	0.14	0.35					
	5.4	13.4	0.25	0.35					
	6.0	13.5	0.20	0.24					
	5.4	11.2	0.15	0.38					
	5.4	11.4	0.06	0.38					
	6.2	13.1	0.15	0.29					
	5.2	13.1	0.10	0.32					
	5.5	12.4	0.06	0.26					
	6.2	11.7	0.11	0.43					
	4.7	13.1	0.13	0.46					
	5.2	12.4	0.15	0.40					
			0.18	0.35					
			0.15	0.37					
			0.28	0.31					
			0.18	0.42					
			0.24	0.42					
			0.27	0.24					
			0.56	0.22					
			0.13	0.18					
				0.27					
				0.36					
				0.36					
				0.20					
				0.27					

Appendix 5.1, continued

	Polar (μm)	Equatorial (μm)	Punctum (μm)	Granulum (μm)	Exine (nm)	Tectum (nm)	Infratectum (nm)	Foot layer (nm)	Endexine (nm)
S. cernuus, continued				0.29					
				0.3					
				0.29					
				0.21					
mean	**5.5**	**11.9**	**0.2**	**0.3**	**488.4**	**142.3**	**144.2**	**154.3**	**42.8**
S. chinensis	4.7	10.0	0.10	0.37	464.3	178.6	59.5	166.7	59.5
	4.6	9.3	0.10	0.24	375.0	125.0	77.4	172.6	59.5
	4.7	11.3	0.10	0.34	464.3	113.1	59.5	250.0	41.7
	4.4	11.6	0.06	0.37	434.5	125.0	89.3	184.5	35.7
	4.2	11.9	0.08	0.24	566.0	150.9	52.8	317.0	37.7
	4.7	12.1	0.20	0.21	513.2	188.7	67.9	218.9	45.3
	4.2	11.7	0.13	0.21	452.8	135.8	37.7	241.5	37.7
	4.9	11.4	0.16	0.34	500.0	140.0	80.0	220.0	60.0
	4.2	10.0	0.16	0.33	520.0	140.0	100.0	220.0	60.0
	4.9	10.8	0.18	0.31	560.0	160.0	40.0	320.0	40.0
	5.0	11.9	0.21	0.27	460.0	140.0	60.0	220.0	60.0
	5.5	10.5	0.09	0.25	440.0	120.0	70.0	220.0	60.0
	4.8	10.7	0.13	0.24	440.0	150.0	80.0	180.0	30.0
	4.7	9.7	0.20	0.27	508.1	225.8	72.6	153.2	64.5
	4.5	11.7	0.20	0.39	419.4	145.2	64.5	161.3	48.4
	4.1	11.2	0.14	0.24	435.5	177.4	64.5	145.2	64.5
	5.2	10.9	0.11	0.28	535.7	208.3	83.3	220.2	23.8
	4.5	11.8	0.25	0.33	571.4	196.4	83.3	261.9	29.8
	4.7	11.1	0.13	0.19	523.8	238.1	71.4	178.6	31.3
	4.4	11.4	0.05	0.44	523.8	196.4	71.4	226.2	39.1
	4.8	10.5	0.10	0.41	445.3	187.5	46.9	164.1	31.3
	4.8	10.8	0.06	0.39	390.6	187.5	54.7	125	31.3
	5.4	10.8	0.05	0.27	406.3	203.1	62.5	109.4	31.3
	4.8	10.8	0.13	0.34	406.3	203.1	62.5	93.8	35.7
	5.5	10.8	0.10	0.15	484.4	218.8	62.5	171.9	29.8
	5.2	10.5	0.10						
	5.2	10.0	0.15						
	5.5	10.3	0.16						
	4.3	10.0	0.19						
	5.0	11.6	0.24						
	5.0	11.0	0.17						
	4.4	10.7	0.11						
	5.6	11.5	0.11						
	5.0	11.3	0.13						
	4.7	10.0	0.20						
	4.5	9.1	0.16						
	5.2	12.2	0.10						
	4.4	11.4	0.08						
	4.4	11.7	0.07						
	4.9	11.7	0.16						
	4.7	12.2							
mean	**4.8**	**11.0**	**0.1**	**0.3**	**473.6**	**170.2**	**67.0**	**197.7**	**43.5**

Appendix 5.1, continued

| S. tuckerae	Polar (µm)	Equatorial (µm)	Punctum (µm)	Granulum (µm)	Exine (nm)	Tectum (nm)	Infratectum (nm)	Foot layer (nm)	Endexine (nm)
	4.4	7.4	0.28	0.24	307.3	87.8	78.0	97.6	53.7
	3.7	8.9	0.15	0.16	322	87.8	97.6	87.8	58.5
	4.0	9.8	0.11	0.20	409.8	107.3	117.1	131.7	53.7
	4.4	9.3	0.09	0.37	400.0	117.1	68.3	151.2	63.4
	4.4	7.0	0.11	0.22	429.3	170.7	46.5	126.8	78
	4.0	7.5	0.08	0.16	452.4	131.0	71.4	190.5	47.6
	4.2	7.6	0.09	0.20	446.4	226.2	83.3	95.2	53.6
	4.4	7.1	0.14	0.16	440.5	214.3	95.2	95.2	29.8
	5.0	6.7	0.2	0.16	464.3	232.1	95.2	107.1	29.8
	5.0	7.5	0.08	0.16	381.0	119.0	131.0	107.1	11.9
	3.5	6.8	0.12	0.16	381.0	131.0	71.4	154.8	23.8
	2.8	7.3	0.12	0.16	248.5	127.3	18.2	72.7	36.4
	4.0	6.6	0.09	0.36	236.4	97.0	36.4	72.7	24.2
	2.9	7.0	0.08	0.29	233.3	60.6	66.7	78.9	30.3
	3.5	6.3	0.10	0.20	230.3	97.0	36.4	72.7	30.3
	3.6	6.7	0.08	0.18	339.6	75.5	90.6	158.5	15.1
	3.9	7.1	0.11	0.18	377.4	143.4	101.9	120.8	11.3
	3.0	7.1	0.09	0.22	362.3	83.0	98.1	173.6	11.3
	3.7	7.2	0.08	0.27	411.3	139.6	113.2	132.1	26.4
	3.5	6.7	0.08	0.18	400.0	128.3	113.2	135.8	22.6
	4.4	8.0	0.11	0.22	400.0	135.8	113.2	143.4	15.1
		6.8	0.06	0.24	384.9	135.8	135.8	90.6	22.6
		7.5	0.05	0.27	407.5	150.9	113.2	128.3	22.6
		7.5	0.07	0.18	422.6	120.8	113.2	173.6	22.6
		7.7	0.22	0.24	384.9	135.8	105.7	120.8	15.1
		7.1	0.24	0.27					
		6.5	0.27	0.14					
		7.7	0.24	0.16					
		8.7	0.18	0.12					
		6.9	0.24	0.14					
		7.2	0.30	0.16					
		7.5	0.11	0.16					
		7.4	0.25	0.17					
			0.15	0.17					
			0.15	0.12					
			0.16	0.12					
			0.20	0.14					
			0.15	0.20					
			0.13	0.12					
			0.28	0.16					
			0.33						
			0.15						
			0.23						
			0.18						
			0.22						
			0.24						
			0.13						
			0.15						
			0.15						
mean	3.9	7.4	0.2	0.2	370.9	130.2	88.4	120.8	32.4

Text-Figure 5.1. Cross-sectional diagram through nonapertural area of a tectate-

perforate, non-acetolyzed angiosperm pollen wall showing layer stratification (redrawn from

Doyle and Walker, 1975). Punctum to right lacks papillae (as seen in *Gymnotheca*) while

that on the left has bordering papillae (as in *Anemopsis, Houttuynia, Saururus*).
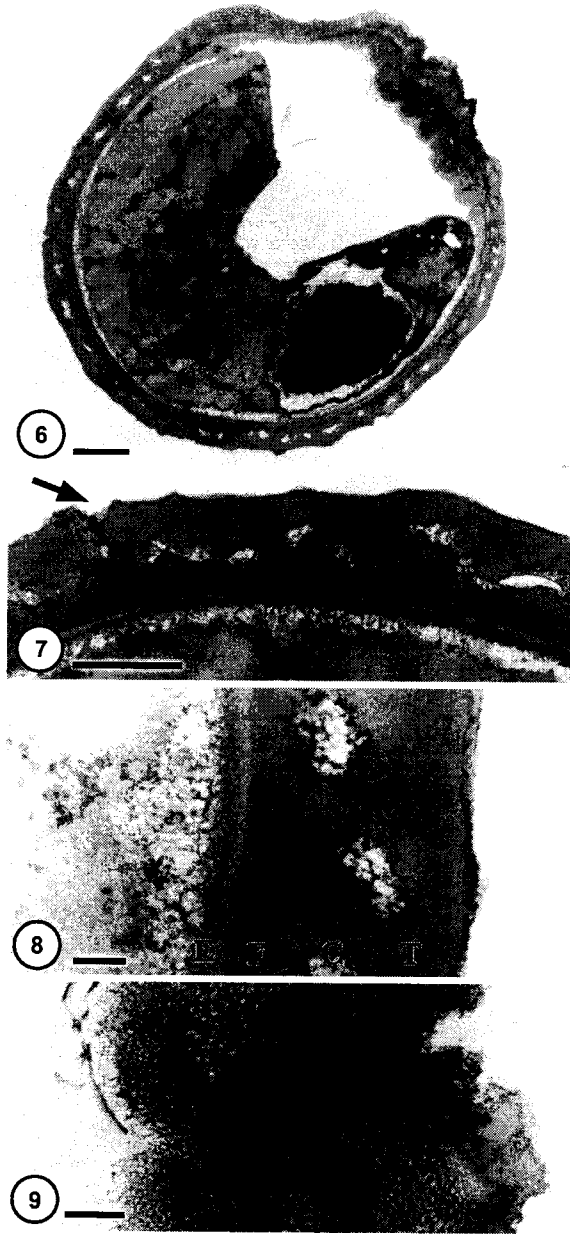
Text-Figure 5.1
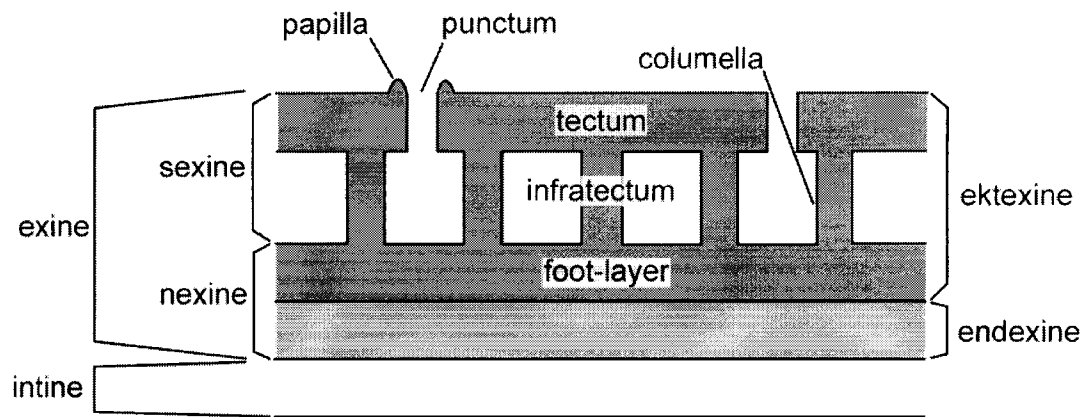
Figures 5.1—5.5. SEM, pollen of *Anemopsis californica* Hook. & Arn. **5.1.** Whole

grain, non-apertural face. **5.2.** Whole grain, apertural face showing monosulcate aperture.

5.3. Whole grain, lateral view. **5.4.** Tectal surface showing puncta with surrounding

papillae. **5.5.** Aperture sculpturing. Scale bars: *5.1, 5.2, 5.3* = 1 µm; *5.4, 5.5* = 500 nm.

Figures 5.1—5.5
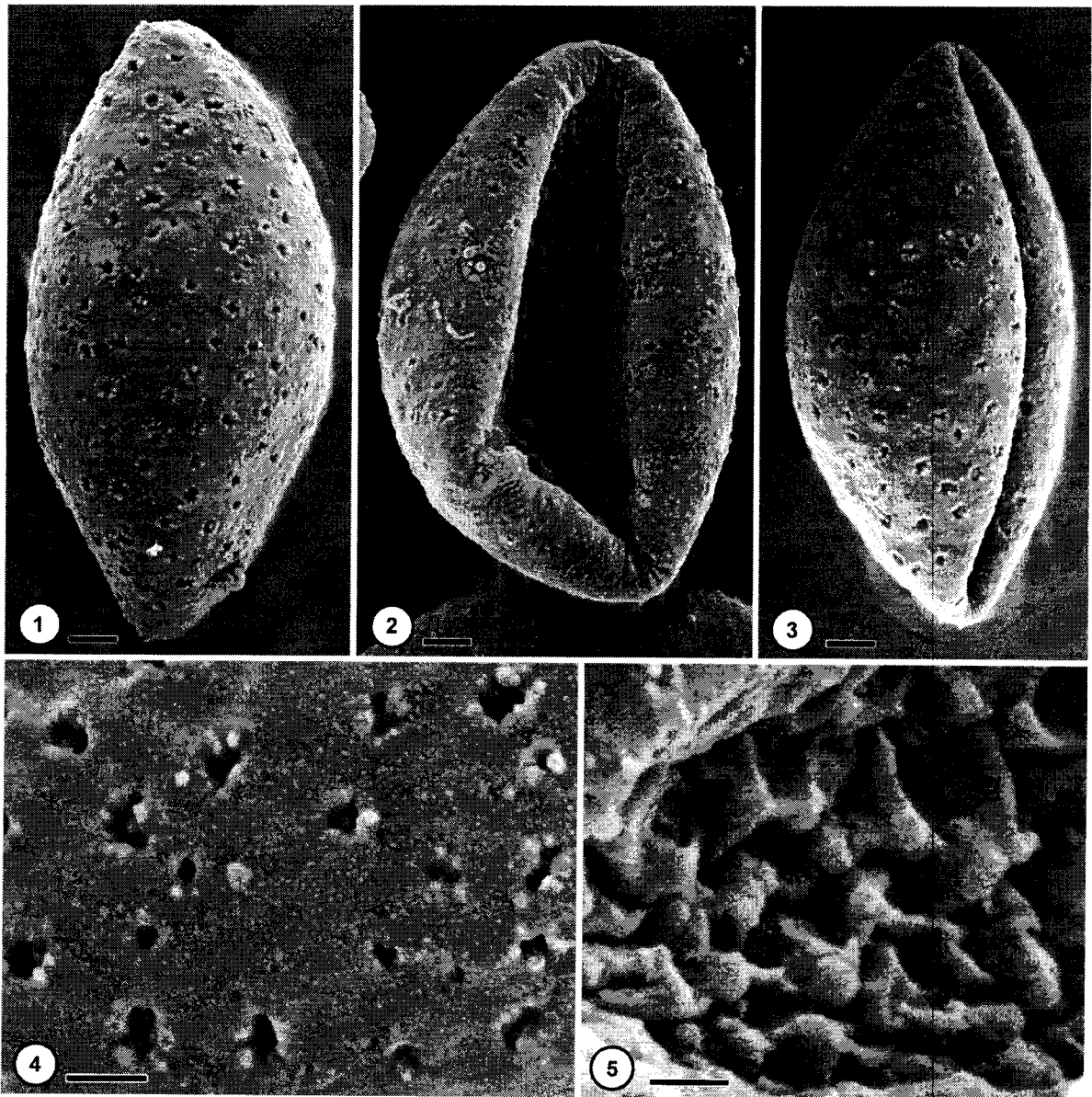
Figures 5.6—5.9. TEM, pollen of *Anemopsis californica* Hook. & Arn. **5.6.** Whole

pollen grain, with aperture at top right showing thicker intine and sculpturing. Scale = 1 µm.

5.7. Cross section of pollen wall, with tectal perforation (arrow). Scale = 500 nm. **5.8.**

Detail of exine structure, showing tectum (T), columellate infratectum (C), thick foot layer

(F) and endexine (E). Scale = 100 nm. **5.9.** Detail of apertural region in cross section,

showing thick intine, laminated endexine, granula (right). Scale = 200 nm.

Figures   5.6—5.9

Figures 5.10—5.14. SEM, pollen of *Gymnotheca chinensis* Decne. **5.10.** Whole grain, non-apertural face. **5.11.** Whole grain, apertural face showing monosulcate aperture with granulate sculpturing. **5.12.** Whole grain, lateral view. **5.13.** Tectal surface showing weakly striate microsculpturing and puncta with no papillae. **5.14.** Aperture sculpturing. Scale bars: 5.10, 5.11, 5.12 = 1 μm; 5.13, 5.14 = 500 nm.
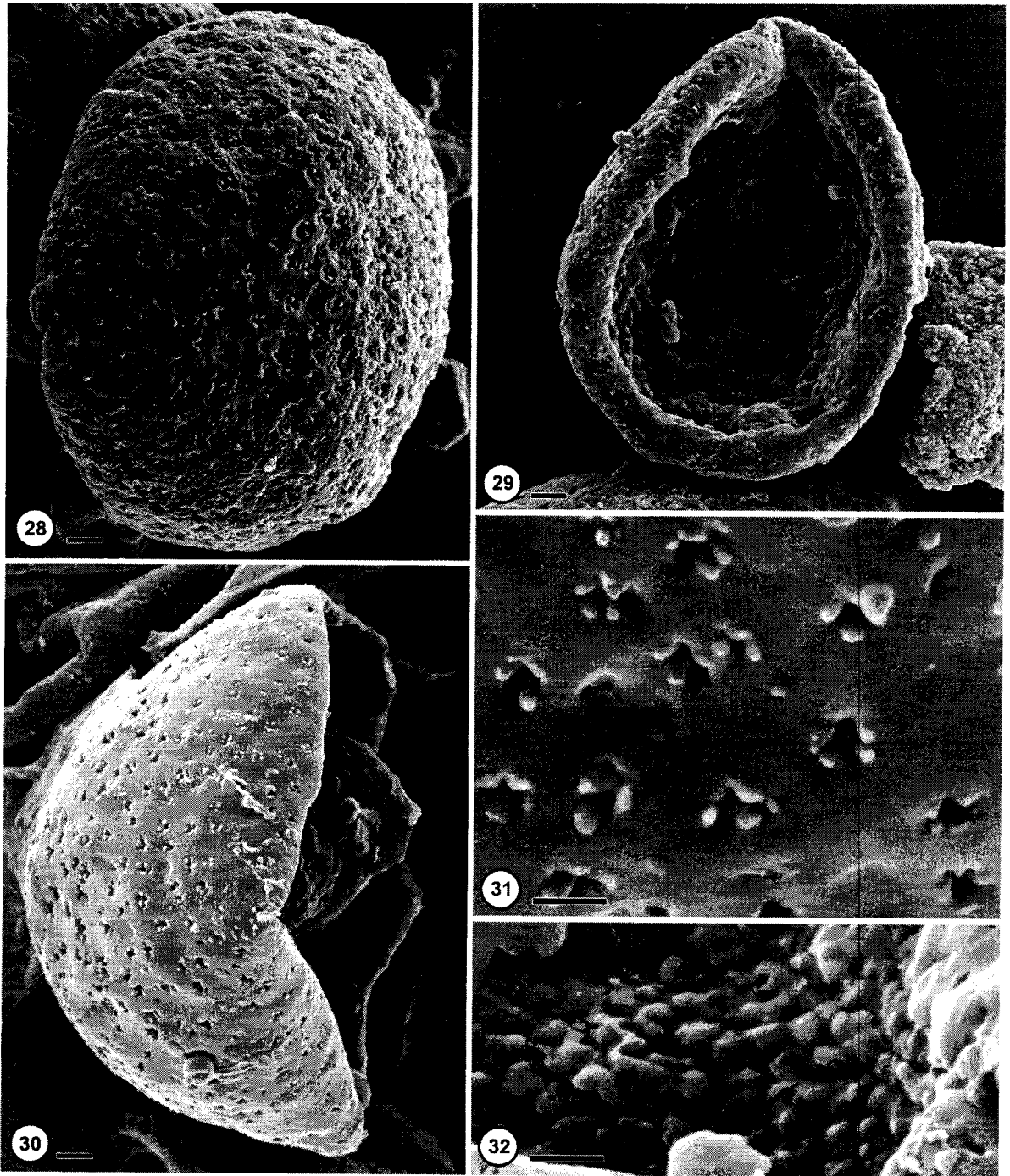
Figures 5.10—5.14
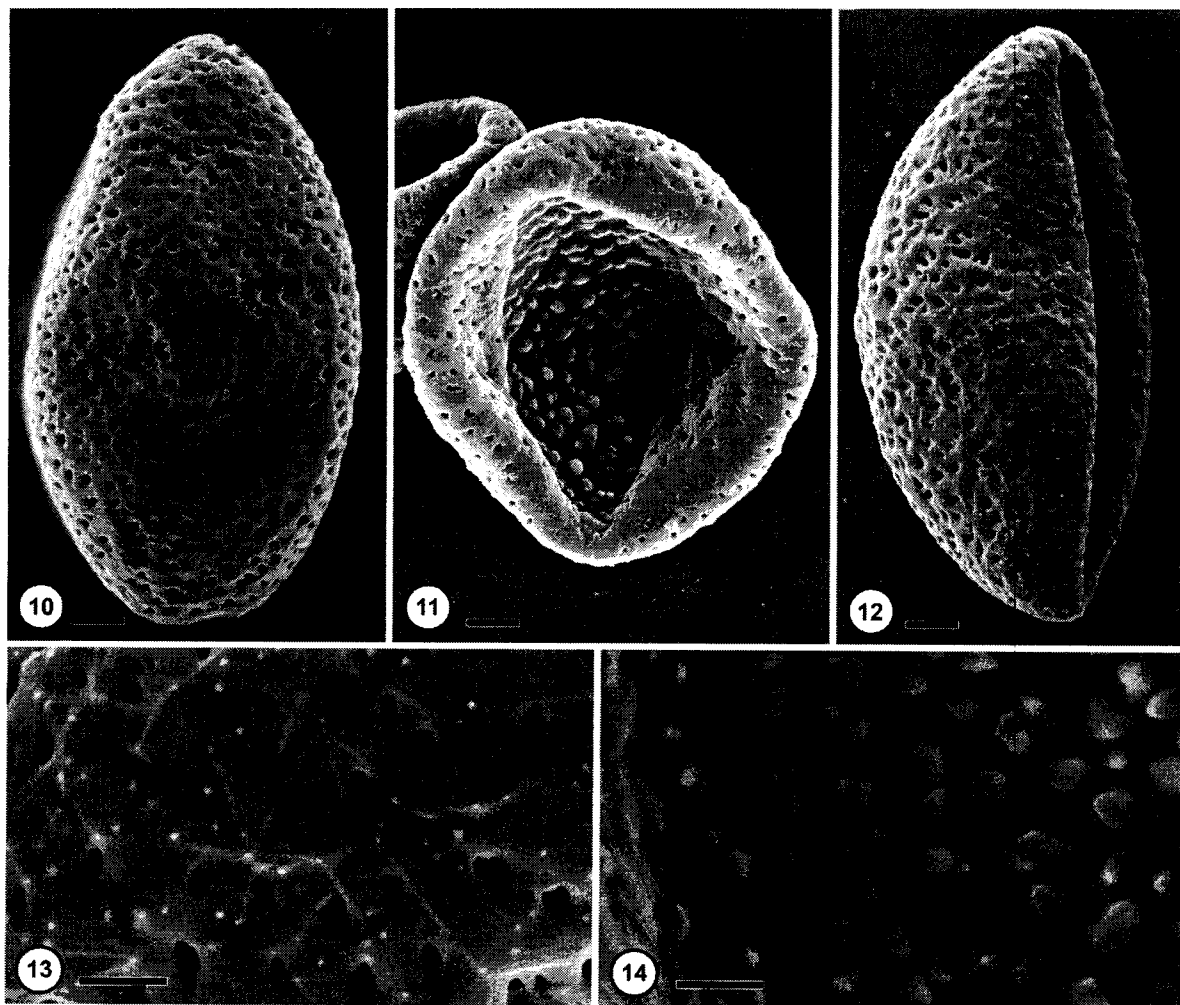
Figures 5.15–5.19. SEM, pollen of *Gymnotheca involucrata* Pei. 5.15. Whole grain, non-apertural face. **5.16.** Whole grain, apertural face showing monosulcate aperture, with granulate sculpturing. **5.17.** Whole grain, lateral view. **5.18.** Tectal surface showing weakly striate microsculpturing and puncta with no papillae. **5.19.** Aperture sculpturing. Scale bars: 5.15, 5.16, 5.17 = 1 µm; 5.18, 5.19 = 500 nm.

Figures   5.15—5.19
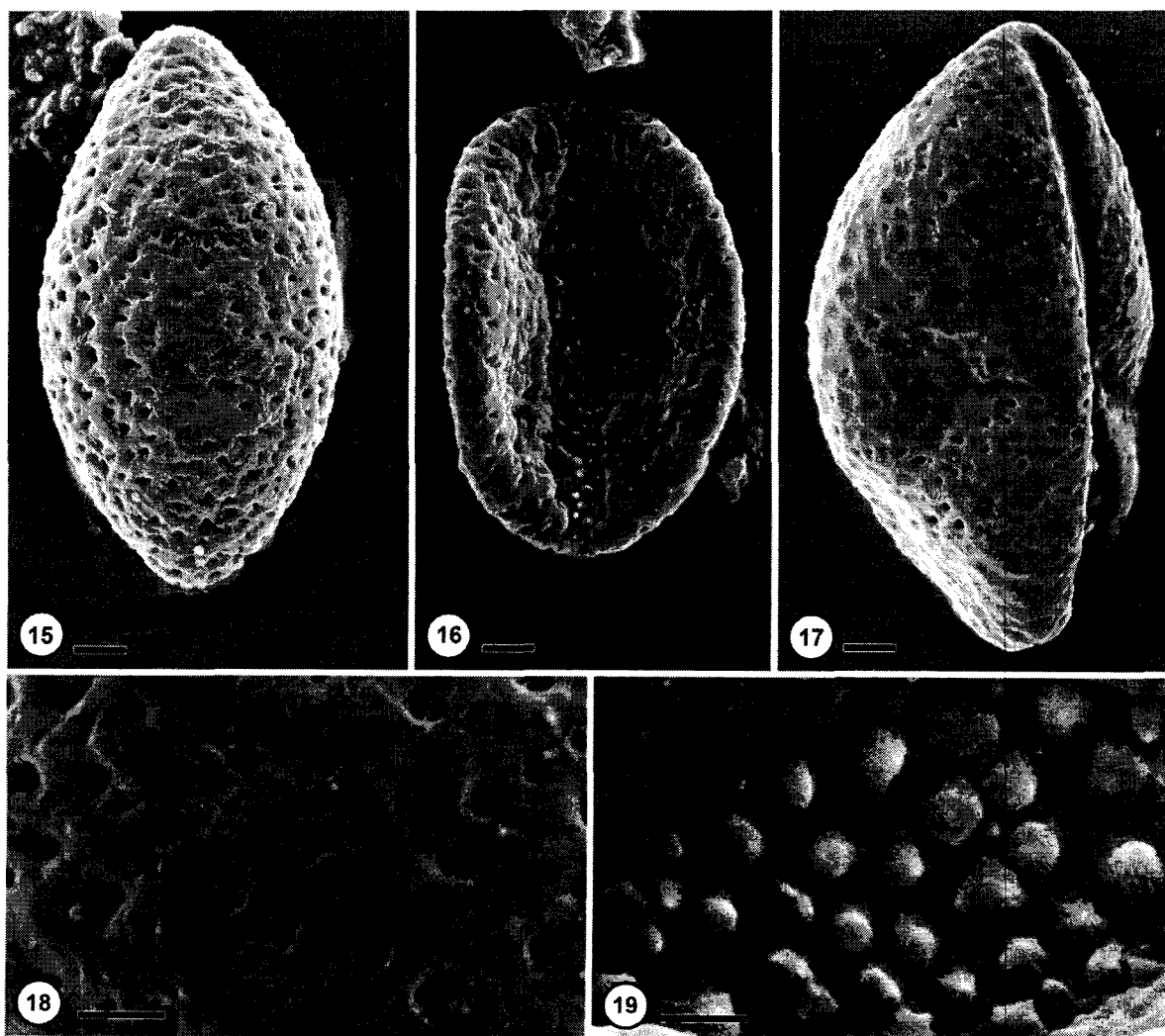
Figures 5.20–5.27. TEM, pollen of *Gymnotheca* Decne. *5.20.* *Gymnotheca chinensis*, whole grain, section parallel to aperture (A). Scale = 1 µm. *5.21.* *Gymnotheca chinensis*, cross section of pollen wall, with tectal perforation (arrow). Scale = 500 nm. *5.22.* *Gymnotheca chinensis*, detail of exine structure, showing tectum (T), columellate infratectum (C), foot layer (F) and endexine (E). Scale = 100 nm. *5.23.* *Gymnotheca chinensis*, detail of apertural region in oblique cross section, showing thick intine, laminated endexine (arrows), and granula (G). Scale = 200 nm. *5.24.* *Gymnotheca involucrata*, whole grain. Scale = 1 µm. *5.25.* *Gymnotheca involucrata*, cross section of pollen wall and tectal perforations (arrows). Scale = 500 nm. *5.26.* *Gymnotheca involucrata*, detail of exine structure, showing tectum (T), columellate infratectum (C), foot layer (F) and endexine (E). Scale = 100 nm. *5.27.* *Gymnotheca involucrata*, detail of apertural region in cross section, showing laminated endexine (arrows) and granula (G). Scale = 200 nm.

Figures 5.20–5.27
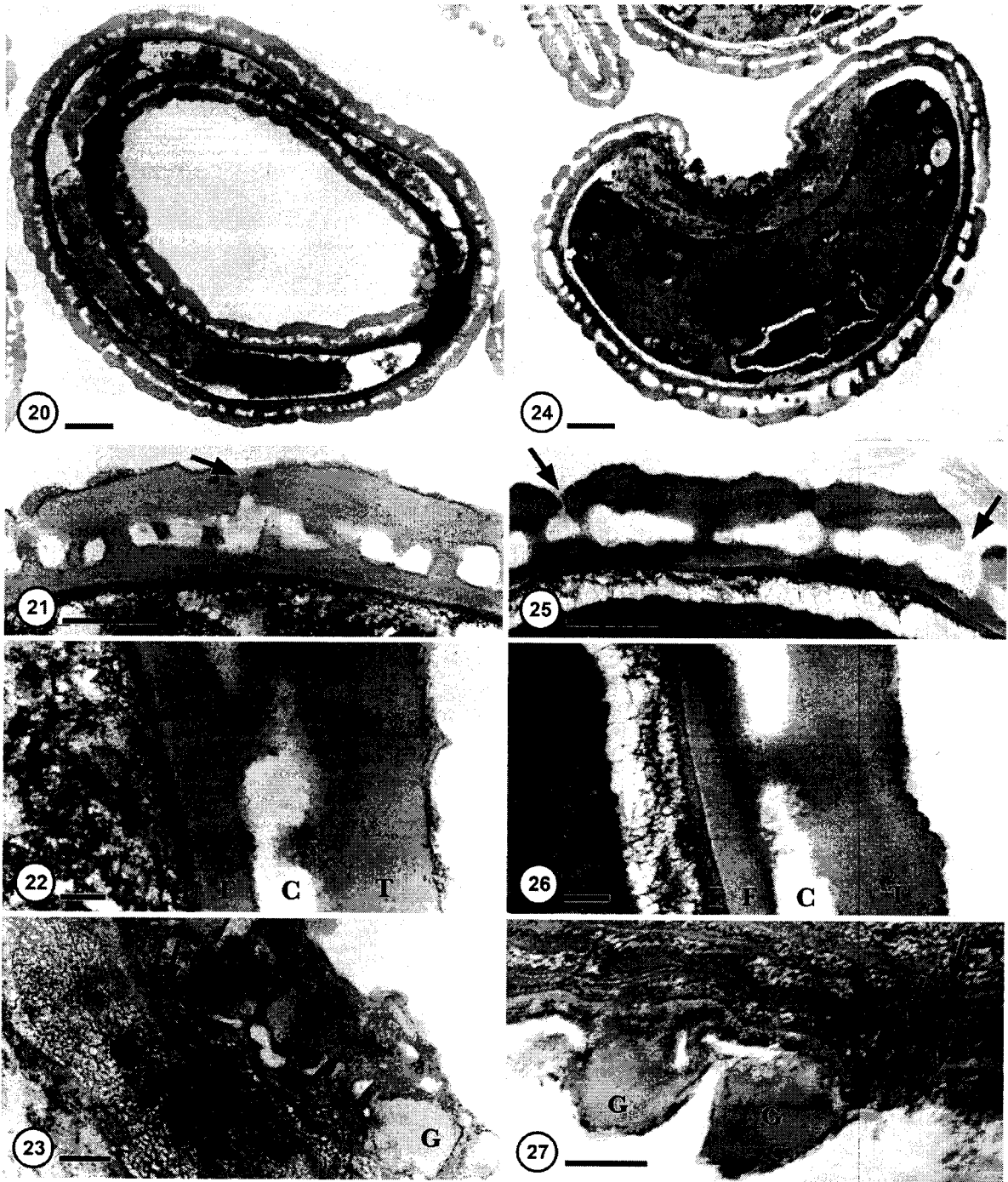
Figures 5.28—5.32. SEM, pollen of *Houttuynia cordata* Thunb. **5.28.** Whole grain,

oblique non-apertural face. **5.29.** Whole grain, oblique apertural face showing

monosulcate aperture, with short granula. **5.30.** Whole grain, lateral view. **5.31.** Tectal

surface showing smooth surface and puncta with three or four surrounding papillae. **5.32.**

Aperture sculpturing. Scale bars: 5.28, 5.29, 5.30 = 1 μm; 5.31, 5.32 = 500 nm.

Figures 5.28—5.32

Figures *5.33—5.36.* TEM, pollen of *Houttuynia cordata* Thunb. *5.33.* Whole grain. *5.34.* Cross section of pollen wall. *5.35.* Detail of exine structure, showing tectum (T), columellate infratectum (C), foot layer (F) and endexine (E). *5.36.* Detail of apertural region in cross section showing laminated endexine (arrows) and granula (G). Scale bars: 5.33 = 1 μm; 5.34, 5.35, 5.36 = 200 nm.

Figures 5.33—5.36
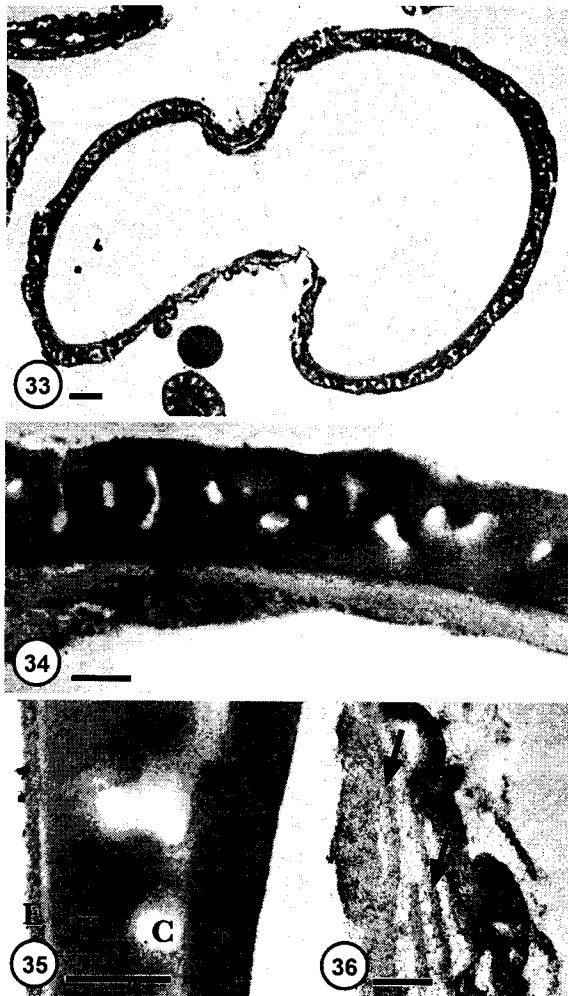
Figures 5.37–5.42. SEM, pollen of *Saururus cernuus* L. **5.37.** Whole grain, non-apertural face. **5.38.** Whole grain, apertural face showing monosulcate aperture with granula. **5.39.** Whole grain, lateral view. **5.40.** Tectal surface showing puncta with three surrounding papillae. **5.41.** Tectal surface of pre-anthesis pollen, showing prominent papillae; note puncta closed. **5.42.** Aperture sculpturing. Scale bars: 5.37, 5.38, 5.39 = 1 µm; 5.40, 5.41, 5.42 = 500 nm.
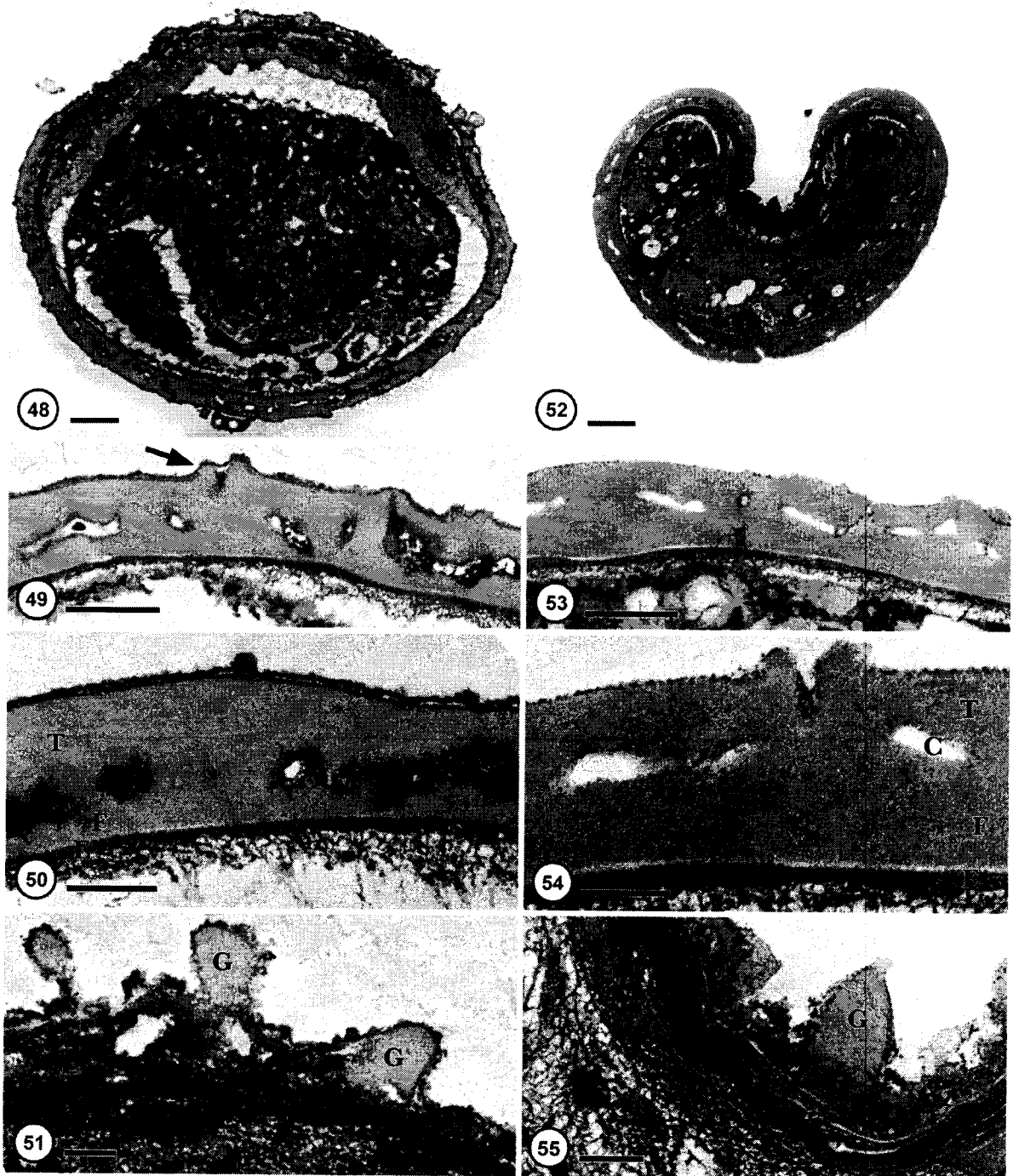
Figures   5.37—5.42
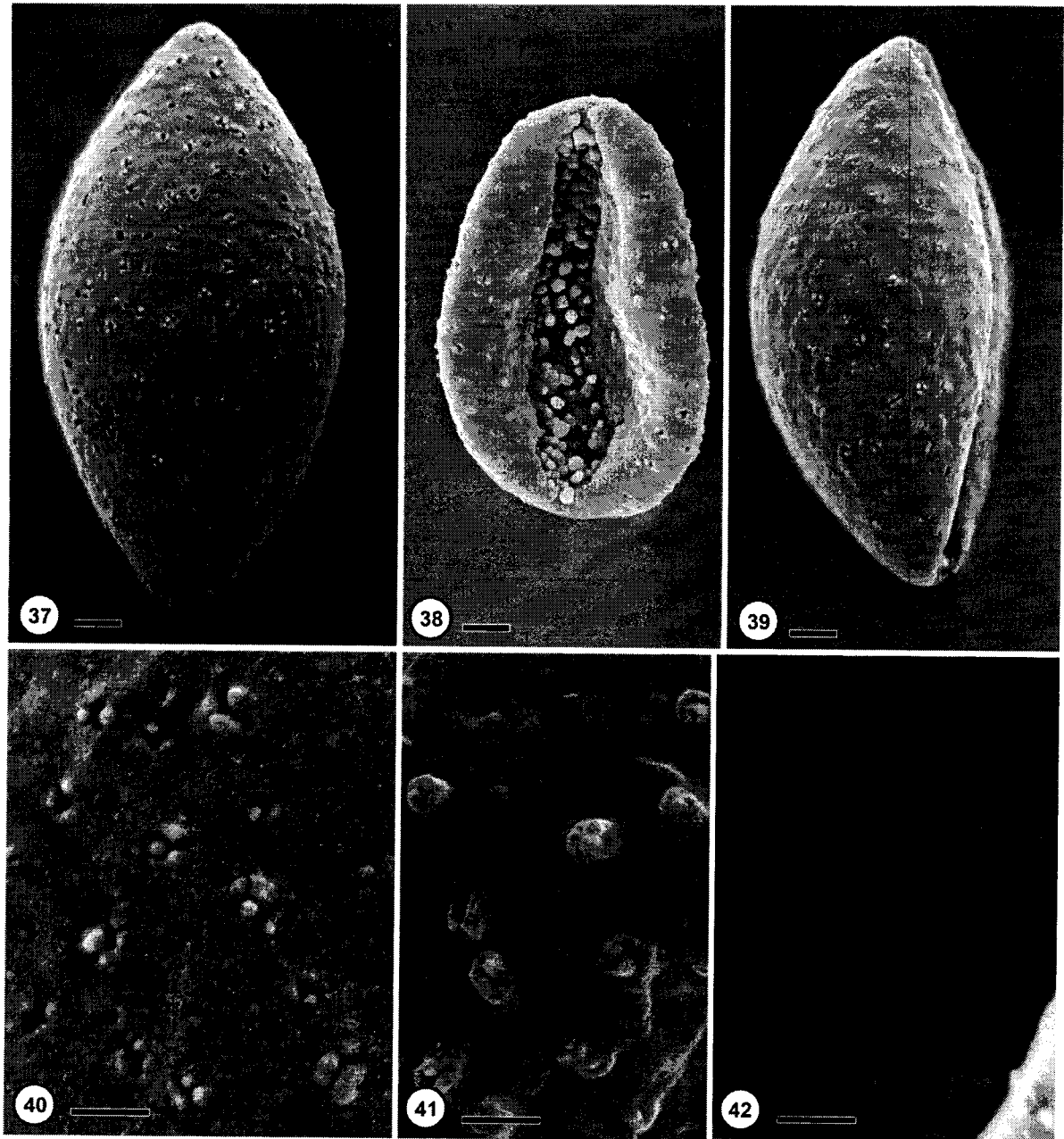
Figures *5.43—5.47*. SEM, pollen of *Saururus chinensis* (Lour.) Baill. **5.43.** Whole

grain, non-apertural face. **5.44.** Whole grain, apertural face showing monosulcate

aperture, with granula. **5.45.** Whole grain, lateral view. **5.46.** Tectal surface showing

smooth surface and puncta with two to four surrounding papillae. **5.47.** Aperture

sculpturing. Scale bars: 5.43, 5.44, 5.45 = 1 μm; 5.46, 5.47 = 500 nm.

Figures 5.43—5.47
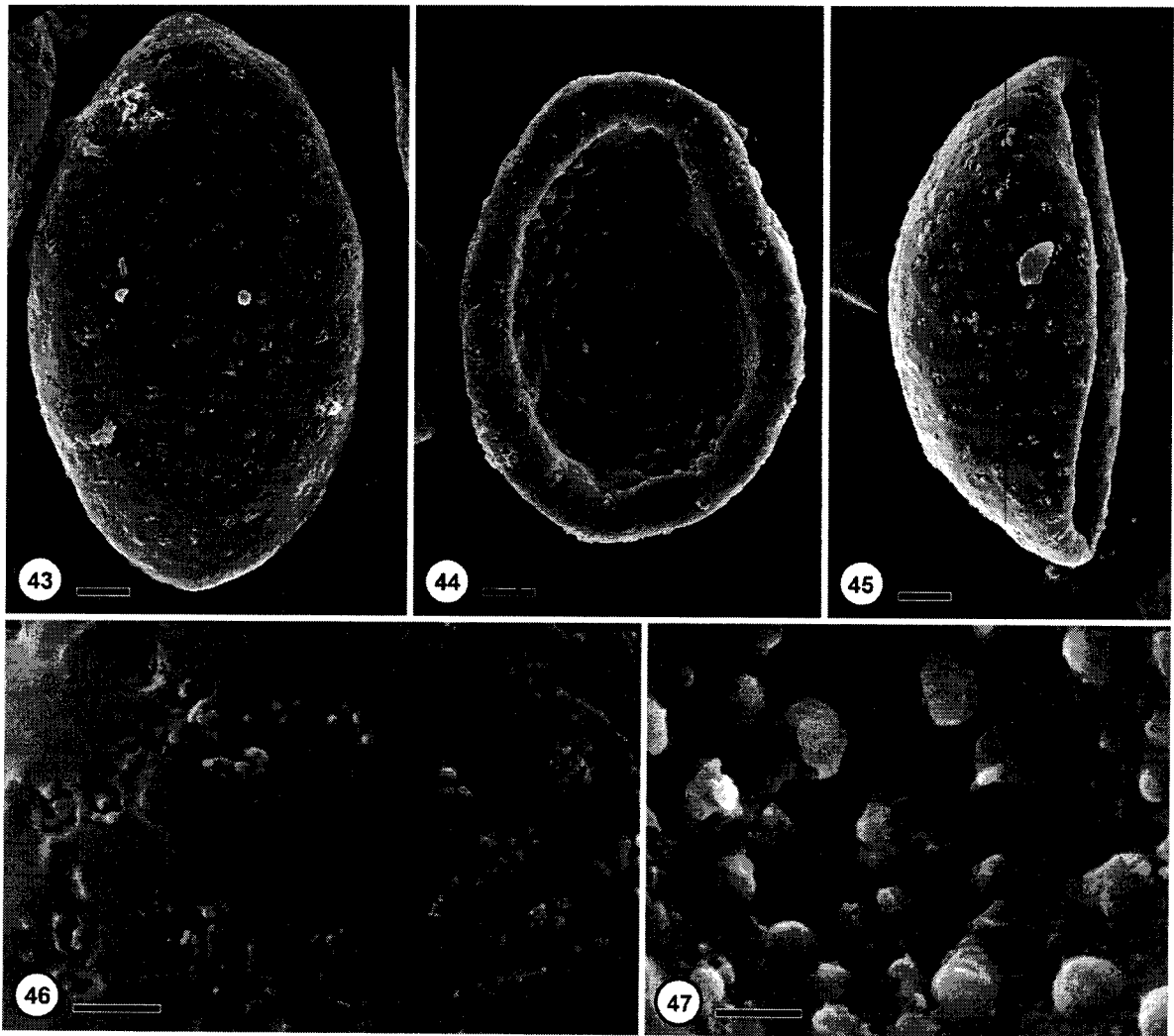
Figures 5.48–5.55. TEM, pollen of *Saururus* L. **5.48.** *Saururus cernuus*, whole grain. **5.49.** *Saururus cernuus*, cross section of pollen wall; note tectal perforation with papilla (arrow). **5.50.** *Saururus cernuus*, detail of exine structure, showing tectum (T), columellate infratectum (C), foot layer (F) and endexine (E). **5.51.** *Saururus cernuus*, detail of apertural region in cross section showing intine, laminated endexine and granula (G). **5.52.** *Saururus chinensis*, whole grain. **5.53.** *Saururus chinensis*, cross section of pollen wall. **5.54.** *Saururus chinensis*, detail of exine structure, showing perforated tectum (T), columellate infratectum (C), foot layer (F) and endexine (E). **5.55.** *Saururus chinensis*, detail of apertural region in cross section showing intine (I), laminated endexine and granulum (G). Scale bars: 5.48, 5.52 = 1 μm; 5.49, 5.53 = 500 nm, 5.50, 5.51, 5.54, 5.55 = 200 nm.

Figures 5.48—5.55

Figures *5.56–5.60.* SEM, pollen of *Saururus tuckerae.* *5.56.* Whole grain, non-apertural face. *5.57.* Whole grain, apertural face showing monosulcate aperture with granula. *5.58.* Whole grain, lateral view. *5.59.* Tectal surface showing smooth surface and puncta with papillae. *5.60.* Aperture sculpturing. Scale bars: *5.56, 5.57, 5.58* = 1 μm; *5.59,* *5.60* = 500 nm.

Figures 5.56—5.60
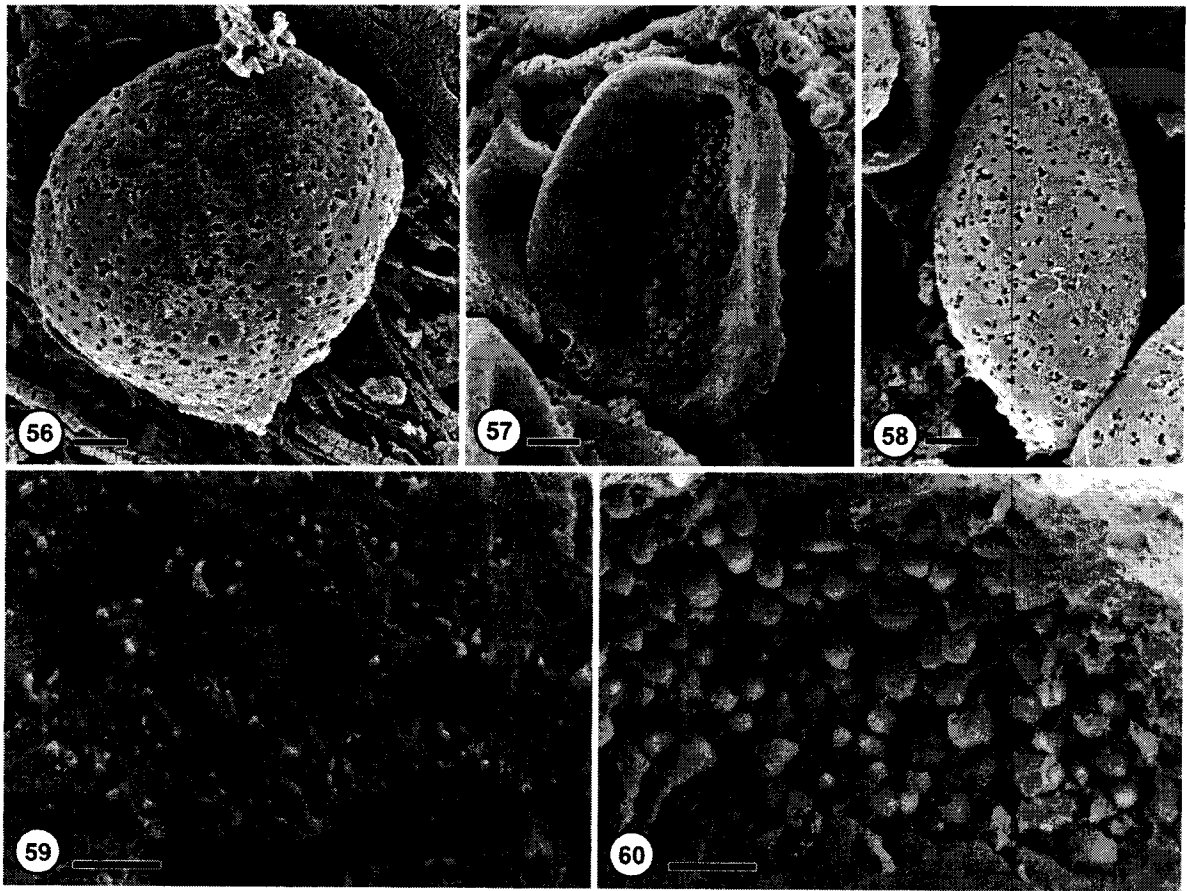
Figures 5.61—5.63. TEM, pollen of *Saururus tuckerae*. **5.61.** Whole grain. Scale bar =

1 μm. **5.62.** Cross section of pollen wall, with numerous tectal puncta (arrows) in tectum.

Scale bar = 500 nm. **5.63.** Oblique cross section of wall showing tectum (T), columellate

infratectum (C), foot layer (F) and endexine (E). Scale bar = 200 nm.

Figures 5.61—5.63
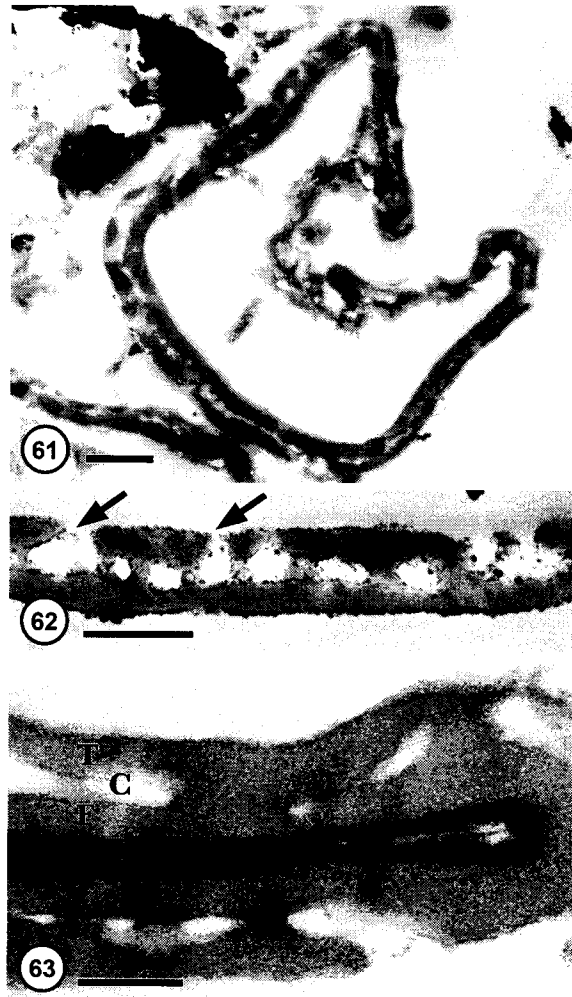
Figures 5.64—5.67. TEM, acetolysed pollen of Saururaceae. **5.64.** *Anemopsis*

californica, oblique cross section of wall showing dark ectexine and thin endexine (arrow).

5.65. *Gymnotheca chinensis*, cross section of wall showing dark ectexine and thin, light-

staining endexine (arrow). **5.66.** *Houttuynia cordata*, cross section of wall showing dark

ectexine and thin, light-staining endexine (arrow). **5.67.** *Saururus chinensis*, cross section

of wall showing dark ectexine with punctum and thin, light-staining endexine (arrow). All

scale bars = 200 nm.

Figures 5.64—5.67
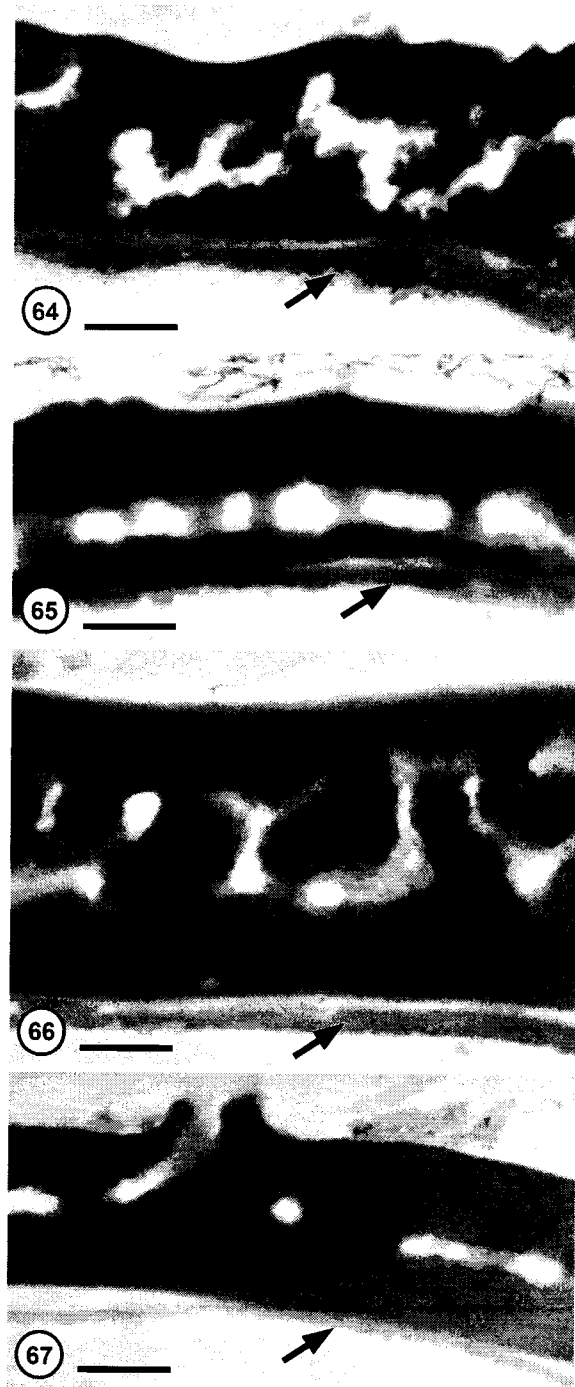
Chapter 6

Conclusions

The Middle Eocene (Allenby Fm.) Princeton Chert of British Columbia, Canada represents a Konservat-Lagerstätte that still has much to reveal. The exceptional preservation of this site means that rare taxa are found and that three-dimensional anatomy is known for plants. Because many plants were preserved in situ, or nearly so, whole-plant reconstructions are possible. During the past 35 years, many taxa have been recognized from the site, including fish, turtles, fungi, ferns, conifers and flowering plants (Cevallos-Ferriz et al., 1991; Pigg and Stockey, 1996; Stockey, 2001). The description of this assemblage has led to the conclusion that the locality represents a shallow, near-shore environment of an ancient pond or lake, similar to environments seen in the southern United States today (Cevallos-Ferriz et al., 1991; Pigg and Stockey, 1996; Stockey, 2001). Evidence for the aquatic nature of the Princeton Chert comes from inferences about the habitat preferences for the nearest living relatives of the extinct taxa, as well as from anatomy. Tissues such as aerenchyma and lacunate phellem that are typical of aquatic plants are common in the Princeton Chert plants (e.g., Erwin and Stockey, 1989; Cevallos-Ferriz et al., 1991; Little and Stockey, 2005; Smith et al., 2006). Petrified flowers are especially rare, but several types have been found at Princeton and that are useful for determining affinities.

In Chapter 2, I describe an inflorescence and flowers from the Princeton Chert that, although they were previously thought to have affinities with alismatid monocots (Currah and Stockey, 1991; Stockey, 1994, 2001, 2006; Pigg and Stockey, 1996; Smith and Stockey, 2004, 2005), strongly resemble *Saururus* (Saururaceae, Piperales). Flowers are minute, ca.

0.8 mm long, perianthless, and have a bract to which the pedicel is fused. Reconstructions of multiple complete flowers show that there are five stamens per flower, adnate to four basally connate carpels. These characters match those of *Saururus* flowers, except that extant flowers typically have six stamens and have trichomes on the bract, flower-bract stalk, and inflorescence axis (Liang and Tucker, 1990; Wu and Kubitzki, 1993; Xia and Brach, 1999). Anther contents were previously described as spores of a smut fungus (Currah and Stockey, 1991; LePage et al., 1994) because of their small size and non-reticulate sculpturing. However, transmission electron microscopy (TEM) done in this thesis shows these anther contents are in fact tectate-columellate pollen grains. The fossil pollen is similar to that of extant *Saururus*, thus providing more evidence for linking these Princeton fossils with the genus.

In this chapter, I also did a phylogenetic analysis using morphological characters. Previous studies have not fully resolved relationships among the four extant genera of Saururaceae (Tucker et al., 1993; Tucker and Douglas, 1996; Nickrent et al., 2002; Meng et al., 2002; Jaramillo et al., 2004; Neinhuis et al., 2005; Qiu et al., 2005). However, Meng et al. (2003) found the same topology of ((*Saururus+Gymnotheca*), (*Anemopsis+Houttuynia*)) using chloroplast, combined molecular, morphological, and combined morphological and molecular data, and this topology has been recovered by other workers (e.g., Tucker et al., 1993; Jaramillo et al., 2004; Neinhuis et al., 2005). My analysis using morphological data resulted in a single most parsimonious with the same topology. The fossil taxon is found sister to extant *Saururus*. I describe the fossils as a new species, *Saururus tuckerae*. The Princeton fossil specimens of *Saururus* are significant

because they represent the oldest fossils to date of Saururaceae, as well as the first fossil flowers and first pollen record in the world of the family.

In Chapter 3, fruit anatomy of the extant species of Saururus (S. cernuus and S. chinensis) was examined. This represents the first anatomical study of these fruits. Fruits were found to be similar in both species, and there is no significant difference in size between the two species. Fruits are schizocarps, with four fruitlets (mericarps) splitting at maturity. Fruitlets have a pleated or wrinkled surface, which are apparent in anatomical sections as projections of the fruit wall. Saururus cernuus fruitlets have more wrinkled exterior than in S. chinensis. The mesocarp is formed of pitted cells with many intercellular spaces that give it a spongy appearance and that probably relate to keeping fruits afloat in water for dispersal (Johnson, 1900; Wood, 1971; Thien et al., 1994). Mesocarp cells of S. cernuus are more strongly pitted than those of S. chinensis. Dried fruitlets of S. cernuus have occasionally persistent styles but not persistent stamens. In contrast, styles and stamens are often persistent on dried fruitlets of S. chinensis. Both species have fruitlets with a thin ventral fruit wall that is broken open at the base, where they were attached to other carpels within a flower, and a ventral ridge that is formed by the carpel margins. Each fruitlet contains a single seed, although rarely two seeds may be found. Ovules have abundant perisperm and, at the micropylar end, endosperm (e.g., Johnson, 1900; Wood, 1971; Corner, 1976; Wu and Kubitzki, 1993; Chapter 3). The integument is composed of four layers. There is an outer layer of thick-walled rectangular cells, two layers that appear to be thick, amorphous deposits, and an innermost integumentary layer that is of thick, possibly tanniniferous, cells. Cells of the innermost layer form a thick area in the chalaza, and at the micropylar end interdigitate with cells of the endosperm and, to a lesser extent,

perisperm. Previously, the outermost integumentary layer has been described as having

large, thin-walled cells (Johnson, 1900; Wood, 1971; Corner, 1976; Friis, 1985). However,

Plisko (1988) interpreted these cells to be part of the fruit wall. In this study I showed that

these cells are in fact those of the endocarp, not the seed integument. In mature fruits

endocarp detaches from the mesocarp and becomes closely associated with the seed coat.

Further studies on ovule development, which follow the integumentary layers to maturity

are needed to determine the exact nature of all the layers currently interpreted as

integument.

In Chapter 4, I describe fossil fruits that are often found in association with the flowers

of *S. tuckerae*. A developmental series with different stages preserved in the same rock

helped to show that these fruits are those of *S. tuckerae*, based on morphological

similarities such as presence of two lateral lobes, a single ovule per carpel and endocarp.

The fossil fruits have strikingly similar anatomy to fruits of extant *Saururus*. Three-

dimensional reconstructions show that the fossil fruits are externally much smoother than

those of extant *Saururus*. Fossil fruits also provide evidence supporting the interpretation of

the presence of a thin-walled endocarp layer, as found in Chapter 3. Other fossil fruits

known as *S. bilobatus* (Nikitin) Mai have been described from the Late Eocene to Pliocene

of Europe and Siberia (Mai and Walther, 1978; Friis, 1985; Lesiak, 1994). Fruits of *S.*

bilobatus are similar to the fossil fruits from Princeton, in that both have relatively smooth

fruit walls compared to extant *Saururus* fruits. However, the mesocarp of *S. bilobatus* has

strongly pitted cells like those found in *S. cernuus*, while in *S. tuckerae* mesocarp the cells

show weak to no pitting. All *Saururus* fruits, living and fossil, fall within the same size range.

By showing that these fruits belong to *S. tuckerae*, an amplified concept of *S. tuckerae* is

provided. Fruits also provide additional evidence that the Princeton fossils clearly represent

an extinct species of *Saururus*. In addition, these fossils show that *Saururus* was once

distributed throughout the Northern Hemisphere. Fossils of *S. bilobatus* and *S. tuckerae*

are now known in Europe, Siberia and western North America (Mai and Walther, 1978;

Friis, 1985; Lesiak, 1994; Chapter 4) where no *Saururus* grows natively today (Wu and

Kubitzki, 1993; Xia and Brach, 1999).

In Chapter 5, I surveyed pollen morphology and ultrastructure of Saururaceae. Pollen

in this family has received little attention, and there are conflicting reports of some details

such as sculpturing type and presence or absence of endexine. I present more detailed

scanning electron micrographs for all species than have been previously published. Chapter

5 also presents the first TEM data for *Anemopsis*, *Saururus chinensis* and both species of

Gymnotheca, and confirms previously reported exine characters for *S. cernuus* and

Houttuynia. There is no known palynological fossil record for Saururaceae except for that

of *Saururus tuckerae* (Chapter 2). Saururaceous pollen is boat-shaped-elliptic to globose

and monosulcate, with granulate sculpturing on the aperture membrane (Walker, 1976a,

1976b; Grayum, 1992; Liang, 1992; Doyle, 2005; Chapter 5). However, in *Anemopsis*

granula are elongate, and in *Houttuynia* aperture sculpturing is not as prominent as in the

other taxa. All taxa have a punctate tectum. In *Anemopsis*, *Houttuynia* and *Saururus*

papillae border the puncta. *Gymnotheca* pollen differs from the other species in having

microstriate tectum (compared to smooth tectum in *Anemopsis*, *Houttuynia* and *Saururus*)

and in lacking papillae around the puncta. Pollen of *Houttuynia* is the largest within the

family, but is still less than 20 μm in size. *Gymnotheca* and the fossil *S. tuckerae* have a

strongly columellate infratectum, while the infratectum of *Anemopsis*, *S. cernuus* and *S.*

chinensis has much wider columellae and thin, irregular spaces between columellae in section view, giving the exine an almost solid look. The presence of endexine in saururaceous pollen has been an issue of some controversy: Walker (1976b) and Doyle and Hotton (1991) reported that endexine is present in Saururaceae, while Sampson (2000) and Takahashi (1986) stated endexine is absent. The results presented in Chapter 5 clearly show endexine is present in all taxa.

It is not too surprising to find saururaceous fossils in the Princeton Chert assemblage, as these are wetland plants (Wu and Kubitzki, 1993; Xia and Brach, 1999) that would have been quite at home growing in the ancient Princeton pond or lake. *Saururus cernuus* is an element in the swampy areas of the southeastern United States, and its neighbours are not unlike plants found in the Princeton Chert assemblage: *Decodon* J. F. Gmel., *Taxodium* Rich., waterlilies, pines and palms. *Decodon allenbyensis* Cevallos-Ferriz and Stockey (1988), *Metasequoia milleri* Rothwell & Basinger (1979), the waterlily *Allenbya collinsonae* Cevallos-Ferriz & Stockey (1989), at least two pines (Miller, 1973; Stockey, 1984) and the palm *Uhlia allenbyensis* Erwin & Stockey (1991, 1994) are all plants found in the Princeton Chert. *Decodon*, *Allenbya* and other angiosperms co-occur with *Saururus tuckerae* in layers 8 and 43, but the conifers and the palm are found in other, not these, layers. The presence of *Saururus* provides additional evidence that the Princeton Chert preserves a shallow, aquatic, near-shore environment (Wilson, 1980; Cevallos-Ferriz et al., 1991; Stockey, 2001).

These fossils represent an important contribution to understanding the history of Saururaceae, and Piperales. The family Saururaceae has a meagre fossil record, and that of the Piperales (sensu APG II, 2003: Piperaceae, Saururaceae, Aristolochiaceae,

Lactoridaceae and Hydnoraceae) is sparse. Thus, in addition to contributing to our understanding of the Princeton Chert environment, the fossil record of Saururaceae and its paleobiogeography are now better understood. It is obvious that *Saururus* was once more widely distributed in the Northern Hemisphere and that the occurrences of *S. chinensis* in eastern Asia and *S. cernuus* in eastern North America are relictual.

Saururus has been said to show the most generalized and primitive floral characters within the family (e.g., Tucker et al., 1993; Liang, 1994, 1995). However, others (Meng et al., 2003; Jaramillo et al., 2004), in the context of ontogenetic data and the sister group (Piperaceae), argued that *Saururus* has a unique floral plan representing derived (not primitive) floral characters within the family. The current work demonstrates that a 48 million year old *Saururus* shows many of the floral characters were present by the Middle Eocene. The position of *Saururus* as sister to the rest of Saururaceae or sister to *Gymnotheca* has not been resolved, but even if the former result is found to be highly supported this does not necessarily mean that the taxon retains the most primitive characters. Basal or fossil taxa do not necessarily show a large number of primitive characters. Future fossil finds of both Piperaceae and Saururaceae that show unique combinations of characters may help clarify how floral characters have evolved within the family. The recognition of this distinct fossil taxon reminds us that we should keep searching for fossils representing poorly known groups.

Pollen of Saururaceae is probably present at many sites, but due to its small size is overlooked or lost using conventional techniques, or simply not recognized due to the previous lack of available illustrative data. The small flowers and fruits in the Princeton Chert will hopefully provide search images for finding more saururaceous fossil material

from North America and elsewhere, and encourage searching for new fossils of this group. Thus will we develop a deeper understanding of piperalean paleobiogeography and floral evolution.

The study of Konservat-Lagerstätten, like the Princeton Chert, is very important on many levels. The exceptional preservation allows for a complete understanding of the fossil plant itself, both in terms of architecture, morphology and/or anatomy as well as some of its ecological preferences. The konservat-lagerstätten also often preserves a fairly complete assemblage, thus enabling accurate paleoenvironmental reconstruction of a locality. In a larger context, well-known fossils can provide paleobiogeographic information, contributing to inferences into the past distribution of groups. Finally, because konservat-lagerstatten often preserve whole or nearly complete plants with many characters, it is possible to make inferences about phylogenetic relationships of plants and their character evolution.

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