

Spore morphology of New Zealand *Azolla filiculoides* Lam. (Salviniaceae)

M. F. LARGE

J. E. BRAGGINS

Department of Botany
University of Auckland
Private Bag 92 019
Auckland, New Zealand

Abstract Sporocarps, megaspores, and microspores of *Azolla filiculoides* are described from fresh fertile material from the South Island of New Zealand. Size estimates are given along with light and scanning electron microscope photographs. A brief discussion of spore morphology, perine substructure, and leaf trichomes is given with regard to the taxonomy of *A. filiculoides* and *A. rubra*. Differences between these taxa with regard to sporoderm substructure and the presence of filaments on the collar of the megaspore support their separation but may be considered insufficient to justify maintaining taxonomic separation beyond varietal status for *rubra*.

Keywords *Azolla filiculoides*; *A. filiculoides* var *rubra*; *Azolla rubra*; Salviniaceae; megaspore; microspore; sporocarp; New Zealand

INTRODUCTION

The heterosporous genus *Azolla* Lam. is represented in New Zealand by two species: an adventive species *A. pinnata* R. Br. (1810), and a native taxon sometimes known as *A. rubra* R. Br. (e.g., Allan 1961) or *A. filiculoides* Lam. var *rubra* (R. Br.) Strasburger (1873) (e.g., Harris 1955). *A. rubra*, which is restricted in distribution to Australia and New Zealand, is usually reduced to synonymy with

the American *A. filiculoides* (e.g., Wakefield 1957; Smith 1966; Willis 1970; Chinnock 1978; Brownsey et al. 1985; Duncan & Isaac 1986; Brownsey & Smith-Dodsworth 1989). Both species are present in Australia and *A. filiculoides* has been collected fertile there (e.g., Fowler & Stennett-Willson 1978; Perkins et al. 1985). In New Zealand, *Azolla* is usually collected sterile. Consequently, there are few descriptions of microspores and no descriptions of megaspores based on New Zealand material. Harris (1955) records, as *A. filiculoides* var. *rubra*, fertile material from one sample (Harris number 3145, Wellington. Slide held by Botany Institute, Manaaki Whenua – Landcare Research, Christchurch) but describes and illustrates only microspores, while Large & Braggins (1991) were unable to obtain fertile material of either taxon.

Bates & Browne (1981) suggested that megaspore ornamentation may provide the only reliable means of identification of *Azolla* species. Consequently, there is a need for an accurate survey of the spore morphology from New Zealand taxa. Bates recently suggested (D. P. Whittier pers. comm.) that sporocarp formation in the American taxon may be related to a preceding cold winter or spring, consequently a search for fertile material of *A. filiculoides* was made in the far south of New Zealand. *A. pinnata* is currently present only in the northern North Island (Brownsey et al. 1985).

MATERIALS AND METHODS

Fertile material of New Zealand *A. filiculoides* was obtained from a site in the South Island (near Oamaru, Canterbury) in February 1992. A voucher specimen (D. J. Blanchon, 1992, AKU 23075) is held at the herbarium, Department of Botany, University of Auckland (AKU). Spore slides obtained from this specimen are held at CHR and AKU. Sterile samples of *A. filiculoides* were also examined from Hawaii (N. Harriman, 1979, AKU 23076), California (E. Lee, 1934, WELT P15342), and Chile (G. Loosez, 1928, WELT P15343).

The leaf epidermis and morphology of the leaf trichomes were studied under the light microscope. The contents of 20 megasporocarps and 20 microsporocarps were fixed in FAA and observed under light and scanning electron microscopy (preparation techniques are as described by Large & Braggins 1991). Samples were dried from 100% analytical grade Ethanol in a critical point dryer using liquid CO₂. These were then mounted onto aluminium stubs with M-Glue (Agar aids, Essex, England). For observation of the inner layers of the perine, spores were split with a blade before sputter coating with gold. Specimens were examined on a Philips 505 SEM.

RESULTS

Trichome hairs: Leaf trichomes, in both the fresh and herbarium specimens studied, are variable, being absent or barely discernible in specimens of both taxa. If discernible, trichomes are unicellular and project slightly from the lower epidermal layer of the leaf.

Sporocarps: Of the material collected, only 0.5–1.0% proved to be fertile. Microsporocarps and megasporocarps are green with a terminal, red pigmented, neck-like structure and are borne in pairs (Fig. 1; either as two mega-, two micro-, or one mega- and one microsporocarp) in the axis of the first leaf of a lateral branch. The large microsporocarps (c. 1–3 mm in diameter) are ovoid to rounded in shape, whereas the smaller megasporocarps are ellipsoid to flask shaped (≤ 1 mm in diameter). The sporocarp wall in both cases is thin and of two cell layers (except towards the neck-like apex where several cell layers may be present).

Microsporangia: Within the microsporocarp are numerous (c. 60, some of which may be aborted) microsporangia, each borne on a short stalk. These sporangia each contain c. 64 microspores, which are embedded in c. 5–6 massulae (Fig. 2A). The formation of these structures is discussed by Eames (1936) and in the review by Perkins et al. (1985). Each massula is rounded to ovoid with tubular outgrowths (glochidia). These (Fig. 2B) may be septate or a-septate and are usually terminated by anchor-shaped tips.

Microspores: Trilete occasionally alete, radially symmetrical, occasionally asymmetrical and

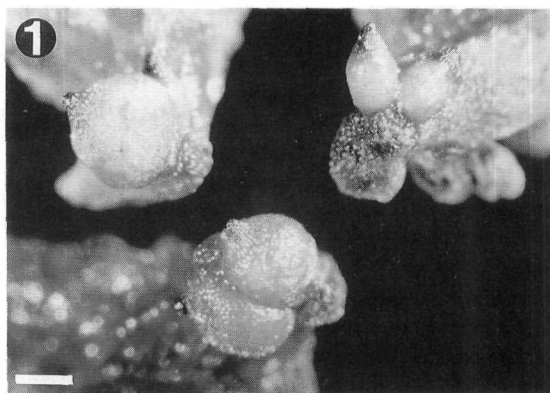


Fig. 1 Sporocarps of *Azolla filiculoides* from Oamaru, South Island of New Zealand (AKU 23075). Top left, microsporocarp and megasporocarp; top right, two megasporocarps; bottom, two microsporocarps. Scale 1 mm.

collapsed, homopolar; polar outline rounded; equatorial view, proximal face hemispherical, distal face hemispherical. Size, 10–30 μ m. Laesurae: reduced or as short branches extending about one-third to three-quarters the radius of the spore. Exine: c. 1.5–2.0 μ m thick, rugulate or smooth to granulate.

Megasporangia: Each megasporocarp contains one megasporangium with one basal megaspore. The morphology of this structure is described below. For a summary of its formation see Eames (1936) or Perkins et al. (1985).

Megaspores: If separated from the structures of the sporocarp and sporangium, the megaspores are irregular, trilete or occasionally alete, radially symmetrical, homopolar; polar outline rounded; equatorial view, proximal face hemispherical, distal face hemispherical. However, they are usually associated with a complex "episore" (see Tryon & Lugardon 1991, pp. 578–582), which covers the upper structure (Fig. 2C). Laesurae: absent or reduced as branches extending one-third to three-quarters the radius of the spore. Episore: (Fig. 2D) comprises c. 3 "floats", a collar, and filaments (which cover the perine ornamentation and the collar). A cap formed from the upper part of the indusium may cover the apex (over the floats). This structure can turn upwards as the floats expand. Size: float apparatus c. 200–300 μ m long; collar c. 80–100 μ m wide; spore c. 200–370 μ m in diameter. Perine: apparently papillate to reticulate under LM; reticulate under SEM (Fig. 2E); muri are thick (c.

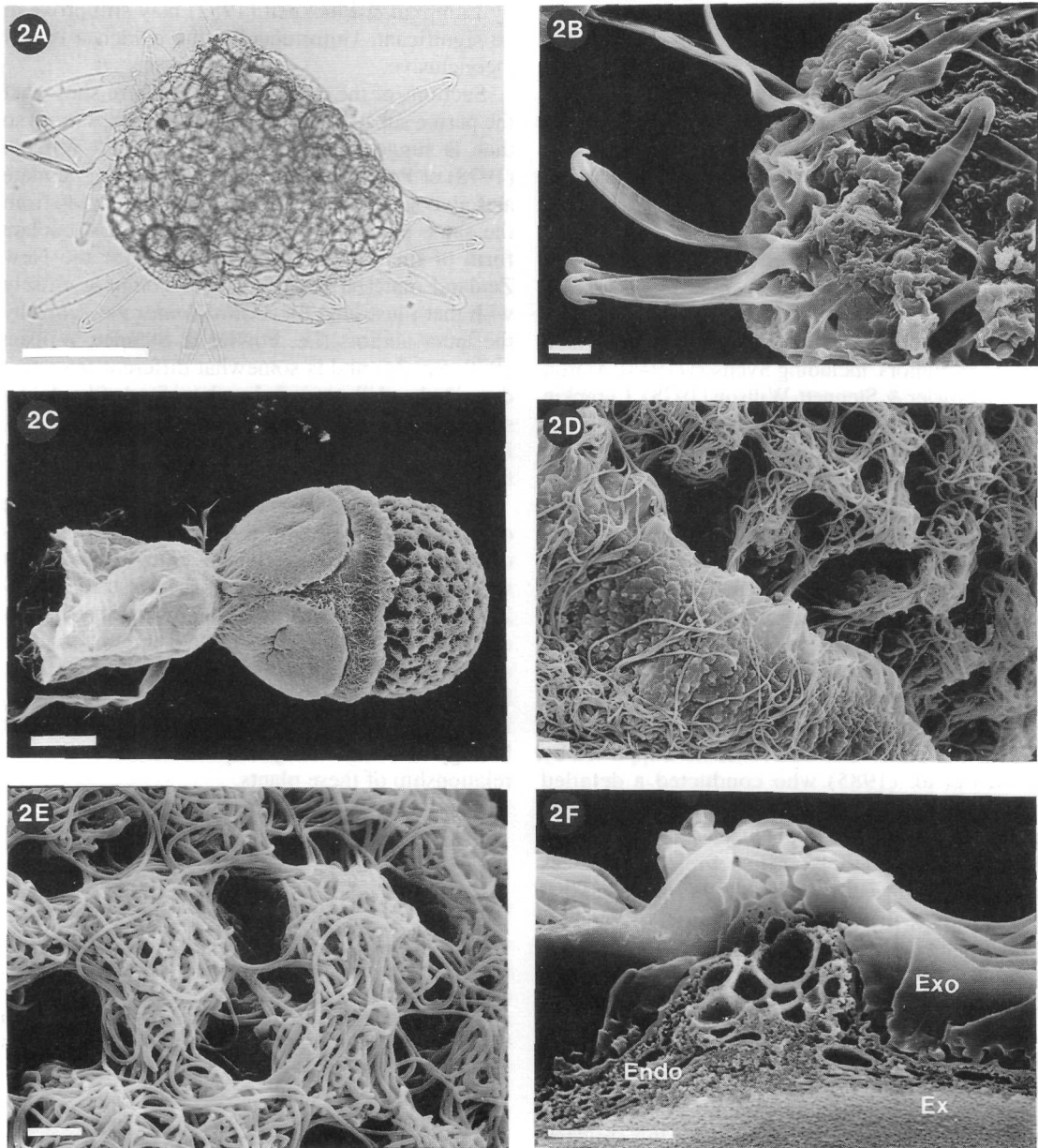


Fig. 2A–F *Azolla filiculoides* from Oamaru, South Island of New Zealand (AKU 23075). **2A**, Light micrograph, ovoid massula with tubular glochidia terminated by anchor-shaped tips, microspores are visible within. Scale 50 μ m. **2B**, Scanning electron micrograph, detail of glochidia and anchor-shaped tip, massula to the right. Scale 10 μ m. **2C**, Scanning electron micrograph, megaspore and episporium with “floats” above a collar and reticulate perine. A cap formed from the upper part of the indusium is visible turned up to the left. Scale 100 μ m. **2D**, Scanning electron micrograph, detail of collar (lower left) with filaments. The reticulate perine is visible on the upper right. Scale 10 μ m. **2E**, Scanning electron micrograph, detail of reticulate perine showing muri composed of fused excrescences. Scale 10 μ m. **2F**, Scanning electron micrograph, cross fracture of the distal perine below muri, showing the vacuolate nature of the endoperine (Exo, exoperine; Endo, endoperine; Ex, exine). Scale 10 μ m.

20–30 µm wide) and composed of fused excrescences, lacunae are small (c. 10–20 µm in diameter). Sporoderm: c. 20–30 µm thick across the raised muri reducing to c. 5–10 µm below the lacunae. Within the muri the perine is variable, occasionally granulate or alveolate (vacuolate) with c. 3–10 alveolae c. 5–10 µm in diameter, the alveolae being irregular and scattered in pockets (Fig. 2F).

DISCUSSION

Spore morphology of *A. rubra* has been described by several authors, including Svenson (1944), Martin (1976), Fowler & Stennett-Willson (1978), Lumpkin & Plucknett (1982), and Perkins et al. (1985).

Martin (1976) noted that the only distinguishing feature for megaspores of this taxon was the presence of filaments on the collar (the collar of *A. filiculoides* sensu stricto is glabrous; see Tryon & Lugardon 1991, fig. 225.9). Lumpkin & Plucknett (1982) based recognition of *A. rubra* on both the extent of hairs on the collar and the morphology of leaf trichomes. Fowler & Stennett-Willson (1978) noted differences in endoperine form (*A. rubra* from N.S.W., Australia, as having granulate wall structure and *A. filiculoides* sensu stricto alveolate). The work of Fowler & Stennett-Willson (1978) is supported by Perkins et al. (1985) who conducted a detailed examination into seven taxa, including *A. rubra* (one sporulating population from Queensland, Australia). While hesitant about supporting or rejecting species status for *A. rubra*, the latter authors concluded that the differences in perine substructure (illustrated for *A. rubra* and *A. filiculoides* in their fig. 4D, 5B, respectively) were distinctive enough to justify further investigation of this taxon.

Results given here for New Zealand material (associated with the name *A. rubra*) support the conclusions about the collar, of Martin (1976) and Lumpkin & Plucknett (1982). In all specimens studied, of various degrees of maturity, this structure was densely covered in filaments.

The latter authors also indicate that although leaf trichomes of *A. filiculoides* and *A. rubra* were similar, those of *A. rubra* were in general more obvious, whereas those of *A. filiculoides* were barely discernible from the epidermis of the leaf. In this study the morphology of the leaf trichomes was found to be reduced or barely discernible in both taxa. As this lack of difference could be related to the comparison of relatively older herbarium material with fresh samples, the differences outlined

by Lumpkin & Plucknett (1982) may still prove to be significant. Unfortunately, the evidence is still inconclusive.

Sections of the megaspore sporoderm show that the perine substructure is variable, perhaps more so than is suggested by Fowler & Stennett-Willson (1978) or Perkins et al. (1985), with both granulate and alveolate/vacuolate forms seen in spores from the same plant. However, the alveolate/vacuolate form of the wall as described here for the New Zealand material (Fig. 2F) does compare closely with that illustrated for *A. filiculoides* var *rubra* by the latter authors (i.e. Fowler & Stennett-Willson 1978, fig. 3g) and is somewhat different from that described and illustrated elsewhere for *A. filiculoides* sensu stricto (e.g., Morbelli 1980, fig. 284, 285; Perkins et al. 1985, fig. 4D; Tryon & Lugardon 1991, fig. 225.14).

Whether the differences seen in the substructure of the perine along with the presence of filaments on the collar are sufficient cause to warrant separation, at species level, of the Australian/New Zealand taxon from *A. filiculoides* sensu stricto, is open to debate. With such a limited amount of fertile material studied, we, like Perkins et al. (1985), hesitate to support or reject the validity of this taxon. Further consideration of the cytology and molecular biology of the taxa may help to illuminate the true relationship of these plants.

ACKNOWLEDGMENTS

We should like to thank D. J. Blanchon and R. F. Beever for collection of the fertile material used in this study, and P. J. Brownsey for his assistance with the herbarium material. We should also like to thank D. P. Whittier for his aid with the American *Azolla*, and the reviewers for their helpful comments on the manuscript.

REFERENCES

- Allan, H. H. 1961: Flora of New Zealand. Vol. I. Wellington, Government Printer.
- Bates, V. M. Jun.; Browne, E. T. Jun. 1981: *Azolla filiculoides* new to the Southeastern United States. *American fern journal* 71: 33–34.
- Brownsey, P. J.; Smith-Dodsworth, J. C. 1989: New Zealand ferns and allied plants. Auckland, David Bateman.

- Brownsey, P. J.; Given, D. R.; Lovis, J. D. 1985: A revised classification of New Zealand pteridophytes with a synonymic checklist of species. *New Zealand journal of botany* 23: 431–489.
- Chinnoek, R. J. 1978: Pteridophyta. In: Jessop, J. P. ed. *Flora of South Australia* (1). 3rd ed. South Australia, Government Printer.
- Duncan, B. D.; Isaac, G. 1986: Ferns and allied plants of Victoria, Tasmania and South Australia. Melbourne, Melbourne University Press.
- Eames, A. J. 1936: Morphology of vascular plants. New York, McGraw Hill Book Co.
- Fowler, K.; Stennett-Willson, J. 1978: Sporoderm architecture in modern *Azolla*. *Fern gazette* 11: 405–412.
- Harris, W. F. 1955: A manual of the spores of New Zealand pteridophyta. *Department of Scientific and Industrial Research bulletin* 116. Wellington, Government Printer.
- Large, M. F.; Braggins, J. E. 1991: Spore atlas of New Zealand ferns and fern allies. Supplement to the New Zealand Journal of Botany. Wellington, SIR Publishing. 168 p.
- Lumpkin, T. A.; Plucknett, D. L. 1982: *Azolla* as a green manure: use and management in crop production. Boulder, Colorado, Westview Press.
- Martin, A. R. H. 1976: Some structures in *Azolla* megaspores and an anomalous form. *Review of palaeobotany and palynology* 21: 141–169.
- Morbelli, M. A. 1980: Morphologia de les Espores de Pteridophyta presentes en la region Fuego-Patagonia, Republica Argentina. *Opera lilloana* 28: 1–138.
- Perkins, S. K.; Peters, G. A.; Lumpkin, T. A.; Calvert, H. E. 1985: Scanning electron microscopy of perine architecture as a taxonomic tool in the genus *Azolla* Lamarck. *Scanning electron microscopy* 4: 1719–1734.
- Smith, G. G. 1966: A census of Pteridophyta of Western Australia. *Journal of the Royal Society of Western Australia* 49: 1–12.
- Svenson, H. K. 1944: The new world species of *Azolla*. *American fern journal* 34: 69–84.
- Tryon, A. F.; Lugardon, B. 1991: Spores of the Pteridophyta. New York, Springer-Verlag.
- Wakefield, N. A. 1957: The Tasmanian representatives of the Pteridophyta. *Papers and proceedings of the Royal Society of Tasmania* 91: 157–162.
- Willis, J. H. 1970: A handbook to the plants in Victoria I. Ferns, conifers and monocotyledons. 2nd ed. Melbourne, Melbourne University Press.