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The invasion of Doñana National Park (SW Spain) by the mosquito fern (Azolla filiculoides Lam)

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ABSTRACT

The invasion of Doñana National Park (SW Spain) by the mosquito fern (Azolla filiculoides Lam)

In 2001, Azolla filiculoides Lam., a floating pteridophyte native to the New World, was found in Doñana National Park (SW Spain), an European protected area well known for its high value from a conservation point of view and for its ecological vulnerability. Until that time, there had been no exotic aquatic macrophyte observations in the National Park. Since then, surfaces covered by Azolla filiculoides have increased explosively forming thick floating mats which eliminate submersed plants, and seriously threaten the aquatic ecosystems in this important protected area.

Key words: Azolla, invasive species, aquatic macrophytes, Doñana, SW Europe.

RESUMEN

La invasion del Parque Nacional de Doñana (SW España) por el helecho flotante (Azolla filiculoides Lam)

En 2001, Azolla filiculoides Lam., una pteridófita acuática nativa del Nuevo Mundo, fue encontrada en el Parque Nacional de Doñana (SW España), un espacio protegido europeo bien conocido por su alto valor desde el punto de vista de la conservación y por su vulnerabilidad ecológica. Hasta ese momento, no se había observado ningún macrófito acuático exótico en el Parque Nacional; desde entonces, las superficies cubiertas por Azolla filiculoides se han incrementado de manera explosiva originando gruesas capas flotantes que eliminan las plantas acuáticas sumergidas y amenazan gravemente los ecosistemas acuáticos de este importante espacio protegido.

Palabras clave: Azolla, especies invasoras, macrófitos acuáticos, Doñana, SW Europa.

INTRODUCTION

Azolla Lam. is a genus of floating heterosporous ferns, native to the New World and invasive in many parts of the world. It includes two species: A. filiculoides Lam. and A. cristata Kaulf. (Evrard & Van Hove, 2004). The first one, Azolla filiculoides, has recently been found in the Doñana National

Park (SW Spain), one of the most outstanding protected spaces in Europe, although its presence has been known for a number of years in other locations in the Iberian Peninsula.

This plant consists of a short branched rhizome (2-5 cm) covered by small, bilobated, alternated, and overlapping leaves and filiform roots, which hang into the water. *Azolla filiculoides* is

able to undergo a rapid vegetative reproduction through rhizome fragmentation. It is also able to reproduce sexually by producing spores.

Like any invasive plant, Azolla filiculoides, shows a very high growing rate. In this way, under ideal conditions, the daily rate of increase may exceed 15 % and doubling time of the fern may be four or five days (Lupkin & Plunket, 1982). In the same way, in the absence of natural enemies and the presence of eutrophic waters, it forms large and dense mats that affect aquatic ecosystems and have an impact on all aspects of water utilization (Van Wilgen et al., 2001). Like other floating plant, mats of Azolla, can affect the water beneath them, eliminating submerged plants and algae, preventing photosynthesis, and blocking oxygen diffusion from the air, causing it to become anaerobic (Gopal, 1987; Janes et al., 1996; Sheppard et al., 2006). In addition, Azolla forms a permanent heredity symbiosis with a nitrogen-fixing cyanobacteria: Anabaena azollae (Van Hove & Lejeune, 2002); this unusual property is the basis for its use in agriculture as a natural fertilizer (Moore, 1969; Wagner, 1997).

Problems caused by *Azolla filiculoides* have been refereed from several places: Africa (Hill, 1999; Hill & Cillier, 1999; Gratwicke & Marshall, 2001; Van Wielgen *et al.*, 2001), Portugal (Carrapiço *et al.*, 1998; Catarino *et al.*, 2001) and United Kingdom (Sculthorpe, 1967; Janes *et al.*, 1996; Sheppard *et al.*, 2006).

Mechanical and herbicide control have been utilised with different success. While mechanical control, removing plants has resulted in failure in larger infestations, it is possible to find many references about the success with herbicides such as: glyfosate, diquat or querosene mixing (Hill & Cilliers, 1999) besides, success in *Azolla* control using a weevil has been cited from South Africa (Hill, 1999; Hill & Cillier, 1999; Gassmann *et al.*, 2006).

The purpose of this note is to notify the invasion by this exotic aquatic fern of a great natural and valuable protected European space, and to present some considerations about the problems this invasive plant can cause in its native ecosystems and the difficulties that its control in a natural preserved area present.



Figure 1. Distribution of Azolla filiculoides Lam in the Iberian Peninsula. [data acquired from Pereira et al. (2001). Real Jardín Botánico (2005) and herbarium material deposited in the herbaria MA (Real Jardín Botánico Madrid), BC (University of Barcelona) and LISU (University of Lisbon)]. ■ First record in Iberian Peninsula. ▲ First Record in Spain. ● Other records. Distribución de Azolla filiculoides Lam. en la Península Ibérica [datos obtenidos de Pereira et al. (2001), Real Jardín Botánico (2005) y material de herbario depositados en los Herbarios MA (Real Jardín Botánico Madrid), BC (Universidad de Barcelona) y LISU (Universidad de Lisboa). ■ Primera recolección en la Península Ibérica. ▲ Primera recolección en España. ● Otras recolecciones.

AZOLLA IN THE IBERIAN PENINSULA

On the basis of a search in different Iberian herbariums, we have concluded that the first record of Azolla in the Iberian Peninsula comes from Portugal. It was collected in 1920 from the lower stretch of the river Sado¹, and soon thereafter, new populations appeared, particularly, in the lower stretches of the rivers Tejo, Sado, Mondego and in the medium stretch of the river Guadiana. In many cases Azolla was found near rice cultures. In Spain, the species' appearance was detected later (1957) and located at the mouth of the river Llobregat¹, NE Spain (also near rice fields). Now, Azolla has spread to many places (Fig. 1), but the majority of the sightings are in the western part of the country.

^{1.} See appendix: herbarium references.

The first recorded sighting of *Azolla* in southern Spain is very recent, in 2000, it was collected by Sanchez Gullón & Macías in a coastal locality from Huelva province.

About the identity of these plants, after a taxonomical controversy compiled by Evrad & Van Hove (2004), Portuguese and Spanish specialists (Salvo, 1990; Pereira *et al.*, 2001) have come to the conclusion that only *Azolla filiculoides* Lam. is in the Iberian Peninsula.

THE HISTORY OF AZOLLA IN DOÑANA

Doñana National Park, located in the SW of Spain, is an area of international importance for

the breeding, staging, and wintering of aquatic birds. It spans, about 65.000 Ha, on the Atlantic Coast in the Gulf of Cádiz, bordering the large estuary of the Guadalquivir River (Fig. 2) and consists of a vast complex coastal marshland in the floodplain of the lower Guadalquivir River, separated from the Atlantic Ocean by an extensive system of both active and stabilised dunes. For a more detailed description see Garcia Novo (1997) and Serrano *et al.* (2006).

The aquatic flora of Doñana National Park is very diverse, especially in the Mediterranean context, and exhibits large populations of interesting species (see García Murillo *et al.*, 2006). Until *Azolla filiculoides* was found, no aquatic exotic plant had ever been observed in Doñana

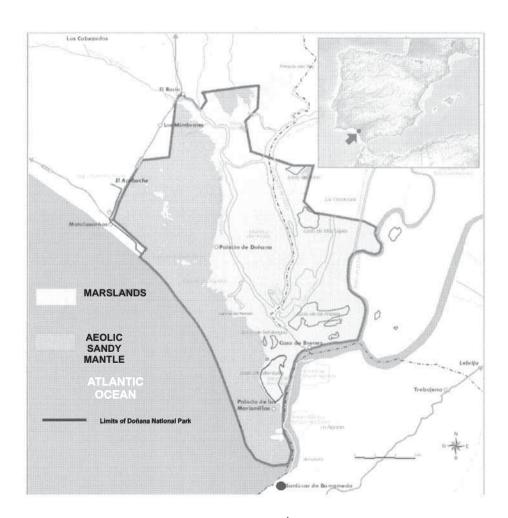


Figure 2. Study area in the south west of the Iberian Peninsula. Área de estudio en el suroeste de la Península Ibérica.

National Park. You can find more details about Doñana aquatic flora and vegetation in: Castroviejo *et al.* (1980), Rivas Martínez *et al.* (1980), García Murillo *et al.* (1993), Espinar *et al.* (2002) and Cobo *et al.* (2003).

In 2001, technicians from Doñana National Park identified some small floating plants that appeared in the marshland of the Park, as Azolla filiculoides Lam. The year before, these plants had been discovered by the Doñana's Biological Station staff, but they were not identified (García Murillo et al., 2004a). Soon thereafter, this plant was found in every part of the marshland of Doñana National Park; from the freshwaters (in the northern part of the park, near Guadiamar stream, further south, near Doñana's Palace) to the saline waters (in the "Lucio Del Membrillo" or near "Casa de Brenes"). Places hidden and isolated as "Laguna Del Hondón", where the only Spanish population of Hydrocharis morsusranae lives (Garcia Murillo et al., 2000), were soon occupied by Azolla (Fig. 2 and 3). Azolla grew with success even in waters with an extremely high level of salinity, where Lemna minor L. can't survive. In 2003, it was possible to observe a dense mat of Azolla (more than 10 cm thicker) covering the water surface in the places where the wind's influence was minor. The mats were easily seen from the plane that the Biological Station uses to take birds census. In May 2004, it had crossed the natural border of the Guadalquivir River and we also found mats of Azolla in some channels near Sanlúcar de Barrameda, Cádiz province, (Fig. 2).

The majority of the Doñana's ecosystems that have been invaded by *Azolla* are rich in nutrients and have the aquatic vegetation rooted to the ground. According to our own field observations, *Azolla* was able to compete the best under the following conditions: when the water surface was unoccupied, when the wind was mild (as on the shoreline or in the neighbouring of helophytes), and when rivals didn't exist. In these cases, *Azolla* quickly grew and covered the water surface. Then it preyed upon the other autochthonous macrophytes, obstructing their growth and ultimately killing them. Without doubt, some severe changes

in water quality (dissolved oxygen decreases, changes in nutrients concentration, etc.) will be produced and, perhaps soon, *Azolla* will be the only macrophyte in the locality.

The staff of Doñana National Park has attempted mechanical control of *Azolla*'s invasion without success. Additionally, the Regional Environmental Ministry is very concerned about the situation, having included the *Azolla* problem in its "Invasive Exotic Species Control Plan" recently started.

DISCUSSION

Azolla filiculoides is an exotic aquatic plant in the Iberian Peninsula. Its presence in this territory began back in the first decades of the XXth century, appearing in the mouth of the Sado River (Portugal) and related to paddy fields. Since then it has spread through many localities from the north to the south of the Iberian Peninsula.

Like in other parts of the world, Iberian water bodies are modified by *Azolla* infestation. The heavy interlocking *Azolla* mat causes light-starvation in the water beneath and prevents weed seed emergence, seriously affecting biodiversity (McConnachie *et al.*, 2003). Its efficacy competing with other aquatic plants is so high that the use of *Azolla* as "natural" herbicide has been suggested in Asia (Moody & Janiya, 1992; Biswas *et al.*, 2005). Furthermore, *Azolla* mats increase saltation, reduce quality of waters, clog canals, and cause drawing of livestock that were unable to differentiate between pasture land and plant covered waters (Hill, 1999).

For these reasons, the recent appearance and rapid expansion of this plant in the wetlands of Doñana National Park is a worry to the people involved in the managing of this natural space. Doñana National Park has been almost completely free of problems caused by exotic plants for many years. The high degree of habitat conservation and Doñana's program for control of exotic plants (García Murillo *et al.*, 2004b), based on early detection and the quick removal of alien plants, have stopped them. However, *Azolla filiculoides* have resisted it. And the problem

has increased because the more effective control measurements refereed: chemical or biological, are undesirable and unpredictable in such a natural area, due to its consequences on other species and aquatic ecosystems.

On the other hand, the growing of *Azolla* is related to phosphorous enriched waters [McConnachie *et al.*, 2003; Arora & Saxena, 2005; and is supported by the results of our own *Azolla*'s laboratory and field experiments (Fernandez Zamudio *et al.*, in press)]. In this way we think that the quick expansion of *Azolla* is due to the nutrients' increasing in Doñana's marshland over the last decades (López *et al.*, 1993; Serrano *et al.*, 2006), as a consequence of agricultural developing in neighbouring areas (Fernández Delgado, 2006)

and high livestock pressure (Soriguer, 1999).

We are not far from reconstructing *Azolla*'s invasive process in Doñana, and we even have an ecological characterisation of its habitat, life cycle and phenology in this area. But, regarding control measurements, there does not seem to be a suitable solution. This case shows a complicate problem in a conservation subject area. How to manage a strong invasive species in a delicate protected and great conservation value area?

At the moment, we can only think about keeping the integrity of native ecosystems (keep few nutrients in the water, keep a low pressure of livestock, prevent eutrofization, etc.) as a better solution to avoid the spread of *Azolla filiculoides*' infestation through all water bodies of Doñana.



Figure 3. Cartography of the *Azolla filiculoides* invasión. ■ 2001 records, ■ 2002 records, ● 2003 records (adapted from García Murillo *et al.*, 2004). *Cartografía de la invasión de* Azolla filiculoides. ■ *Recolecciones de 2001*, ■ *Recolecciones de 2002*, ● *Recolecciones de 2003* (adaptado de García Murillo et al., 2004).

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REFERENCES

- ARORA, A. & S. SAXENA. 2005. Phosphorus requeriments of Azolla microphylla. *I. R. R. N.*, 30: 25-26
- BISWAS, M., S. PAR VEEN, H. SHIMOZAWA & N. NAKAGOSHI. 2005. Effects of Azolla species on weed emergence in a rice paddy ecosystem. *Weed Biology and Management*, 5: 176-183.
- CARRAPIÇO, F., M. L. COSTA, G. TEIXEIRA & M.V. BAIOA. 1998. A new evaluation of some Azolla blooms: The Guadiane Cases. *Proc.* 10th *EWRS Aquat. Weeds. Lisboa*. 249-252.
- CASTROVIEJO, S., E. VALDÉS-BERMEJO, S. RI-VAS MARTÍNEZ & M. COSTA. 1980. Novedades florísticas de Doñana. *Anales Jard. Bot. Madrid*, 36: 203-244.
- CATARINO, L., I. MOREIRA, T. FERREIRA & M.C. DUARTE. 2001. *Plantas aquaáticas infestantes de valas e canais*. Instituto Superior de Agronomia. Lisboa. 161 pp.
- COBO, M. D., E. SÁNCHEZ GULLÓN & P. GAR-CÍA MURILLO. 2002. Flora y Vegetación. In: *Parque Nacional de Doñana*: 109-174. Canseco Editores, Talavera de la Reina.
- ESPINAR, J. L., L. V. GARCÍA, P. GARCÍA MURI-LLO & J. TOJA. 2002. Submerged macrophyte zonation in a Mediterranean salt marsh: a facilitation effect from established helophytes? *Journal of Vegetation Science*, 13: 1-15.
- EVRARD, C. & C. VAN HOVE. 2004. Taxonomy of the American Azolla species(Azollaceae) a critical review. *Syst. Geogr. Pl.*, 74: 301-318.
- FERNÁNDEZ DELGADO, C. 2006. Conservation Management of an European Area: Doñana National Park, Spain. In: *Principles of Conservation Biology*. M.J. Groom, G.K. Meffe & C. Ronal

- Carroll (Eds.): 533-543. Sinauer Associates, Inc. Sunderland. USA.
- FERNÁNDEZ ZAMUDIO, R., S. CIRUJANO, J. M. L. ESPINAR, A. RUBIO OLMO, M. I. L. LÓPEZ BRAVO, M. D. COBO & P. GARCÍA MURILLO. *In press*. Datos preliminares sobre la biología y ecología de Azolla filiculoides en el Parque Nacional de Doñana. Actas 2º Congreso Nacional sobre Especies Exóticas Invasoras. León.
- GARCÍA MURILLO, P., M. BERNUÉS & C. MONTES. 1993. Los macrófitos del Parque Nacional de Doñana (SW de España). Aspectos florísticos. *Actas IV Congreso Español de Limnología*. Granada. España: 261-267.
- GARCÍA MURILLO, P., S. CIRUJANO, L. ME-DINA & A. SOUSA. 2000. ¿Se extinguirá Hydrocharis morsus-ranae L. en la Península Ibérica? *Portugaliae Acta Biol.*, 19: 149-158.
- GARCÍA MURILLO, P., M. D. COBO, E. SÁN-CHEZ GULLÓN & H. GARRIDO. 2004a. Una planta acuática americana invade Doñana. *Quercus*. 218: 46-47.
- GARCÍA MURILLO, P., M. D. COBO, E. SÁN-CHEZ GULLON, H. GARRIDO. 2004b. Plantas Exoticas e Invasoras en el Parque Nacional de Doñana. *Medio Ambiente*, 46: 47-53.
- GARCÍA MURILLO, P., R. FERNÁNDEZ ZAMU-DIO, S. CIRUJANO & A. SOUSA. 2006. Aquatic macrophytes in Doñana protected area (SW. Spain): an overview. *Limnetica*, 25: 71-80.
- GARCÍA NOVO, F. 1997. The ecosystems of Doñana National Park.- In: *The Ecology and Conservation of European Dunes*. García Novo, F, R. M. M. Crawford & M. C. Díaz Barradas (Eds.): 97-116. Universidad de Sevilla, Sevilla.
- GASSMANN, A., M. J. W. COCK, R. SHAW & H. C. EVANS. 2006. The potential for biological control of invasive aquatic weeds in Europe: a review. *Hydrobiologia*, 570: 217-222.
- GOPAL, B. 1987. *Water hyacinth*. Elsevier Science Publishers. Amsterdam. 471 pp.
- GRATWICKE, B. & B. E. MARSHALL. 2001. The impact of Azolla filiculoides Lam. on animal biodiversity in Zimbawe. *Afr. J. Ecol*, 39: 216-218.
- HILL, M. P. 1999. Biological control of red water fern Azolla filiculoides Lam. (Pteridophyte: Azollaceae) *African Entomol. Mem.*, 1: 119-124.
- HILL, M. P. & C. J. CILLIERS. 1999. Azolla filiculoides Lamarck (Pteridophyte: Azollaceae), its status in South Africa and control. *Hydrobiologia*, 415: 203-206.

- JANES, R. A., J. W. EATON & K. HARDWICK. 1996. The effects of floating Azolla filiculoides Lam. and Lemna minuta Kunth on the growth of submerged macrophytes. *Hydrobiologia*, 340: 23-26.
- LÓPEZ, T., J. ROMÁN & J. TOJA. 1993. Diatomeas de los sedimentos de las lagunas de santa Olalla y Dulce (P.N. Doñana). In: *Actas VI Congreso Español de Limnología*. L. Cruz-Pizarro, R. Morales-Baquero, P. Sánchez-Castillo & P. Carrillo (eds.): 291-298. Facultad de Ciencias, Granada.
- LUMPKIN, T. A. & D. L. PLUMKETT. 1982. Azolla as green manure: Use and Management in crop production. Westview Press. Boulder, Colorado. USA. 230 pp.
- McCONNACHIE, A. J., M. P. DE WIT, M. P. HILL & M. J. BYRNE. 2003. Economic evaluation of the successful biological control of Azolla filiculoides in South Africa. *Biol. Control*, 28, 25-32.
- MOODY, K. & J. D. JANIYA. 1992. The role of Azolla in weed control in rice. *Philipp. J. Weed Sci.*, 19: 79-102.
- MOORE, A. W. 1969. Azolla: biology and agronomic significance. *Bot Rev.*, 63: 1-26.
- PEREIRA, A. L., G. TEIXEIRA, I. SEVIANATE-PINTO, T. ANTUNES & F. CARRAPIÇO. 2001. Taxonomic re-evaluation of the Azolla genus in Portugal. *Plant Byosistematics*, 135: 285-294.
- REAL JARDÍN BOTÁNICO DE MADRID 2005. Proyecto Anthos. Sistema de información sobre plantas de España. Available from http://www. programanthos.org.
- RIVAS MARTINEZ, S., M. COSTA, S. CASTRO-VIEJO & E. VALDES. 1980. Vegetación de Doñana (Huelva, España). *Lazaroa*, 2, 5-190
- ROMERO, M. I., P. RAMIL, J. AMIGO, M. A. RODRÍGUEZ GUITIAN & M. RUBINOS. 2004. Notas sobre la flora de humedales del noroeste ibérico. *Bot. Complot.*, 28: 61-66.
- SALVO, E. 1990. Guía de los helechos de la Península Ibérica y Baleares. Pirámide SA. Madrid. 377 pp.
- SÁNCHEZ GULLÓN, E. & F. J. MACÍAS. 2000.

- Nuevos pteridófitos de la provincia de Huelva. I. *Acta Botánica Malacitana*, 25, 229-230.
- SCULTHORPE, C. D. 1967. *The Biology of Aquatic Vascular Plants*. Edward Arnold (Publishers) Ltd., London. 610 pp.
- SERRANO, L., M. REINA, G. MARTÍN, I. REYES, A. ARECHEDERRA, D. LEÓN & J. TOJA. 2006. The aquatic systems of Doñana (SW Spain): watersheds and frontiers. *Limnetica*, 25(1-2): 11-32.
- SHEPPARD, A. W., R. H. SHAW & R. SFORZA. 2006. Top 20 environmental weeds for classical biological control in Europe: a review of opportunities, regulations and other barriers to adoption. *Weed Res.*, 46: 93-117.
- SORIGUER, R. C., A. RODRÍGUEZ & L. DOMÍN-GUEZ. 2001. Análisis de la incidencia de los grandes herbívoros en la marisma y vera del Parque Nacional de Doñana. Parques Nacionales. Ministerio de Medio Ambiente. Madrid. 231 pp.
- VAN HOVE, C. & A. LEJEUNE. 2002. The Azolla-Anabaena simbiosis. *Biology and Environnment*. *Procc. Irish Acad.*, 102: 23-26.
- VAN WILGEN, B. W., D. M. RICHARDSON, D. C. LE MAITRE, C. MARAIS & D. MAGADLELA. 2001. The economic consequences of alien plant invasions: examples of impacts and approaches to sustainable management in South Africa. *Environment, Development and Sustainability*, 3: 145-168.
- WAGNER, G. M. 1997. Azolla: A review of its biology and utilization. *Bot. Rev.*, 63: 1-26.

APPENDIX. HERBARIUM REFERENCES

- PORTUGAL. Baixo Alentejo. Alcocer da Sal, arrozaes, V-1920, L. Fernandez (LISU 2786); Alcocer da Sal, V-1920, L. Fernandez (LISU 2783).
- SPAIN. Barcelona. Prat de Llobregat, in oryzetes et estany de l'Ulla, 16-IX-1957, A. & O. de Bolós,
 F. Masclans, A. López & M. Montferrer (BC 117173).