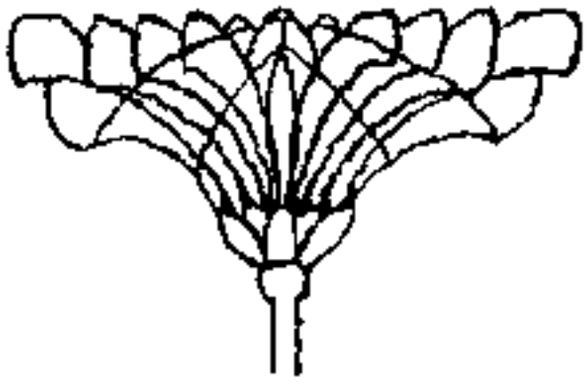

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Taxonomy of the American *Azolla* species (Azollaceae): a critical review

C. Evrard & C. Van Hove*

Catholic University of Louvain, Faculty of Sciences, Laboratory of Plant Biology,
Place Croix du Sud 5 bte 14, B–1348 Louvain-la-Neuve, Belgium
*author for correspondence [vanhove@bota.ucl.ac.be]

Abstract. *– The taxonomy of the New World species of* Azolla *has been the subject of much debate and remains unsatisfactory. Since 1944 most authors, mainly Americans, recognize four species:* A. caroliniana, A. filiculoides, A. mexicana *and* A. microphylla. *The present study is based on a comprehensive review of the literature and on original observations, by optical and scanning electron microscopy, of numerous samples cultivated in our laboratory as well as herbarium exsiccata. Observation of the type specimens confirms the opinion of some authors that* A. caroliniana *and* A. microphylla *are synonyms of the previously described* A. filiculoides. *The ferns named* A. caroliniana *and* A. microphylla *by most authors, including the American taxonomists in their recent works, are different from their type specimens. The study also shows that the Mettenius conception, proposed as early as 1867, has to be rehabilitated: two species only exist in America. According to the priority rule they must be named* A. cristata *and* A. filiculoides.

1 Introduction

The genus *Azolla* is cosmopolitan. A recent treatment (Saunders & Fowler 1993) divides it in two subgenera: *Azolla* and *Tetrasporocarpia.* According to this treatment subgenus *Azolla* includes two sections: *Azolla* and *Rhizosperma.* The subgenus *Tetrasporocarpia* and the section *Rhizosperma* are distributed in the tropical and subtropical regions of the Old World. The section *Azolla* is present in America, Australia and Asia. It is introduced in Europe since the nineteenth century. It is a matter of fact that numerous scientists, especially field scientists, are not satisfied with the present taxonomic treatment of the extant species of the genus. Concerns are, among others, the relations between *A. nilotica* (subg. *Tetrasporocarpia*) and *A. pinnata* (sect. *Rhizosperma*), and the characterization of subspecific taxa in *A. pinnata* (Saunders & Fowler 1992). The taxonomical status of *A. filiculoides*, *A. rubra* and *A. japonica* (sect. *Azolla*) in Australia and in Asia are also debated (Lumpkin & Plucknett 1982, Chinnock 1998, Watanabe & al. 1999).

The present study aims at clarifying still another problem, the taxonomy of the New World species, some of which are subspontaneous or naturalized in Europe. Since the revision of Svenson (1944) most authors recognize four species: *A. caroliniana*, *A. filiculoides*, *A. mexicana* and *A. microphylla*. Recent data suggest that this opinion is not adequate. We have therefore performed a critical review of the literature on the taxonomy of *Azolla* species from American origin, including physiology, hybridization and molecular biology data. We have examined the type specimens as well as numerous others, either herbarium exsiccata or fresh material cultivated in our laboratory, by optical microscopy and/or by scanning electron microscopy.

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2 Material and methods

2.1 Cultivation

Numerous *Azolla* strains are routinely cultivated in our laboratory (Van Hove & al. 1987), under a wide diversity of ecological conditions, in controlled culture rooms and in greenhouses.

2.2 Observations

Azolla voucher specimens have been examined under a light microscope. Herbarium abbreviations follow Holmgren & al. 1990. The megasporocarps of specimens from BR have been observed with a scanning electron microscope and photos are deposited at BR.

2.3 Branching pattern

Fresh fronds are immersed in ethanol 95° for 1 h, rinsed in water, immersed in concentrated bleaching water under vacuum for 15 minutes, rinsed in water, mounted in lactophenol and photographed under a Wild M 420 macroscope.

2.4 Leaf trichomes

Dry *Azolla* fragments are immersed in ethanol 95° for 10 seconds, rinsed in water, immersed in concentrated bleaching water under vacuum for 15 minutes, rinsed in water and observed in a depression slide.

2.5 Megasporocarps

Dry megasporocarps are gently rolled on double face scotch tape for discarding their indusium, fixed on an aluminium stub, gold coated and observed (Philips, SEM 501 B).

2.6 Glochidia

Dry microsporangia are immersed for 24 hours in water/ethanol/ glycerol (1/1/1) and squashed under a cover glass for dissociating the massulae before microscopic observation.

3 Review of the literature

With the view of avoiding unnecessary citations, only papers having raised divergent opinions or which are necessary for a good understanding of the problem are discussed here. Taxa indisputably considered as synonymous are only mentioned.

3.1 From a prelinnean record to 1944

The first description of *Azolla* in the taxonomic literature is from Feuillée (1725), who figured a Peruvian plant, *Muscus squamosus aquaticus elegantissimus*. Even if the roots are represented bifid (a character never observed at present!), the drawing clearly represents *Azolla*. Being anterior to Linné the name cannot be considered. The plant was mentioned to be used for improving chicken eggs production, which shows that not only in Asia, as generally reported (Lumpkin & Plucknett 1982; Shi & Hall 1988), but also in South America, farmers had recognized the agronomic value of *Azolla* centuries ago.

It is Lamarck (1783) who introduced *Azolla* as a genus, *A. filiculoides* being the type species, described on a sterile voucher collected by Commerson. Lamarck also illustrated *Azolla* in 1823.

In 1810 Willdenow named the same species, mentioning the same type, *A. magellanica*. He cited other specimens, from Chile and Peru, some of which fertile. Even if adopted by some authors this name is therefore superfluous. In the same paper Willdenow also described *A. caroliniana,* which "habitat in aquis Carolinae", on a sterile specimen from the Richard herbarium, actually a duplicate of the Michaux herbarium, present at B, BM, BR and P. The distinction between the two species was based only on the position of their leaves, respectively spreading and imbricate.

Kaulfuss (1824) described *A. microphylla* on a fertile voucher specimen collected in California by Chamisso, but without precise data on the reproductive structures. His distinction from *A. magellanica* was based on plant shape and leaf morphology. Kaulfuss also described *A. cristata* on sterile material from Demerary (Guyana), which more than probably corresponds to voucher 255 from Parker, precisely collected in Demerary. The original is at BM and a duplicate at BR, from the Kaulfuss herbarium, even if the collector is not mentioned in the protologue.

In its Prodromus Filicum, Desvaux (1827) recognized *A. magellanica* and *A. caroliniana.* He described *A. densa* ("in aquis Carolinae et Virginiae") and *A. arbuscula* ("in America calidiori").

Martius (1827) represented specimens identified as *A. microphylla.* Their glochidia are pluriseptate and their megasporocarps, immature and thus still covered by their indusium, seem rather warty. Unfortunately Martius, who disposed of the type from Chamisso (California) as well as of a specimen from Pöppig (Cuba) and other specimens from Brazil and from Chile, did not identify the material used for his descriptions and figures.

Meyen (1836) considered two American species. *A. magellanica* (syn.: *A. filiculoides*) has unseptate glochidia, warty perines and unicellular trichomes on the abaxial surface of the upper leaf lobe. *A. microphylla* Kaulf. sensu Martius has septate glochidia.

A. mexicana, a *nomen nudum* mentioned in 1830 by Schlechtendal & Chamisso, was validated independently and the same year by Presl (1845) and by Kunze (1845); it is traditionally fathered to Presl but available data do not allow to settle this question. The type specimen, fertile, had been collected by Schiede in Mexico.

In his first monograph of the genus, Mettenius (1847) recognized four American species; unfortunately some descriptions and illustrations cannot be clearly attributed to given specimens:
– *A. magellanica* (syn.: *A. filiculoides*; *A. arbuscula*), from Chile, Brazil, Uruguay, Australia;
– *A. microphylla* (syn.: *Salvinia azolla*; *A. magellanica* Willd. p.p. sec. Martius 1827; *A. portoricensis*), from Brazil, Cuba, Puerto-Rico, California. Figures cannot be definitely attributed but, according to the text, they are based on a sample from Cuba and therefore not on the type, collected in California by Chamisso. They correspond probably to material from the Martius herbarium, collected by Pöppig in Cuba, which belongs to a different species (see below);
– *A. cristata*, described with detailed illustrations of a fertile specimen from the Kunze herbarium collected in French Guyana and not of the type specimen, from Demerary (Guyana);
– *A. caroliniana* (syn.: *A. densa*; *A. mexicana*), from North America; the description and illustrations are probably based on the type specimen of *A. mexicana*, collected by Schiede, and surely not on the type of Michaux, described by Willdenow.

Mettenius characterized *A. magellanica* by its megaspores bearing specific protuberances and by glochidia unseptate or sometimes with one septum; the other species were considered difficult to distinguish through their megaspore surface and all have pluriseptate glochidia.

Subsequently, when we cite *A. caroliniana* or *A. microphylla* it is sensu Mettenius and not sensu Willdenow or Kaulfuss, except when explicitly mentioned.

Twenty years after his first monograph, Mettenius (1867) reduced to two the number of American species:
– *A. filiculoides* (syn.: *A. magellanica*; *A. rubra*; *A. arbuscula*), from New Granada, Bolivia, Peru, Brazil, Chile, Patagonia, Australia, New Zealand, Tasmania; megaspore surface presenting great annular or crater-like tubercles (tuberculis maioribus annularibus s. crateriformibus).
– *A. caroliniana* (syn.: *A. densa*; *A. mexicana*; *A. portoricensis*; *A. microphylla*; *A. cristata*; *A. bonariensis*; *Salvinia azolla*), from Eastern United States, Mexico, Caribbean, Central America, Venezuela, Guyana, Brazil; megaspore surface nearly evenly granulated (subequaliter granulata).

Strasburger (1873) followed Mettenius (1867) and presented a remarkable description, with many figures, of the two species. *A. caroliniana* has regularly granulated (gleichmässig granuliert) perines and pluriseptate glochidia, whereas *A. filiculoides* presents annular, prominent warts (ringförmig vorspringenden Warzen) on its perine and has glochidia unseptate or with an apical septum. Strasburger was also the first author having described the internal, two-layered structures of the perine from the two species. He has moreover distinguished *A. caroliniana* from *A. filiculoides* through a vegetative character, the number of cells of the trichomes present on the abaxial surface of the upper leaf lobe (one cell in *A. filiculoides*, two in *A. caroliniana*). Lastly he has described the branching pattern of *A. caroliniana* as pseudodichotomous, the one of *A. filiculoides* as most often racemose.

The taxonomy proposed by Mettenius (1867) for the American species has been adopted until 1944 (Strasburger 1873; Kuhn 1884; Baker 1886; Campbell 1893; Wettstein 1935). The three criteria proposed by Strasburger (trichome, perine and glochidia morphologies), or at least two of them, have been utilized for identifying *A. filiculoides* and/or *A. caroliniana* from America (Kuhn 1884; Baker 1886; Campbell 1893; Clausen 1940; Duncan 1940) as well as from Europe (Bernard 1904; Béguinot & Traverso 1906; Hannig 1911; Marsh 1914).

3.2 The revision of Svenson

In his revision of the New World species of *Azolla*, Svenson (1944) partially rehabilitated the classification proposed by Mettenius in 1847, but he did not even cite Mettenius (1867) and has not seen type specimens. He considered that Strasburger's work "contributed nothing to taxonomy" without mentioning the original observations of this author concerning leaf trichomes, stem branching and perine architecture. Svenson recognized four species in the subgenus *Azolla*.

– *A. filiculoides* (syn.: *A. magellanica*; *A. squamosa*; *A. bonariensis*). Guatemala to Alaska, Andean and Southern America, occasionally introduced in the Eastern United States, Hawaii and Europe. Svenson considered that glochidia are not septate, except occasionally, and that the megasporangia (sic) have raised, irregularly hexagonal markings. Plants are elongate.

– *A. caroliniana* sensu Willd. (syn.: *A. portoricensis*). Eastern United States and West Indies. Svenson deemed that Mettenius had mistaken this species with *A. mexicana*. In his opinion *A. caroliniana* has unseptate glochidia similar to those of *A. filiculoides*. The only fertile material observed by the author concerns microsporocarps from South Carolina and Florida; he had not seen megasporocarps and apparently ignored that foliar trichomes can be uni- or bicellular.

– *A. mexicana* (syn.: *A. caroliniana* sensu Mettenius; *A. densa*). Mexico and of scattered occurrence in the lowlands southward to French Guyana and Bolivia, northward to Utah and British Colombia and eastward to Wisconsin and Illinois. Glochidia pluriseptate, megaspores pitted. Plants isodiametric and dichotomous.

– *A. microphylla* (syn.: *Salvinia azolla*). Chiefly in the lowlands of Brazil and British Guyana, of scattered distribution in Western South America and northward to Central America, the West Indies and California. Glochidia pluriseptate, megaspores smooth. Plants pinnately branched.

What means *A. microphylla* for Svenson is not clear. He based his description on a specimen from the Galapagos Islands and not on the type. He doubted about the Californian origin of the type, considering that he has never seen any trace of *A. microphylla* in the traditional sense of Mettenius among the numerous collections he has examined from this State. On the other hand he made reference to Martius (1827), who described the megaspore as not smooth but reticulate, and to Mettenius (1847) who, according to Svenson, represented the megaspore as smooth (even if this is not evident).

3.3 From 1944 to 1971

Since 1944 only West (1953), Moore (1969) and Moran (1995) have completely followed Svenson. Most authors also recognized four American species, but with *A. caroliniana* and *A. microphylla* sensu Mettenius and not sensu Svenson, a very confusing situation. It is perhaps useful to repeat that when we cite these two species it is always sensu Mettenius, except if explicitly mentioned.

In the Netherlands (van Ooststroom 1948) and in Belgium (Lawalrée 1950), two species from American origin were reported: *A. filiculoides*, with unicellular leaf trichomes, warty perines and glochidia unseptate or with one or two apical septae, and *A. caroliniana*, with bicellular trichomes, finely granular perines and pluriseptate glochidia. All the Belgian *Azolla* specimens from BR have nevertheless been shown to be *A. filiculoides* (Sotiaux 1979).

West (1953) followed Svenson (1944).

In 1956 and 1961 di Fulvio considered *A. caroliniana* sensu Svenson as probably *A. filiculoides*. She recognized *A. caroliniana* sensu Mettenius and *A. filiculoides*, the two species having leaf trichomes, glochidia and perine architectures conform to Strasburger (1873), and *A. mexicana*, very similar to *A. caroliniana* but with a perine architecture intermediate between *A. caroliniana* and *A. filiculoides*. She suggested that *A. mexicana* could be a hybrid between the two species. For di Fulvio *A. microphylla* sensu Svenson is synonym of *A. caroliniana* sensu Mettenius.

According to Bonnet (1957), *A. filiculoides* has warty perines and glochidia unseptate or uniseptate, whereas *A. caroliniana* has glochidia with one or two septae.

Mason (1957) considered two species in California: *A. filiculoides* and *A. mexicana*, with glochidia respectively unseptate and septate. Both were said to possess pitted perines but the figures are ambiguous, the megaspore of *A. mexicana* being naked whereas the one from *A. filiculoides* is still covered by its indusium.

Godfrey & al. (1961) questioned the use of glochidia for distinguishing *A. filiculoides* and *A. caroliniana*. Their point of view was based on the examination of *Azolla* plants collected in a given locality in Florida and identified, on this sole geographical basis, as *A. caroliniana*. The glochidia of a given massula were described as both septate and unseptate without exception. No quantitative data were nevertheless provided.

According to Hills & Gopal (1967) *A. caroliniana, A. filiculoides, A. mexicana* and *A. microphylla* have glochidia septate.

In his review on *Azolla*, Moore (1969) followed Svenson.

Morton & Wiggins (1971) described the perine of *A. microphylla* as smooth or faintly pitted and their glochidia as pluriseptate.

3.4 The contribution of electron microscopy

Bertelsen (1972) provided the first scanning electron micrographs of megaspores and massulae, from *A. filiculoides*.

Kempf (1976) presented the smooth perine from a fossil *Azolla* identified as *A. microphylla*. Transmission electron micrographs show it divided into a foot layer, columellae and a tectum.

Martin (1976) subdivided the *A. filiculoides* endoperine described by Strasburger (1873) into an endo- and a mesoperine. He distinguished *A. caroliniana* and *A. filiculoides* by the position of the hairs (filosum) covering the exoperine and by the shape and surface of the floats.

Having observed fifteen populations from the Netherlands, Pieterse & al. (1977) identified *A. filiculoides*, with unicellular leaf trichomes, unseptate glochidia and warty perines bearing dispersed clusters of hairs, some apparently originating from within the protuberances. A second species, with bicellular trichomes, septate glochidia and perines with small papillae emerging between an evenly spread mass of hairs, was tentatively identified as *A. mexicana* or *A. microphylla*.

Fowler & Stennett-Willson (1978) described two perine surfaces and internal structures, respectively attributed to *A. filiculoides* and *A. microphylla*. For the first species the perine surface and internal structure clearly correspond to those represented by Strasburger (1873). As for *A. microphylla,* it does not seem possible to differentiate its perine from the *A. caroliniana* perine described by the same author.

Lin (1980) considered two American species, *A. filiculoides* and *A. caroliniana*, differing by their unseptate or septate glochidia.

According to Lucas & Duckett (1980) *A. filiculoides* glochidia are devoid of internal structures except at the tip and base, where a reticulum of wall material is present. The perine is warty.

Bates (1980), having observed the surface of megasporocarps from fifty-three *Azolla* specimens collected from Mexico to Alaska and from the East to the West Coast of North America, recognized only two patterns. One typically corresponds to *A. filiculoides*, the other one,

admittedly more variable, being attributed to *A. caroliniana.* Bates critically questioned the reintroduction by Svenson of the name *A. mexicana* which, for him, is more than probably synonym of *A. caroliniana.* On the other hand he stated, without argumentation, that vegetative characters and glochidia septation are too variable and generally not considered meaningful. This is surprising, since all the authors who had previously considered leaf trichome structures and/or glochidia septation had emphasised their usefulness, except Godfrey & al. (1961), who had questioned the validity of the septation criterion for distinguishing *A. caroliniana* from *A. filiculoides* (table 1).

Lumpkin & Plucknett (1982) also estimated, without more argumentation, that glochidia septation is not useful for identifying species into the *Azolla* section. They maintained nevertheless four American species, with the leaf trichomes unicellular in *A. filiculoides*, bicellular in *A. caroliniana*, bi- or sometimes tricellular in *A. mexicana* and *A. microphylla.* They introduced the concept of maturity growth habit, *A. filiculoides* and *A. microphylla* being characterized by an erect habit at maturity. The perine has large foveae in *A. mexicana*, and foveae partially masked by a thin weft of hair in *A. caroliniana.* It is scrobiculate but with smooth appearance caused by an even cover of hair in *A. microphylla*, whereas in *A. filiculoides* it presents wart-like excrescences, each covered with a weft of hair.

Tryon & Tryon (1982) largely followed Svenson (1944), except concerning the branching pattern, described as subdichotomous in *A. caroliniana* and *A. mexicana* but pinnately branched in *A. filiculoides* and *A. microphylla.*

Calvert & al. (1983) provided detailed scanning electron micrographs of the reproductive structures of *A. mexicana*; they described its perine markedly pitted "unlike other Euazolla species", and concluded that this character is useful for species identification.

One year later nevertheless Calvert & Peters (1984) characterized the perines of *A. mexicana* by their dimple-like depressions. They considered that the distinction between *A. caroliniana* and *A. mexicana* based on perine structure is difficult if not impossible.

The following year finally, the same authors (Perkins & al. 1985) described five distinct types of perines: regular, unpitted, densely covered by the filosum (*A. caroliniana*), with distinct raised hexagonal markings interconnected by narrow ridges (*A. filiculoides*), pitted, coarsely rugulate, with many dimple-like depressions (*A. mexicana*), rugulate-verrucate, with many irregular foveae of varying sizes (*A. microphylla*) or intermediate between those of *A. microphylla* and *A. filiculoides* (*A.* sp.) No correlation was established between these perine types and other taxonomic criteria. Moreover the observations bear on only one population of *A. caroliniana* and one of *A.* sp. For *A. mexicana* and *A. microphylla* the number of populations observed was not explicitly mentioned, but does not exceed four and three respectively. This is definitely not enough for making any taxonomic conclusion, as acknowledged by the authors themselves.

According to Tan & al. (1986) perines are distinctly and regularly pitted or foveolate (*A. filiculoides* and *A. mexicana*), indistinctly and irregularly pitted (*A. caroliniana*), and weakly and irregularly pitted, with pits small, mostly on the distal face (*A. microphylla*). Glochidia are unseptate or with only a few septae in *A. filiculoides* and *A. caroliniana*, pluriseptate in the two other species.

From preliminary results of their investigation on the taxonomy of *Azolla,* Dunham & Fowler (1987) recognized four New World species, essentially characterized by their specific perine structure. *A. filiculoides* has unicellular leaf trichomes, warty perines and glochidia mainly unseptate, sometimes with one, two or three septae. *A. caroliniana* Willd. and *A. microphylla* Kaulf. are considered as synonyms of *A. filiculoides*, since their type specimens possess one-celled leaf trichomes and are native from localities where only *A. filiculoides* is present. Moreover, if the type of *A. caroliniana* is sterile, the one from *A. microphylla* has megaspore and glochidia characters of *A. filiculoides.* The three other species possess leaf trichomes with more than one cell and glochidia mainly pluriseptate. One was identified as *A. mexicana.* The internal structure of its

Table 1. Leaf trichome cell number (T), perine surface morphology (P) and number of septae per glochidia (G) of *Azolla* according to the literature.

Boxes with ■: species considered as synonyms; g: perine surface granular; grey boxes: European material; n: perine surface variously described but not warty; uc: character not considered useful; v: perine surface warty; +: glochidia pluriseptate.

[1] *A. caroliniana* sensu Willd.

[2] For Pieterse & al. (1977) the non-*filiculoides* species is either *A. mexicana* or *A. microphylla.*

[3] Perkins & al. (1985) observed moreover one *A.* sp. whose perine was similar partly to *A. filiculoides*, partly to *A. microphylla.*

[4] These authors also consider two other species, differing from *A. mexicana* through their sporocarp apparatus.

Publication	*A. filiculoides*			*A. caroliniana*			*A. cristata*			*A. microphylla*			*A. mexicana*		
	T	P	G	T	P	G	T	P	G	T	P	G	T	P	G
Martius (1827)											v ?	+			
Meyen (1836)	1	v	0									+			
Mettenius (1847)		v	0-1		n	+		n	+		n	+	■	■	■
Mettenius (1867)		v			g		■	■	■	■	■	■	■	■	■
Strasburger (1873)	1	v	0-1	2	g	+	■	■	■	■	■	■	■	■	■
Kuhn (1884)	1	v	0	2	g	+	■	■	■	■	■	■	■	■	■
Baker (1886)		v	0		g	+				■	■	■	■	■	■
Campbell (1893)		v	0-1-2												
Bernard (1904)	1	v	0-1-2	2	g	+									
Béguinot & Traverso (1906)		v	0		g	+									
Hannig (1911)		v	0-1												
Marsh (1914)	1	v	0	2	g	+									
Duncan (1940)		v	0												
Clausen (1940)		v	0		g	+									
Svenson (1944) [1]		v	0			0					n	+		n	+
van Oostroom (1948)	1	v	0-1-2	2	g	+									
Lawalrée (1950)	1	v	0-1-2	2	g	+									
West (1953) [1]		v	0-1-2			0					n			n	+
di Fulvio (1956)	1	v	0-1-2	2	g	+				■	■	■	2	n	+
Bonnet (1957)		v	0-1			1-2									
Mason (1957)		n	0											n	+
di Fulvio (1961)		v			g					■	■	■		n	
Godfrey & al. (1961)			uc			uc									
Hills & Gopal (1967)			+			+						+			+
Morton & Wiggins (1971)											n	+			
Bertelsen (1972)		v	0-1-2												
Kempf (1976)											n				
Martin (1976)		v			n										
Pieterse & al. (1977) [2]	1	v	0							2	n	+			
Fowler & Stennett-Willson (1978)		v									n				
Lin (1980)			0			+									
Bates (1980)	uc	v	uc	uc	n	uc				■	■	■	■	■	■
Lucas & Duckett (1980)		v	?												
Lumpkin & Plucknett (1982)	1	v	uc	2	n	uc				2-3	n	uc	2-3	n	uc
Tryon & Tryon (1982) [1]		v	0-1-2			0-1-2					n	+			+
Calvert & al. (1983)														n	uc
Calvert & Peters (1984)			uc		n	uc					n	uc		n	uc
Perkins & al. (1985) [3]		v	uc		n	uc					n	uc		n	uc
Tan & al. (1986)	uc	n	0-1-2-3	uc	n	0-1-2-3				uc	n	+	uc	n	+
Dunham & Fowler (1987) [4]	1	v	0-1-2-3	■	■	■	■	■	■	■	■	■	≥2	n	+
Zimmerman & al. (1989)	1			2						2			2		
Tryon & Lugardon (1991)		v			n										
Lumpkin (1993)	1	v	uc	2	n	uc							2	n	uc
Kahn & al. (1993)			0-1-2									+			+
Saunders & Fowler (1993)	1			2						2			2		
Jermy (1993)	1		0	■	■	■							2		+
Moran (1995)		v	0-1-2			0-1-2					n	+		n	+
Jonsell (2000)	1	v											≥2	n	
Prelli (2001)	1		0										2		+

perine is variable and its surface regularly pitted. The two other species, which were not named, have smooth perines covered by a dense filosum, which perhaps masks pits. Unfortunately some sentences are incomprehensible, due apparently to typing mistakes. The three species are distinguished through their megaspore apparatus: float surface perforation, collar morphology and perine structure. Scanning electron micrographs of these structures were provided, nevertheless without any comment.

In 1989 and 1990 Stergianou & Fowler mentioned *A. caroliniana* and *A. microphylla* as currently recognized within section *Azolla*, without mentioning the previous paper. The authors recorded chromosome numbers and caryotypes of fifty two *Azolla* strains identified respectively as *A. caroliniana* (16), *A. filiculoides* (13), *A. mexicana* (9) and *A. microphylla* (14). All these strains were found to be 2n = 44 except four, which were 2n = 66. The caryotypes were also rather uniform. Caryology provides therefore no contribution to identify different species in the subgenus *Azolla*.

In their "Spores of Pteridophyta" atlas, Tryon & Lugardon (1991) focused on the morphology of the megasporocarps at the generic level. They reproduced previous SEM photographs from Lugardon & Husson (1982) and from Perkins & al. (1985). No new data concerning species diagnostic are provided.

Saunders & Fowler (1993) recognized *A. filiculoides*, *A. mexicana*, *A. caroliniana* auct. non Willd. and *A. microphylla* auct. non Kaulf., attributing the paternity of this nomenclature to Dunham & Fowler (1987) (but see above). The branching pattern of *A. caroliniana* and *A. mexicana* was described as dichotomous isotomous, the two other species being dichotomous anisotomous. The trichomes of *A. filiculoides* are unicellular, those from the three other species are bicellular. No differentiating criteria are provided for *A. caroliniana* and *A. mexicana*.

3.5 Present-day floras

Jermy (1993) considered two species introduced in the European flora, *A. filiculoides* (syn. *A. caroliniana* Willd.), with trichomes unicellular and glochidia non-septate and *A. mexicana* (syn. *A. caroliniana* auct. non Willd.), with trichomes bicellular and glochidia septate.

In Flora of North America North of Mexico, Lumpkin (1993) followed the nomenclature proposed by Svenson. He considered that *A. microphylla* is absent from the territory of the flora. Referring to Godfrey & al.(1961), he maintained his previous opinion (Lumpkin & Plucknett 1982) that the number of septae in the glochidia is not constant, either within a species or in a given individual. He distinguished *A. filiculoides* by the unicellular hairs of the upper leaf lobe and *A. filiculoides, A. caroliniana* and *A. mexicana* by the structure of their megaspores, reproducing the figures of Perkins & al. (1985), without adding new arguments.

Kahn & al. (1993) followed Tryon & Tryon (1982).

Marticorena & Rodriguez (1995) recognized only *A. filiculoides* in Flora of Chile. The usual diagnostic characters (leaf trichomes, glochidia and megasporocarps morphologies) are nevertheless not mentioned.

Moran (1995) followed the Svenson treatment.

Bennert (1998) followed Jermy (1993) for the synonymy of *A. caroliniana* and *A. filiculoides*. He doubted about the presence of a second species in Germany.

Jonsell (2000) recognized two species in Scandinavia, *A. filiculoides* and *A. mexicana*, following Jermy (1993) for the nomenclature and the characters used.

Prelli (2001) adopted the same opinion for the territory of France and Western Europe, but the presence of *A. mexicana* in France seems doubtful to him.

3.6 The advent of physiological and molecular biology methods

Zimmerman & al. (1989) compared forty-nine neotropical strains from the International Rice Research Institute collection as for their leaf trichome morphology and the electrophoresis pattern

of twelve enzymes. Sixteen accessions, classified as *A. filiculoides* in the collection, are clearly distinct enzymatically and by their unicellular trichomes. All the other strains, classified as *A. caroliniana* (16), *A. mexicana* (6) and *A. microphylla* (11) have bicellular trichomes and are phenetically similar, with *A. microphylla* nevertheless identifiable by the banding pattern of some enzymes. These three species cluster closely and are not easily defined.

In 1991 the same authors, on the basis of isoenzyme, RFLP, phosphorus deficiency symptoms and breeding experiments analyses, suggested that *A. caroliniana*, *A. mexicana* and *A. microphylla* could be regarded as one species, so favouring Mettenius (1867) over Svenson (1944).

RAPD and RFLP data from *Azolla* and from *Anabaena azollae* (Van Coppenolle & al. 1993, 1995; Zheng & al. 1999) as well as fatty acid composition of *Anabaena azollae* (Caudales & al. 1995) also showed great similarities between *A. caroliniana*, *A. mexicana* and *A. microphylla*. It may be argued that considering *Anabaena azolla*e characters for comparing various *Azolla* taxa constitutes a surprising method. The probable permanent linkage between the two partners of the symbiosis (Plazinski & al. 1988; Caudales & al. 1995; Van Hove, unpublished data) justifies it.

Most data based on molecular and physiological approaches therefore plead in favour of a great similarity between *A. caroliniana*, *A. mexicana* and *A. microphylla*. Some (Zimmerman & al. 1989, 1991; Caudales & al. 1995) suggest a closer proximity of *A. caroliniana* and *A. mexicana* as compared to *A. microphylla*.

Recently nevertheless (Chen & al. 2002) similar techniques suggested different groupings, with *A. mexicana* and *A. microphylla* (as well as their cyanobacterial symbionts) on one side, *A. caroliniana* and *A. filiculoides* on the other.

4 Discussion

This review of the literature indicates that objections to the classification proposed by Svenson (1944) are accumulating. It brings out the main characters which have been used for identifying American species, namely leaf trichome structure, megaspore perine structure, glochidia septation and stem branching pattern. It is true that a number of other morphological characters have been advocated, namely leaf shape, size, colour or position, leaf margin size or colour, root anatomy or size, root hair position, stem vascularisation, collar structure, float shape or structure, abundance and localisation of filosum on the perine or the collar, number of microsporangia per sporocarp. The taxonomic value of these criteria has nevertheless not received any substantial confirmation until now. A detailed analysis of the data concerning the other characters is presented below. It is based on the literature and on personal observations.

4.1 Leaf trichome structure

Since Meyen (1836) there is a large consensus (table 1) concerning the unique status of *A. filiculoides*, which has only unicellular trichomes on the abaxial epidermis of its upper leaf lobe. When trichome structure has been utilized for characterizing *A. caroliniana*, *A. mexicana, A. microphylla* or *A.* sp. it has nearly always been identified as bicellular (table 1). According to Lumpkin & Plucknett (1982) *A. mexicana* and *A. microphylla* moreover bear tricellular trichomes. Dunham & Fowler (1987) and Jonsell (2000) also described the trichomes of *A. mexicana* as having two or more cells. Only Bates (1980) and Tan & al. (1986) estimated that trichome structure has no taxonomic value, without presenting any evidence.

From hundreds of microscope observations of numerous *Azolla* strains cultivated in diverse conditions, we confirm that a given strain always bears either bicellular trichomes, eventually accompanied by tricellular ones, or unicellular trichomes. On the other hand we have observed that strains producing some 3-celled trichomes in given conditions have only 2-celled ones in other conditions. It is important to specify that this criterion only bears on trichomes present on the

abaxial epidermis of the upper lobe of the leaf, not those present on its margin, which can be bicellular even in *A. filiculoides*.

4.2 Perine structure

There is one very constant perine surface type, which may be shortly described as warty, attributed to *A. filiculoides* (table 1). Only Mason (1957) and Tan & al. (1986) consider the perine of this species as pitted. The description of the internal structure of this perine by Strasburger (1873) has been confirmed by all the authors who have considered this character (Campbell 1893; Hannig 1911; Duncan 1940; di Fulvio 1956; Bonnet 1957; Demalsy 1958; di Fulvio 1961; Martin 1976; Fowler & Stennett-Willson 1978; Perkins & al. 1985; Dunham & Fowler 1987; Tryon & Lugardon 1991).

For the authors who recognize only one species beyond *A. filiculoides*, its perine is generally described as granular (Mettenius 1867; Strasburger 1873; Kuhn 1884; Baker 1886), or variable, from pitted to smooth (Bates 1980).

When only "European" *Azolla* have been considered (Bernard 1904; Béguinot & Traverso 1906; Marsh 1914; van Ooststroom 1948; Lawalrée 1950; Pieterse & al. 1977; Jonsell 2000) the perines from the non-*A. filiculoides* samples were also described as granular (or at least not warty for the last author).

For those who recognize more than two species in America, perines attributed to *A. caroliniana* are diversely described: finely granular (di Fulvio 1956,1961), foveate with foveae partially masked by a thin weft of hair (Lumpkin & Plucknett 1982), presenting dimple-like depressions (Calvert & Peters 1984), regular, unpitted and densely covered with filosum (Perkins & al. 1985; Lumpkin 1993), irregularly pitted (Tan & al. 1986), rugulate-verrucate covered by a dense filosum (Tryon & Lugardon 1991). The perines of *A. mexicana* are stated to be pitted (Svenson 1944; West 1953; Mason 1957), granular (di Fulvio 1956), intermediate between the *A. filiculoides* and the *A. caroliniana* perine (di Fulvio 1961), presenting large foveae, especially near collar (Lumpkin & Plucknett 1982), markedly pitted, presenting dimple-like depressions (Calvert & al. 1983; Calvert & Peters 1984), pitted, coarsely rugulate with many dimple-like depressions (Perkins & al. 1985), regularly pitted or foveolate (Tan & al. 1986), pitted, sparsely covered with a few long filaments (Lumpkin 1993), foveolate (Moran 1995). The perines from *A. microphylla* are described as smooth (Svenson 1944; Moran 1995), scrobiculate with smooth appearance (Lumpkin & Plucknett 1982), rugulate-verrucate with many foveae of varying sizes (Fowler & Stennett-Willson 1978; Perkins & al. 1985), weakly irregularly pitted, with pits small, mostly on the distal face (Tan & al. 1986).

Considering that the qualificatives pitted, foveate, foveolate, scrobiculate and dimple-like are practically synonymous (Kremp 1965), the usefulness of this terminology seems at least doubtful. More generally there is a clear lack of consensus concerning possible differences between perine surfaces of American *Azolla* other than *A. filiculoides*. Nevertheless the recent works of Tryon & Lugardon (1991) and of Lumpkin (1993) follow the opinion of Perkins & al. (1985).

Perine cross-sections of *A. caroliniana* (Strasburger 1873; di Fulvio 1956, 1961), *A. mexicana* (Calvert & al. 1983; Calvert & Peters 1984; Perkins & al. 1985), *A. microphylla* (Kempf 1976; Fowler & Stennet-Willson 1978; Perkins & al. 1985; Dunham & Fowler 1987; Tryon & Lugardon 1991) and, partly, of *A.* sp. (Perkins & al. 1985) are very similar. Those from *A. caroliniana* (Perkins & al. 1985), *A. mexicana* (di Fulvio 1961; Dunham & Fowler 1987) and *A.* sp. (Dunham & Fowler 1987) are different from each other. The last authors recognize the variability of the *A. mexicana* perine internal structure, and present two pictures illustrating this point of view. They also consider that *A.* sp. is more variable, with characters intermediate between those of *A. mexicana* and *A. microphylla*.

We have personally observed fifty-seven herbarium specimens bearing megasporocarps and eight cultivated ones (SEM photographs at BR). Thirty-eight specimens had typical warty perines

characteristic of *A. filiculoides*. The diversity of granulation and of filosum density of the others does not allow the identification of clear-cut categories among them.

In conclusion, neither perine surface nor perine internal structure seem useful differentiating characters among the non-*filiculoides* American *Azolla*.

4.3 Glochidia septation

We found thirty-nine papers in which the septation of glochidia is considered (table 1). *A. filiculoides* is characterized by glochidia mainly unseptate or uniseptate, sometimes accompanied by some glochidia with two or three septae, in twenty-nine papers. On the other hand all the microsporocarps bearing mainly pluriseptate glochidia are identified as belonging to *A. caroliniana*, *A. cristata*, *A. mexicana*, *A. microphylla* or *A.* sp. None of these species is described with unseptate glochidia, except *A. caroliniana* sensu Willd. (Svenson 1944; West 1953; Tryon & Tryon 1982; Tan & al. 1986). One paper (Hills and Gopal 1967) considers the glochidia of *A. filiculoides* as septate, but without giving details.

Bates (1980), Lumpkin & Plucknett (1982), Calvert & al. (1983), Calvert & Peters (1984), Perkins & al. (1985) and Lumpkin (1993) follow Godfrey & al. (1961): without presenting any further evidence, they state indeed that glochidia septation is too variable and generally not recognized as meaningful.

We have therefore examined the Godfrey & Houk voucher specimen (60474) mentioned by Godfrey & al. (1961). Thirty-six massulae, belonging to five microsporocarps, have been observed. Only well observable glochidia (varying from 6 to 26 per massula) were considered. Among 503 glochidia observed, 427 were unseptate, 63 possessed one septum and 13 were biseptate.

In conclusion it appears that glochidia septation is a useful character for distinguishing *A. filiculoides* from the other American *Azolla* but does not allow the identification of different types among these last.

4.4 Branching pattern and habit

Four branching patterns have been ascribed to *Azolla*: racemose or pinnate (Strasburger 1873; Kuhn 1884; Baker 1886; Svenson 1944; Lawalrée 1950; Bonnet 1957; Tryon & Tryon 1982; Kahn & al. 1993; Moran 1995; Marticorena & Rodriguez 1995), pseudodichotomous (Strasburger 1873; Kuhn 1884; Lawalrée 1950), subdichotomous (Tryon & Tryon 1982; Tan & al. 1986; Kahn & al. 1993; Lumpkin 1993) and dichotomous (Svenson 1944; Saunders & Fowler 1993; Moran 1995). It is nevertheless well established that the branches of *Azolla* arise from one superficial cell of the stem (Strasburger 1873; Bonnet 1957; Demalsy 1958; Konar & Kapoor 1972). The branching pattern is therefore typically lateral and not dichotomous (Troll 1937: 465-478). Depending on the relative development of the terminal and lateral apexes, this branching can give rise to a racemose, more or less elongate and erected pattern or to a pseudodichotomous, more or less isodiametric and horizontal pattern. It is generally recognized that in some environmental conditions all the American *Azolla* present this late pattern, associated with relatively small sizes. In other circumstances some *Azolla*, always identified either as *A. filiculoides* or as *A. microphylla*, acquire a larger size and the first type of pattern.

Our observations of cleared fronds of diverse *A. filiculoides* and non-*filiculoides* strains, cultivated in various conditions, confirm these views. For *A. filiculoides* the pattern varies according to environmental conditions and developmental stage from nearly symmetrical (pseudodichotomous) to clearly asymmetrical (racemose), with an easy to identify main axis. The same is true for some other *Azolla*, especially for those usually identified as *A. microphylla*. All the others are always pseudodichotomous.

4.5 Correlations between trichome, perine and glochidia structures

We found nine papers in which trichomes, perine and glochidia structures have been considered together for identifying species (Strasburger 1873; Kuhn 1884; Bernard 1904; Marsh 1914; van Ooststroom 1948; Lawalrée 1950; di Fulvio 1956; Pieterse & al. 1977; Dunham & Fowler 1987). In all of them unicellular trichomes are associated with warty perines and with glochidia mainly unseptate. All the plants presenting these characters were identified as *A. filiculoides*. Those possessing bicellular trichomes, non- warty perines and glochidia mainly pluriseptate were most often identified as *A. caroliniana,* but also as *A. mexicana, A. microphylla* or *A.* sp.

On the other hand a number of papers associate perine and glochidia structures without mentioning the leaf trichomes. Fourteen of them characterize *A. filiculoides* by warty perines and glochidia unseptate or with only a few septae at the apex (Mettenius 1847; Baker 1886; Campbell 1893; Béguinot & Traverso 1906; Hannig 1911; Clausen 1940; Duncan 1940; Svenson 1944; West 1953; Bonnet 1957; Bertelsen 1972; Lucas & Duckett 1980; Tryon & Tryon 1982; Moran 1995). For Mason (1957) and Tan & al. (1986) nevertheless, *A. filiculoides* has pitted perines and mainly unseptate glochidia. Non-warty perines and pluriseptate glochidia characterize *A. caroliniana, A. cristata* and *A. microphylla* (Mettenius 1847), *A. mexicana* and *A. microphylla* (Svenson 1944; Tan & al. 1986; Moran 1995), *A. caroliniana* (Baker 1886; Béguinot & Traverso 1906; Clausen 1940), *A. mexicana* (West 1953; Mason 1957) and *A. microphylla* (Morton and Wiggins 1971; Tryon & Tryon 1982).

One paper (Lumpkin & Plucknett 1982) associated unicellular trichomes with warty perines in *A. filiculoides* and bicellular trichomes with non-warty perines in *A. caroliniana, A. mexicana* and *A. microphylla*. Lumpkin (1993) confirmed this opinion, excluding nevertheless *A. microphylla* from the area of his study.

For Jermy (1993) and Prelli (2001) at last, *A. filiculoides* has unicellular trichomes and unseptate glochidia, *A. mexicana* has bicellular trichomes with pluriseptate glochidia.

Among all the herbarium specimens we have examined, fifty-seven presented micro- and megasporocarps. No exception was found to the correlations mentioned above between leaf trichomes, perines and glochidia morphologies.

5 Conclusion

All the papers having described leaf trichomes on the upper leaf lobe have presented them as unicellular in *A. filiculoides*, bicellular (sometimes accompanied by tricellular) in *A. caroliniana, A. mexicana, A. microphylla* and *A.* sp. Only Bates (1980) and Tan & al. (1986) contest, without argumentation, the usefulness of this character.

With the exception of Lucas & Duckett (1980) all the papers having characterized *A. filiculoides* by the structure of their glochidia described them as unseptate or mainly unseptate. On the other hand most authors having described glochidia of *A. caroliniana, A. cristata, A. mexicana, A. microphylla* or *A. spp.* presented them as mainly septate. Only Svenson (1944), followed by West (1953), Tryon & Tryon (1982) and Tan & al. (1986) described *A. caroliniana* as having unseptate glochidia, with sometimes a few apical septae. It is nevertheless noteworthy that the first four authors did neither consider the leaf trichomes nor the perine structure of their material, whereas Tan & al. doubt about the validity of this species. As for the authors who do not recognize the taxonomic value of glochidia septation (Godfrey & al. 1961; Bates 1980; Lumpkin & Plucknett 1982; Calvert & Peters 1984; Perkins & al. 1985), we have seen (§ 4.3) that their position is not justified.

The only known remaining morphological character susceptible to discriminate possible *Azolla* taxa among the American *Azolla* is the perine architecture. There is one very constant, warty perine, attributed to *A. filiculoides*. We have shown that there is no consensus about the possible

existence of two or eventually several stable types besides this one. Even if some constant differences existed, which is at least seriously questionable, few taxonomists, if any, could agree to recognize different species on this sole criterion, so rarely observable and, what is more, requiring such elaborate investigation techniques (Stace, 1980: 201; Cronquist, 1988: 72; Judd & al., 2002: 144-147).

We conclude therefore that there is no reason for considering more than two *Azolla* species in the American flora. One, *A. filiculoides*, has unicellular leaf trichomes, glochidia mainly unseptate or uniseptate, some with only a few, generally apical septae, and its perine is warty. The other species is characterized by bicellular leaf trichomes, glochidia mainly septate and a perine structure, quite variable, but not warty. Considering the priority rule this species must be named *A. cristata*. The observation of leaf trichomes under a light microscope is therefore the necessary and sufficient condition for identifying American sterile specimens.

In other words the taxonomy proposed by Mettenius (1867) and so remarkably documented by Strasburger (1873) has to be rehabilitated, with nevertheless *A. caroliniana* Willd. sensu Mettenius replaced by *A. cristata* Kaulf.

6 Taxonomic treatment

Azolla filiculoides Lam., Encycl. 1: 343 (1783). — *Azolla magellanica* Willd., Sp. Plant. 5: 541 (1810). — *Azolla squamosa* Molina, Saggio s. storia nat. Chili: 301 (1810). — *Salvinia azolla* Raddi, Plant. Bras.: 2 (1825) Pars I Fil. Florentinae Fol. 84, Tab. 1, Fig.3. — Type: "plante rapportée de Magellan par M. de Commerson" (in herb. Lamarck, P holo-; BM, P iso-). See note 1.

Azolla caroliniana Willd., Sp. Plant. 5: 541 (1810). — Type: "habitat in aquis Carolinae" *N. Michaux*, s.n., s.d. (in herb. Willdenow 20260, B holo-; BM, BR, P iso-). See note 2.

Azolla microphylla Kaulf., Enum. Fil.: 273 (1824). — Type: "habitat in California" Chamisso s.n., s.d. (B holo-?; BM iso-; BR iso- or holo-). See note 3.

Azolla arbuscula Desv., Ann. Soc. Lin. Paris VI: 177 (1827). — Type: " Habitat in America calidiori " sine col., s.n., s.d. (in herb. Desvaux, P holo-). See note 4.

Azolla densa Desv., Ann. Soc. Lin. Paris VI: 177 (1827). — Type: "Crescit in aquis Carolinae et Virginiae" sine col., s.n., s.d. (in herb. Desvaux, P holo-). See note 4.

Azolla bonariensis Bertol., Miscel. Bot. XXI, 4: 18 (1861). — Type: Buenos Ayres, Fox-Strangwais s.n., s.d. (BOLO holo-? n.v.).

Diagnostic characters: leaf trichomes unicellular; glochidia mostly 0-1 - septate; megaspore perine warty.

Notes. (1). Microscopic observation of the type, sterile, shows unicellular leaf trichomes. The isotypes at P (herb. Jussieu 1601 B) and at BM are each annotated: "1) Montevideo, 2) Buenos Ayres". A handwritten note from Jussieu on his voucher stipulates that " Lamarck l'a annoncé du détroit de Magellan par erreur".

(2). Microscopic examination of the type, sterile and with unicellular leaf trichomes, clearly demonstrates its synonymy with *A. filiculoides*, as previously mentioned e.a. by Alston (mss in BM), Dunham & Fowler (1987) and Jermy (1993).

(3). The specimen at BR, a duplicate from the Kaulfuss herbarium, is annotaded by Alston: "This appears to be the type of *Azolla microphylla* Kaulf., as there is no specimen in Berlin. It is *A. filiculoides*". Microscopic examination shows unicellular leaf trichomes and unseptate glochidia. Dunham & Fowler (1987) had recognized this synonymy.

(4). The type specimens of both *A. arbuscula* and *A. densa* are sterile and have unicellular leaf trichomes and the two species are thus synonyms of *A. filiculoides*.

Specimens examined

South America

Country not indicated: Habitat in America calidiori, in Herb. *Desvaux*, s.n., s.d., s.col. (holotype of *A. arbuscula*) (P).

Bolivia: Cochabamba, *M. Bang* 1032-1033, 1891 (BM); Miske (Santa Cruz), *W. M. A. Brooke* 5902, 1949 (BM); San Luis, *R. Pearce* s.n., Apr. 1864 (BM).

Brazil: Santarem (prov. Para), *R. Spruce* s.n., Apr. 1850 (BM); Sao Pedro do rio Grande do Sul, *Lindman* s.n., Nov. 1892 (BM, K); Etat de Santa Catharina, *E. Ule* s.n., Oct. 1899 (BR).

Uruguay: Montevideo, *Archevaleta* 471, 1874 (BM); Montevideo, *d'Orbigny* 63, s.d. (BM, BR); Maldonado, *Felippone* 1247, s.d. (K).

Chile: Coquimbo (N. Chile), *C. Elliott* 546, 1929 (K); Central Chile, prov. Cautin, *Calvert & Reed* s.n., 1914 (BM); Valdivia, *E.C. Reed* s.n., Jan. 1892 (BM); Andes, 37° lat. austr., *Pöppig* s.n., Jan. 1829 (BR); Chile, *J. Triana* s.n., received 1891 (BM).

Argentina: Guanchin (prov. La Rioja), *S. Venturi* 8230, s.d. (BR); Cordoba, *P.G. Lorentz* 745, s.d. (BM); Cordoba, *Hieronymus* s.n., Sept. 10 and Sept. 19, 1877 (BR); La Plata, *A. Lefebvre* s.n., 1890 (BR); Buenos-Ayres, *Balansa* s.n., Dec. 1870 (BM) and Nov. 1875 (BR); 1) Montevideo, 2) Buenos-Ayres, *Commerson* s.n., s.d., (isotype of *A. filiculoides*) (P, BM); Buenos-Ayres, *J. Smith* s.n., s.d. (BM).

North America

USA: California: San Francisco, *Lenormand* s.n., s.d. (BR); San Bernardino Valley, *S.B. & W.F. Parish* 915, 1882 (BR); Lake Hemet, for. res. San Bernardino, *Evrard* 11686, 1993 (BR); California, *von Chamisso* s.n., s.d. (BM photo, BR) (isotype of *A. microphylla*), *Bolander* s.n., 1866 (BM), Yellow Creek valley, *R.M. Austin* s.n., June 1894 (BM), *J.C. Nevin* s.n., 1881 (BM); Aptos Santa Cruz Co., *Le Roy Abrams* 1833, Oct. 1902 (P). Carolina: in aquis Carolinae, *N. Michaux* s.n., s.d. (BR, P) (isotypes de *A. caroliniana*); Carolina, in Herb. *Desvaux* s.n., s.d. (holotype of *A. densa*) (P); Sardinia, Clarendon county, S. Carolina, *R.M. Tryon & R.K. Godfrey* 907, 1939 (BR, K). Florida: south of Perry, Taylor county, *R. K. Godfrey & R.D. Houk* 60474, 1960 (FLAS); Lake Monroe, *A. P. Garber* s.n., March 1876 (BM).

Europe

United Kingdom: S.W. England, *Hubbard* s.n., Jul. 1955 (K); S.E. England, *Mathew* s.n., Oct. 1915 (K); Essex, S. E. England, *Middleton* s.n., 1961 (K).

Netherlands: Canaux en Hollande, *Marsdo* s.n., Sept. 1900 (BR); Boskoop (S. Holland), *Pulle* in Herb. *van Ooststroom* 5951, Jul. 1932 (BM).

Belgium: Koksijde, *Magnel* s.n., Jul. 1916 (BR); Jabbeke, *Vanhecke* 3282, Jul. 1973 (BR); Gistel, *W. Robyns* s.n., Dec. 1927, 1928 (BR); Damme, *Van Hove* 4, Sept. 1977 (BR); Oostkamp, *Hostie* s.n., Jul. 1931 (BR); Zwijnaarde, *Fr. Rogier* s.n., Sept. 1953 (BR); Afsné, *Vanden Berghen* s.n., Aug. 1948 (BR); Gent, *J. Lebrun* s.n., Jul. 1924 (BR), *Magnel* s.n., Aug. 1919 (BR); Gent-Drongen, *Magnel* s.n., Aug. 1923 (BR); Harchies, *Fr. Macédone* s.n., Sept. 1955 (BR), 350, Apr. 1961 (BR), *Fr. Macédone & Buxant* s.n., Sept. 1955 (BR), *Sotiaux* s.n., Oct. 1978 (BR); Bauche, *Lefèvre* s.n., Oct. 1912 (BR).

France: Cherbourg (Manche), *Corbière* s.n., Jul. 1886 (BM), *Corbière* s.n., Aug. 1890 (BR); Noyelles/s/Mer (Somme), *Perin* s.n., Jul. 1910 (BR), *Bécourt* s.n., Jul. 1923 (BR); Royan (Charente inf.), *Duffort* s.n., Aug. 1886 (BR); Drusenheim (Bas-Rhin), *Walter* 153, Aug. 1938 (BR); Mus. Hist. Nat. Paris, *Delacour* s.n., Aug. 1893 (BM, BR); Niort au Vivier (Deux-Sèvres), *de Litardière* s.n., June 1900 (P); Grande Brière (Loire inf.), *Gadeceau* s.n., Aug.1907 (BM); Ponts-de-Lé (Maine et Loire) *Thériot* s.n., Aug. 1888 (BR); Viaduc de Guétrin (Cher), *Prudhomme* s.n., Oct. 1948 (BR); Batz (Loire inf.), *Houdaille* s.n., Aug. 1914 (BR); Germigny (Nièvre), *Loiseau* s.n., Sept. 1972 (BM); Limoges (Hte Vienne), *Héribaud* s.n., Jul. 1893 (BM); Clermond-Ferrand (Puy-de-Dôme), *Malinvaud & Héribaud* s.n., May 1889 (BR); Coutras (Gironde), *Cassat* s.n., Jan. 1901 (BR); Bordeaux (Gironde), *Neyraut* in herb. *Magnier* s.n., 1890-91 (BM, BR), *Jeanjean & Bouchon* s.n., June 1926 (BR), *Bouchon & Tempère* s.n., Jul. 1947 (BR), *Clavaud* 749, Dec. 1883 (BR); Argelès/s/Mer (Pyr. Orient.) *Conill* 454, Aug. 1906 (BR).

Germany: Nürnberg, *Kaulfuss* s.n., Oct. 1903 in herb. *Wirtgen* 441 (BM, BR); *Semler* s.n., Sept. 1906 (BM); Regnitz, Bezirk Erlangen, *Poeverlein* s.n., Oct. 1899 (BR).

Portugal: Ilhavo (Beira), *Matos & al.* 4766, May 1954 (BR).

Cultivated in the *Azolla* collection of the Catholic University of Louvain: origin: Dijon Botanical Garden, *Van Hove* 3, Jun. 1986 (BR); origin: Colombia, *Van Hove* 39, Sept. 1984 (BR); origin: Hangzhou, China, originally from Germany, *Van Hove* 173, 1985 (BR).

Azolla cristata Kaulf., Enum. Fil.: 274 (1824). – Type: Demerary, sine col., s.n., s.d. (in herb. Kaulf. M holo-? n.v.; BM , BR iso-). See note 1.

Azolla caroliniana auct. non Willd. See note 2.

Azolla microphylla auct. non Kaulf. See note 3.

Azolla portoricensis Spreng., Syst. Veg. 1: 9 (1827). — Type: Portorico, *Bertero*, s.n., s.d. (in herb. Sprengel and Mettenius, B holo-, n.v.). See note 4.

Azolla mexicana K. Presl, Abh. Böhm. Ges. Wiss. V, 3: 150 (1845). — Type: "Habitat in Mexico ubi legit clar. *Schiede*. Inter Serpillo et Estero" (BM iso-; PRU holo-, n.v.). See note 5.

Diagnostic characters: leaf trichomes bicellular sometimes tricellular; glochidia mostly 2-pluriseptate; megaspore perine variously granular.

Notes. (1). The Kaulfuss holotype has not been located. The BR isotype is a fragment of the Kaulfuss herbarium, supplied by Roemer from the Martius herbarium. The BM isotype is the original specimen: Demerary, *C.I. Parker* 255, s.d., which has been duplicated for Kaulfuss. The sterile material is characterized by bicellular leaf trichomes.

(2). Most authors have confused *A. caroliniana* with *A. cristata*. The origin of this confusion is probably attributable to Mettenius (1847), whose description and illustrations of *A. caroliniana* are based on a specimen from Mexico, more than likely the type of *A. mexicana*, and not on the type of Michaux. This paper has been considered as the reference by the subsequent authors, including Mettenius (1867).

(3). Most authors have also confused *A. microphylla* with A. *cristata*. The first descriptions and illustrations of Martius (1827) and of Mettenius (1847) are indeed probably based on the specimen of Pöppig, collected in Cuba, which belongs to *A. cristata*, and not on the Chamisso type. The subsequent authors have maintained this confusion.

(4). Since Mettenius (1847) this species has been unanimously considered a synonym of *A. cristata* (*sub A. caroliniana* auct. non Willd.).

(5). The specimen from BM is sterile, characterized by bicellular leaf trichomes. Mettenius (1847) and di Fulvio (1956) have presented illustrations of glochidia and perines from material collected by Schiede in Mexico.

Specimens examined

South America

Guyana: Demerary, *C. J.Parker* 255, s.d. (BM, BR) (isotypes of *A. cristata*); near Georgetown, *Jenman* 22/2, Aug. 1886 (BM).
Surinam: Paramaribo, *Wullschlägel* 661, 1852 (BR).
French Guyana: Guyana gallica, in Herb. *Kunze* s.n., s.d. (BR).
Ecuador: in littore Mari Pacifici, *Spruce* 6549, s.d. (BM).
Bolivia: San Ignacio-Florida (prov. Santa Cruz), *Evrard* 8372, 1977 (BR).
Brasil: Maranhao, Santa Lucia (N.E.Brasil), *Silva & al.* 1050B, 1983 (BM); Flumen Ilheos, prov. Bahia, *de Neuwied* s.n., Dec. 1816 (BR); Prov. Minhas Geraes, *A. F. Regnell III* 1507, 1869 (BR); Rio de Janeiro, *Glaziou* 2442, 1868 (BR), *Luschnatt* s.n., June 1833 (BR); sine loco, *Glaziou* 14416, 1889 (BR).
Paraguay: Nueva Asuncion, *Billiet & Jadin* 3229, May 1984 (BR); prope Ingavi, *Hassler* 1422, s.d. (K); Asuncion, *Balansa* 1121, Sept. 1875 (K, BM, BR); Rio Pilcomayo, *Kerr* s.n., 1890-91 (K).
Argentina: Dpt. S. Fernando, prov. Chaco, *Renvoize & al.* 3588, Nov. 1878 (K); Dpt. Mburucuya, prov. Corrientes, *J. M. Petersen* 1357, Nov. 1951 (BR); Reconquista-Resistencia, prov. Corrientes, *Evrard* 8052 bis, Oct. 1977 (BR).

Central America

Honduras: Dpt. El Paraiso, *A. Molina* 3962, s.d. (BM).
San Domingo: Cochoa, prov. Barahoma, *M. Fuertes* 913 b, Aug. 1911 (BM).
Cuba: Las Piedras, *Pöppig* s.n., Apr. 1824 (BM, BR); sine loco, *C. Wright* 1797, 1860-64 (BM).

North America

Canada: British Columbia, *Sweet & Sweet* s.n., Oct. 1968 (K).
USA: Oregon, sine loco, *E. Hall* 628, 1871 (BM); Wisconsin, Confl. Wisconsin-Mississipi, *Nuttal* s.n., s.d., sent Jul. 1915 (BM); Illinois, Savanna, *M. B. Waite* s.n., Oct. 1887 (BM); Missouri, Wabashi, *de Neuwied* s.n., 1832 (BR).
Mexico: Inter Serpillo et Estero, *Schiede* s.n., s.d. (BM) (isotype of *A. mexicana*).

Europe

Netherlands: Oostgeest (S. Holland), *Suringar* s.n., Sept. 1897 (BM).
Cultivated in the *Azolla* collection of the Catholic University of Louvain: origin: Univ. Davis collection, California, *Van Hove* 70, May 1984 (BR); origin: Galapagos, *Van Hove* 69, Jul. 1984 (BR); origin: Kogoni, Mali, originally from Brazil, *Van Hove* 195, Jan. 1986 (BR); origin: Mwera, Zanzibar, originally from Paraguay, *Van Hove* 175, May 1986 (BR).

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