Pl. Syst. Evol. 184: 175 – 193 (1993)

Plant
Systematics
and
Evolution

The supraspecific taxonomy and evolution of the fern genus *Azolla* (*Azollaceae*)

RICHARD M. K. SAUNDERS and KEITH FOWLER

Received March 13, 1992; in revised version September 9, 1992

Key words: Pteridophytes, *Rhizosperma, Tetrasporocarpia*, water ferns. – Chromosome number, cladistics, cytology, morphology, systematics.

Abstract: The systematics of the aquatic fern genus *Azolla* LAM. is revised at the supraspecific level. Published morphological data are reviewed, and cytological and cladistic analyses of the genus conducted, enabling a discussion of the putative evolutionary trends in the genus, and a revision of the current taxonomy. A phylogenetically more acceptable subgeneric and sectional classification is proposed, viz. subg. *Azolla* sect. *Azolla* (comprising *A. filiculoides* LAM., *A. rubra* R. BR., *A caroliniana* auct. non WILLD., *A. microphylla* auct. non KAULF. and *A. mexicana* PRESL), subg. *Azolla* sect. *Rhizosperma* (MEY.) METT. (comprising *A. pinnata* R. BR.), and subg. *Tetrasporocarpia* subg. nov. (comprising *A. nilotica* DECNE. ex METT.).

Azolla LAM. is a genus of small aquatic ferns of mainly tropical to warm temperate distribution. The free-floating aquatic habit is manifested as an extreme morphological reduction of the sporophyte, which consists of a profusely branched stem that floats horizontally on the water surface and bears alternately arranged imbricate leaves and pendulous roots. *Azolla* possesses an advanced heterosporous reproductive cycle, with distinct micro- and megaspores which develop in leptosporangiate micro- and megasporangia. These sporangia are borne in separate micro- and megasporocarps, although *Azolla* is monoecious since both types of sporocarp are borne on the same individual.

Azolla is proving to be agronomically very important as a result of the symbiotic association it maintains with the cyanobacterium (blue-green alga) *Anabaena azollae* STRAS. *Anabaena* is able to fix atmospheric nitrogen that would otherwise be unavailable to the fern, and consequently the *Azolla-Anabaena* association can be used as a source of organic nitrates in the flooded paddy fields of tropical lowland rice production (MOORE 1969; ASHTON & WALMSLEY 1976; LUMPKIN & PLUCKNETT 1980, 1982; SHI & HALL 1988).

Recent research aimed at increasing the effective use of *Azolla* has been severely hampered by the lack of a sound taxonomic framework for the genus. Although earlier taxonomic studies have addressed this problem (LIN 1980, TAN & al. 1986, DUNHAM & FOWLER 1987, SAUNDERS & FOWLER 1992), little attention has been directed towards the supraspecific classification.

Taxonomic history. The genus was established in 1783 when Lamarck published the protologue of the type species, *A. filiculoides*. The name *Azolla* is derived from the Greek words αζω (to dry) and οδδυω (to kill), reflecting the plants inability to survive dry conditions (Ashton & Walmsley 1984).

Azolla is phylogenetically very isolated (Sporne 1975, Schneller 1990), and although it appears to be related to *Salvinia* Ség., the degree of similarity is not clear. These obscure phylogenetic affinities are mirrored by the differing familial classifications proposed: *Azolla* has been variously placed in the *Marsileaceae* R. Brown (1810), *Salviniaceae* Dumortier (1829), and *Azollaceae* Wettstein (1903).

Meyen (1836) redefined the genus *Azolla* to include only those species with three floats in the megaspore apparatus and with massula processes with barbed apices ("glochidia"). This excluded species which have nine floats per megaspore apparatus and massula processes with simple apices; these species were included in a newly described genus *Rhizosperma* Mey.

The distinct nature of these genera is no longer accepted because of their similar vegetative morphology, although both have been awarded sectional (Mettenius 1847) or subgeneric (Strasburger 1873) status within the genus *Azolla* sensu lato. The most widely accepted supraspecific classification of the genus recognises two sections, viz. sect. *Azolla*, with five species (*A. filiculoides* Lam., *A. rubra* R. Br., *A. caroliniana* auct. non Willd., *A. microphylla* auct. non Kaulf. and *A. mexicana* Presl), and sect. *Rhizosperma* Mey. (Mett.) with only two (*A. pinnata* R. Br. and *A. nilotica* Decne. ex Mett.).

The taxonomic distinctions between sects. *Azolla* and *Rhizosperma* are well established and have been widely accepted (e.g., Mettenius 1847, 1867; Baker 1886, 1887; Sadebeck 1902; Christensen 1906; Tan & al. 1986), and numerous vegetative and reproductive characters have been shown to be diagnostically important.

The species that comprise sect. *Azolla* are morphologically very similar, and the section is taxonomically very coherent (Svenson 1944, Dunham 1986, Dunham & Fowler 1987). In marked contrast, however, the two species in sect. *Rhizosperma, A. pinnata* and *A. nilotica*, are widely recognised as being morphologically distinct (Mettenius 1867; Strasburger 1873; Baker 1886, 1887; Sadebeck 1902; Christensen 1906; Tan & al. 1986; Saunders & Fowler 1992). A wide range of both vegetative and reproductive characters have been shown to be taxonomically useful in distinguishing between the two species (Saunders & Fowler 1992).

Many other sections of *Azolla* have been created in addition to sects. *Azolla* and *Rhizosperma* to incorporate the wide range of extinct species, viz. sects. *Antiqua* Dorofeev (1959), *Filifera* Hall (1968), *Kremastospora* Jain & Hall (1969), *Simplicispora* Hall (1970), and *Trisepta* Fowler (1975).

Material and methods

Material. Living material was obtained from the wild and from cultures maintained at the International Rice Research Institute (IRRI), Los Baños, Philippines; the specimens studied are listed in Table 1. The plants were subsequently grown in a nitrate-free P, K, Ca, and Mg based liquid culture medium with Mn, Mo, B, Zn, Cu, and Fe trace elements, buffered at pH 5.5 (adapted from Watanabe & al. 1977). A 12 h day photoperiod was maintained, using a combination of fluorescent and tungsten illumination (17,600 lux); day/night temperatures were regulated at 26/18 °C, with c. 75% relative humidity.

Table 1. Plant material studied and its chromosome numbers. Culture code and collector acronyms as follows: *CRRI* Central Rice Research Institute, India; *Hunan AAS* Hunan Academy of Agricultural Sciences, China; *IRRI* International Rice Research Institute, Philippines; and *PPBS* University of Portsmouth, U.K. Intraspecific classification of *A. pinnata* follows proposals by SAUNDERS & FOWLER (1992)

Taxon, culture code	Origin	Collector, date	Chromosome number
A. pinnata* R. BR. subsp. *pinnata			
IRRI 39	Australia: Murrumbidgee, New South Wales	IRRI, 1980	2n = 44
IRRI 101	Australia: Condamine River, Warwick, Queensland	DENNING, 1986	2n = 44
IRRI 102	Japan: Okinawa	MOCHIDA, 1987	2n = 44
IRRI 7004	Australia: Fog Dam, Kakadu National Park, Northern Territory	IRRI, 1982	2n = 66
IRRI 7524	Australia: Perth, Western Australia	Anon., February 1985	2n = 44
***A. pinnata* R. BR. subsp. *asiatica* SAUNDERS & FOWLER**			
IRRI 4	Philippines: Ifugao	IRRI, 1977	2n = 44
IRRI 23	India: Cuttack, Orissa	CRRI, 1978	2n = 44
IRRI 29	China: Changsha, Hunan	Hunan AAS, 1980	2n = 66
PPBS 1	Thailand: 100 km NE of Bangkok	J. BOGNER, March 1971	2n = 88
***A. pinnata* R. BR. subsp. *africana* (DESV.) SAUNDERS & FOWLER**			
IRRI 7506	Sierra Leone	C. A. DIXON, August 1982	2n = 44
IRRI 7507	Zaïre: Simisimi	L. PAUWELS, July 1983	2n = 44
IRRI 7511	Guinea Bissau: Contuboel	H. F. DIARA, August 1983	2n = 44
IRRI 7528	Mali: Baguineda	T. TRAORE, March 1987	2n = 44
***A. nilotica* DECNE. ex METT.**			
IRRI 5002	Sudan: Kosti	T. A. LUMPKIN, 1984	2n = 52

Cytology. *Azolla* is widely recognised as being difficult cytological material due to its extremely small chromosomes; the techniques employed here follow the methods developed by STERGIANOU & FOWLER (1989, 1990). Mitotic preparations were achieved using primordial leaf meristematic tissue rather than root apices since the roots of *Azolla* are highly reduced. The material was pretreated with a cycloheximide-hydroxyquinoline solution with added "Tween 80" (polyoxyethylene sorbitan mono-oleate) to increase chemical penetration. The material was fixed in Farmer's fixative (3 : 1, absolute alcohol : acetic acid) and hydrolysed with HCl before being transferred to Schiff's reagent (Feulgen); the material was then macerated and compressed in acetocarmine. Photographs were taken using standard light microscopy.

Thirteen different *A. pinnata* and one *A. nilotica* cultures were examined cytologically (Table 1). Voucher specimens of these cultures have been deposited in the Natural History Museum, London (BM).

Cladistics. All extant species of *Azolla* were incorporated in the analysis, with data abstracted from Svenson (1944), Dunham (1986), Dunham & Fowler (1987), Saunders (1990), Saunders & Fowler (1992), and other sources. The fossil species *A. schopfii* Dijk. (belonging to sect. *Kremastospora*) was also incorporated; this species is known to have existed during the Cretaceous and Palaeocene (Dijkstra 1961, Snead 1969, Sweet & Chandrasekharam 1973). Following proposals by Sweet & Chandrasekharam (1973), *A. fragilis* Jain & Hall (1969) and *A. extincta* Jain (1971) have been considered synonymous with *A. schopfii*. The related fossil genus *Azollopsis* Hall subg. *Azollopsis* (Hall 1968, 1974; Stough 1968; Sweet & Hills 1974) was used to root the tree as it is the closest outgroup taxon.

The following characters were employed in the study: **(1)** Branching pattern: 0 = isotomous dichotomous, 1 = anisotomous dichotomous, 2 = elongate alternate, or 3 = sub-pinnate (see Fig. 1). **(2)** Habit at maturity: 0 = sub-erect, or 1 = planar. **(3)** Stem vasculature: 0 = protostele, or 1 = siphonostele. **(4)** Occurrence of lacunae in stem cortex: 0 = absent, or 1 = present. **(5)** Stem indumentum: 0 = glabrous, or 1 = pubescent. **(6)** Dorsal leaf lobe venation: 0 = lacking midrib, or 1 = with prominent midrib. **(7)** Leaf trichome type: 0 = unicellular (basally contiguous with epidermis), or 1 = bicellular (upper cell superficially positioned on lower cell contiguous with epidermis). **(8)** Occurrence of central chlorophyllous zone with stomata on ventral leaf lobe: 0 = present, or 1 = absent. **(9)** Root arrangement: 0 = solitary, or 1 = in fascicles. **(10)** Root hair arrangement: 0 = in oblique partial whorls, or 1 = irregular or in pairs. **(11)** Grouping of sporocarps: 0 = in pairs, or 1 = in fours ("tetrads"). **(12)** Number of floats per megaspore apparatus: 0 = three, 1 = nine, 2 = 24, or 3 = > 32. **(13)** Separation of floats into three sectors: 0 = occurring, or 1 = not occurring. **(14)** Occurrence of megaspore collar: 0 = ± absent, 1 = small collar present, or 2 = large collar present. **(15)** Occurrence of megaspore infrafilosum: 0 = absent, 1 = present. **(16)** Massula trichome shape: 0 = simple, 1 = glochidiate (single-barbed), or 2 = multibarbed.

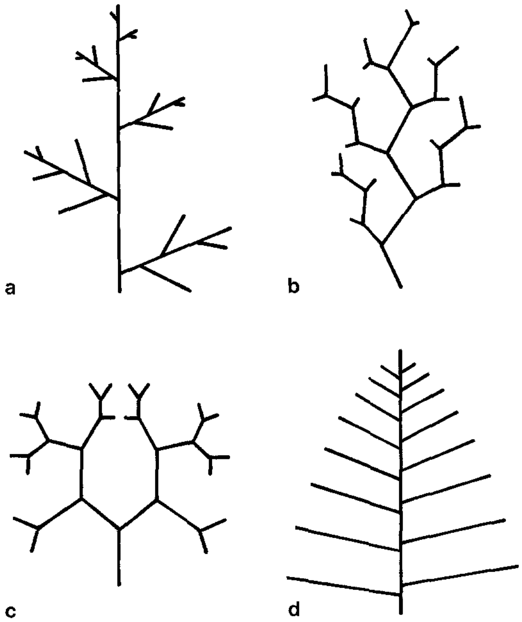

Fig. 1. Diagrammatic representation of stem branching patterns in *Azolla*. *a* Elongate alternate branching. *b* Anisotomous dichotomous branching. *c* Isotomous dichotomous branching. *d* Sub-pinnate alternate branching

A data matrix of the above character states is presented in Table 2. These data were then analysed using the parsimony computer program Hennig 86 (Farris 1988). Characters 12 and 14 were coded as additive transformation series (ccode +), and characters 1 and 16 as non-additive series (ccode −). The cladograms were constructed using the implicit enumerator (ie) option, with the strict consensus tree computed using the Nelsen option.

Results

Cytology. *Azolla* was found to have extremely small chromosomes, with an estimated size of only 1–2 µm. Even after heavy staining, they are only just visible at the limits of the resolution of light microscopy, and it was not possible to make detailed size comparisons or determine centromeric positions. This severely restricted the range of cytotaxonomic characters that could be employed.

A range of different chromosome counts were found for *A. pinnata*, with 44, 66 or 88 chromosomes (Table 1, Fig. 2 b–d). Only one collection of *A. nilotica* was analysed; this was found to have 52 chromosomes, which are markedly larger than those of *A. pinnata* (Table 1, Fig. 2 a).

Cladistics. Analysis of Table 2 resulted in the construction of 12 different but equally parsimonious cladograms. An example of one of these cladograms is presented as Fig. 3; this has a consistency index of 91 (indicating the amount of homoplasy in the data) and a length of 24. The twelve cladograms only differ in the suggested relationships between the five species of sect. *Azolla*; in each case, the relationships between *A. pinnata, A. nilotica, A. schopfii,* and the outgroup taxon *Azollopsis* subg. *Azollopsis* are identical.

The strict consensus tree derived from these cladograms is presented as Fig. 4. Sect. *Azolla* is shown to be a coherent taxon, although the evolutionary relationships between the five species cannot be distinguished. Of the extant species of *Azolla, A. nilotica* is shown to be the most plesiomorphic, although *A. pinnata* is also shown to be more primitive than the five species of sect. *Azolla.*

Table 2. Data matrix of character states for each cladistic character analysed. Taxon codes as follows: AZO *Azollopsis* subg. *Azollopsis*, SCH *Azolla schopfii*, NIL *A. nilotica*, PIN *A. pinnata*, MIC *A. microphylla*, CAR *A. caroliniana*, MEX *A. mexicana*, RUB *A. rubra*, FIL *A. filiculoides*. ? Data absent; − character not applicable

Characters:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
AZO	?	?	?	?	?	?	?	?	?	?	?	3	1	0	−	2
SCH	2	0	?	?	1	1	?	0	1	?	?	2	1	0	0	0
NIL	2	0	1	1	1	0	1	0	1	1	1	1	0	1	0	0
PIN	3	1	0	0	1	0	1	1	0	0	0	1	0	1	0	0
MIC	1	1	0	0	0	0	1	1	0	0	0	0	0	2	1	1
CAR	0	1	0	0	0	0	1	1	0	0	0	0	0	2	1	1
MEX	0	1	0	0	0	0	1	1	0	0	0	0	0	2	1	1
RUB	0	1	0	0	0	0	0	1	0	0	0	0	0	2	1	1
FIL	1	1	1	0	0	0	0	1	0	0	0	0	0	2	1	1

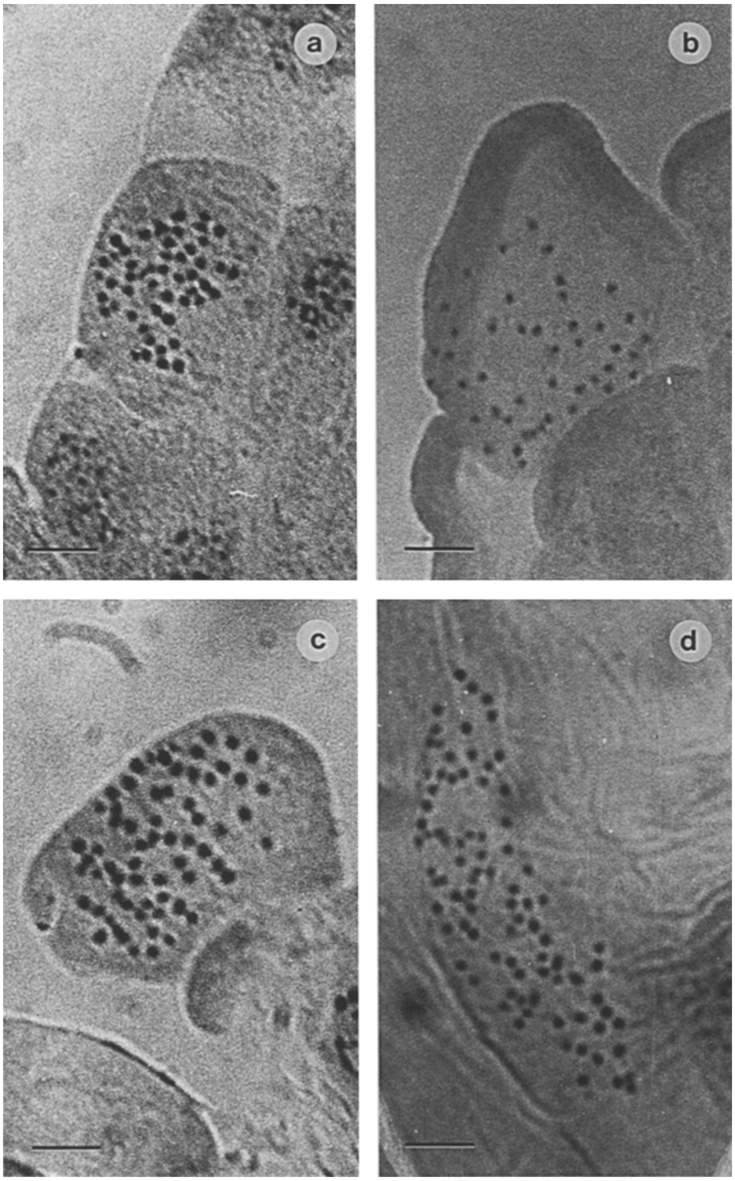

Fig. 2. Photomicrographs of mitotic metaphase karyotypes of *Azolla*. *a* Diploid karyotype (2n = 52) of *A. nilotica* (T. A. LUMPKIN s.n., 1984, Sudan, live culture IRRI 5002). *b* Diploid karyotype (2n = 44) of *A. pinnata* (C. A. DIXON s.n., August 1982, Sierra Leone, live culture IRRI 7506). *c* Triploid karyotype (2n = 66) of *A. pinnata* (Hunan AAS s.n., 1980, Hunan, China, live culture IRRI 29). *d* Tetraploid karyotype (2n = 88) of *A. pinnata* (J. BOGNER s.n., March 1971, Thailand, live culture PPBS 1). Bars: 5 μm

Discussion

Cytology. The numerical relationship between the chromosome counts for *A. pinnata* suggests that they represent diploid, triploid and tetraploid populations: i.e., 2n = 2x = 44, 2n = 3x = 66, and 2n = 4x = 88. The diploid count of 44 confirms

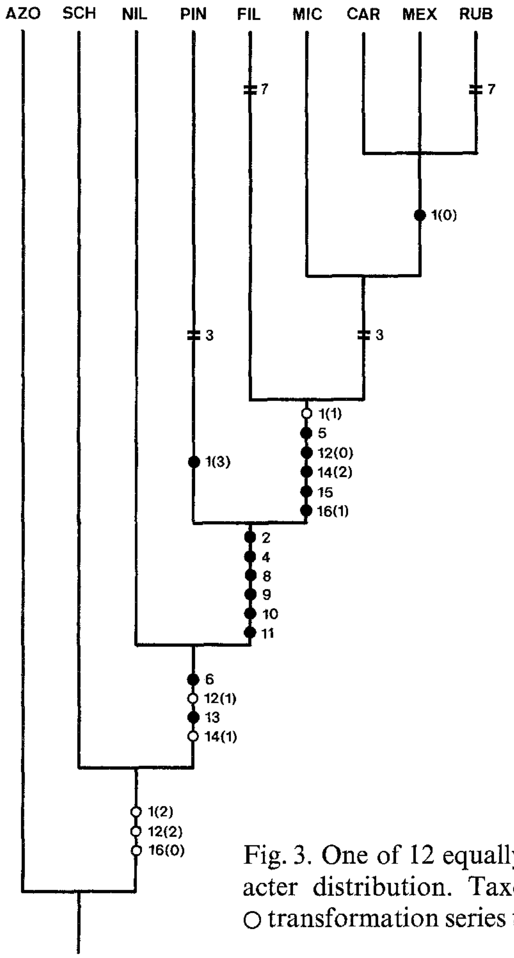

Fig. 3. One of 12 equally parsimonious cladograms, illustrating character distribution. Taxon codes as in Table 2. ● Synapomorphy, ○ transformation series transforming on the cladogram, = parallelism

previously published numbers for this species (Loyal 1958, 1975; Loyal & al. 1982; Singh & al. 1984; Nayak & Singh 1989; Stergianou & Fowler 1989, 1990). The same diploid chromosome number has also been reported for all species of sect. *Azolla* (Stergianou & Fowler 1989, 1990).

Only one collection of *A. nilotica* was analysed, with a diploid number of 2n = 52. This confirms the only previously published chromosome number for this species (Stergianou & Fowler 1989, 1990), although the present count was based on one of the same clones investigated by these authors.

The occurrence of polyploidy in *Azolla* is very limited, with only one tetraploid clone ever reported (Stergianou & Fowler 1989, 1990; and confirmed in the present publication); the chromosomes of *Azolla* are furthermore the smallest recorded for ferns (Loyal 1975). Stergianou & Fowler (1990) have suggested that this is related to the negative correlation that exists between the total amount of DNA per genome and the duration of the mitotic cycle; this also has the effect of altering cell dimensions, and has been shown to have a major influence on ecological factors such as sensitivity to altitude and climate. The phylogenetic trends in *Azolla* appear to have been towards smaller, faster growing colonising species (see below). The low total DNA contents per genome in *Azolla* have possibly been achieved

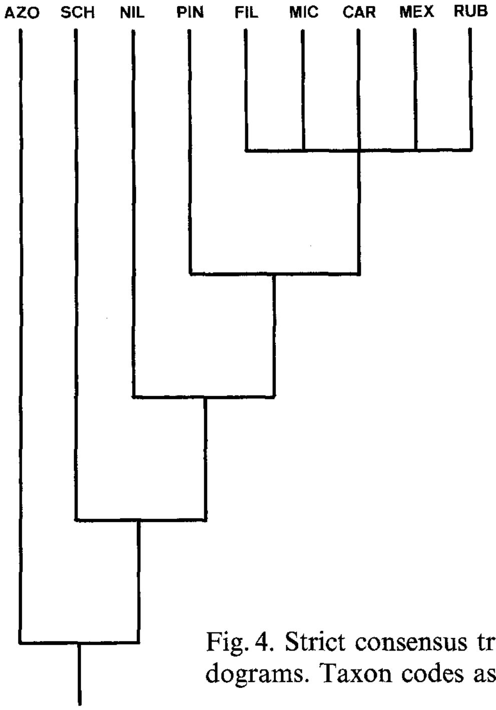

Fig. 4. Strict consensus tree, derived from 12 equally parsimonious cladograms. Taxon codes as in Table 2

by the maintenance of low ploidy levels and by the overall reduction in chromosome size (STERGIANOU & FOWLER 1990).

Evolutionary trends in vegetative morphology. Evolutionary adaptations to a free-floating aquatic habit are often manifested as an extreme morphological reduction of the sporophyte. This is observed in hydrophytes of many diverse plant groups, including monocotyledons (e.g., *Pistia* L., *Lemna* L., and *Wolffia* HORKEL ex SCHLEIDEN), dicotyledons (e.g., *Utricularia* L. and *Trapa* L.), and pteridophytes (e.g., *Salvinia* SÉG.) (SCULTHORPE 1967: 176–217).

Azolla has similarly undergone considerable evolutionary reduction of the sporophyte, thereby achieving greater buoyancy. The diminutive size of mature plants also enables more rapid growth and colonisation of new bodies of water by means of vegetative reproduction. Plesiomorphic *Azolla* species such as *A. nilotica* are less well adapted to free-floating conditions, however, because of their large overall size. To increase buoyancy, *A. nilotica* has large lacunae derived from an arrangement of stem cortical cells immediately beneath the epidermis (SAUNDERS & FOWLER 1992). Although the presence of lacunae is likely to be an adaptation to aquatic habitats, it can be regarded as a plesiomorphic character in *Azolla*, since the more apomorphic taxa tend to be considerably smaller, with little need for such buoyancy aids.

Buoyancy is also achieved in *Azolla* by pockets of air that become trapped around leaf and stem trichomes if the plants are submerged. An equivalent buoyancy system is observed in *Salvinia* (SCULTHORPE 1967: 197), although the trichomes are considerably more complex in this genus. The presence of leaf trichomes is a consistent feature of all known *Azolla* species, although it appears that there has been an evolutionary trend towards the reduction and subsequent loss of stem trichomes. Stem trichomes have been observed in the Cretaceous-Palaeocene species *A. schopfii* (Sweet & Chandrasekharam 1973), the Oligocene species *A. prisca* Reid & Chandler (1926) and the Miocene species *A.* aff. *ventricosa* Nik. (Bůžek & al. 1988).

The elongate alternate stem branching pattern (Fig. 1 a) has been indicated as plesiomorphic. This is corroborated by the fossil record, where it has been observed in both the Cretaceous-Palaeocene species *A. schopfii* (Sweet & Chandrasekharam 1973) and the Eocene species *A. berryi* R. W. Brown (1934). It can be hypothesised that later evolutionary changes could have enabled the development of anisotomous dichotomous branching (Fig. 1 b) by the occurrence of dichotomies rather than alternate and opposite divisions in lateral branches; this could subsequently have given rise to isotomous dichotomous branching (Fig. 1 c), where the dichotomies are all equally sized. The sub-pinnate branching pattern observed in *A. pinnata* could also be derived from the elongate alternate pattern, however, by the shortening of the main stem so that pairs of opposing lateral branches become attached very close to one another on the main stem, and by the loss of divisions in all but the most basal lateral branches (Fig. 1 d).

Consonant with these suggested changes in stem branching pattern has been an evolutionary trend towards planar rather than sub-erect growth habits. The plesiomorphic sub-erect habit observed in *A. nilotica* is associated with an assurgent leaf arrangement, which allows more light to reach the stem and ventral leaf lobes. This enables these structures to function photosynthetically, and both consequently possess chloroplasts and stomata in *A. nilotica* (Saunders & Fowler 1992). This suggested evolutionary trend is corroborated by the fossil record. Whilst the Cretaceous-Palaeocene species *A. schopfii* has a sub-erect habit (Sweet & Chandrasekharam 1973), the later Eocene-Oligocene species *A. primaeva* (Penh.) Arn. (Arnold 1955, Hills & Gopal 1967) and *A. prisca* (Reid & Chandler 1926) have been described with distinctly planar habits.

The continuous availability of water removes many of the stresses experienced by terrestrial plants, and it is suggested that the vascular system has consequently become highly reduced: the complex amphiphloic siphonosteles of the stems of species such as *A. nilotica* have possibly become reduced to haplostelic protosteles, and the vascularisation of both dorsal and ventral leaf lobes have similarly become residual. Evidence for the plesiomorphic nature of this prominent dorsal leaf lobe venation is provided by its presence in the Cretaceous-Palaeocene species *A. schopfii* (Sweet & Chandrasekharam 1973), the Palaeocene species *A. stanleyi* Jain & Hall (Melchoir & Hall 1983) and the Oligocene species *A. prisca* (Reid & Chandler 1926). Continuous water availability has also lessened the requirement for large numbers of well developed roots. Corroborative evidence for this phylogenetic trend is again provided by the Cretaceous-Palaeocene species *A. schopfii*, which has large fascicles of roots similar to those of the extant species *A. nilotica* (Sweet & Chandrasekharam 1973).

Evolutionary trends in reproductive morphology. Although Mahabalé (1963) recognised the evolutionary reduction of the stele in *Azolla*, many discussions concerning the phylogenetic trends of the genus are restricted to reproductive structures. This is largely due to the limited number of fossil *Azolla* species with adequate vegetative descriptions. The presumed ancestors of *Azolla, Glomerisporites*

POT. (HALL 1974, 1975) and *Azollopsis* (HALL 1968, 1974; SWEET & HILLS 1974), are furthermore only described from reproductive remains, and nothing is known of their vegetative morphology.

WAGNER (1973) hypothesised that the origin of heterospory in aquatic ferns such as *Azolla* might have been initiated by xeric habitats, which required the rapid completion of the life cycle during brief seasonal rains. He regarded the occurrence of extant heterosporous ferns in primarily aquatic habitats as representing "pre-adaptation", since once the plants had become adapted to growth in small temporary pools, they would subsequently be able to maintain their growth in aquatic conditions throughout the year.

The cladistic analysis conducted can be used to infer that the formation of sporocarps in fours (or "tetrads") is plesiomorphic. Verification of this by reference to extinct species is precluded, however, by the rarity of fossilised vegetative remains with sporocarps attached.

One of the main evolutionary trends in the morphology of the megaspore apparatus appears to have been the reduction in the number of floats. The ancestral genus *Azollopsis* possessed more than 32 small floats (HALL 1968, 1974; SWEET & HILLS 1974), whereas *Azolla* species have no more than 24. Within the genus *Azolla*, furthermore, there appears to have been a reduction in the number of floats from 24, through 18, 15, 12, and nine, to three floats: numerous multi-floated species have been reported from the Cretaceous (see FOWLER 1975: 485, Table 1; and COLLINSON 1980: 222–224, Table 1 for references), with the earliest reported three floated species, *A. indica* TRIVEDI & VERMA (1971) and *A. intertrappea* SAHNI & RAO (SAHNI 1941, SAHNI & RAO 1943), occurring in deposits considered to be of Cretaceous/Tertiary boundary age (COURTILLOT & al. 1988, DUNCAN & PYLE 1988).

There are several anomalous descriptions of early *Azolla* species with only one or three floats, however, viz. *A. capricornica* FOSTER & HARRIS (1981), *A. geneseana* HILLS & WEINER (1965), *A. primaeva* (ARNOLD 1955, HILLS & WEINER 1965, HILLS & GOPAL 1967, HALL 1969 b) and *A. simplex* HALL (1969 b). Several authors have suggested that these reports need confirmation, however, since the floats might consist of a greater number of less well defined floats, or else the "single float" might be columellate in origin and thus not homologous with massulae (JAIN 1971; SWEET & CHANDRASEKHARAM 1973; FOWLER 1975, 1981; MARTIN 1976 b; COLLINSON 1980). *A. simplex*, for example, was originally considered to have a single float (HALL 1969 b), although FOWLER (1981) has suggested that it may have an undifferentiated suprafilosum or may be multi-floated. Similarly, *A. primaeva* has been described as both three floated (HILLS & WEINER 1965, HILLS & GOPAL 1967) and single floated (HALL 1969 a), but is now considered to have nine floats that are obscured by a filosum (SWEET & HILLS 1976).

The evolutionary reduction in the number of floats per megaspore apparatus has been generally accepted by most researchers (JAIN & HALL 1969; JAIN 1971; HALL & BERGAD 1971; HALL 1974; FOWLER 1975, 1981; MARTIN 1976 b; COLLINSON 1980), although the evolutionary pattern may be reticulate, with several different lineages. One of the earliest discussions of the evolutionary trend in float number (HILLS & WEINER 1965) was based on evidence that suggested that sect. *Azolla* had a longer fossil record than sect. *Rhizosperma*, and was therefore more ancient. An evolutionary sequence was therefore proposed, with an increase in float number from three to nine being achieved by either the fusion of three megaspore apparatuses and the subsequent abortion of two megaspores, or by the segmentation of floats (Hills & Gopal 1967).

The structure of the floats of early multi-floated species such as *A. montana* Hall (Hall & Swanson 1968, Jain & Hall 1969) appears to have been virtually indistinguishable from that of the suprafilosum. Later species, including *A. distincta* Snead (1969, Hall & Bergad 1971), *A. fragilis* (Jain & Hall 1969), *A. stanleyi* (Jain & Hall 1969, Sweet & Hills 1976, Melchoir & Hall 1983) and *A. velus* (Dijk.) Jain & Hall (Dijkstra 1961, Jain & Hall 1969, Martin 1969 b), possess floats that lack hairs and are apparently more readily removed from the suprafilosum (Jain & Hall 1969, Hall & Bergad 1971). The earliest megaspore apparatuses described with alveolate floats belong to *A. indica* (Trivedi & Verma 1971) and *A. intertrappea* (Sahni 1941, Sahni & Rao 1943) from Cretaceous/Tertiary boundary deposits (Courtillot & al. 1988, Duncan & Pyle 1988), and *A. stanleyi* from the Palaeocene (Melchoir & Hall 1983).

Another important evolutionary change of the megaspore apparatus has been the increased complexity of float arrangement and attachment. The floats of the ancestral genus *Azollopsis* were loosely entangled in a mesh of perinous hairs over all surfaces of the spore (Hall 1968, 1974; Sweet & Hills 1974). With the evolution of *Azolla*, however, the floats became aggregated over the proximal pole of the megaspore (Jain & Hall 1969, Jain 1971), often arranged in one, two or three tiers. Other evolutionary developments have enabled a more intimate attachment between the floats and megaspore. The apomorphic development of an acrolamella that divides the floats into three sectors is associated with the occurrence of suprafilosum hairs that become entangled with small hairs on the inner surfaces of the floats, and hence help maintain the float arrangement. Martin (1976 a, b) observed large hook-like processes ("maniculae") that extend from the acrolamellae of *A. teschiana* Florsch. and *A. anglica* Dor.; these structures apparently performed a similar function to the suprafilosum, by becoming entangled with the hairs on the inner surfaces of the floats. The peltate suprafilosum also helps maintain float attachment when it covers the upper parts of the floats beneath the indusial cap (Fowler 1975). The floats have also become more firmly attached to the megaspore by means of a perinous collar around the proximal pole of the megaspore (Fowler 1975, Martin 1976 b). This is often contoured to fit the shape of the floats, and is most highly developed in the extant species of sect. *Azolla* (Fowler 1981, Dunham 1986), whereas the Cretaceous-Palaeocene species *A. simplex* and *A. schopfii* lack a significant collar (Dijkstra 1961, Hall 1969 b, Snead 1969, Sweet & Chandrasekharam 1973).

The infrafilosum of the megaspore has presumably evolved as a means of enhancing the likelihood of massula attachment to the megasporoderm, and hence increasing the chances of successful fertilisation. There is correspondingly a relationship between the occurrence of barbed trichomes (glochidia) on the massulae and the infrafilosum hairs on the megaspore, since the fluked apices of glochidia function by becoming entangled in the hairs of the infrafilosum. The fine perinous hairs that cover the megaspores of *Azollopsis* species are a means of attaching floats rather than massulae, and are therefore analogous to the suprafilosum of *Azolla* rather than the infrafilosum.

The structure of the massula trichomes is very variable, with simple, circinate and single-barbed forms all reported in *Azolla*, and both circinate and multi-barbed forms in *Azollopsis*. As noted by JAIN (1971) and COLLINSON (1980), the complex stratigraphic occurrence of these various massula trichome types makes phylogenetic interpretation difficult. Other authors have suggested a reductive evolutionary trend, with simple massula trichomes being derived from barbed glochidia (MAHABALÉ 1963, HILLS & WEINER 1965, HILLS & GOPAL 1967); this trend is correlated with the evolutionary increase in float numbers proposed by the latter authors.

Conflicting views also exist concerning the septation of massula trichomes: whilst MAHABALÉ (1963) regards the septate condition as plesiomorphic, HILLS & GOPAL (1967) have hypothesised the reverse.

Comparisons between *Azollopsis* and *Azolla* reveal several other phylogenetic trends of the male reproductive structures. The increased reproductive specialisation in the *Azollaceae* has been achieved in part by a reduction in the size of the microspores: *Azollopsis* microspores are generally more than 40 µm in diameter (HALL 1968, SWEET & HILLS 1974), whereas those of *Azolla* are considerably smaller. Consonant with this phylogenetic decrease in spore size has been a possible increase in the number of microspores in each massula: massulae of *Azollopsis* species contain only 1–7 microspores (HALL 1968, SWEET & HILLS 1974), whereas those of early *Azolla* taxa such as the Cretaceous species *A. extincta* contain up to 15 (SWEET & CHANDRASEKHARAM 1973). There appears to have been a subsequent phylogenetic reduction in the number of microspores per massula in *Azolla*, however, with the largest numbers occurring in early multi-floated species; the occurrence of fewer than eight microspores per massula has been a consistent feature in *Azolla* since the Oligocene (FOWLER 1975).

Several different phylogenetic trends can therefore be deduced in *Azolla*. There appears to have been a reduction of somatic structures in response to the aquatic habit, and an increased specialisation of both micro- and megasporocarpic reproductive structures.

Cladistic taxonomy. The five species that comprise sect. *Azolla* form a monophyletic taxon, defined by the following five synapomorphies: (1) glabrous stems; (2) three floats per megaspore apparatus; (3) well developed megaspore collars; (4) the presence of a megaspore infrafilosum; and (5) the presence of barbed massula trichomes (glochidia) (Figs. 3, 4).

Although sect. *Azolla* is therefore an acceptable cladistic taxon, problems are encountered with regard to sect. *Rhizosperma*. No synapomorphies exist for *A. pinnata* and *A. nilotica* that are unique for these species collectively, and *A. pinnata* appears to be evolutionarily more closely related to sect. *Azolla* than to *A. nilotica* (Figs. 3, 4). Sect. *Rhizosperma* as currently defined is therefore paraphyletic, and is inadmissable in cladistic taxonomy.

Taxonomic conclusions

The genus *Azolla* is phylogenetically very isolated, with no clearly related sister genera; this has been reflected by its classification in the *Marsileaceae* R. BROWN (1810), *Salviniaceae* DUMORTIER (1829) and *Azollaceae* WETTSTEIN (1903). It is significant, however, that the *Marsileaceae* sensu stricto have sporocarps which are believed to be evolutionarily derived from pinnae, whereas in *Azolla* they are hypothesised to be highly adapted indusia (SPORNE 1975). Similarly, the *Salviniaceae* sensu stricto contrast strongly with *Azolla* in having multisporangiate megasporocarps and lacking true roots. It therefore seems most appropriate to classify *Azolla* in the monotypic family *Azollaceae*.

Previous analysis of the pattern of morphological variation within *Azolla* (DUNHAM & FOWLER 1987, SAUNDERS & FOWLER 1992) has indicated that there are three distinct groups that can be recognised in the genus, viz.: (1) the five morphologically similar species that comprise sect. *Azolla*; (2) *A. pinnata* (currently part of sect. *Rhizosperma*); and (3) *A. nilotica* (also currently part of sect. *Rhizosperma*). This pattern of variation is also revealed by the cladistic analysis, although *A. pinnata* is shown to have a closer evolutionary relationship with the species of sect. *Azolla* than with *A. nilotica*. These relationships are also corroborated by the cytological data, which shows that *A. pinnata* shares the same chromosome number of $2n = 44$ with the species of sect. *Azolla*, but that *A. nilotica* has $2n = 52$ (STERGIANOU & FOWLER 1989, 1990).

It is proposed that this pattern of supraspecific variation be classified by using both subgenera and sections, viz.: (1) subg. *Azolla*, divided into (a) sect. *Azolla* (containing *A. filiculoides* LAM., *A. rubra* R. BR., *A. mexicana* PRESL, *A. caroliniana* auct. non WILLD. and *A. microphylla* auct. non KAULF.) and (b) sect. *Rhizosperma* (MEY.) METT. (containing only *A. pinnata* R. BR.); and (2) subg. *Tetrasporocarpia* subg. nov. for the inclusion of *A. nilotica* DECNE. ex METT. The new subgeneric name *Tetrasporocarpia* is derived from the unique habit its constituent species has of producing sporocarps in fours.

The intrageneric taxonomic rank traditionally used in *Azolla* is the section, following the initial descriptions of sects. *Azolla* and *Rhizosperma* by METTENIUS (1847), although STRASBURGER (1873) later elevated these taxa to the subgeneric level. SWEET & HILLS (1974, 1976) recommended a dual hierarchy in *Azolla*, using both subgenera and sections, whereby the subgenera are defined by the type of glochidia or homologous structures that are borne by the massulae, and the sections are defined by the gross morphology of the megaspore apparatuses. This was intended to allow fossil species with circinate massula processes to be accommodated easily. A classification of this type, however, involves a reversion to an Aristotelean "essentialist" philosophy that requires the a priori designation of certain characters as being of greater importance than others, and is therefore unacceptably artificial.

Identificatory key and taxon descriptions

1 Plants either rounded or elongate; main stems glabrous; three floats per megaspore apparatus; large megaspore collar; infrafilosum present on megaspore; massula trichomes with barbed apices (glochidiate) .... Subg. *Azolla* sect. *Azolla*.

1′ Plants either triangular, trapezoidal or elongate; main stems with trichomes; nine floats per megaspore apparatus, arranged in three sectors of three; small megaspore collar; infrafilosum absent from megaspore; massula trichomes with simple apices (eglochidiate) .... 2.

2 Deltoid to trapezoidal plants, up to 4 cm long; plants red under adverse environmental conditions; stem trichomes bicellular; protostelic stem vascular system;

ventral leaf lobe one cell thick and translucent, lacking stomata and trichomes; roots solitary; root hairs arranged in oblique partial whorls; sporocarps grouped in twos .... Subg. *Azolla* sect. *Rhizosperma.*

2′ Elongate plants, up to 40 cm long; plants never red; stem trichomes multicellular at maturity; amphiphloic siphonostelic stem vascular system; ventral leaf lobes > 1 cell thick and chlorophyllous, with stomata and trichomes; mature plants with roots in fascicles; root hairs irregularly arranged or in pairs; sporocarps grouped in fours .... Subg. *Tetrasporocarpia.*

Azolla **LAM.,** Encycl. Méth. Bot. **1(1)**: 343 (1783).

Type species: *A. filiculoides* LAM.

Synonyms: *Salvinia* [MICH.] ADANS. emend. A. L. DE JUSSIEU, Gen. Pl. **5**: 16 (1789), pro parte. *Azolla* LAM. emend. MEYEN, in Nova Acta **18**: 523 (1836). *Rhizosperma* MEYEN, in Nova Acta **18**: 523 (1836).

Description: Plants small free-floating aquatics or rooted in mud. Flabellate branched stems bearing roots and leaves. Leaves 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* STRAS. Ventral leaf lobe resting on water surface. Roots either solitary or in fascicles, growing from stem branching points; with numerous root hairs. 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. Sporocarps lack dehiscence zone. Megasporocarp contains solitary megasporangium, which contains solitary megaspore attached by placenta. Megaspore with trilete mark, and three or nine proximally positioned alveolate floats (up to 24 in fossil taxa) attached by filosum of megasporoderm. Microsporocarp contains numerous microsporangia that develop successively from apex to base; each microsporangium contains ± 64 microspores. Microspores aggregated together in alveolate structures (massulae), analogous to floats. Massulae partially or fully covered with trichomes.

1. Subg. *Azolla* sensu lato, non sensu STRAS., Über *Azolla*, 79 (1872).

Type species: *A. filiculoides* LAM.

Description: Stem branching pattern dorsiventrally flattened. Protostelic stem vascular system. Ventral leaf lobes unistratose and translucent (except at base), lacking stomata and trichomes. Plants red under adverse environmental conditions, due to anthocyanin pigments. Roots solitary, growing from stem branching points. Sporocarps arranged in pairs. Basic chromosome number: n = 22 (derived from STERGIANOU & FOWLER 1989, 1990).

1 a. Sect. *Azolla* sensu METT., in Linnaea **20**: 273 (1847) (syn. sect. *Euazolla*).

Type species: *A. filiculoides* LAM.

Synonyms: *Azolla* LAM. emend. MEYEN, in Nova Acta **18**: 523 (1836), pro gen. Subg. *Azolla* sensu STRAS., Über *Azolla*, 79 (1872) (syn. subg. *Euazolla*).

Description: Dichotomous stem branching patterns, giving rise to rounded plants, occasionally elongate in mature specimens of some species. Stems glabrous. Megaspore apparatus with three floats, and large collar. Megasporoderm with infrafilosum. Massulae with trichomes with barbed apices (glochidiate).

Extant species included: *A. filiculoides* Lam., *A. rubra* R. Br., *A. mexicana* Presl, *A. caroliniana* auct. non Willd. and *A. microphylla* auct. non Kaulf. (nomenclature according to Dunham & Fowler 1987).

Distribution: Warm temperate to tropical areas of North, Central and South America, Australia, and New Zealand (Brown 1810, Herzog 1938, Svenson 1944).

1b. Sect. *Rhizosperma* (Mey.) Mett., in Linnaea **20**: 273 (1847), emend. R. M. K. Saunders & K. Fowler.

Type species: *A. pinnata* R. Br.

Synonyms: *Rhizosperma* Mey., in Nova Acta **18**: 523 (1836), pro gen. Sect. *Rhizosperma* (Mey.) Mett., in Linnaea **20**: 273 (1847), pro parte. Subg. *Rhizosperma* (Mey.) Stras., Über *Azolla*, 79 (1872), pro parte.

Description: Main stem with alternately arranged lateral branches; branching pattern dorsiventrally flattened. Stems with bicellular trichomes at maturity (occasionally absent in juvenile plants). Megaspore apparatus with nine floats, and small collar. Megasporoderm without infrafilosum. Massulae with simple spiniform trichomes.

Extant species included: *A. pinnata* R. Br.

Distribution: Tropical Africa and Madgascar, Asia and Australia (Saunders & Fowler 1992).

2. Subg. *Tetrasporocarpia* R. M. K. Saunders & K. Fowler, subg. nov.

Type species: *A. nilotica* Decne. ex Mett.

Synonyms: Sect. *Rhizosperma* (Mey.) Mett. emend. Mett., in Kotschy & Peyr., Pl. Tinn., 54 (1867), pro parte. Subg. *Rhizosperma* (Mey.) Stras., Über *Azolla*, 79 (1872), pro parte.

Descriptio: Caulis principalis cum ramos laterales alternos; plantae non plano-compressae, cum folium dispositio assurgens. Caules trichomatibus multicellulosis (interdum unicellulosis aut absentibus in plantis juvenalibus). Systema vascularia caulis siphonostela amphiphlebia. Lobi foliorum ventrales > 1 cellulam latam. Plantae nunquam rubrae. Radicibus in fasciculis magnis e ramus caulibus. Sporocarpiis dispositis in tetradibus. Megasporarum apparatus cum organes natatibus novem. Collum megasporae parvum. Megasporodermate sine infrafilosio. Massulis cum trichomatibus simplicibus filiformibus, aut interdum absentibus. Numerus basicus chromosomatum: n = 26.

Description: Main stem with alternate lateral branches; plants not dorsiventrally flattened, with assurgent leaf arrangement. Stems with multicellular trichomes (occasionally unicellular or absent in juvenile plants). Amphiphloic siphonostelic stem vascular system. Ventral leaf lobes > 1 cell thick, chlorophyllous, with stomata and trichomes. Plants never red. Roots in large fascicles from stem branching points. Sporocarps arranged in fours. Megaspore apparatus with nine floats. Megaspore collar small. Megasporoderm without infrafilosum. Massulae with simple filiform trichomes, or occasionally absent. Basic chromosome number: n = 26.

Extant species included: *A. nilotica* Decne. ex Mett.

Distribution: Tropical Africa, in Great Rift Valley and extending west into the Congo Basin (Saunders & Fowler 1992).

This study was supported by the Overseas Development Administration (grant R 4359 awarded to K. Fowler). We are also grateful to: Dr K. K. Stergianou (East Birmingham

Hospital) for advice with cytological techniques and for the use of Fig. 2 d; Dr I. WATANABE (International Rice Research Institute, Philippines) and Mr H. W. E. VAN BRUGGEN (Heemskerk, Netherlands) for providing living specimens; Mr C. DERRICK (University of Portsmouth) for photographic assistance; and Dr K. BREMER (Department of Systematic Botany, University of Uppsala), Dr M. E. COLLINSON (Royal Holloway and Bedford New College, University of London), and Mr A. C. JERMY (Natural History Museum, London) for critically reading the manuscript.

References

ARNOLD, C. A., 1955: A Tertiary *Azolla* from British Columbia. – Contr. Univ. Mich. Mus. Paleontol. **12**: 37–45.

ASHTON, P. J., WALMSLEY, R. D., 1976: The aquatic fern *Azolla* and its *Anabaena* symbiont. – Endeavour **35**: 39–43.

– – 1984: The taxonomy and distribution of *Azolla* species in southern Africa. – Bot. J. Linn. Soc. **89**: 239–247.

BAKER, J. G., 1886: A synopsis of the *Rhizocarpeae*. – J. Bot. **25**: 97–101.

– 1887: *Azolla* LAM. – In BAKER, J. G.: Handbook of the fern-allies, pp. 137–138. – London: George Bell.

BROWN, R., 1810: Prodromus Florae Novae Hollandiae et Insulae Van-Dieman. – London: Johnson.

BROWN, R. W., 1934: The recognisable species of the Green River Flora. – U.S. Geol. Surv. Prof. Pap. **185 C**: 45–68.

BŮŽEK, Č., KONZALOVÁ, M., KVAČEK, Z., 1988: *Azolla* remains from the Lower Miocene of the North-Bohemian Basin, Czechoslovakia. – Tertiary Res. **9**: 117–132.

CHRISTENSEN, C., 1906: Index Filicum. – Copenhagen: Hagerup.

COLLINSON, M. E., 1980: A new multiple-floated *Azolla* from the Eocene of Britain with a brief review of the genus. – Palaeontology **23**: 213–229.

COURTILLOT, V., FÉRAUD, G., MALUSKI, H., VANDAMME, D., MOREAU, M. G., BESSE, J., 1988: Deccan flood basalts and the Cretaceous/Tertiary boundary. – Nature (London) **333**: 843–846.

DIJKSTRA, S. A., 1961: Some Palaeocene megaspores and other small fossils. – Mededel. Geol. Sticht., n.s., **13**: 5–11.

DOROFEEV, P. I., 1959: New species of *Azolla* LAM. in Tertiary flora of USSR. – Bot. Ž. **44**: 1756–1763.

DUMORTIER, B. C. J., 1829: Analyse des familles des plantes. – Tournay.

DUNCAN, R. A., PYLE, D. G., 1988: Rapid eruption of the Deccan flood basalts at the Cretaceous/Tertiary boundary. – Nature (London) **333**: 841–843.

DUNHAM, D. G., 1986: Taxonomic re-evaluation and species recognition of *Azolla* LAM., with particular reference to section *Azolla*. – Ph.D. thesis, C.N.A.A., Portsmouth Polytechnic, U.K.

– FOWLER, K., 1987: Taxonomy and species recognition in *Azolla* LAM. – In International Rice Research Institute, (Eds): *Azolla* Utilization, Proc. Workshop *Azolla* Use, Fuzhou, Fujian, China, 31 March–5 April 1985, pp. 7–16. – Manila: International Rice Research Institute.

FARRIS, J. S., 1988: Hennig 86 Reference, version 1.5. – Port Jefferson Station, New York: published by the author.

FOSTER, C. B., HARRIS, W. K., 1981: *Azolla capricornica* sp. nov. first Tertiary record of *Azolla* LAM. (*Salviniaceae*) in Australia. – Trans. Roy. Soc. South Australia **105**: 195–204.

FOWLER, K., 1975: Megaspores and massulae of *Azolla prisca* from the Oligocene of the Isle of Wight. – Palaeontology **18**: 483–507.

– 1981: Reproductive structures in fossil and extant species of the genus *Azolla*. – In Fowler, K.: Aspects of palynology in the Palaeogene of the Hampshire Basin, Southern England, pp. 1–100. – Ph.D. thesis, C.N.A.A., Portsmouth Polytechnic, U.K.

Hall, J. W., 1968: A new genus of *Salviniaceae* and a new species of *Azolla* from the late Cretaceous. – Amer. Fern J. **58**: 77–88.

– 1969 a: A reappraisal of the megaspores of two Eocene species of *Azolla*. – J. Palaeont. **43**: 528–531.

– 1969 b: Studies on fossil *Azolla*: primitive types of megaspores and massulae from the Cretaceous. – Amer. J. Bot. **56**: 1173–1180.

– 1970: A new section of *Azolla*. – Taxon **19**: 302–303.

– 1974: Cretaceous *Salviniaceae*. – Ann. Missouri Bot. Gard. **61**: 354–367.

– 1975: *Ariadnesporites* and *Glomerisporites* in the late Cretaceous: ancestral *Salviniaceae*. – Amer. J. Bot. **62**: 359–369.

– Bergad, R. D., 1971: A critical study of three Cretaceous salviniaceous megaspores. – Micropaleontology **17**: 345–356.

– Swanson, N. P., 1968: Studies on fossil *Azolla: Azolla montana*, a Cretaceous megaspore with many small floats. – Amer. J. Bot. **55**: 1055–1061.

Herzog, R., 1938: Geographische Verbreitung der Gattungen *Salvinia* und *Azolla*. – Bot. Arch. **39**: 219–225.

Hills, L. V., Gopal, B., 1967: *Azolla primaeva* and its phylogenetic significance. – Canad. J. Bot. **45**: 1179–1191.

– Weiner, N., 1965: *Azolla geneseana* new species, and revision of *Azolla primaeva*. – Micropaleontology **11**: 255–261.

Jain, R. K., 1971: Pre-Tertiary records of *Salviniaceae*. – Amer. J. Bot. **58**: 487–496.

– Hall, J. W., 1969: A contribution to the early Tertiary fossil record of the *Salviniaceae*. – Amer. J. Bot. **56**: 527–539.

Lamarck, J. B. A. P. M. de, 1783: Encyclopedia méthodique botanique. 1. – Paris: Panchonke.

Lin, Y. X., 1980: A systematic study of the family *Azollaceae* with reference to the extending utilisation of certain species in China. – Acta Phytotax. Sinica **18**: 450–456.

Loyal, D. S., 1958: Cytology of two species of *Salviniaceae*. – Curr. Sci. **27**: 357–358.

– 1975: Chromosome size and structure in some heterosporous ferns with a bearing on evolutionary problems. – In Kachroo, P., (Ed.): Advancing frontiers in cytogenetics, pp. 293–298. – Delhi: Hindustan.

– Gollen, A. K., Ratra, R., 1982: Morphological and cytotaxonomic observations on *Azolla pinnata*. – Fern Gaz. **12**: 230–232.

Lumpkin, T. A., Plucknett, D. L., 1980: *Azolla*: botany, physiology and use as a green manure. – Econ. Bot. **34**: 111–153.

– – 1982: *Azolla* as a green manure: use and management in crop production. – Westview Tropical Agricult. Ser. No. 5. – Boulder, Colorado: Westview Press.

Mahabalé, T. S., 1963: Evolutionary tendencies in the genus *Azolla*. – Mem. Indian Bot. Soc. **4**: 51–54.

Martin, A. R. H., 1976 a: Upper Palaeocene *Salviniaceae* from the Woolwich/Reading beds near Cobham, Kent. – Palaeontology **19**: 173–184.

– 1976 b: Some structures in *Azolla* megaspores, and an anomalous form. – Rev. Palaeobot. Palynol. **21**: 141–169.

Melchoir, R. C., Hall, J. W., 1983: Some megaspores and other small fossils from the Wannagan Creek site (Paleocene), North Dakota. – Palynology **7**: 133–145.

Mettenius, G. H., 1847: Über *Azolla*. – Linnaea **20**: 259–282.

– 1867: *Filicinae*. – In Kotschy, T., Peyritsch, J., (Eds): Plantae Tinneanae, pp. 51–54. – Wien.

MEYEN, F. J. F., 1836: Beiträge zur Kenntniss der Azollen. – Nova Acta Acad. Caesar. Leop. Carol. **18(1)**: 505–524.

MOORE, A. W., 1969: *Azolla*: biology and agronomic significance. – Bot. Rev. **35**: 17–34.

NAYAK, S. K., SINGH, P. K., 1989: Cytological studies in the genus *Azolla*. – Cytologia **54**: 275–286.

REID, E. M., CHANDLER, M. E. J., 1926: Catalogue of Cainozoic plants in the Department of Geology. 1. The Bembridge Flora. – London: British Museum (Natural History).

SADEBECK, R., 1902: *Salviniaceae*. – In ENGLER, A., PRANTL, K., (Eds): Die Natürlichen Pflanzenfamilien, 2nd edn. 1, pp. 383–402. – Leipzig: Engelmann.

SAHNI, B., 1941: Indian silicified plants. 1. *Azolla intertrappea* SAHNI and H. S. RAO. – Proc. Indian Acad. Sci. **B 14**: 489–601.

– RAO, H. S., 1943: A silicified flora from the Intertrappean cherts round Saugar in the Deccan. – Proc. Nat. Acad. Sci. India **B 13**: 36–75.

SAUNDERS, R. M. K., 1990: The systematics of the aquatic fern genus *Azolla* LAM., with particular reference to section *Rhizosperma* (MEY.) METT. – Ph.D. thesis, C.N.A.A., Portsmouth Polytechnic, U.K.

– FOWLER, K., 1992: A morphological taxonomic revision of *Azolla* LAM. section *Rhizosperma* (MEY.) METT. (*Azollaceae*). – Bot. J. Linn. Soc. **109**: 329–357.

SCHNELLER, J. J., 1990: *Azollaceae*. – In KRAMER, K. U., GREEN, P. S., (Eds): The families and genera of vascular plants. I. Pteridophytes and gymnosperms, pp. 57–60. – Berlin: Springer.

SCULTHORPE, C. D., 1967: The biology of aquatic vascular plants. – London: Edward Arnold.

SHI, D.-J., HALL, D. O., 1988: *Azolla* and immobilized cyanobacteria (blue-green algae): from traditional agriculture to biotechnology. – Plants Today **1**: 5–12.

SINGH, P. K., PATRA, R. N., NAYAK, S. K., 1984: Sporocarp germination, cytology and mineral nutrition of *Azolla* species. – In SILVER, W. S., SCHRÖDER, E. C., (Eds): Practical applications of *Azolla* for rice production. Proc. Intern. Workshop, Mayaguez, Puerto Rico, Nov. 17–19, 1982, pp. 55–65. – The Hague: Nijhoff Junk.

SNEAD, R. J., 1969: Microfloral diagnosis of the Cretaceous-Tertiary boundary, Central Alberta. – Res. Council Alberta Bull. **25**: 1–148.

SPORNE, K. R., 1975: The morphology of Pteridophytes, 4th edn. – London: Hutchinson.

STERGIANOU, K. K., FOWLER, K., 1989: Preliminary report of chromosome counts in the genus *Azolla* (*Pteridophyta*). – Fern Gaz. **13**: 317–319.

– – 1990: Chromosome numbers and taxonomic implications in the fern genus *Azolla* (*Azollaceae*). – Pl. Syst. Evol. **173**: 223–239.

STOUGH, J. B., 1968: New late Cretaceous palynomorphs from southern South America. – Univ. Kansas Paleontol. Contr. **32**: 1–7.

STRASBURGER, E., 1873: Über *Azolla*. – Jena: Hermann Dabis.

SVENSON, H. K., 1944: The New World species of *Azolla*. – Amer. Fern J. **34**: 69–84.

SWEET, A. R., CHANDRASEKHARAM, A., 1973: Vegetative remains of *Azolla schopfii* DIJKSTRA from Genesee, Alberta. – Canad. J. Bot. **51**: 1491–1496.

– HILLS, L. V., 1974: A detailed study of the genus *Azollopsis*. – Canad. J. Bot. **52**: 1625–1642.

– – 1976: Early Tertiary species of *Azolla* subg. *Azolla* sect. *Kremastospora* from western and arctic Canada. – Canad. J. Bot. **54**: 334–351.

TAN, B. C., PAYAWAL, P., WATANABE, I., LACDAN, N., RAMIREZ, C., 1986: Modern taxonomy of *Azolla*: a review. – Philipp. Agric. **69**: 491–512.

TRIVEDI, B. S., VERMA, C. L., 1971: Contributions to the knowledge of *Azolla indica* sp. nov. from the Deccan Intertrappean Series M. P. India. – Palaeontographica **B 136**: 71–82.

WAGNER, W. H., 1973: Some future challenges of fern systematics and phylogeny. – In JERMY, A. C., CRABBE, J. A., THOMAS, B. A., (Eds): The phylogeny and classification of the ferns. – Bot. J. Linn. Soc. (Suppl. 1) **67**: 245–256.

WATANABE, I., ESPINAS, C. R., BERJA, N. S., ALIMAGNO, B. V., 1977: Utilization of the *Azolla-Anabaena* complex as a nitrogen fertilizer for rice. – Intern. Rice Res. Inst. Res. Pap. Ser. **11**: 1–15.

WETTSTEIN, R. VON, 1903: Handbuch der Systematischen Botanik. 2. – Leipzig, Wien: Deuticke.

Address of the authors: Dr RICHARD M. K. SAUNDERS* and Dr KEITH FOWLER, School of Biological Sciences, University of Portsmouth, King Henry I Street, Portsmouth PO1 2DY, U.K. – * Present address: Department of Botany, University of Hong Kong, Pokfulam Road, Hong Kong.

Accepted September 9, 1992 by F. EHRENDORFER