

TAXONOMY OF THE GENUS *AZOLLA* LAM. IN PORTUGAL

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The Portuguese and European floras refer the presence of two or three *Azolla* species in Portugal: *A. caroliniana* and/or *A. mexicana* and *A. filiculoides*. According to recent observations in light and scanning electron microscopes, the taxonomy of *Azolla* in Portugal is reviewed using the two most important characters that distinguish the several *Azolla* species: papillae of the dorsal leaf lobes and the perine of the megaspore. We also observed other characters such as the glochidia septa, the shape of sporophyte and the number of hyaline border cells, which are considered as accessory ones. From our observations, all of the *Azolla* specimens collected in Portugal have the characters of *A. filiculoides*: unicellular papillae and perine with raised hexagonal marks tied by the ends. In conclusion, we believe that there is only an *Azolla* species in Portugal, which is *Azolla filiculoides* Lam.

Key words: *Azolla filiculoides* Lam., papillae, megaspore perine, taxonomy.

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As Floras Portuguesas e Europeias referem a existência de duas ou três espécies de *Azolla* em Portugal: *A. caroliniana* e/ou *A. mexicana* e *A. filiculoides*. De acordo com recentes observações efectuadas em microscopia óptica e microscopia electrónica de varrimento, a taxonomia do género *Azolla* em Portugal é revista tendo em consideração os dois caracteres mais importantes que permitem distinguir as várias espécies: as papilas dos lobos dorsais e a perina do macrósporo. Também se observaram outros caracteres, considerados

secundários para o processo classificativo das espécies de *Azolla*, tais como o número de septos nos gloquídeos, a forma do esporófito e o número de células da margem hialina da folha. Todos os espécimes de *Azolla* recolhidos nos vários locais de Portugal apresentam as características identificativas de *A. filiculoides* como as papilas unicelulares e perina com elevações hexagonais ligadas pelas extremidades. Em conclusão, todos estes dados indicam a presença em Portugal de apenas uma só espécie de *Azolla*, que é *Azolla filiculoides* Lam.

Palavras chave: *Azolla filiculoides* Lam., papilas, macrósporo, perina, taxonomia.

INTRODUCTION

Azolla Lam. is a small heterosporic aquatic fern with deeply bilobed leaves: the ventral lobe, very thin and without chlorophyll and the dorsal lobe, aerial and with chlorophyll. Each dorsal lobe has an extracellular cavity in which exists a population of the cyanobacteria *Anabaena azollae* Strasb. able to fix nitrogen, associated with several bacterial strains (WAGNER, 1997). This fern is widespread throughout the world and became naturalised wherever introduced..

For many years *Azolla* was classified as a Salviniaceae, but at present it is considered an Azollaceae with only one genus – *Azolla*. The genus is divided in two subgenus: *Azolla* with two sections – *Azolla* and *Rhizosperma* – and subgenus *Tetrasporocarpia* (SAUNDERS & FOWLER, 1993). In Portugal, according to several works, there are two or three *Azolla* species: *A. filiculoides* Lam., *A. caroliniana* Willd. and/or *A. mexicana* Presl (FLORA IBERICA, 1986; FLORA EUROPAEA, 1964 & 1993; FRANCO, 1971; VASCONCELLOS, 1968).

The taxonomy of *Azolla* is based primarily on vegetative characters such as plant dimension, leaf characters (shape and hyaline border of the dorsal leaves lobes) and on the sexual characters such as the number of massulae and microsporangia (SVENSON, 1944). Since these characters are highly variable, this may lead to misinterpretations. Current species delimitation is based on features of the megaspore apparatus, especially the architecture of the perine megaspore wall (PERKINS *et al.*, 1985; PETERS & MEEKS, 1989), which is a more reliable character.

In this work we used and observed the two most important characters – papillae of the dorsal leaves lobes and the ornamentation of megaspore perine – to identify the *Azolla* specimens collected in several places in Portugal.

MATERIAL AND METHODS

Plant material. Fresh *Azolla* specimens with sporocarps were collected from six different sites in Portugal between 1992 and 1998 (Table 1). Two voucher specimens were deposited in the Herbarium of Lisbon Botanical Garden (LISU).

Light Microscopy (LM). After dissociation or cross section, the dorsal leaves lobes and glochidia were observed and photographed in a Leitz Wetzlar Dialux and Nikon Labophot2 light microscope.

Scanning Electron Microscopy (SEM). Sporophyte and sporocarps were fixed in 3% glutaraldehyde in 0,1 M sodium cacodylate buffer, pH 7,2 for 2 h at 4°C. The material were rinsed thoroughly in the same buffer and post-fixed with 2% osmium tetroxide for 1 h. After dehydration in a graded ethanol series, the material was dried by the critical point drying method in a Polaron E3500. Dried specimens were sputter coated with gold in a Polaron E5350 and the observations were made at 15-20 kV using a JEOL JSM 5200 LV or JEOL JSM T220 scanning electron microscope.

Table 1 - *Azolla* specimens collected in Portugal used in our study and their identification.

Origin	Date	Collector	Voucher	Taxa
Adema	3/92	F. Carrapiço	/	<i>A. filiculoides</i>
Guadiana	3/93	F. Carrapiço	LISU 166489	<i>A. filiculoides</i>
Golegã	6/95	G. Teixeira	LISU 166486	<i>A. filiculoides</i>
Comporta (J.Bot.Lx)	7/97	G. Teixeira	/	<i>A. filiculoides</i>
Pancas (J.Bot.Lx)	8/97	A. L. Pereira	/	<i>A. filiculoides</i>
Nisa	3/98	M. Bastos	/	<i>A. filiculoides</i>

Legend : J. Bot. Lx – Jardim Botânico de Lisboa (plants kept in reservoirs); / - specimen not deposited in Herbarium

RESULTS AND DISCUSSION

Currently, in the taxonomy of the genus *Azolla* the shape of sporophyte, the number of hyaline border cells, the shape of glochidia and the number of glochidia septa are considered. The *Azolla* specimens collected in Portugal have an orbicular sporophyte (Fig. 1), typical of *A. filiculoides*. However when the plant density is higher the sporophyte shows a filiform shape and a vertical growth not similar to the normal prostate habit. In the dorsal lobe leaf the number of hyaline border cells varies between two and four. Many authors consider the number of glochidia septa as a valuable taxonomic character. However in our observations we found out that the glochidia apex could have no septa, one, two or three in the same massulae. This character is not very reliable to distinguish the *Azolla* species. In this sense our study only consider the papillae and the perine architecture as the main distinctive characters.

All *Azolla* specimens had unicellular papillae on the upper dorsal lobes (Fig. 2), *i.e.*, with one cell above the epidermal cells and the perine showing distinct raised hexagonal markings, sometimes tied by the ends and covered with few filaments (Fig. 3). In cross section all the specimens show a highly alveolated

endoperine in the raised area alternating with less alveolated endoperine in the depressions areas. In contrast, the exoperine is granular but is much thicker and denser in the depression areas than in the raised ones (Fig. 4). The surface and stratification of the megaspore cell wall are considered as distinct characters, which enable taxonomists to distinguish between *Azolla* species, and is considered like a fingerprint (PERKINS *et al.*, 1985). Our observations of perine surface match those made by PERKINS *et al.* (1985) and SVENSON (1944) for *A. filiculoides*.

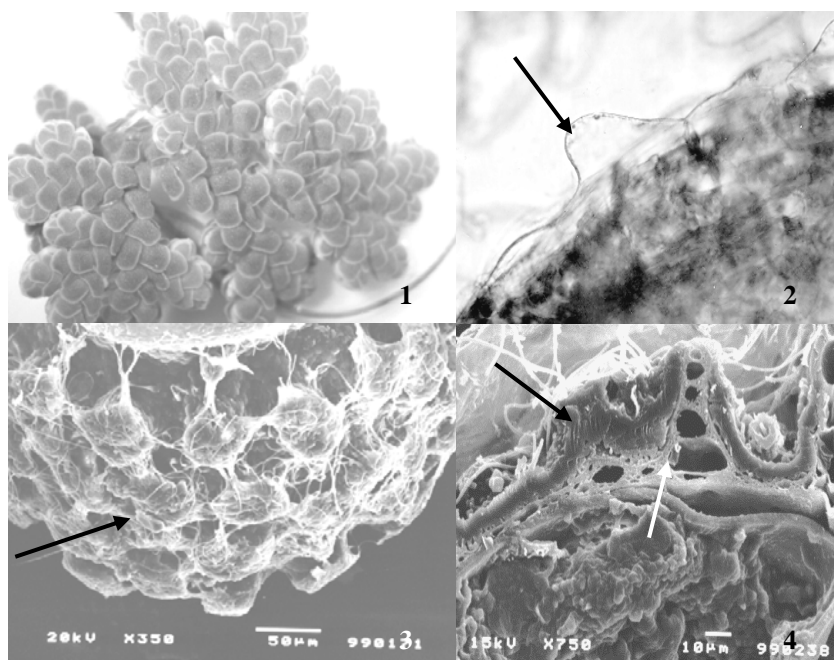


Figure 1 – Orbicular sporophyte of *Azolla filiculoides* Lam. Figure 2 – Unicellular papilae in the dorsal leaf lobe (arrow). Figure 3 – Surface of megaspore perine with hexagonal marks (arrow) tied by the ends. Figure 4 – Cross section of perine with highly alveolated endoperine (white arrow) and granular exoperine (black arrow).

A. caroliniana from Pancas, maintained and collected in the Botanical Garden of Lisbon is in fact *A. filiculoides* since it has the same characters as the other specimens. Probably the identification was made using the vegetative characters such as the shape of the sporophyte, which varies from region to region and lead to misinterpretations; the same applies to the number of glochidia septa. Also in a previous work the observation of *A.*

filiculoides and *A. caroliniana* from herbaria shows that they have all the typical characters of *A. filiculoides* (G. TEIXEIRA, pers. comm.; PEREIRA *et al.*, 1998).

The review in FLORA EUROPAEA (1993) does not mention an important character such as the perine ornamentation but states the presence of *A. mexicana* in Portugal. The main characters to distinguish the *Azolla* species are: *A. filiculoides* with unicellular papillae and distinct hexagonal markings in perine with few filaments; *A. caroliniana* with bicellular papillae and unpitted perine densely covered with filaments; and *A. mexicana* with bicellular papillae and pitted perine almost without filaments. The perine and papillae are reliable characters to distinguish between *A. filiculoides*, *A. caroliniana* and *A. mexicana*. In our opinion, the presence of *A. mexicana* in Portugal has no support from field and lab research data. The same can be applied to *Azolla caroliniana*.

All of these data question the taxonomy and the distribution of this aquatic fern in Portugal. In conclusion, we believe that there is only one *Azolla* species in Portugal, which is *Azolla filiculoides* Lam.

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