AZOLLACEAE

(R.M.K. Saunders, Hong Kong)

Azollaceae Wettst., Handb. Syst. Bot. 2 (1903) 77; C. Chr. in Verdoorn, Man. Pterid. (1938) 550; Wettst., Trat. Bot. Sist. (1944) 416; Reed, Bol. Soc. Brot. II, 28 (1954) 15.

Small aquatic plants with flabellate branched stems bearing roots and leaves. *Leaves* sessile, alternate, imbricate, bilobed. *Dorsal leaf lobe* fleshy and chlorophyllous, held above water surface; with uni- or bicellular trichomes, and anomocytic stomata; with cavity containing mucilage and filaments of the cyanobacterium *Anabaena azollae*. *Ventral leaf lobe* generally unistratose and translucent (except at base), resting on water surface. *Roots* either solitary or in fascicles, growing from stem branching points; with numerous root hairs and two semi-persistent root caps and a basal root sheath. *Sporocarps* borne in pairs or fours at base of branches, initially covered by involucre of dorsal leaf lobe. Plants monoecious, with separate mega- and microsporocarps. *Megasporocarp* containing a solitary indehiscent megasporangium, which contains a single megaspore attached by placenta. Megaspore with trilete mark, and 3 or 9 proximally positioned alveolate 'floats' (up to 24 in fossil taxa) attached by filosum of megasporoderm. Megasporoderm sculpturing and stratification variable and often highly complex. Megagametophyte endosporic, forcing megaspore open at laesura, bearing several archegonia. *Microsporocarp* containing numerous indehiscent microsporangia that develop successively from apex to base; each microsporangium with c. 64 trilete microspores. Microspores aggregated together in alveolate structures ('massulae'), analogous to floats. *Massulae* partially or fully covered with simple or glochidiate trichomes. Microspores germinating within massula; microgametophyte reduced, with one antheridium; antherozoids multiflagellate, released through flask-shaped cavities in massula.
One genus only.

AZOLLA

Azolla Lam., Encycl. Méth. Bot. 1 (1) (1783) 343; Mett. in Kotschy & Peyritsch, Pl. Tinn. (1867) 51–52; Strasb., Über Azolla (1873) 76–77; Baker, J. Bot. 25 (1886) 99–100, reprinted in Handb. Fern Allies (1887) 137; Reed, Bol. Soc. Brot. II, 28 (1954) 15–16; Schneller in Kubitzki (ed.), Fam. & Gen. Vasc. Pl. 1 (1990) 57; Saunders & Fowler, Pl. Syst. Evol. 184 (1993) 175–193. — Type species: *Azolla filiculoides* Lam.

Characters of the family. — **Fig. 1.**

Distribution — Almost cosmopolitan, throughout wet tropics and warm temperate regions (about 6 or 7 species); throughout *Malesia* (1 species).

Habitat — Free-floating aquatics on stagnant or slow-flowing freshwater sites, or else rooted on wet mud that is not subject to drying.

Ecology — *Azolla* maintains a symbiotic association with the cyanobacterium (blue-green alga) *Anabaena azollae* Strasb., which is able to fix atmospheric nitrogen; this allows the association to grow in nitrate-poor environments that cannot easily be colonised by other hydrophytes (Moore 1969; Lumpkin & Plucknett 1980, 1982; Shi & Hall 1988).

Reproduction in *Azolla* is often vegetative, by fragmentation of the stem; this enables rapid population growth under favourable environmental conditions, and consequently *Azolla* can often become an aggressive weed, clogging waterways and drainage systems. *Azolla* also possesses an advanced heterosporous life cycle, however, with distinct micro- and megaspores. The gametophytes are endosporic (retained within the spore), and are consequently protected from desiccation of the environment. If the megagametophyte has been fertilised, it can therefore survive periods of seasonal drying and allow continuity of the population.

Literature: Lumpkin, T.A. & D.L. Plucknett, Econ. Bot. 34 (1980) 111–153; Azolla as green manure (1982); Moore, A.W., Bot. Rev. 35 (1969) 17–34; Shi, D.-J. & D.O. Hall, Bot. Rev. 54 (1988) 353–386.

Fossils — There is an extensive fossil history of *Azolla*, with the earliest record that of *A. simplex* Hall from the late Cretaceous of North America (Hall 1969a). The soft herbaceous structure of the sporophytic tissues has generally precluded fossilisation, and most records are of isolated massulae or megaspores. Significant exceptions to this, however, are *A. schopfii* Dijkstra from the Cretaceous to Palaeocene of North America (Sweet & Chandrasekharam 1973), and *A. stanleyi* Jain & Hall, from the Paleocene of North America (Rothwell & Stockey 1994), for which vegetative remains have been described in detail.

Five exclusively fossil sections have been described, viz. Sect. *Antiqua* P.I. Dorof. (1959), Sect. *Filifera* J.W. Hall (1968), Sect. *Kremastospora* R.K. Jain & J.W. Hall (1969), Sect. *Simplicispora* J.W. Hall (1970) and Sect. *Trisepta* K. Fowler (1975). Important reviews of extinct *Azolla* species can be found in J.W. Hall (1969b, 1974); R.K. Jain & J.W. Hall (1969), R.K. Jain (1971), M.E. Collinson (1980) and R.M.K. Saunders & K. Fowler (1993).

Several fossil genera have also been described which are believed to be closely related to or ancestral to *Azolla*, viz. *Glomerisporites* Pot. (J.W. Hall 1974, 1975), *Azollopsis* J.W. Hall (J.W. Hall 1968, 1974; Sweet & Hills 1974), *Parazolla* J.W. Hall (J.W. Hall 1969a, 1974; Collinson 1991), and *Hydropteris* Rothwell & Stockey (1994).

References: Collinson, M.E., Palaeontology 23 (1980) 213–229; in S. Blackmore & S.H. Barnes (eds.), Pollen and Spores: Patterns of diversification (1991) 119–150. — Dorofeev, P.I., Bot. Zh. SSSR 44 (1959) 1756–1763. — Fowler, K., Palaeontology 18 (1975) 483–507. — Hall, J.W., Amer. Fern J. 58 (1968) 77–88; Amer. J. Bot. 56 (1969a) 1173–1180; J. Palaeont. 43 (1969b) 528–531; Taxon 19 (1970) 302–303; Ann. Missouri Bot. Gard. 61 (1974) 354–367; Amer. J. Bot. 62 (1975) 359–369. — Jain, R.K., Amer. J. Bot. 58 (1971) 487–496. — Jain, R.K. & J.W. Hall, Amer. J. Bot. 56 (1969) 527–539. — Rothwell, G.W. & R.A. Stockey, Amer. J. Bot. 81 (1994) 479–492. — Saunders, R.M.K. & K. Fowler, Pl. Syst. Evol. 184 (1993) 175–193. — Sweet, A.R. & A. Chandrasekharam, Can. J. Bot. 51 (1973) 1491–1496. — Sweet, A.R. & L.V. Hills, Can. J. Bot. 52 (1974) 1625–1642.

Phylogeny — Cladistic analysis has elucidated several different phylogenetic trends in the genus [R.M.K. Saunders & K. Fowler, Pl. Syst. Evol. 184 (1993) 175–193]. There appears to have been a reduction of somatic structures in response to the aquatic habit, including overall plant size, adaptations to enhance buoyancy, and the simplification of leaf and stem vascular anatomy. There also appears to have been increased specialisation of both micro- and megasporocarpic reproductive structures, including a reduc-

tion in the number of floats per megaspore apparatus, an increase in the complexity of float arrangement and attachment, and a reduction in the size of the microspores.

Vegetative structure — Evolutionary adaptations favouring a free-floating aquatic habit have resulted in a highly reduced vegetative morphology, reviewed by Saunders & Fowler (1993).The diminutive sporophyte consists of a branched stem that floats horizontally on the water surface and bears alternately arranged imbricate bilobed leaves and pendulous roots (Fig. 1a, b). The absence of xeric stresses has resulted in the reduction of the stem vascular system to a haplostelic protostele in most species, and the leaves are almost devoid of a vascular trace.

Contrary to many descriptions, the ventral lobes rest on the water surface, and are not submerged (Svenson 1944). The pockets of air trapped between the two leaf lobes, together with the forces of surface tension acting between the ventral lobe and the water, enable the plants to remain buoyant. Buoyancy is also maintained by numerous hydrophobic papillae (trichomes) on the upper surface of the dorsal leaf lobes. These trichomes have been shown to be diagnostically important (Dunham & Fowler 1987), being composed of either a single erect cell which is basally contiguous with the epidermal layer, or of two cells, with one cell superficially positioned on an epidermal cell.

The cyanobacterium *Anabaena azollae* is maintained in a cavity of the dorsal leaf lobe; a specialised 'cavity trichome' has been shown to be involved in metabolite exchange between the fern and its endosymbiont (Calvert et al. 1985a). As new leaves develop at the meristem, the cavity needs to be inoculated with *Anabaena* cells. This is achieved by means of the 'apical trichome', which becomes entwined with strands of *Anabaena*; as leaf development progresses, the apical trichome becomes incorporated within a depression which represents the initial stage in leaf cavity formation (Calvert & Peters 1981; Peters & Calvert 1983; Calvert et al. 1985b).

The occurrence of *Anabaena* in the dorsal leaf lobes of *Azolla* is very widespread, although the association is not obligate since rare naturally occurring *Anabaena*-free populations have been reported (Huneke 1933; Shen 1960). Bacteria belonging to the genus *Arthrobacter* have recently been recognized as a possible third partner in the symbiosis (Wallace & Gates 1986; Petro & Gates 1987; Carrapico 1991).

Rapid vegetative reproduction is achieved through fragmentation of the stem by abscission of branches. Uheda et al. (1995) have shown that this is achieved by the degradation of the middle lamella between the cells in mature regions of the stem.

References: Calvert, H.E., M.K. Pence & G.A. Peters, Protoplasma 129 (1985a) 10–27. — Calvert, H.E., S.K. Perkins & G.A. Peters, Amer. J. Bot. 72 (1985b) 808. — Calvert, H.E. & G.A. Peters, New Phytol. 89 (1981) 327–335. — Carrapico, F., Pl. Soil 137 (1991) 157–160. — Dunham, D.G. & K. Fowler in: Intern. Rice Res. Inst., Azolla Utilization (1987) 7–16. — Huneke, A., Beitr. Biol. Pflanz. 20 (1933) 315–341. — Peters, G.A. & H.E. Calvert in: L.J. Goff, Algal Symbiosis: A continuum of interaction strategies (1983) 109–145. — Petro, M.J. & J.E. Gates, Symbiosis 3 (1987) 41–48. — Saunders, R.M.K. & K. Fowler, Pl. Syst. Evol. 184 (1993) 175–193. — Shen, E.Y.-F., Taiwania 7 (1960) 1–7. — Svenson, H.K., Amer. Fern J. 34 (1944) 69–84. — Uheda, E., et al., Int. J. Pl. Sci. 156 (1995) 756–763. — Wallace, W.H. & J.E. Gates, Appl. Environ. Microbiol. 52 (1986) 425–429.

Reproductive structure and life cycle — *Azolla* is heterosporous and monoecious, with separate leptosporangiate micro- and megasporangia, which are borne in enclosed indusial micro- and megasporocarps. The megasporocarp contains a single megasporangium, which contains a single megaspore; the microsporocarp, however, contains numerous microsporangia, each of which contains many aggregates of microspores.

Sexual reproduction in *Azolla* involves the rupturing of the sporocarps to liberate the sporangia, which subsequently sediment out. The microsporangia subsequently rupture, although the microspores are not released into the water directly, but are aggregated together into complex structures known as 'massulae' (Fig. 1c), which consist of hardened mucilage. Each massula bears trichomes which enable attachment to the megaspore prior to antherozoid release; the massula trichomes are either spiniform or barbed ('glochidiate'). The antherozoids are multiflagellate (Dunham & Fowler 1987) and are released from the antheridia in situ within the massula by means of specialized flask-shaped cavities (Fowler 1975).

The megaspore (Fig. 1d) has a highly sculptured sporoderm, and has a 'filosum' of fine perinous hairs. The filosum on the distal surface of the megaspore and collar region is known as the 'infrafilosum' and is an adaptation to enhance the likelihood of massula attachment; this is absent from some species, however. The filosum on the proximal megaspore surface, the 'suprafilosum', is involved in the attachment of the 'floats' (Fig. 1d). The floats are analogous to the massulae, and contain seven aborted megaspores; there are three or nine floats in extant species, although extinct species often have more. The megaspore and the proximally positioned float structures are collectively referred to as the 'megaspore apparatus'.

Antherozoid release from the antheridia in the male gametophyte results in the fertilisation of the oospore in the archegonium (in situ within the female gametophyte). The developing sporophyte emerges from the proximal pole of the megaspore apparatus, and ruptures the megasporangial wall before pushing aside the remains of the sporocarp wall (indusial cap).

Strands of *Anabaena* are located between the megasporocarp and megasporangial walls, allowing maintenance of the symbiotic associaton (Perkins & Peters 1993). As the funnel-shaped first leaf emerges, it dislodges the *Anabaena* and establishes a colony around an apical trichome that is incorporated into the dorsal leaf cavity (Becking 1987; Dunham & Fowler 1987; Peters & Perkins 1993).

References: Becking, J.H., Pl. Soil 100 (1987) 183–212; Dunham, D.G. & K. Fowler, Bot. J. Linn. Soc. 95 (1987) 43–53. — Fowler, K., Amer. Fern J. 65 (1975) 7–10. — Perkins, S.K. & G.A. Peters, New Phytol. 123 (1993) 53–64. — Peters, G.A. & S.K. Perkins, New Phytol. 123 (1993) 65–75.

Chromosomes — The chromosomes of *Azolla* are the smallest recorded for pteridophytes (Loyal 1975), and this has often resulted in the publication of inaccurate chromosome counts, reviewed in Stergianou & Fowler (1990). All *Azolla* species have a diploid chromosome number of 44, except *A. nilotica* Decne. ex Mett. which is $2n = 52$ (Stergianou & Fowler 1989, 1990). Triploids ($2n = 66$) have been discovered in four species (Stergianou & K. Fowler, l.c.), and one tetraploid ($2n = 88$) population has also been reported (Stergianou & Fowler, l.c.; Saunders & Fowler 1993).

References: Loyal, D.S. in P. Kachroo, Advancing Frontiers in Cytogenetics (1975) 293–298. — Saunders, R.M.K. & K. Fowler, Pl. Syst. Evol. 184 (1993) 175–193. — Stergianou, K.K. & K. Fowler, Brit. Fern Gaz. 13 (1989) 317–319; Pl. Syst. Evol. 173 (1990) 223–239.

Phytochemistry and chemotaxonomy — A limited amount of research has been conducted using anthocyanins (Shimura & Terada 1967; Holst 1977; Ishikura 1982) although this was not considered in a taxonomic context, and only involved the identification of the compounds occurring. Related studies of the phenolic compounds in *Azolla*, including flavonoids, have been interpreted taxonomically (Van Hove et al. 1987). The chemical composition of the spore apparatus has also been studied (Toia et al. 1985; Van Bergen et al. 1993).

The phytochemical research that has proved most valuable for deducing taxonomic and phylogenetic relationships in *Azolla* has involved studies of isozyme variation (Zimmermann et al. 1989a, b, 1991a, 1994) and molecular data (Zimmerman et al. 1991b, 1993, 1994; Eskew et al. 1993; Van Coppenolle et al. 1993). Restriction fragment length polymorphism (RFLP) studies of the endosymbiont clearly indicate co-evolution with the *Azolla* host (Van Coppenolle et al. 1995).

References: Eskew, D.L., et al., Pl. Mol. Biol. 21 (1993) 363–373. — Holst, R.W., Amer. Fern J. 67 (1977) 99–100. — Ishikura, N., Bot. Mag. (Tokyo) 95 (1982) 303–308. — Shimura, Y. & S. Terada, J. Jap. Bot. 42 (1967) 266–271. — Toia, R.E., et al., Amer. Fern J. 75 (1985) 38–43. — Van Bergen, P.F., et al., Grana, Suppl. 1 (1993) 18–30. — Van Coppenolle, B., et al., Genome 36 (1993) 686–693; Theor. Appl. Genet. 91 (1995) 589–597. — Van Hove, C., et al., Intern. Rice Res. Inst., Azolla Utilization (1987) 77–87. — Zimmerman, W.J., et al., Euphytica 42 (1989a) 163–170; Euphytica 43 (1989b) 223–232; Pl. Soil 137 (1991a) 161–170; New Phytol. 119 (1991b) 561–566; Amer. Fern J. 83 (1993) 97–104; Amer. Fern J. 84 (1994) 86–93.

Uses — As a result of the nitrogen fixing capability of the *Anabaena* endosymbiont, *Azolla* is useful as an organic fertilizer is tropical lowland rice cultivation and has been used in Chinese and Vietnamese agriculture for over two thousand years (Moore 1969; Lumpkin & Plucknett 1980, 1982). Research and effective management practices have increased the potential for higher rice yields when grown with *Azolla*. The average nitrogen fixing activity of *Azolla* is 1–2 kg N ha^{-1} day^{-1} (Watanabe 1982); this is sufficient to meet the nitrogen requirement of rice if the *Azolla* is grown for the period of one rice cropping. Liu (1979) has estimated that the effective use of *Azolla* in paddy fields can increase rice yields by an average 600–700 kg ha^{-1}. Within Malesia, the use of *Azolla* in rice cultivation has mainly been restricted to the Philippines (Mabbayad 1987) where considerable agronomic research on *Azolla* has been conducted at the International Rice Research Institute (IRRI) at Los Baños. Recent attempts at hybridizing *Azolla* species (Do et al. 1989; Watanabe et al. 1993) have revealed positive heterosis in growth and nitrogen fixation abilities.

Azolla has also been grown with water bamboo (*Zizania aquatica* L.), arrow head (*Sagittaria sagittifolia* L.) and taro [*Colocasia esculenta* (L.) Schott], and has been used in aquatic weed control and as a fodder for pigs, cattle, poultry and fish (Lumpkin & Plucknett 1982). *Azolla* is also proving to be an important antipollutant: its ability to extract phosphorus from eutrophic water, even after complete denitrification, has resulted in many investigations assessing its use as a decontaminant in sewage treatment

(Shiomi & Kitoh 1987; De Wet et al. 1990). The formation of dense mats of *Azolla* on the surface of stagnant bodies of water has also led to an evaluation of its efficacy in mosquito control (Ansari & Sharma 1991; Rajendran & Reuben 1991).

References: Ansari, M.A. & V.P. Sharma, Ind. J. Malariology 28 (1991) 51–54. — De Wet, L.P.D., et al., Water S.A. (Pretoria) 16 (1990) 281–286. — Do, V.C., et al., Can. J. Bot. 67 (1989) 3482–3485. — Liu, C.C., in Intern. Rice Res. Inst., Nitrogen and Rice (1979) 375–394. — Lumpkin, T.A. & D.L. Plucknett, Econ. Bot. 34 (1980) 111–153; Azolla as green manure (1982). — Mabbayad, B.B., in Intern. Rice Res. Inst., Azolla Utilization (1987) 101–108. — Moore, A.W., Bot. Rev. 35 (1969) 17–34. — Rajendran, R. & R. Reuben, Med. Vet. Entomol. 5 (1991) 299–310. — Shiomi, N. & S. Kitoh, in Intern. Rice Res. Inst., Azolla Utilization (1987) 169–176. — Watanabe, I., in Y.R. Dommergues & H.G. Diem, Microbiology of Tropical Soils and Plant Productivity (1982) 169–185. — Watanabe, I., et al., Soil Sci. Pl. Nutr. 39 (1993) 669–676.

Taxonomy — Extant species of *Azolla* have historically been classified into two sections, *Azolla* (4 or 5 species) and *Rhizosperma* (Mey.) Mett. (2 species) (Mettenius 1847), although these taxa have also been regarded as subgenera (Strasburger, 1873). A phylogenetically more acceptable supraspecific classification of the genus has recently been proposed by Saunders & Fowler (1993), however, as follows.:

Subgenus *Azolla*
    sect. *Azolla* (4 or 5 species)
    sect. *Rhizosperma* (1 species, also in Malesia)
Subgenus *Tetrasporocarpia* Saunders et Fowler (1 species).

References: Mettenius, G.H., Linnaea 20 (1847) 259–282. — Saunders, R.M.K. & K. Fowler, Pl. Syst. Evol. 184 (1993) 175–193. — Strasburger, E., Über Azolla (1873).

Azolla pinnata R. Br.

Azolla pinnata R. Br., Prodr. Fl. Nov. Holl. (1810) 167; Mett., Linnaea 20 (1847) 273; Strasb., Uber Azolla (1873) 79; Baker, J. Bot. 25 (1886) 100, repr. in Handb. Fern Allies (1887) 138; Sweet & Hills, Amer. Fern J. 71 (1971) 3; Fowler & Stennett-Willson, Brit. Fern Gaz. 11 (1978) 409; Zhou, Rev. Palaeobot. Palyn. 39 (1983) 111; Ashton & Walmsley, Bot. J. Linn. Soc. 89 (1984) 242; Saunders & Fowler, Bot. J. Linn. Soc. 109 (1992) 348. — Types: *R. Brown 134*, Oct. 1804 (BM lecto; E, K), Richmond, Hawkesbury, Australia; *R. Brown 135*, Oct. 1804 (BM, E, K, para), Paterson's River, Australia.
Salvinia imbricata Roxb. ex Griff., Calc. J. Nat. Hist. 4 (1844) 470. — *Azolla pinnata* R. Br. var. *imbricata* (Roxb. ex Griff.) Bonap., Notes Ptérid. 7 (1918) 130. — *Azolla imbricata* (Roxb. ex Griff.) Nakai, Bot. Mag. (Tokyo) 39 (1925) 185. — Type: *Roxburgh s.n.* (BR lecto; BM, G), Bengal, India.
Azolla decomposita Zoll., Syst. Verz. (1854) 51, nom. nud., based on *Zollinger 408* from Java (P).

Plants triangular to trapezoidal; branching alternate, with basal branches sometimes similarly branched. *Main stem* with protostelic vascular system. Devoid of leaves except for those that subtend branches; trichomes present, although often sparse and aggregated near base of leaf lobes. *Dorsal leaf lobes* with variable degree of imbrication; hyaline margins generally of (2–)3(–4) cells diameter. Leaf trichomes consisting of a cell superficially positioned on an 'epidermal' cell. Red anthocyanin pigments evident under adverse environmental conditions. *Ventral leaf lobes* devoid of stomata and trichomes. *Roots* solitary, with obliquely arranged groups of (3–)5–6 root hairs; (9–)12
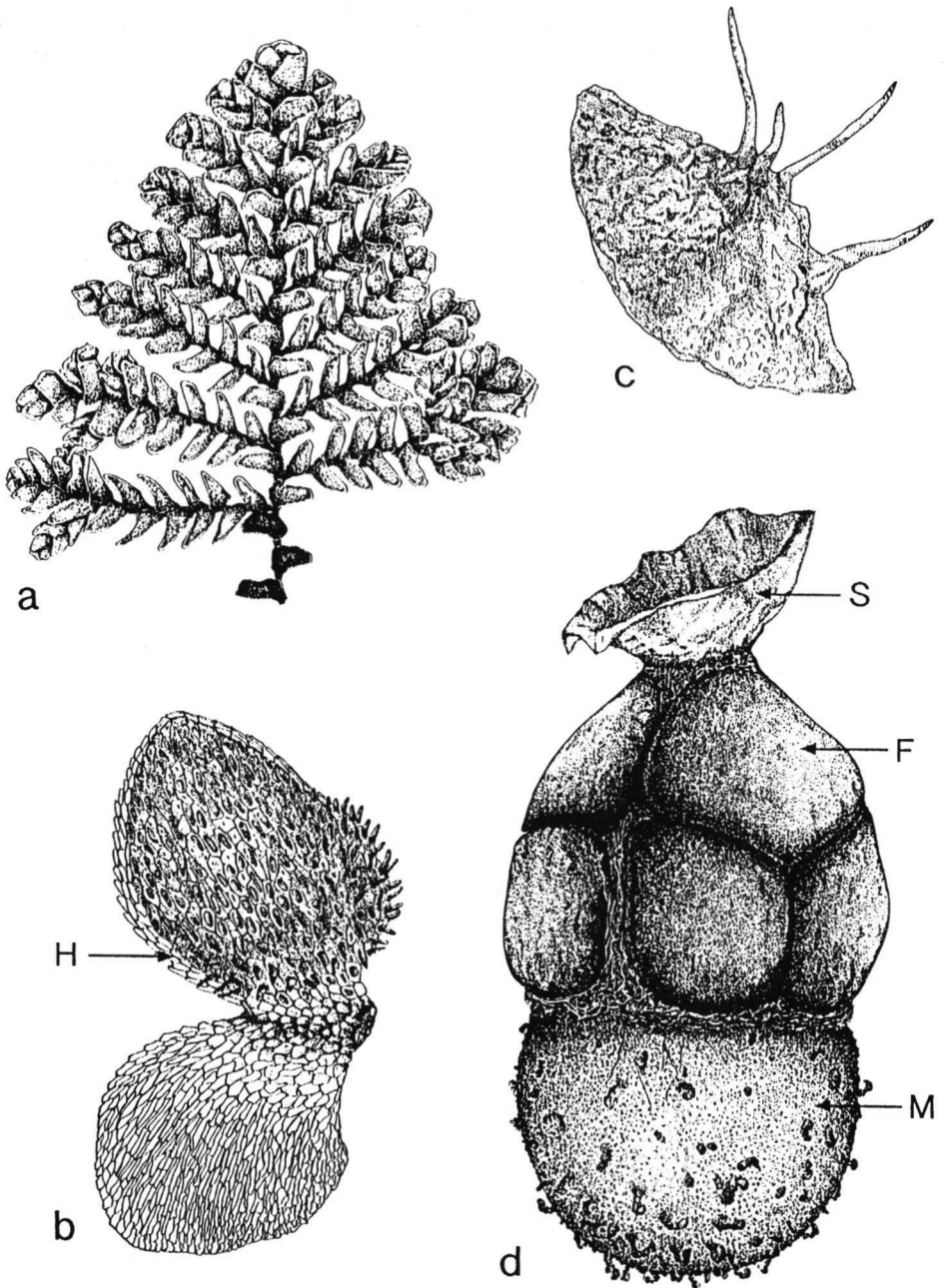
Fig. 1. *Azolla pinnata* R. Br. a. Entire plant (viewed from above), × 7; b. leaf with dorsal and ventral lobes, with hyaline margin (H), × 30; c. massula with simple trichomes, × 450; d. megaspore apparatus, with suprafilosum (S) retaining floats (F) in position over proximal pole of megaspore (M), × 100. Del. S.J. Stanley.

outer cortical cells and (13–)18(–19) epidermal cells in transverse section. *Megaspore* sporoderm surface of anastomosing baculae, often fused to form a surface of rounded nodules; baculae aggregated into prostrate or erect elongate or tuberculate excrescences, especially pronounced near distal pole. Excrescences of anastomosing baculae with interstices, or with smooth surface. Collar of megaspore apparatus comparatively small, without flange; both internal and external surfaces covered with filosum. *Massulae* with simple spiniform trichomes without glochidiate apex; 2–4(–12) trichomes on inner surface only, aseptate with occasional irregular internal cavities, sometimes with small irregular protuberances. — **Fig. 1.**

Distribution — Wet tropical Africa, Madagascar, India, Sri Lanka, southern and eastern China, SE Asia, northern and eastern Australia, and New Caledonia; in *Malesia:* throughout.

Infraspecific taxa — In a recent revision of the taxonomy of *Azolla pinnata* [R.M.K. Saunders & K. Fowler, Bot. J. Linn. Soc. 109 (1992) 329–357] three subspecies were accepted, of which only one is indigenous to Malesia: *A. pinnata* R. Br. subsp. *asiatica* Saunders et Fowler, Bot. J. Linn. Soc. 109 (1992) 349 [Type: *T. Sørensen, K. Larsen & B. Hansen 1829*, 2 March 1958, N Phayao, Thailand (K holo)]. This subspecies possesses lax to slightly imbricate dorsal leaf lobes with narrow hyaline margins (width of leaf/width of margin = c. 9.5/1), and a megasporoderm surface of prostrate or erect elongate excrescences.

Chromosomes — The chromosome number has been reported to be n = 22 [D.S. Loyal, Curr. Sci. 27 (1958) 357–358] or 2n = 44 [D.S. Loyal in P. Kachroo, Advancing Frontiers in Cytogenetics (1975) 293–298; D.S. Loyal et al., Br. Fern Gaz. 12 (1982) 230–232; P.K. Singh et al. in W.S. Silver & E.C. Schröder, Practical Applications of Azolla for Rice Production (1984) 55–65; S.K. Nayak & P.K. Singh, Cytologia 54 (1989) 275–286; K.K. Stergianou & K. Fowler, Brit. Fern Gaz. 13 (1989) 317–319; Pl. Syst. Evol. 173 (1990) 223–239; R.M.K. Saunders & K. Fowler, Pl. Syst. Evol. 184 (1993) 175–193]. Triploidy (2n = 66) and tetraploidy (2n = 88) are also reported (K.K. Stergianou & K. Fowler, l.c.; R.M.K. Saunders & K. Fowler, l.c.).