An Anatomical Study of the Hawaiian Fern
Adenophorus sarmentosus[1]

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THE ENDEMIC HAWAIIAN FERN, *Adenophorus sarmentosus* (Brack.) K. A. Wilson, occurs on all of the major islands, growing on moss-covered trees and occasionally also on mossy rocks. As a representative of the large but poorly understood fern family Grammitidaceae, *A. sarmentosus* was chosen for anatomical studies in order to contribute some information on the family which may be of value in later systematic studies of the group. Anatomical or morphological studies of the grammitids are rare. A series of recent papers by Nozu (1958–1960) presents the only anatomical investigation of members of this family except for a few notes published earlier by Ogura (1938).

The plant material used was collected at Waianae Iki on the island of Oahu in 1954 by Earl T. Ozaki. A voucher specimen has been deposited in the herbarium of San Fernando Valley State College. The fern was killed and fixed in F.A.A.; sectioning was done according to standard paraffin techniques. Leaves were cleared by the sodium hydroxide technique (Foster, 1949) and stained in tannic acid and ferric chloride (Wilson, 1958).

OBSERVATIONS

The rhizome of *A. sarmentosus* is erect or ascending, short, usually less than 1 cm in length, 0.5–1 mm thick, and clothed in reddish-brown, concolorous, linear-lanceolate scales (Fig. 1, 8). The roots are wiry, dark brown, and few-branched, often bearing propagules which develop into new plants (Fig. 1, 2). This vegetative means of propagation is known not only in *A. sarmentosus,* but also in the closely related *A. haalilioanus,* and should be looked for in the rare *A. pinnatifidus.* Similar reproductive behavior has been reported recently in *Asplenium plenum* of Florida (Wagner, 1963).

The petioles are short, less than 2 cm long, crowded on the rhizome, and bearing simple or branched deciduous, reddish-brown hairs. The blades are pinnatifid, elliptic-lanceolate, 8–15 cm long, 1–2.5 cm wide, and narrowing gradually at both ends, often becoming prolonged into a long caudate apex (Fig. 1, 3). The venation is pinnate, with free simple (rarely branched) veins with clavate or punctiform ends which do not extend to the margins of the blade (Figs. 1, 3 and 4).

The outline of the stem is almost a perfect circle in cross-section. The vascular cylinder is protostelic in the young stages and becomes solenostelic in older portions of the rhizome. During the transition from the protostele, a parenchymatous pith develops (Fig. 2, 1), within which a central phloem strand differentiates in slightly later stages (Figs. 2, 2 and 3). In the older portions of the stem the inner phloem of the solenostele is cylindrical and surrounds a central pith (Figs. 2, 13–17). The single leaf traces which arise from the main stele and lead to the leaves are surrounded by the sclerenchyma of the cortex which extends into the petiole itself. Each leaf trace is generally associated with two root traces which arise either below the juncture of the leaf trace with the stelar gap (Figs. 2, 2–5) or somewhat above this level (Figs. 2, 7, 29, 30). One instance was observed in which a leaf gap was associated with only a single root trace which arose directly opposite the gap rather than perpendicular to the plane of the gap (Fig. 2, 21). Not all roots, however, arise in association with leaf traces. Pairs of root traces were observed departing from the main stele in regions between

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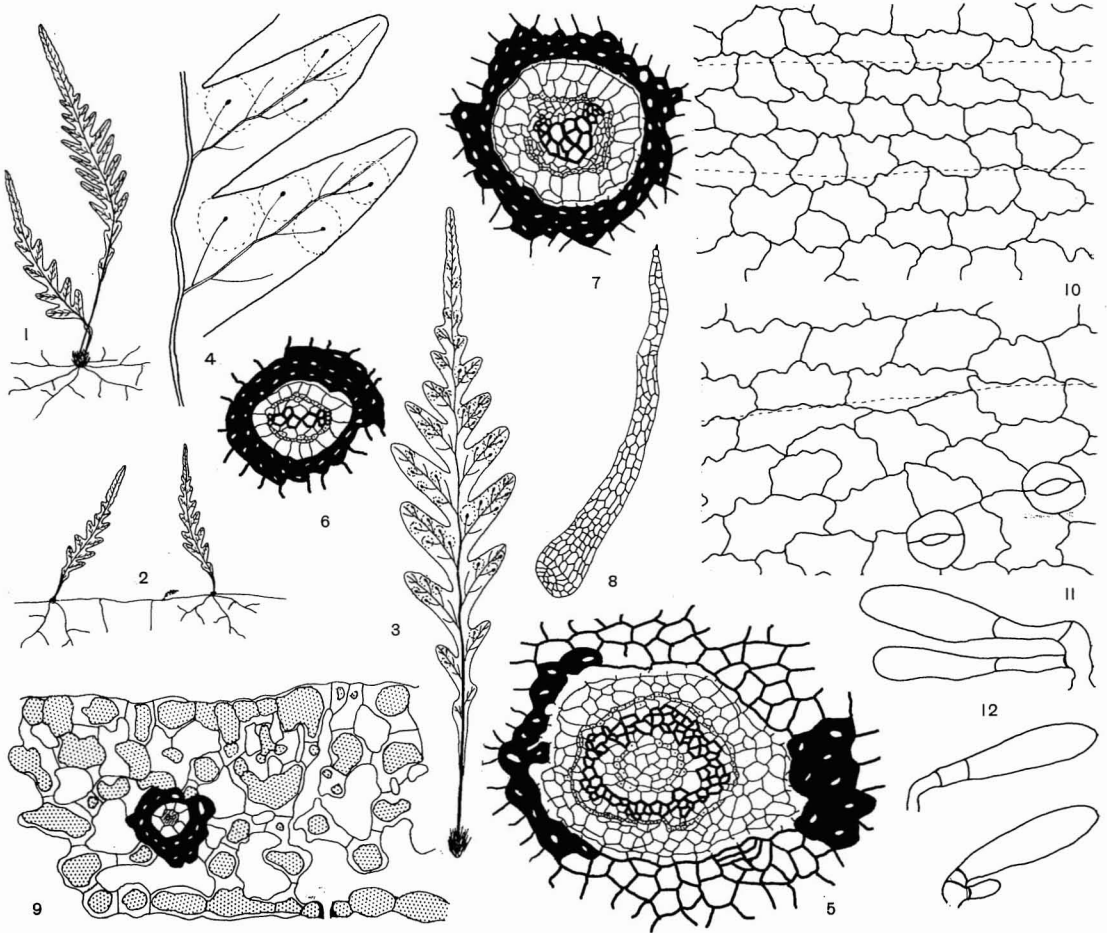
FIG. 1. *Adenophorus sarmentosus.* *1,* Habit × ⅓; *2,* habit showing vegetative reproduction by root prop-agules, × ⅓; *3,* frond, × ⅔; *4,* portion of frond showing venation, × 3½; *5,* cross-section of stele of rhizome × 100; *6,* cross-section of stele of root, × 100; *7,* cross-section of stele of petiole, × 100; *8,* rhizome scale, × 17; *9,* cross-section of lamina, × 100 (stippled portions indicate cut surfaces); *10,* upper epidermal cells, × 100; *11,* lower epidermal cells, × 100; *12,* epidermal hairs, × 100.

successive leaf traces, showing no particular relation with the vascular supply to the leaf (Figs. 2, *13–16*). The orientation of the root traces is horizontal as they arise from the stele as well as during their course through the cortex. The leaf gaps do not become closed in all cases before the departure of the next leaf trace. In our material the gap was observed to close in the lower portion of the rhizome (Figs. 2, *10–12*), while in the upper portions no such closure was observed (Figs. 2, *19–31*).

The stele is surrounded by an endodermis composed of small, thin-walled cells with con-

spicuous, bright-staining Casparian strips on the radial walls. In all of our material the stele is pulled away from the outer tissues of the stem at the region of the endodermis (Fig. 1, 5). This separation results in the destruction of most of the endodermal cells and apparently is caused by the differential action of killing and fixing agents on the tissues of the stele *versus* the surrounding cortical tissues.

The cortex is composed of 10–12 layers of cells. The cells of the innermost 5–7 layers consist of heavily thickened, often completely oc-cluded, sclerenchyma cells. However, patches
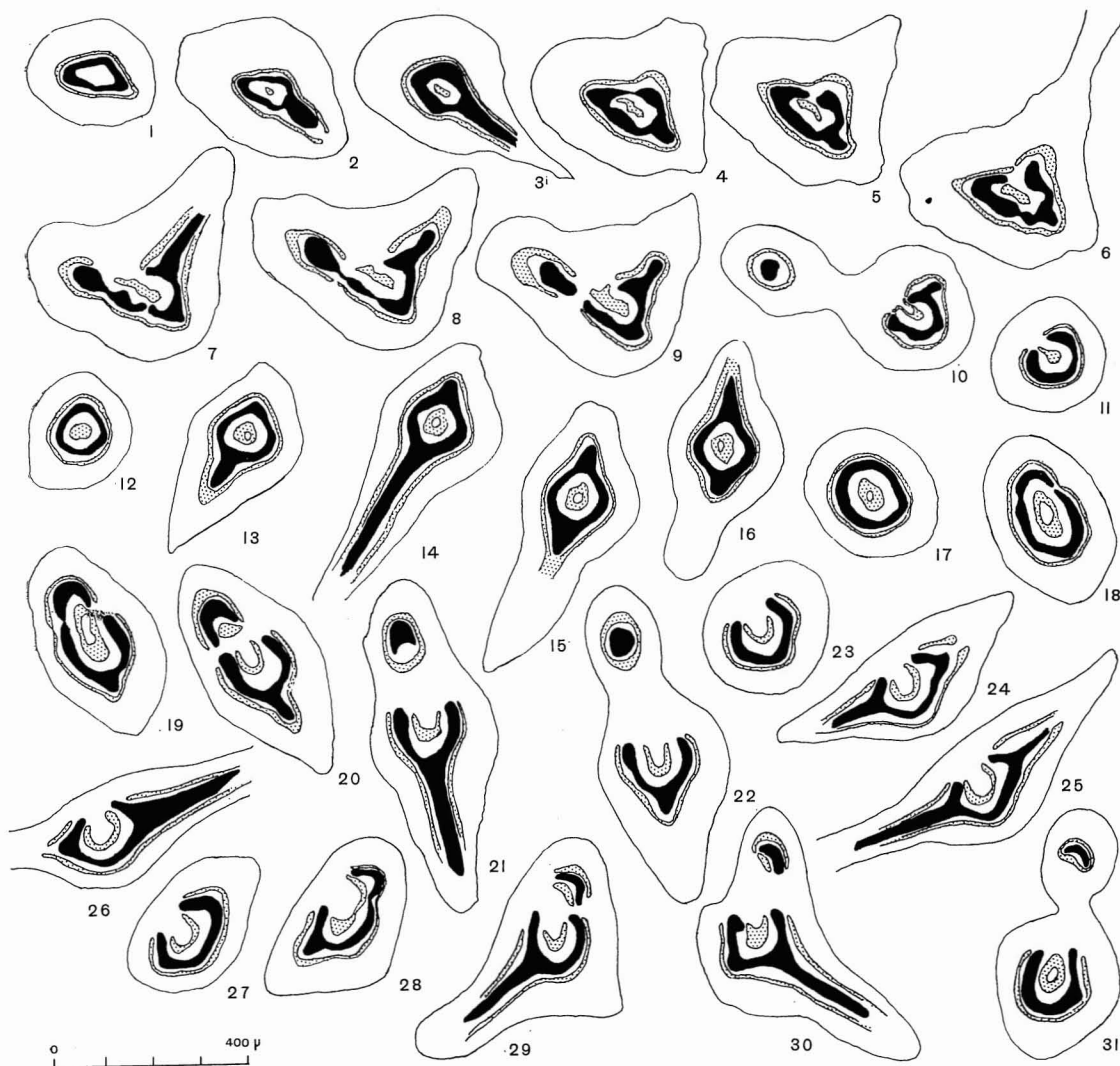
FIG. 2. *Adenophorus sarmentosus.* *1–31,* Selected serial cross-sections of the stele of the rhizome showing origin of leaf traces and root traces.

of slightly thinner-walled, protoplast-containing cells often occur, usually directly opposite a departing leaf trace. The outer 2 or 3 cortical cell layers are composed of uniformly large parenchyma cells. The cells of the single-rowed epidermis are thin-walled.

The xylem cylinder is from one to three cells thick. Its tracheids are of relatively uniform size. The protoxylem tracheids are only about ½ the diameter of the average metaxylem elements and are grouped in more or less distinc-

tive areas on the outer margin of the xylem cylinder. The xylem, therefore, is apparently exarch. Within the cylinder of xylem, the cells of the one-layered or occasionally two-layered internal phloem are separated in most instances from the xylem by a layer of parenchyma cells similar in structure to the cells of the central pith. Another layer of parenchyma also occurs between the xylem and the outer phloem. The cells of the outer phloem are conspicuously smaller than those of the inner phloem and

have a diameter of only ½–⅓ of the average of the elements of the inner phloem. In the outermost tissue of the stele, outside the narrow strip of outer phloem, occur the numerous large cells of the pericycle (Fig. 1, *5*).

The stele of the petiole is circular in cross section and is surrounded by flat endodermal cells which separate it from the heavily sclerified layers of the cortex. The center part of the stele is occupied by the single xylem strand which, in cross section, is roughly reniform (Fig. 1, *7*). The protoxylem elements are in groups of about six at each of two points forming the adaxial lobes of the strand. The xylem is separated from the phloem by two or three layers of irregularly shaped, nucleated parenchyma cells. The phloem area is made up of one or two rows of tiny, thin-walled cells, and surrounds the entire xylem core except at its protoxylem poles. Between the phloem and the endodermis occur the large, nucleated, thin-walled cells of the pericycle containing plastids. The cortex is clearly differentiated from the epidermis and is composed of six to eight layers of thick-walled sclerenchyma cells. The thickness of the cell walls of the cortical cells increases gradually, so that the cells in the outermost layer have extremely thick walls. There is also a gradual decrease in the diameter of the cells toward the periphery of the cortex. The large thin-walled cells of the epidermis are subtended by a layer of slightly smaller thin-walled cells containing numerous plastids which separate the cortical sclerenchyma from the epidermis itself. In the lateral marginal wings of the petiole the epidermis, containing occasional pairs of guard cells, is subtended by a tissue of loosely arranged parenchyma cells with large intercellular spaces.

The adaxial laminar surface lacks stomata. In face view the epidermal cells are approximately 1–3 times as long as wide, and their general outline is somewhat oblong. The longitudinal walls are moderately wavy (Fig. 1, *10*). The abaxial laminar surface is characterized by more irregular cells, generally with pronounced undulate walls (Fig. 1, *11*). Both epidermal surfaces bear scattered suberect or erect hairs. The epidermal hairs are two- to four-celled, uniseriate, simple or frequently branched, and with a large, reddish-brown terminal cell (Fig. 1, *12*).

In cross section, the cells of the upper epidermis are seen to have irregular vertical projections which reach below the epidermal layer itself and come in contact with the spongy tissue of the leaf (Fig. 1, *9*). The lower epidermal cells rarely display such projections. The mesophyll is composed entirely of irregular, loosely arranged, spongy parenchyma cells with large intercellular spaces. The round sori are borne terminally on the veins. The sporangia have a single row of cells at the base of the stalk, and an annulus with 11–13 thickened bow cells (Wilson, 1959a). The spores are green, tetrahedral, trilete, without exospore, and have an average measurement of $34 \times 12 \mu$ (Selling, 1946).

The roots have a diarch stele with two protoxylem groups, one at each end of the xylem tract (Fig. 1, *6*). The one- or two-layered phloem surrounds the xylem except at the protoxylem poles, and is separated from it by a layer of parenchyma cells. The endodermal cells are small, and thin-walled, and were difficult to observe in our preparations. This many-celled endodermis is surrounded by the inner layer of the two layers of heavily sclerified cortical cells. The outer two or three layers of cortical cells are conspicuously thinner-walled.

DISCUSSION

In general, the structure of *Adenophorus sarmentosus* agrees with the observations of Nozu (1958–1960) made on species of *Micropolypodium (Xiphopteris), Scleroglossum,* and *Grammitis.* On the basis of these studies and of general systematic surveys (Holttum, 1954; Copeland, 1947), the solenostele of *A. sarmentosus* seems to be characteristic of the rhizome of most of the Grammitidaceae, although in some groups a dissected solenostele is not uncommon. The absence of an internal phloem in the younger portions of the rhizome of *Adenophorus sarmentosus* is noteworthy. A similar medullated protostele was described in *Micropolypodium okuboi* by Nozu. The stele of *A. sarmentosus,* however, soon becomes soleno-

stelic, interrupted occasionally by leaf gaps. In this it resembles the species of *Grammitis* investigated by Nozu. The dictyostelic vascular cylinder which he reported for *Scleroglossum pusillum* evidently is not repeated in *A. sarmentosus*. However, the failure of some leaf gaps to close may perhaps be interpreted as a trend towards the further dissection of the solenostele. Conclusive evidence cannot be presented at this time to establish the evolutionary trend in the vascular structure of the rhizome in this family. The hypothesis that the family consists of a reduction series seems to us to be most acceptable. With this point of view, we would interpret the solenostele as having been derived from a dictyostele, although it seems probable that some dictyostelic grammitid groups in turn have evolved secondarily from solenostelic ancestors. The temptation to make broad generalization about evolutionary trends is one to which we would rather not yield until more comparative data are available.

A leaf supplied by a single vascular trace with a U- or V-shaped xylem in the petiole seems to typify members of this group. Ogura (1938) designated this particular type of petiolar vascular structure as the "Grammitis" type and subsequent investigators have found similar patterns in other grammitids (Holttum, 1947; Nozu, 1958–1960). The xylem strand of *A. sarmentosus* adds still another species to this list.

It is difficult to attempt to interpret the phylogenetic relationship of the Grammitidaceae on the basis of anatomical investigations of so few members of the group. Numerous studies have been published which indicate that these ferns represent a natural group. Other than anatomical studies of the sporophyte, evidence from the gametophyte generation (Stokey, 1959; Stokey and Atkinson, 1958) and from the morphology of the sporangium (Wilson, 1959*a*; 1959*b*) strongly support the distinctness of the family. Broad phylogenetic conjectures would best await additional studies within the Grammitidaceae. Systematic problems within the family will also require detailed studies before a satisfactory taxonomic arrangement can be proposed. Some of these problems have been discussed recently for the genus *Adenophorus*

by Wilson (1964). We have undertaken this present study in order to obtain some information which may be of value in later systematic considerations of the grammitid ferns. Other similar studies are badly needed.

REFERENCES

COPELAND, E. B. 1947. Genera Filicum, the Genera of Ferns. Chronica Botanica, Waltham, Massachusetts.

FOSTER, A. S. 1949. Practical Plant Anatomy. 2nd ed. D. Van Nostrand, New York.

HOLTTUM, R. E. 1947. A revised classification of the leptosporangiate ferns. J. Linn. Soc. (Bot.) 53:123–158.

———— 1954. Flora of Malaya, II. Ferns of Malaya. Government Printing Office, Singapore.

NOZU, Y. 1958–1960. Anatomical notes on *Micropolypodium, Scleroglossum,* and *Grammitis,* 1–3. J. Geobot. 7:102–107; 8:1–5; 9:4–9.

OGURA, Y. 1938. Anatomie der Vegetationsorgane der Pteridophyten. In: Karl Linsbauer, Handbuch der Pflanzenanatomie. Band 7, Abt. 2 (Teil 2). Berlin.

SELLING, O. H. 1946. Studies in Hawaiian Pollen Statistics, Part I. The Spores of the Hawaiian Pteridophytes. Bishop Mus. Spec. Publ. 37:1–87, pls. 1–7.

STOKEY, A. G. 1959. *Polypodium pectinatum* and *P. plumula*—Polypodiaceae or Grammitidaceae? Am. Fern J. 49:142–146.

———— and L. R. Atkinson. 1958. The gametophyte of the Grammitidaceae. Phytomorphology 8:391–403.

WAGNER, W. H., JR. 1963. A biosystematic survey of United States ferns—preliminary abstract. Am. Fern J. 53:1–16.

WILSON, K. A. 1958. Ontogeny of the sporangium of *Phlebodium (Polypodium) aureum.* Am. J. Bot. 45:483–491.

———— 1959*a*. Sporangia of the fern genera allied with *Polypodium* and *Vittaria.* Contrib. Gray Herb. 185:97–127.

———— 1959*b*. The sporangia of three problematic species of *Polypodium.* Am. Fern J. 49:147–151.

———— 1964. Notes on the Hawaiian fern genus *Adenophorus.* Am. Fern J. 54:68–70.