


1976

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Anatomical Observations on Floating Leaves

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Manuscript received January 20, 1975.

Uploaded to the University of Nebraska-Lincoln institutional repository July 2016.

ABSTRACT

An examination of 24 genera of aquatic plants having floating leaves and leaf-like thalli has been made. Functional stomata occur on the adaxial sides of floating leaves of angiosperms and some ferns, and in some floating leaves there are stomata on the abaxial surface as well. Most floating leaves have prominently chambered mesophyll, and in some instances the chambers are locally enlarged and form buoys.

INTRODUCTION

Floating leaves have evolved independently in many ferns, monocotyledons, and dicotyledons, and even some bryophytes have floating leaf-like thalli. Sometimes floating leaves are produced only during juvenile stages (some *Sagittaria* spp.) or only at flowering time (*Cabomba*), while sometimes they are elicited by inundation of normally emerged plants (*Ranunculus sceleratus* L., *Sparganium eurycarpum* Engelm.). For many plants floating leaves are the main type produced, while for others they share prominence with submersed or emerged leaves.

The evidence from the study of the evolution of vascular plants is that the aquatic taxa have terrestrial ancestors, and that vegetative organs have preceded flowers in adapting to life in the water. The movement into the water is still occurring in some groups and there is some evidence to suggest that the trend is also reversing in some others. Various degrees of adaptation to the water are shown by different aquatic plant taxa and it appears that those with floating leaves are intermediate in many respects between the emerged and permanently submersed taxa.

Plants with floating leaves are found in fresh water throughout the world. Most grow in sunny, still waters but a few can withstand some turbulence. None of the marine angiosperms have leaves adapted specifically for floating.

It has been suggested (Hiern, 1872; Jahn, 1897) that cordate, ovate, or circular shapes are optimal for floating leaves. Other advantageous features are entire margins that resist tearing, surface drainage systems and water-repellent adaxial surfaces that remove water rapidly, upturned margins that prevent flooding, and stomata on the

adaxial side of the leaf. Other adaptations include the ability of petioles of bottom-rooted plants to accommodate fluctuating water levels and the ability of some species to produce aerial leaves above crowded mosaics of floating leaves. These phenomena are discussed at length by Sculthorpe (1967). It is my purpose here to review some of the anatomical features of floating leaves and to present new observations where needed. I have chosen floating leaves and leaf-like thalli from taxa which produce them as regular features of their adult lives, either alone or in combination with submersed or aerial leaves.

MATERIALS AND METHODS

I have collected all specimens studied from the wild, except that leaves of *Aponogeton*, *Hydrocleis*, *Nymphoides*, *Victoria*, and *Nymphaea* hybrids were obtained from cultivated plants.

In most cases samples were removed from each fresh or preserved leaf between the median plane and the margin, but in some instances a section of the entire leaf was removed and embedded in wax and sectioned at 8-25 μ . Entire leaves or leaf segments were cleared in 5% NaOH and stored in lactic acid for darkfield observation; some were stained and stored in xylene as well. Stomatal frequencies were counted on parts of the leaf free from median planes and margins. Data presented are from specimens appearing to represent the average condition of those studied.

OBSERVATIONS

Cryptogamic plants

Ricciaceae. — The liverwort *Ricciocarpus natans* (L.) Corda grows luxuriantly as a floating plant, but it also grows well when stranded on mud. Multicellular ventral scales bearing chloroplasts (Figures 1 and 2) develop fully in the aquatic form and, according to Parihar (1965), continue to grow by a basal meristem. Their functions have been suggested to be primarily maintaining the balance or effecting the absorption of water and nutrients (Parihar, 1965). Most of the thallus is occupied by numerous schizogenously-originated polyhedral chambers separated by walls one cell thick (Figure 2). The walls, including the upper epidermal layer, are rich with chloroplasts, and the upper chambers open to the air by small pores bounded by five or more small cells (cf. Bold, 1967, Figure 15-4). There are several layers of parenchyma toward the ventral side, especially near the median axis.

The structure of terrestrial liverworts, with their air chambers and dorsal pores, seems admirably preadapted to an aquatic existence and it is surprising that there are so few aquatic liverworts. The dorsiventral structure is probably derived in the Hepaticae (Schuster, 1966) and so it is likely that the aquatic liverworts are derived from terrestrial ancestors, particularly in view of Schuster's (1966) theory of terrestrial evolution of the bryophytes as a whole.

Salviniaceae. — *Salvinia molesta* Mitchell has floating and aerial leaves up to 5 cm broad and submerged leaves as much as 30 cm long. When growing vigorously in strong light the leaves are partially folded up from the water surface, but in dimmer light they lie flat on the surface (Figure 6).

The upper and lower epidermis of irregularly shaped cells are held apart by one-cell-thick girders of elongate parenchyma cells (Figure 7). Internally the girders delimit polyhedral chambers. Large multicellular stipitate trichomes are dispersed evenly over the upper epidermis, although those towards the margins are usually somewhat reduced. A function of these trichomes, or egg-beater hairs, is clearly the prevention of wetting. Water falling on the leaf coalesces into beads on the hairs and flows off. Thus the leaf is dorsally almost unwettable. The buoyancy, water-repellency, and stability conferred by the internal architecture and the hairs, as well as the large size and rapid growth, have been factors favoring this plant to become a serious weed in the tropics.

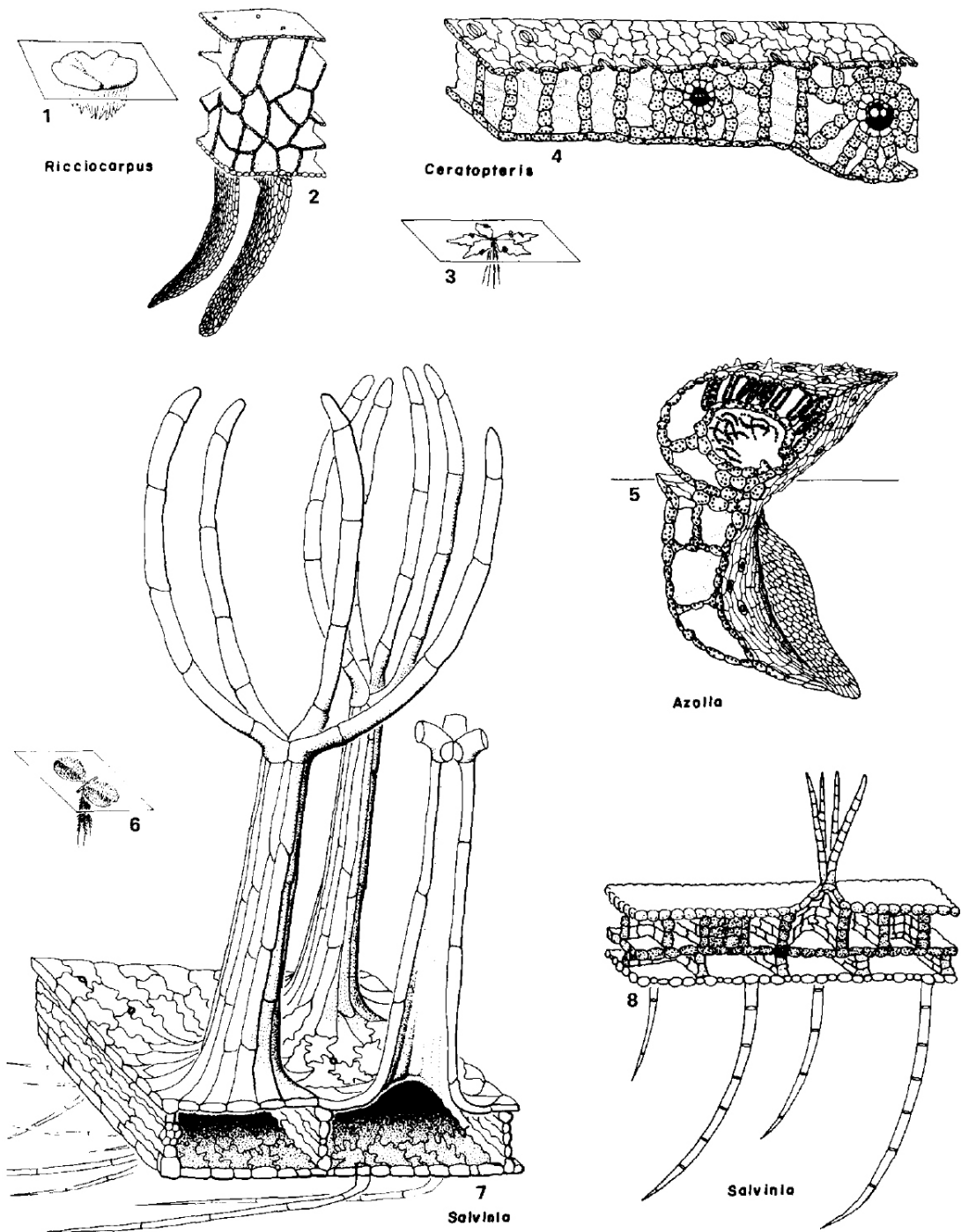
Small stoma-like pores, about $100/\text{mm}^2$, are scattered evenly over the upper epidermis which, like the lower epidermis, the girders, and the trichomes, is rich in chloroplasts. The pores are shown in Figure 7 and can be seen in more detail in the work of Van Cotthem (1970). Their nature as true stomata is doubtful, although each is bordered by a pair of tiny guard-like cells.

The lower epidermis is invested with multicellular achlorophyllous filaments, each terminating in an acicular cell. They might function in absorption (Mayr, 1915) or in stabilizing the plant, but they are wettable in contrast to the upper hairs. There are no pores or stomata in the lower epidermis but there are hydropote-like cells (See Drawert, 1937, Figures 5 and 6).

Salvinia rotundifolia Willd. is smaller than *S. molesta* and differs from it in leaf structure. Instead of a single layer of chambers there are two layers (Figure 8; see also Ogura, 1972, Figure 452). All parenchymatous cells of the leaf except the abaxial hairs are chlorophyllous. The multicellular trichomes on the upper side of the leaf are generally estipitate but provide water-repellency. Toward the margins the hairs are smaller. Multicellular filaments are abundant on the lower epidermis (Figure 8).

Stoma-like pores occur only on the upper epidermis, and Inamdar et al. (1971) found an average of $128/\text{mm}^2$. They are shown in detail by Inamdar et al. (1971, Figure 34) and by Ogura (1972, Figure 121). Rajan et al. (1971) found no response of these pores to light or dark in their experiments and they concluded that there is no defined system of guard cells. According to De la Sota (1964) stomata of *S. sprucei* Kuhn are vestigial.

Azollaceae. — *Azolla mexicana* Presl. is typical of the genus and will serve as an example of its leaf architecture (Figure 5). The leaves are usually interpreted as bilobed, with one lobe aerial and the other submersed, but Ogura (1972) refers to floating and submersed leaves. Both lobes contain large chambers and the largest in



Figures 1-8. Hepaticae and Pteridophyta, habit sketches and leaf segments. Figure 1. *Ricciocarpus natans* ($\times 2.5$). Figure 2. *R. natans* ($\times 10$). Figure 3. *Ceratopteris pteridoides* ($\times 0.12$). Figure 4. *C. pteridoides* ($\times 100$). Figure 5. *Azolla mexicana* ($\times 30$). Figure 6. *Salvinia molesta* ($\times 0.3$). Figure 7. *S. molesta* ($\times 110$). Figure 8. *Salvinia natans* ($\times 120$).

the upper lobe contains a colony of *Anabaena azollae* Strasb. and opens to the outside by a large pore. The upper lobe also has a palisade layer of columnar cells rich with chloroplasts, making it darker green than the adjacent epidermal and mesophyll cells, which also contain chloroplasts.

Stomata occur on both sides of both lobes. Inamdar et al. (1971) found 112 stomata/mm² in *A. pinnata* R.Br. There are prominent papillae on the upper side of the upper lobe (Figure 5; see also Ogura, 1972, Figure 453) which probably help keep the leaf dry, as does the imbrication of the aerial leaves.

Parkeriaceae. — *Ceratopteris pteridoides* (Hook.) Hieron. produces mostly floating sterile fronds and upright fertile fronds when growing rooted or floating freely in shallow water. It survives also completely submersed or when stranded on mud. The pinnately-lobed floating leaf is one of the few of that shape to be found in plants with floating leaves. Adventitious vegetative buds arise in the axils of the lobes and young plants remain attached until they are quite large (Figure 3).

Upper and lower epidermis are separated by an undifferentiated mesophyll of thin parenchymatous girders delimiting large chambers (Figure 4). Several layers of parenchyma cells sheath the vascular bundles. All of these tissues are chlorophyllous.

The stomata on the upper surface average 122/mm² and there are scattered stomata on the lower surface as well. The sterile fronds of *C. thalictroides* (L.) Brongn. were reported by Pal and Pal (1962) to have 52 and 64 stomata/mm² on their upper and lower surfaces respectively; these data may have been from aerial leaves. Details of stomatal structure are shown by Ford (1902) and Pal and Pal (1962) for *C. thalictroides*; those of *C. pteridoides* are similar.

Epidermal cells on both sides are similar in shape and size but Drawert (1937) showed by differential staining the presence of scattered hydropote cells, mostly on the lower surface.

Dicotyledons

Nymphaeaceae. — *Nymphaea* has the most species with floating leaves in the dicotyledons. The leaves range in size from 3 cm in diameter in *N. tetragona* Georgi and the so-called pygmy species, to 60 cm or more in some tropical species. The leaves of most species are orbicular but they are oval in *N. caerulea* Savigny, *N. elegans* Hook., *N. pubescens* Willd., and *N. tetragona* (Conard, 1905). Smaller leaves are entire-margined but the larger leaves of some tropical species have crenate or dentate margins. The floating leaves of most species lie flat on the surface but in a few species with very large leaves the margins are turned up as much as several centimeters. In general, the larger the leaf the more prominent are the ribs on the underside, a trend culminating in this family in the large ribs of the huge leaves of *Euryale* and *Victoria* (Figures 15-17). The ribs contain not only the major vascular

bundles but also extensive air canals. Their architecture provides strength, elasticity, and buoyancy. The leaves are somewhat brittle despite their leathery texture, and the networks of ribs associated with the major veins are factors in keeping them from breaking apart in agitated water. The leaf sclereids may also function in this way. Many species will produce aerial leaves when crowded or when stranded by lowering of the water level.

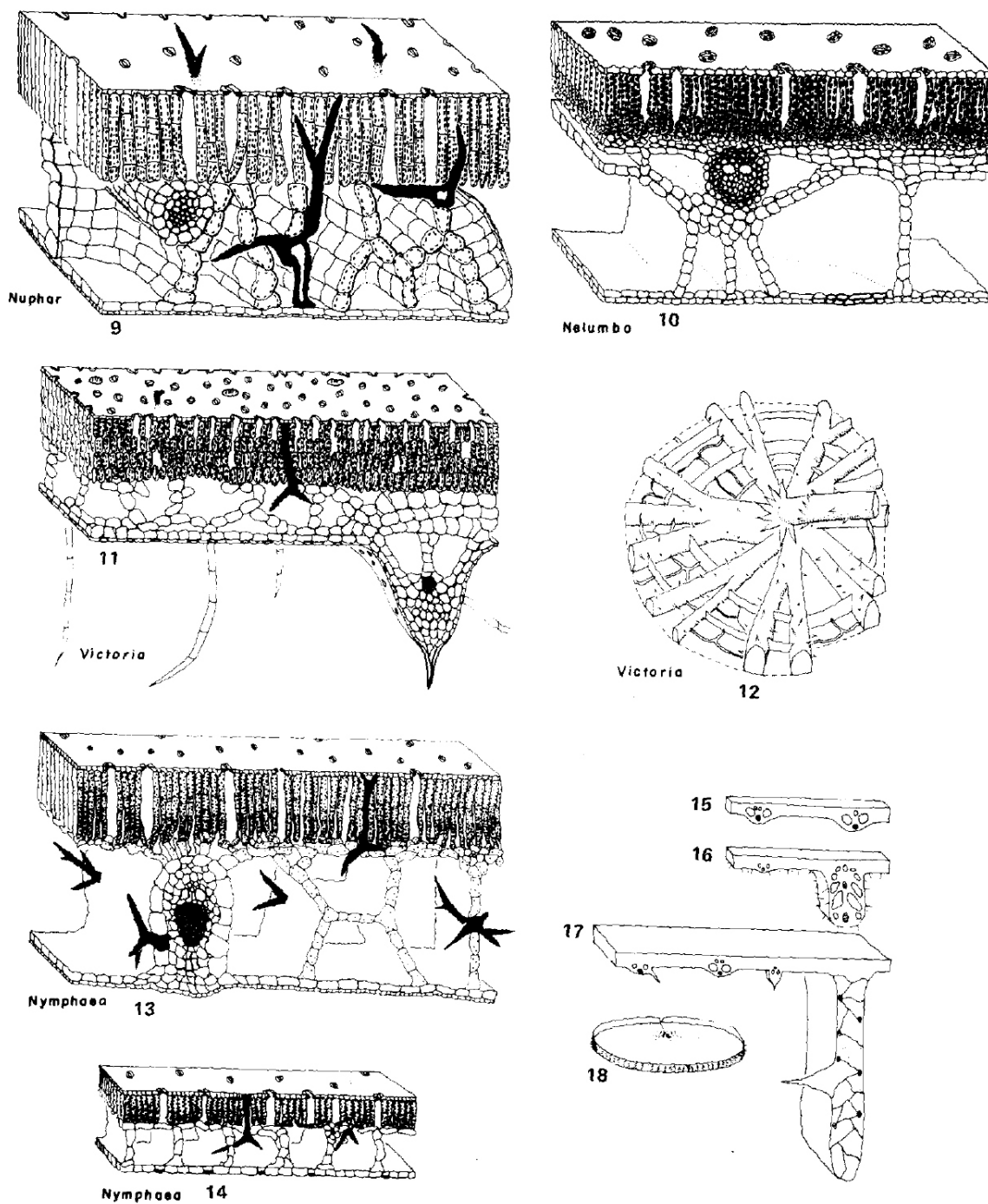
Internally the various species show much uniformity of structure. In the smaller leaves, as in dwarf *N. × daubeniana* hort. (Figure 14), the palisade layer occupies about the same proportion of the thickness of the leaf as it does in larger leaves such as those of *N. tuberosa* Paine (Figure 13). Conard (1905) noted constancy in size of palisade cells regardless of size of leaf. The larger leaves are thicker and consequently the chambers are not only larger but also in some places there are several layers of them (Figures 13 and 14). The stomata on the upper epidermis vary greatly in density. In *N. tuberosa* I found an average of 196/mm² away from the margins and the sinus; in *N. cultivar* August Koch the average is 276/mm²; while in *N. cultivar* Missouri and *N. × daubeniana* the averages are 784 and 625/mm² respectively.

In thinner leaves (Figure 14) the lower epidermis is the only abaxial layer, but in thicker leaves (Figure 13) there is a hypodermis as well. Scattered cells in the lower epidermis may function as hydropotes; they are shown in detail by Gruss (1927). Basal cells from dehiscent mucilaginous hairs can be found also in the lower epidermis of mature leaves. According to Conard (1905), citing various authors, different interpretations exist of the functions of the hairs and the mucilage. They have been suggested to provide protection from animals, to prevent contact of young leaves in the bud, and to prevent water from penetrating embryonic tissues.

Of the four taxa of *Nymphaea* that I have examined only *N. cultivar* Missouri, of the night-flowering group, lacks sclereids in its leaves. The sclereids are variable in shape but always arise as idioblasts from cells adjacent to the chambers. Their arms often reach to the upper and lower epidermis but they do not penetrate it. Sclereids do not occur below the stomata. Their function as supporting structures or as repositories for waste products is not clear, nor is their adaptive value, if any, to the floating habit apparent. Their ontogeny and distribution have been discussed by Conard (1905), Gaudet (1960), and Malaviya (1962). Similar sclereids occur in the floating leaves of other Nymphaeaceae and in *Nymphoides* (Gentianaceae s. l.).

The peltate leaves of *Victoria* are up to 2 m in diameter and are among the largest simple leaves of any plant, aquatic or terrestrial. The margins are turned up as much as 8 cm all around the leaf, except that there are two opposite sinuses in the median plane. These allow water to drain from the surface, while the system of depressions over the major vascular bundles channels the water to them (Figure 18).

The underside of the leaf has a spectacular system of spine-covered girders radiating from the petiolar attachment (Figure 12; Fitch and Hooker, 1851; Anderson, 1965). These girders are presaged by the smaller ones in some species of *Nymphaea* (Figures 15 and 16) but in *Victoria* they are much larger. Systems of smaller, thinner



Figures 9-18. Dicotyledons: Nymphaeaceae. Figure 9. *Nuphar variegatum* ($\times 115$). Figure 10. *Nelumbo lutea* ($\times 180$). Figure 11. *Victoria* sp. ($\times 15$). Figure 12. *Victoria* sp. ($\times 0.2$). Figure 13. *Nymphaea tuberosa* ($\times 55$). Figure 14. *Nymphaea daubeniana* ($\times 55$). Figures 15 and 16, *Nymphaea* spp. ($\times 1.5$). Figure 17. *Victoria* sp. ($\times 0.4$). Figure 18. *Victoria* sp. ($\times 0.03$).

girders cross the large ones at right angles, dividing the leaf into approximately equal segments. The girders are not rigid but instead are flexible enough to give elastic support. Internally they are divided by parenchymatous walls into irregular chambers. Numerous small bundles pass through these walls. Larger spines have large chambers in them too, but smaller spines are solid. The spines are sharp and rigid and may serve as protection from herbivores, as suggested by Blake (1887). Masses of multicellular hairs cover the lower epidermis.

The lamina is surprisingly thin for so large a leaf. The palisade layer occupies about half of the laminal thickness, and below it is a series of irregular chambers separated by parenchymatous walls (Figure 11). Scattered sclereids arise adjacent to the chambers, and some of them grow up through the palisade layer to the upper epidermis.

Stomata on the upper surface average about 450/mm² in the *Victoria* hybrid which I have examined, and hydropote-like cells on the lower epidermis average 190/mm².

The lamina is perforated by cylindric pores, the stomatodes, which are about 0.5 mm in diameter and are spaced 3-6 mm apart. They do not occur over veins or girders. They function as drains for rainwater (Unger, 1854; Gessner, 1950, 1969). They develop by necrosis of maturing leaf tissue, and they do not occur on the upturned margin of the leaf or in the region of petiolar attachment (Gessner, 1969).

Nuphar variegatum Engelm., like other members of the genus, produces submersed, floating, and aerial leaves. Floating leaves are produced under optimal conditions while aerial leaves develop with crowding; submersed leaves form in spring and fall when water temperatures are low.

Stomata average 224/mm² on the upper surface and there are none on the lower surface. However, hydropotes occur in the lower epidermis at 140/mm², and there are widely scattered hairs there too. These structures are shown in detail by Grilss (1927).

Large branched sclereids originate in the spongy layer and ramify throughout the leaf, reaching but not penetrating the upper and lower epidermis (Figure 9). The spongy layer forms irregular chambers, some of which are continuous with stomatal chambers above. Smaller vascular bundles run just below the palisade layer and larger ones occupy the entire space between the palisade layer and the lower epidermis.

The large peltate leaves of *Nelumbo lutea* (Willd.) Pers. are often held well above the water but floating leaves are usually present. The upper surface of the leaf is remarkably water-repellent, as might be expected from the waxy appearance. Closer examination shows that the epidermal cells are papillate (Figure 10) and that the cuticle is thicker over the papillae than it is between them. These features are absent from the lower epidermis.

Stomata occur only on the upper surfaces in both floating and aerial leaves. In floating leaves they average 180/mm². Occasional groups of cells on the lower surface (Figure 10) may be hydropotes, although Kristen (quoted in Napp-Zinn, 1973) believes them to be unlike those of other Nymphaeales.

Thin parenchymatous walls separate large and irregular chambers in the mesophyll. The chambers occupy about two-thirds of the thickness of the leaf and are bordered basally by a hypodermis.

The presence of stomata only on the upper surfaces of both floating and aerial leaves suggests that the latter are derived from the former (Conard, 1905). *Nelumbo* is capable of living and flowering for years when stranded on mud, where it produces only aerial leaves. Thus it appears to be in the early stages of adaptation to a more emersed or even to a terrestrial existence. Similar transitional features occur in some species of *Nuphar* and *Nymphaea*, too.

Brasenia schreberi Gmel. has oval peltate floating leaves up to 12 cm long. The upper epidermis occupies about one-third of the thickness of the leaf (Figure 21) and consists of large columnar cells with irregular walls. The irregularities of one cell are complemented by those of adjacent cells so there are no intercellular spaces. Numerous mucilage droplets occur in them. Stomata average 270/mm² in the upper epidermis; they are absent from the petiolar attachment region. Schrenk (1888) noted an average of 325/mm².

The palisade layer is less remarkable, being 2-4 layers of typical cells (Figure 21). The stomatal cavities are unusually prominent because of the great combined depth of the epidermis and palisade layers. The spongy layer is formed of irregularly arranged branched cells often covered with calcium oxalate. There are sizable intercellular spaces but no defined chambers.

The cells of the lower epidermis are quite normal when compared with those of the upper epidermis. Occasional hydropote-like cells occur. Numerous hairs project downward from the lower epidermis; Schrenk (1888) counted up to 560/mm². They consist of a single large cell on a stalk of two disc-shaped basal cells attached to an epidermal cell (Figure 21). They secrete copious mucilage that makes a thick coating on the underside of the leaf, the petiole, and the stem. The accumulating mucilage from each hair bulges the cuticle, which eventually breaks. Schrenk (1888) suggested the function of the mucilage to be protection from herbivores and from being covered by algae. The mucilage also adds greatly to the strength of the organs. Of the floating leaves I have observed those of *Brasenia* are most often heavily infested with insects. However, the insects appear to gain entrance through the upper surface, which is the only area not protected by mucilage.

Cabomba is the closest relative of *Brasenia* (Li, 1955) and the comparative structure of their floating leaves shows both similarities and differences. *Cabomba* is primarily adapted for the submersed habit and it produces floating leaves only at flowering time. The submerged leaves are highly dissected, like those of so many aquatic dicotyledons, while a raft of small (<1 cm) floating leaves buoys the emersed flowers (Figure 20).

The upper epidermis of *C. caroliniana* Gray consists of polygonal cells arranged stellately around the stomata (Figure 19), which average about 200/mm². The palisade layer is a single row of slightly columnar cells occupying one-third of the

thickness of the leaf. There is a weakly defined spongy layer. The lower epidermis is thickly invested with hairs similar to those of *Brasenia*: two small disc-shaped basal cells support a large attenuate apical cell. As in *Brasenia*, these hairs produce mucilage which forms a thick temporary coating on the underside of the leaves and on the petioles and young stems. Hydropote cells have been observed in *C. australis* Spegazz. (Napp-Zinn, 1973).

Polygonaceae. — The floating leaves of *Polygonum coccineum* Muhl. form a raft which holds the spike upright (Figure 23). There are terrestrial and semi-aquatic forms of the species.

The rather large stomata show a relatively low frequency of 66/mm² on the adaxial surface, and among them are curious scutiform hairs of four cells, each attached to the basal cell. The two-layered palisade is interrupted by large stomatal chambers and its cells are loosely arranged. The spongy layer is more open than it is in aerial leaves, but adjacent to the midrib there are larger chambers separated by walls one cell thick (Figure 22). Extra buoyancy is provided by this enlarged float. In contrast to buoys of *Trapa*, *Hydrocleis*, and others, those of *Polygonum* are occupied mostly by vascular bundles rather than by chambers.

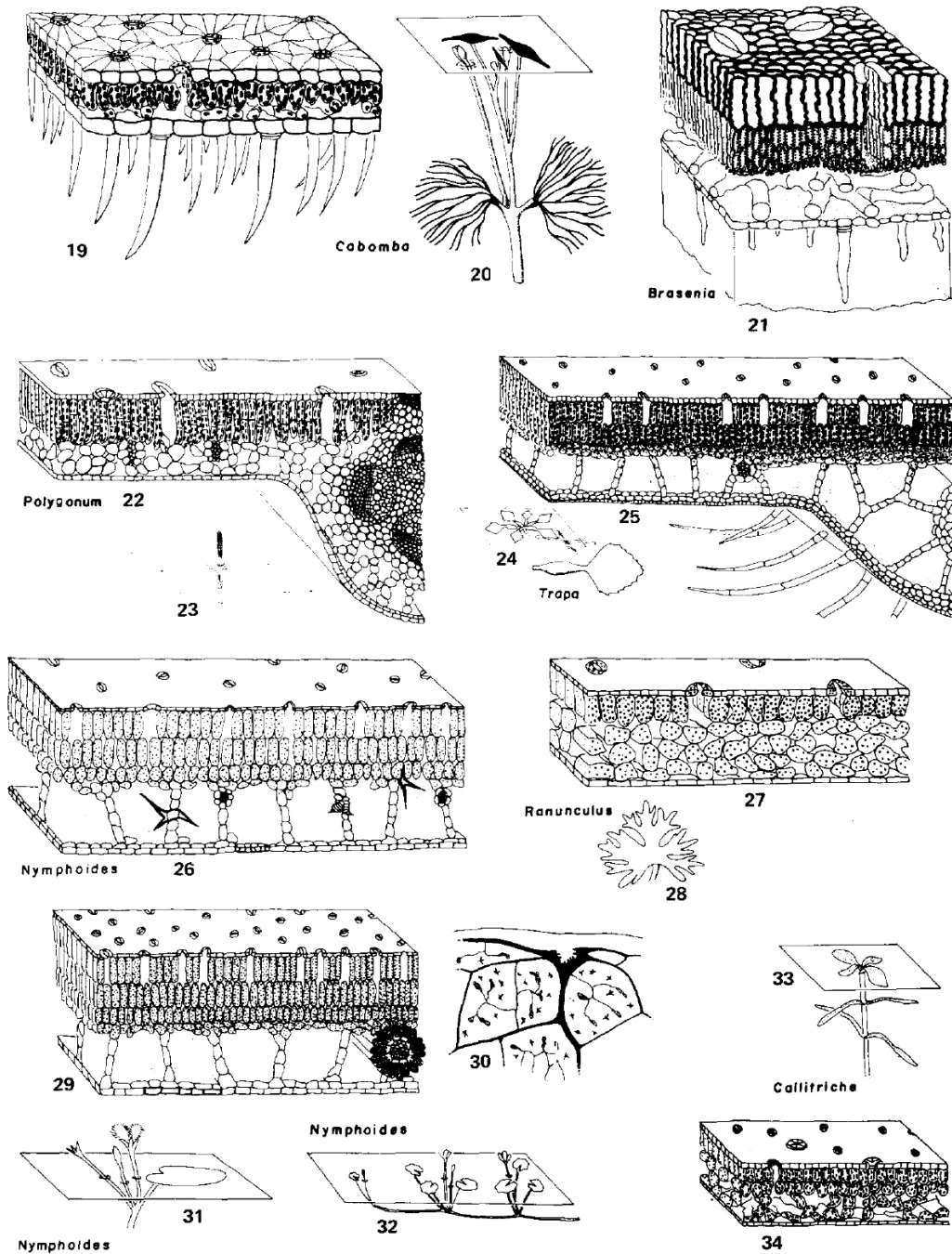
Trapaceae. — The floating rosettes of rhomboid leaves of *Trapa natans* L. make geometric patterns on the surface. They are buoyed by the prominent swollen petioles of the floating leaves (Figure 24) and by the chambers in the blades, particularly by the large chambers in the inflated median plane (Figure 25).

A thick palisade layer of two rows of heavily chlorophyllous columnar cells is penetrated by prominent stomatal chambers. Beneath it is a single layer of polyhedral chambers separated from each other by walls one cell thick. Minor vascular bundles occur just below the palisade layer (Figure 25) and major bundles occupy the full depth of the spongy layer, except that the median bundle is surrounded by prominent chambers. Numerous multicellular hairs occur on the abaxial side, especially along the swollen axis.

Callitrichaceae. — Unlike many dicotyledons with both floating and submersed leaves, the two kinds of leaves of *Callitriche palustris* L. are entire (Figure 33). The floating leaves are spatulate while the submersed ones are linear.

Stranded individuals produce all spatulate leaves (Fassett, 1957). The rosette of floating leaves is not always present, but its occurrence correlates with flowering, even though the flowers are not all produced in the leaf axils of the rosette.

The anatomy of the floating leaf does not suggest the floating habit except for the presence of adaxial stomata (Figure 34). Peltate hairs are scattered among the stomata. The palisade and spongy layers are weakly differentiated and there are no sizable chambers or specialized inflated areas. The internal architecture is so similar to that of the submersed leaves, and so unlike that of most floating leaves,



Figures 19-34. Dicotyledons. Figure 19. *Cabomba caroliniana* ($\times 75$). Figure 20. *C. caroliniana* ($\times 1$). Figure 21. *Brasenia schreberi* ($\times 80$). Figure 22. *Polygonum coccineum* ($\times 130$). Figure 23. *P. coccineum* ($\times 0.2$). Figure 24. *Trapa natans* ($\times 0.08$), 0.3. Figure 25. *T. natans* ($\times 150$). Figure 26. *Nymphoides indica* ($\times 120$). Figure 27. *Ranunculus flabellaris* ($\times 130$). Figure 28. *R. flabellaris* ($\times 0.5$). Figure 29. *Nymphoides peltata* ($\times 110$). Figure 30. *N. peltata* ($\times 80$). Figure 31. *N. indica* ($\times 0.3$). Figure 32. *N. peltata* ($\times 0.1$). Figure 33. *Callitriche palustris* ($\times 1$). Figure 34. *C. palustris* ($\times 60$).

as to support the conclusion that the floating leaves are recently derived from the submersed leaves.

Ranunculaceae. — Like many species of the genus, *Ranunculus flabellaris* Raf. is variable in growth habit and leaf shape. The submersed leaves are highly dissected but the floating leaves are less so (Figure 28). They are notable in their departure from the rounded shapes so typical of most floating leaves, including those of other *Ranunculus* species.

The anatomy of the floating leaf of *R. flabellaris* does not suggest its habit. There is a single layer of weakly developed palisade cells and a spongy layer of randomly arranged cells with large intercellular spaces (Figure 27). Stomata in the upper epidermis average 72/mm²; there are none in the lower epidermis. In *R. sceleratus*, an emersed species producing floating leaves only when in deeper water, Arber (1920) found stomata on both sides of both the aerial and the floating leaves, but they were fewer on the lower surface of the floating leaves. Their absence from the lower surface of the floating leaves of *R. flabellaris* indicates a stronger adaptation to the floating habit than *R. sceleratus*. However, the similarity of shapes of the submersed and the floating leaves of *R. flabellaris*, as well as the poorly differentiated internal architecture of the floating leaves, suggest that the two kinds are phylogenetically more closely related than is the case in most aquatics with floating leaves. A precedent for this interpretation is shown by the experimental work of Cook (1969) on *R. aquatilis* L.

Gentianaceae. — The floating leaves of *Nymphoides (Limnanthemum) indica* (L.) O. Kuntze, *N. aquatica* (Walt.) O. Kuntze, and *N. cordata* (Ell.) Fern. form buoys for the inflorescences which join the leaves just below the surface (Figure 31). If the leaves are cut off the flowers sometimes cannot emerge into the air.

The leaf of *N. indica*, like so many other floating leaves, is adaxially unwettable. Stomata occur in the upper epidermis at about 170/mm². The palisade layer is very dark green and consists of two layers of columnar cells above a layer of shorter cells (Figure 26). The spongy layer consists of partitions delimiting chambers of approximately equal size. Idioblastic sclereids of many shapes form in the partitions and in the palisade layer adjacent to the chambers, in the manner of *Nymphaea*. Their development in *N. cristata* (Roxb.) O. Kuntze has been studied by Malaviya (1963). Terminal idioblasts occur in the partitions at the veinlet endings (Figure 30). Many of the major veins empty into prominent hydathodes spaced every 5 mm on the leaf periphery (Figure 30). Groups of hydropote cells occur at about 8/mm² in the lower epidermis (Figure 26).

Nymphoides peltata (Gmel.) O. Kuntze spreads across the water by long stolonous internodes, but it does not root at the nodes as do *N. indica* and its relatives. Two or more leaves produced at the crowded nodes subtend the flowers (Figure 32), and thus the entire plant apart from the rooted base is supported by the floating leaves.

The leaf is nearly round but has an irregular angular margin interrupted by a single sinus. A transverse section (Figure 29) reveals an extraordinarily dark palisade layer of two rows of columnar cells above shorter cells. The stomatal chambers, 256/mm², do not penetrate the palisade layer entirely. As in *N. indica*, the partitions of the spongy layer block off chambers of about equal size. Rather evenly spaced groups of hydropote cells occur in the lower epidermis and its adjacent hypodermis (Figure 29) but there are no stomata there. Hydathodes like those of *N. indica* (Figure 30) are dispersed along the margin but there are no sclereids or terminal idioblasts.

Monocotyledons

Butomaceae. — *Hydrocleis nymphoides* (H. & B. ex Willd.) Buchenau, the water poppy, spreads through still tropical and sub-tropical waters by producing long internodes (Figure 43). The leaves from the crowded nodes form a raft supporting the colony, and roots from the nodes anchor it in shallow water.

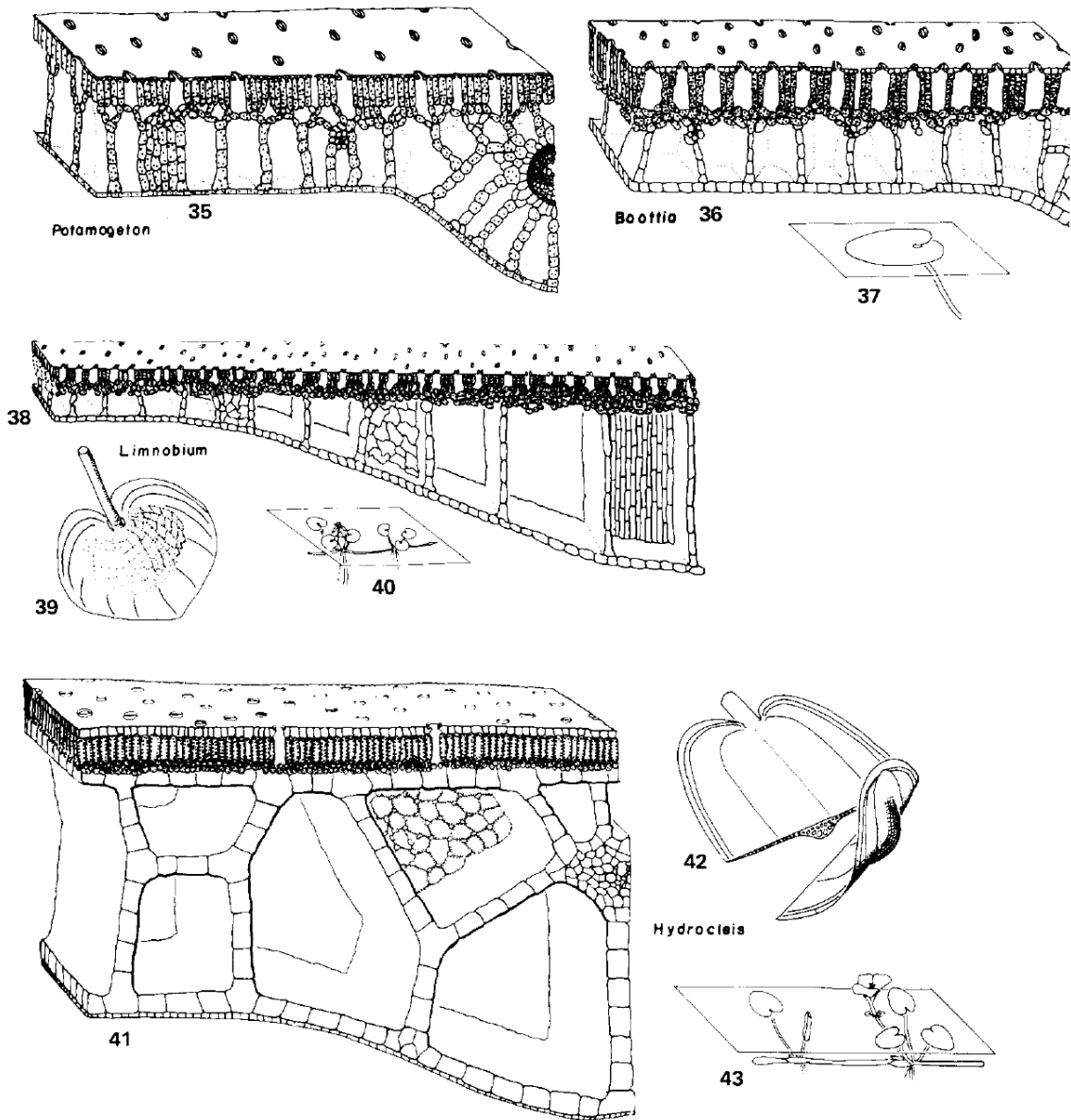
The leaves are characterized by a swollen area, the buoy, parallel to the median bundles (Figure 42). It is made of large chambers in the spongy layer (Figure 41) which are crossed here and there by diaphragms.

Stomata in the upper epidermis average 82/mm². The palisade consists of a single layer of columnar cells above small, spherical, loosely arranged cells (Figure 41). There are a few stomata in the lower epidermis, as well as hydropote cells (Stant, 1967, Figure 20).

Hydrocharitaceae. — One of the few genera of Hydrocharitaceae with floating leaves is *Boottia* (*Ottelia*). This genus has primitive flowers for the family (Kaul, 1968) and, since most genera in the family have strictly submersed foliage, the *Boottia* genus is vegetatively among the most primitive too. *Boottia cordata* Wall. produces only floating leaves after the linear submersed juvenile leaves disappear. They are rather thick and water-repellent. Their shape is ovate, with entire margins and overlapping basal lobes (Figure 37).

Stomata on the upper surface average 90/mm², and there are occasional stomata on the lower side too. The stomatal chambers in the palisade layer are large (Figure 36) and may function in allowing light to penetrate the thick leaf. More than half the leaf's thickness is occupied by irregular chambers separated by parenchymatous partitions. Smaller vascular bundles run through the partitions just below the palisade layer, while larger ones occupy the entire distance between the palisade layer and the lower epidermis. There are no hydropote cells in the lower epidermis.

Limnobium spongia (Bosc.) Steud. is often found in shallow water but it grows well on mud too. It often produces aerial as well as floating leaves. The two differ most prominently in the presence of an expanded aerenchymatous buoy on the lower side of the floating leaf (Figure 39). These buoy not only individual leaves but also,



Figures 35-43. Broad-leaved monocotyledons. Figure 35. *Potamogeton nodosus* ($\times 28$). Figure 36. *Boottia eordata* ($\times 45$). Figure 37. *B. eordata* ($\times 0.3$). Figure 38. *Limnobium spongia* ($\times 15$). Figure 39. *L. spongia* ($\times 0.8$). Figure 40. *L. spongia* ($\times 0.08$). Figure 41. *Hydrocleis nymphoides* ($\times 45$). Figure 42. *H. nymphoides* ($\times 1.2$). Figure 43. *H. nymphoides* ($\times 0.1$).

collectively, the entire colony, which can spread for considerable distances across the water by long stolonetic internodes (Figure 40).

The transverse section of a floating leaf (Figure 38) shows that the float is simply an enlarged part of the spongy layer in which not only the chambers but the cells

of the partitions separating them are vertically elongate. The prominent cross-walls between chambers are shown in cellular detail in Figure 38. There are no diaphragms. Small stomata in the upper epidermis average $82/\text{mm}^2$. Each opens into a prominent cavity in the palisade layer. There are no stomata or hydropote cells in the lower epidermis.

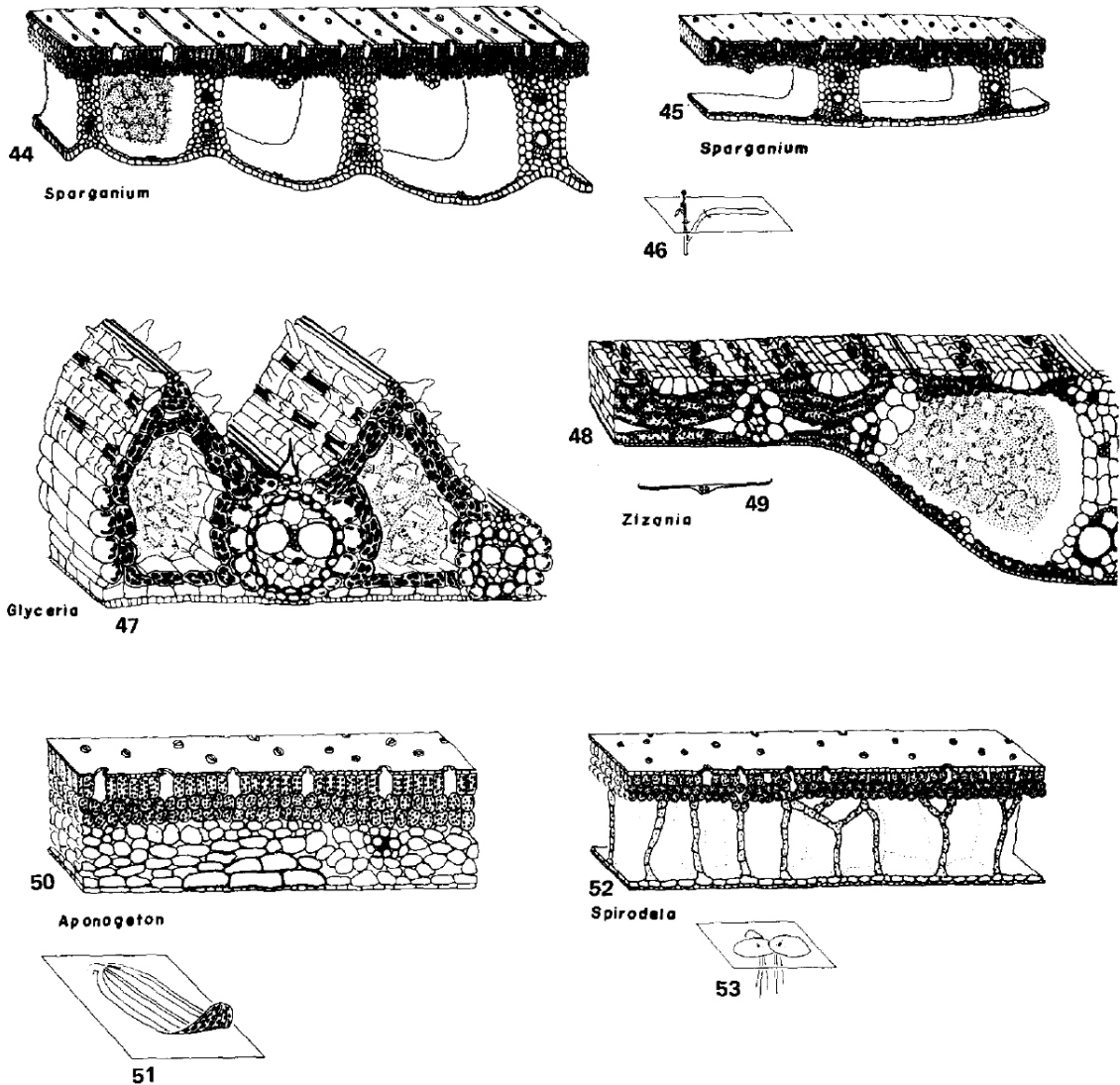
Potamogetonaceae. — Many species of *Potamogeton* produce floating as well as submersed leaves. The floating leaves invariably have entire margins and are ellipsoidal, ovate, or otherwise elongate, but never round. The structure of the floating leaf of *P. nodosus* Poiret is typical of the genus, with allowance for size difference in other species. Stomata on the upper surface average $120/\text{mm}^2$ and they open into rather large chambers in the palisade layer (Figure 35). The spongy layer is divided into irregular but approximately equal sized compartments by walls one cell thick. Along the median axis, around the vascular bundle, the chambers are somewhat enlarged and form a buoy. There are no stomata in the lower epidermis.

Lemnaceae. — The lemnaceous frond is variously interpreted as entirely shoot, entirely leaf, or a combination of the two. Whatever its nature it is distinctly leaf-like in anatomy and function. The frond of *Spirodela polyrrhiza* (L.) Schleid. (Figure 53) will serve as an example of the anatomy of floating fronds in the family, detailed illustrations of which are given by Hegelmaier (1868). Stomata occur only in the upper epidermis (Figure 52) and average $170/\text{mm}^2$. The palisade layer is densely packed with chloroplasts, and the one-cell-thick walls separating the chambers in the spongy layer have a few chloroplasts as well. There is a hypodermal layer adjacent to the lower epidermis.

Aponogetonaceae. — Most species of *Aponogeton* produce only submersed leaves but the South African *A. distachyos* L. fil. produces only floating leaves when mature. They are elliptical and rather leathery, but they lack obvious strengthening tissues. Arber (1920), quoting Sergueeff, notes that the leaves are heliotropic, growing flat on the surface in bright light, but growing upright in darker places.

In transverse section the leaf belies its floating habit except for the existence of stomata on the upper surface, which average $120/\text{mm}^2$, and for the median swollen area with prominent chambers bridged by diaphragms. The palisade layer occupies nearly half the thickness of the leaf (Figure 50) and consists of a single columnar layer over two layers of spherical cells. The spongy layer is rather densely packed and the intercellular spaces are smaller than in leaves of many land plants. The lower surface is densely and obviously covered with large patches of hydropote cells (Figure 51). They are shown in detail by Drawert (1937).

Sparganiaceae. — I have suggested (Kaul, 1972) that floating-leaved species of *Sparganium* are neotenous forms derived from emersed types. This is supported by the observation that the floating seedling leaves of emergent species are anatomically



Figures 44-53. Narrow-leaved monocotyledons and Lemnaceae. Figure 44. *Sparganium fluctuans* ($\times 60$). Figure 45. *S. minimum* ($\times 60$). Figure 46. *S. minimum* ($\times 0.3$). Figure 47. *Glyceria borealis* ($\times 300$). Figure 48. *Zizania aquatica* ($\times 100$). Figure 49. *Z. aquatica* ($\times 1$). Figure 50. *Aponogeton distachyos* ($\times 100$). Figure 51. *Aponogeton distachyos* ($\times 100$). Figure 52. *Spirodela* ($\times 100$). Figure 53. *Spirodela* ($\times 100$).

similar to the floating leaves of adult specimens of *S. fluctuans* (Morong) Robinson and *S. minimum* Wallr. among others. In adult leaves of emerged species there is a dense chlorophyllous layer on both sides, while in floating-leaved species only the upper side has one. Emerged leaves are more fibrous at the margins and are subdivided into more layers of compartments than are floating leaves. Where emerged leaves have large numbers of stomata on both sides, floating leaves have them abundantly on the upper side (*S. fluctuans*, $250/\text{mm}^2$; *S. minimum*, $110/\text{mm}^2$), with only abortive stomata widely spaced on the lower side.

In both *S. fluctuans* and *S. minimum* one or more floating leaves form a raft supporting the upright inflorescence above the water surface (Figure 46). Most of the floating leaves, however, do not function this way. The leaves of *S. fluctuans* (Figure 44) are much larger and thicker than those of *S. minimum* (Figure 45), but the basic architecture is the same. Most of the leaf is composed of a single layer of compartments separated from each other by multicellular walls carrying vascular bundles. Small bundles also occur over the chambers in the palisade layer. The median compartments are crossed at regular intervals by diaphragms of two kinds: vascular and non-vascular. I have shown these and their distributions elsewhere (Kaul, 1972, 1973).

The linear floating leaves of *Sparganium* are better adapted to life in running water than those of most aquatic plants. I have observed masses of floating leaves on *S. angustifolium* Michx. in rapid brooks, and floating leaves on *S. eurycarpum* in deep running water. Floating-leaved aquatic grasses can occupy similar sites.

Gramineae. — When *Glyceria borealis* (Nash) Batch. grows in water it produces floating and aerial leaves. The two are anatomically almost indistinguishable. The stomata are in rows on the sides of the steep-walled parallel ridges (Figure 47). The ridges are hollow and are bridged at regular intervals by diaphragms or large stellate cells with prominent intercellular spaces. The upper epidermal cells are papillate but those in the lower epidermis are not.

Zizania aquatica L., wild rice, also produces floating and aerial leaves. In the transverse view of the floating leaf (Figures 48 and 49) the large median chambers, and the diaphragms that bridge them, are the two most obvious indicators of adaptation to water. The chambers run the entire length of the floating part of the blade. The first leaves produced after germination are submersed and have chambers along their entire length between all bundles. They have no stomata (Weir and Dale, 1960). In floating leaves Weir and Dale (1960) reported stomata only on the upper side, but in my material they occur on both sides. Air trapped between the hairs and papillae probably aids in preventing wetting of the upper side.

In grasses diaphragms occur only in aquatic taxa, e.g. *Glyceria*, *Zizania*, and *Oryza*, all of which can produce floating leaves, as well as in the terrestrial genus *Dactylis*.

CONCLUSIONS

Some features of aerial leaves are also adaptive for the floating habit and have been retained: the dorsiventral structure, the adaxial palisade layers, the water-repellent adaxial surface. The most consistent anatomical innovations have been the development of functional stomata on the adaxial surface and, in most taxa, the formation of the chambered mesophyll. These innovations have occurred independently in the transition to the floating-leaved condition by distantly related taxa. In some groups, e.g. Gramineae and Sparganiaceae, these characteristics were present in the aerial leaves of the forms ancestral to the floating-leaved species.

The densities of stomata on the adaxial sides of floating leaves vary greatly, just as they do on the abaxial sides of aerial leaves. It is likely that the frequency of stomatal opening is at least as significant as stomatal density. It is known that stomatal frequency and position are greatly influenced by leaf development above, on, or in the water in *Polygonum* (Massart, 1902), *Potamogeton* (Shinobu, 1952), and *Ranunculus* (Sculthorpe, 1967). The presence of some stomata, whether functional or not, on the lower surface of some floating leaves may be viewed as an intermediate condition in the transition from the aerial habit, while the complete absence of stomata in most submersed leaves, at least some of which have evolved from floating leaves, is then the final product of this evolutionary sequence. Floating leaves which have been derived from submersed leaves are likely to have no stomata at all on their abaxial sides, but the evidence for this derivation is at present meager.

The chambered mesophyll is most often generalized across the leaf, but in some instances it is confined to, or emphasized in, the median plane. Its function in buoying individual leaves and in some instances entire plants is clear. In some cases the chambered mesophyll is elicited only when the leaves develop as floating leaves, e.g. *Limnobiium*. The absence of chambers in the floating leaves of *Callitriche palustris* and *Ranunculus flabellaris* can be interpreted as evidence that these leaves are derived from submersed leaves which, in these taxa, also lack prominent chambers.

Many floating leaves have individual or grouped hydropote cells where they contact the water. Little is known of hydropotes and their functions and structure as assimilatory structures need further investigation. The literature on this subject has recently been reviewed by Napp-Zinn (1973). It is likely that hydropotes have had different origins and functions in different taxa, and for the present they must be classified only by structure. Their occurrence in so many unrelated taxa is additional evidence of the convergent evolutionary tendencies found in aquatic plants.

A trend toward the development of aerial from floating leaves appears to be occurring in some Nymphaeaceae. The evidence is best in *Nelumbo*, where the aerial leaves have only adaxial stomata, as do the floating leaves. In *Nuphar*, where some species regularly produce aerial leaves, the evidence is less obvious but it does suggest adaptation to a more terrestrial existence. *Nuphar* and *Nymphaea* need further investigation to determine such trends.

Another phenomenon needing further investigation is the apparent trend toward the development of floating from submersed leaves in *Potamogeton*, *Callitriche*, and *Ranunculus*. The study of as many species as possible should provide instructive information about evolutionary trends in these genera. The genus *Ranunculus*, and especially its Section *Batrachium*, with its plastic heterophylly and its variety of submersed, floating, and emerged leaves, is likely to be of particular interest.

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