

**FLORAL ANATOMY AND SYSTEMATIC POSITION OF *DIPLARRHENA*
(*IRIDACEAE*): A NEW TRIBE *DIPLARRHENEAE***

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ABSTRACT - This paper describes floral anatomy and pollen morphology in *Diplarrhena latifolia* in relation to the systematic position of the genus, which is taxonomically isolated and unique in *Iridaceae* in having only two stamens. Although the loss of a stamen is autapomorphic for *Diplarrhena*, some characters, especially presence of septal nectaries and pollen sculpturing, indicate an association with *Nivenioideae*. However, since *Diplarrhena* lacks the main synapomorphy for *Nivenioideae*: inflorescences in binate rhipidia, and also lacks the repeated codon of the *rps4* gene found in the Nivenioid-Ixioid clade, its subfamilial classification is problematic, and a new monogeneric tribe *Diplarrheneae* is proposed.

KEY WORDS - *Iridaceae*, orbicules, pollen, septal nectaries, staminode, systematics

INTRODUCTION

Diplarrhena Labill. is a genus of two species from SE Australia and Tasmania. It is unique in *Iridaceae* in having only two stamens rather than the usual three; flowers are bilaterally symmetrical, and the anterior (lower) stamen is lacking (Cooke, 1986; Goldblatt, 1998).

Diplarrhena is taxonomically isolated within *Iridaceae*, and its systematic position has been much debated. From morphological characters, many previous authors (e.g. Pax, 1888; Diels, 1930; Hutchinson, 1934; Weimarck, 1940; Goldblatt, 1990) have grouped it with the Australian/south American genus *Libertia* Spreng. and other *Sisyrinchieae* (e.g. *Orthrosanthus* Sweet and *Sisyrinchium* Eckl.). Goldblatt (1990) placed it in *Iridoideae-Sisyrinchieae*, but later (1998) discussed a possible association with *Irideae* (*Iridoideae*). Leaf anatomy to some extent supports a link with *Libertia* and other *Sisyrinchieae*, as they have similar leaf margin structure, but differ in other features (Rudall, 1986; 1994; 1995). Williams *et al.* (1986), based on phenolic patterns, considered that *Diplarrhena* should be in *Irideae*, near *Dietes* Salisb. The three-gene molecular analysis of Reeves *et al.* (1997 and this volume) placed *Diplarrhena* as sister to the remaining *Iridoideae* (including *Irideae* and *Sisyrinchieae*), with *Isophysis* T.Moore sister to all other *Iridaceae*, and *Nivenioideae* plus *Ixiodeae* sister to *Diplarrhena* plus *Iridoideae*.

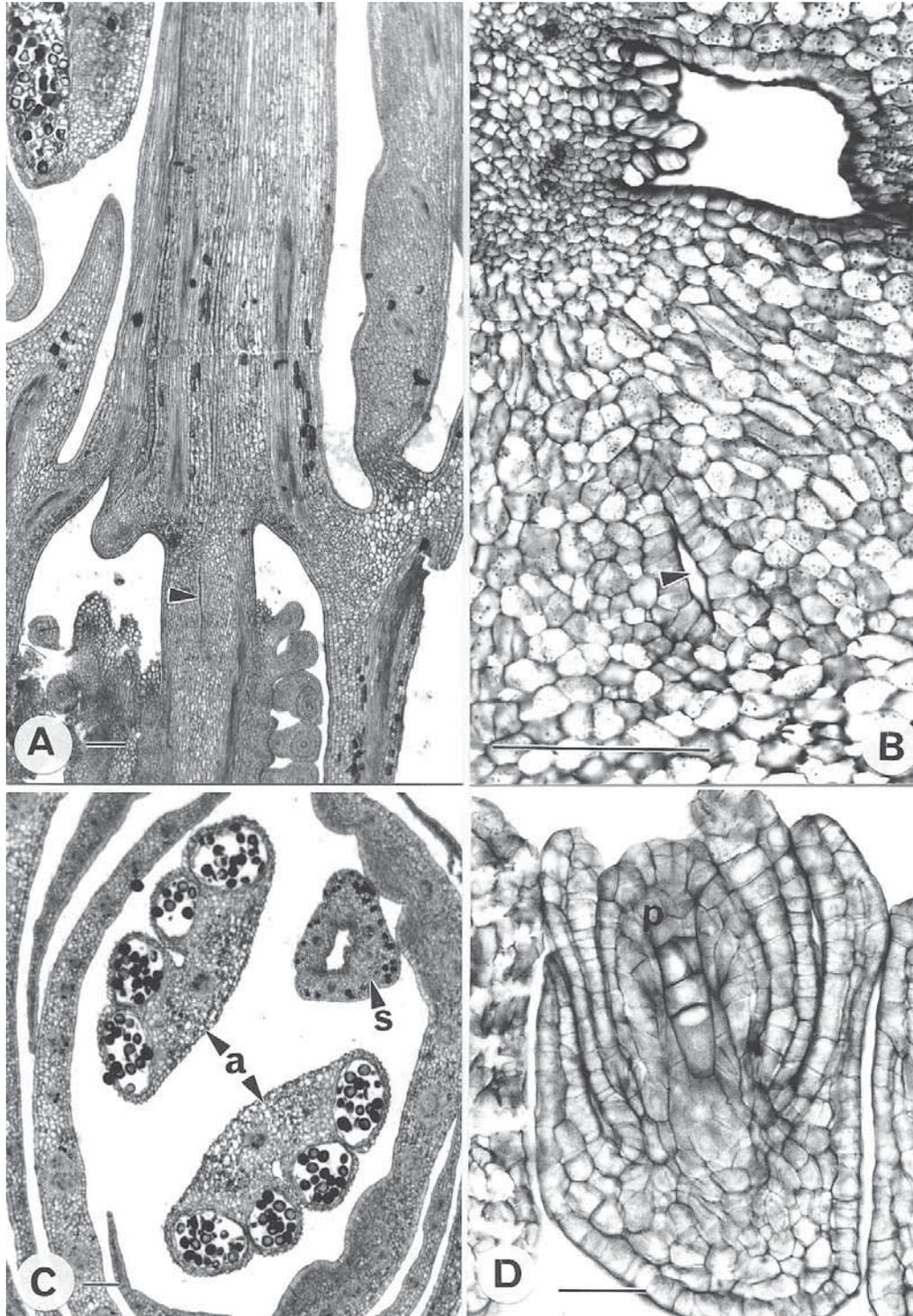


Figure 1 - *Diplarrhena latifolia*, flower sections (scale bars: 100 μ m). A: LS flower at top of ovary and base of style, with septal nectary arrowed; B: TS ovary, with septal nectary arrowed; C: TS upper part of flower through two fertile anthers (a) and style (s); D: TS ovule at tetrad stage, with two integuments and parietal tissue (p).

MATERIAL AND METHODS

Flowers of *Diplarrhena latifolia* Benth. were obtained from the living collections at the Royal Botanic Gardens, Kew (accession number: 1980-2283). They were fixed in FAA (formalin acetic alcohol) and stored in 70% ethanol before processing. Flowers were embedded in Paraplast using standard methods of wax embedding, and serially sectioned using a rotary microtome. Paraffin sections were stained with safranin O and Alcian blue 8GX, dehydrated through an alcohol series to 100% ethanol then HistoClear, mounted in Euparal, and examined using normal bright-field optics on a Leitz Dialux 20 photomicroscope. For SEM examination of pollen, flowers were critical-point-dried using a Balzers CPD 020, then anthers were dissected to expose the pollen, which was mounted onto SEM stubs on double-sided sellotape. Material was coated with platinum using a Emscope SC 500 sputter coater and examined using a Cambridge Instruments Stereoscan 240 SEM.

RESULTS

As in most *Iridaceae*, flowers of *Diplarrhena latifolia* have an inferior trilocular ovary with axile placentation and numerous ovules per locule (Fig. 1A). Ovules are bitegmic and tenuinucellate (Fig. 1D). There are three short septal nectaries at the top of the ovary, opening near the base of the style (Fig. 1A, B); these are not ‘septal slits’, which are open at one side for their entire length. The tepals and stamens are fused into a short tube, separating at about the same level (Fig. 2A). The perianth consists of two whorls of tepals (i.e. 3 + 3), an outer whorl of subequal, spreading, uniformly white tepals and an inner whorl of unequal tepals. The posterior tepal of the inner whorl is suberect and uniformly white but the two anterior (lower lateral) tepals of the inner whorl arch outward and are yellow marked with transverse dark streaks in the proximal half. The perianth is thus zygomorphic and more or less bilabiate, with a nectar guide on the lower lateral tepals. There are no perigonal nectaries.

The androecium consists of a highly reduced anterior staminode and two fertile stamens (Figs. 1C, 2A, B). The staminode diverges from the perianth slightly before the two fertile stamens, and lacks an anther. The two posterior stamens are unequal, one filament being slightly shorter than the other. The anthers are fixed obliquely on the filaments and the one on the shorter filament is slightly smaller than the other. The two stamen filaments and the staminode each have a single vascular bundle. The gynoecium is largely radially symmetrical, except that the style is distorted (swollen) on the side nearest to the staminode (Fig. 2B), presumably as a result of more space being available. The style has three vascular bundles for its entire length. The style divides above the anthers into three unequal stigmatic lobes, one much larger and obscuring the other two. The stigma lobes are ciliate and have lightly serrate margins.

The tapetum is secretory and there are numerous dark bodies (orbicules) in the anther locules, associated with the pollen grains (Fig. 3C). Pollen is inaperturate (Fig. 3A), although the exine thins to a smooth area on one face, which may be a germination zone (Goldblatt and Le Thomas, 1992a). The exine of *Diplarrhena* is almost vermiform (Fig. 3B), as in *Patersonia* (Fig. 3D); it was also described by Goldblatt and Le Thomas (1992a) as ‘bearing discrete pieces of sexine of more or less reticulate structure, lying fairly closely together’.

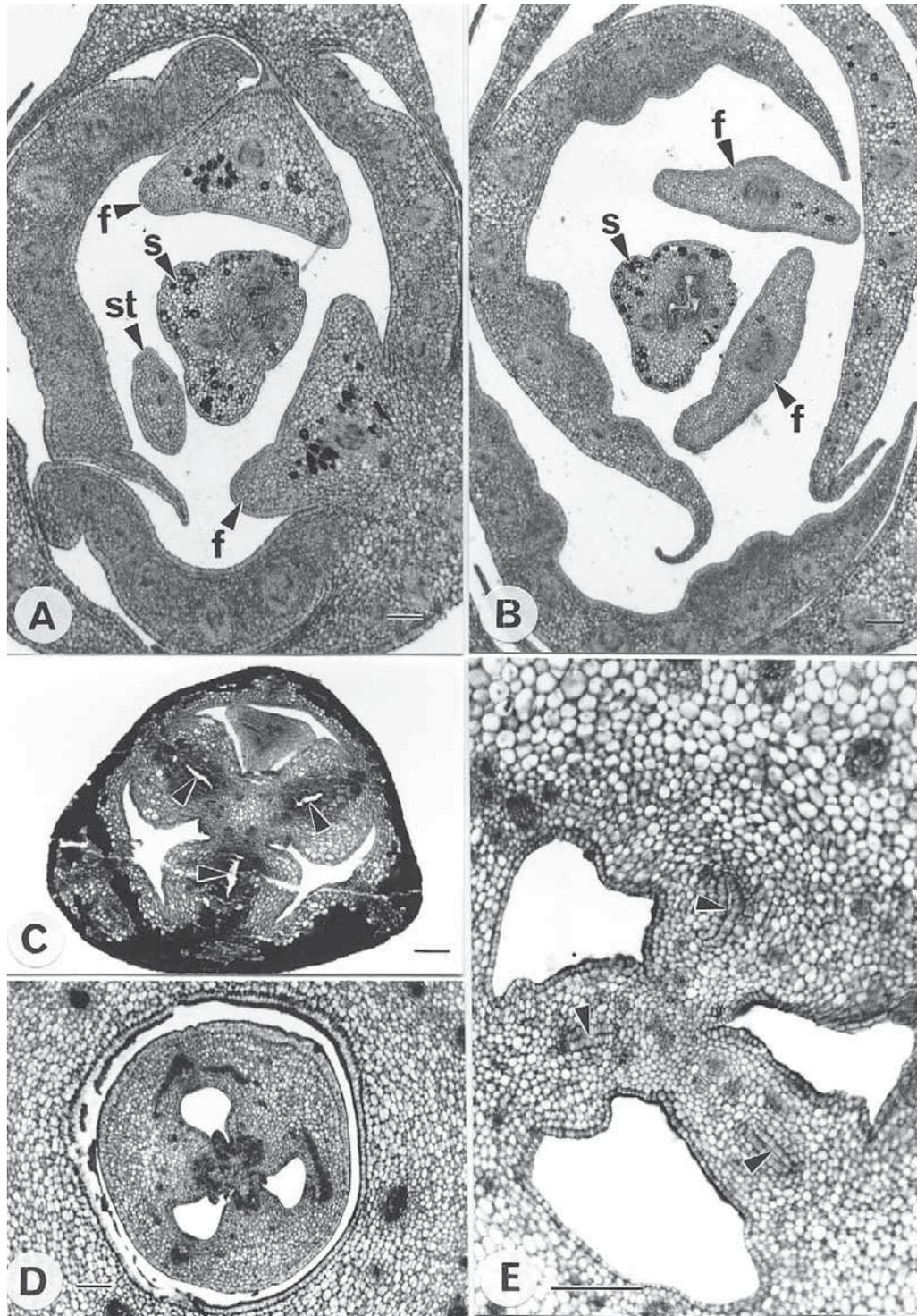


Figure 2 - Flower sections (scale bars: 100 μ m). A, B: *Diplarrhena latifolia*, TS flower near base of style and just above region of perianth fusion (f = filament, s = style, st = staminode); C: *Nivenia binata* Klatt (*Nivenioideae*), TS ovary, with septal nectaries arrowed; D: *Gelasine azureus* Herb. (*Iridoideae*), TS ovary, septal nectaries absent. E: *Crocus sieberi* Gay (*Ixioideae*), TS ovary, with septal nectaries arrowed.

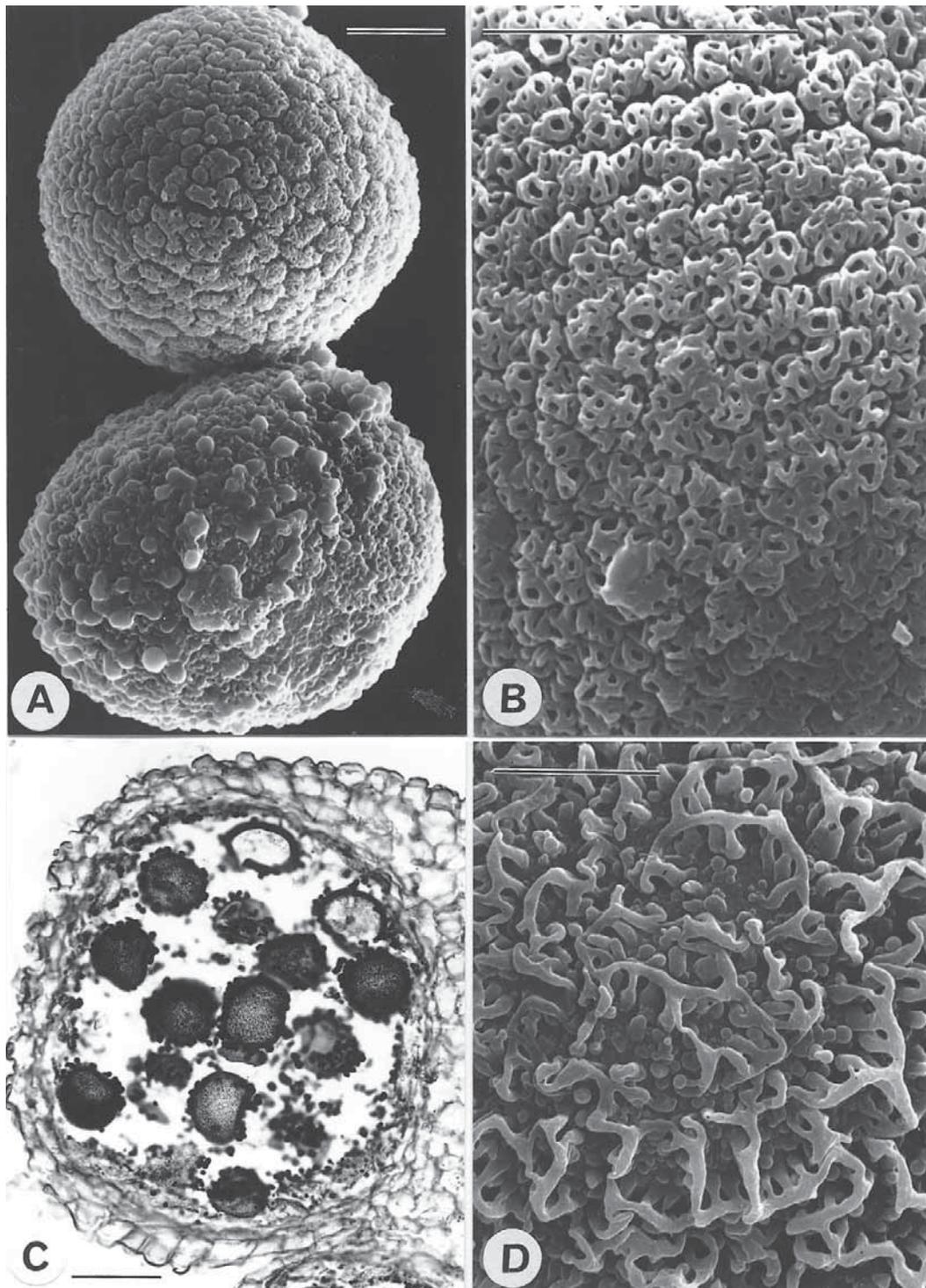


Figure 3 - Pollen (scale bars: in A, B, D = 10 μ m, in C = 100 μ m). A: *Diplarrhena latifolia*, inaperturate pollen grains (SEM), lower grain with orbicules attached; B: *Diplarrhena latifolia*, pollen surface (SEM); C: *Diplarrhena latifolia*, TS anther locule showing pollen grains with orbicules; D: *Patersonia* sp., pollen surface (SEM).

DISCUSSION

In general, floral and pollen characters in *Diplarrhena* indicate an association with *Nivenioideae*, although the loss of a stamen is autapomorphic for *Diplarrhena* within *Iridaceae* (loss of one or more stamens also occurs in the related family *Tecophilaeaceae*) and a secretory tapetum is almost ubiquitous in *Asparagales*, with the exception of *Hypoxidaceae* (Furness and Rudall, 1998). The zygomorphy of the perianth is homoplasious, many genera of *Ixioideae* also having a zygomorphic perianth and the condition may be ancestral for that subfamily (Goldblatt, this volume).

Septal nectaries occur in *Diplarrhena*, many *Nivenioideae* (including *Nivenia*: Fig. 2C, *Klattia*, *Witsenia* and *Patersonia* but not *Geosiris* or *Aristea*) and nearly all *Ixioideae* (e.g. *Crocus*, Fig. 2E), but apparently not in *Isophysis* (pers. obs.) or *Iridoideae* (e.g. *Orthrosanthus*, *Gelasine*, Fig. 2D). Septal nectaries are also absent from some taxa closely related to *Iridaceae*, such as some *Tecophilaeaceae* (Simpson and Rudall, 1998); in *Doryanthes*, although copious nectar is secreted, the precise location of the nectary is not clear (Newman, 1928 and pers. obs.). If the molecular topology (Reeves et al., 1997 and this volume) is correct, then there has either been a *de novo* development of septal nectaries in *Nivenioideae*/*Ixioideae* plus *Diplarrhena*, and a subsequent secondary loss in *Iridoideae* or (as seems more likely) a suppression of the character in *Isophysis*, *Aristea* and *Iridoideae*. The presence of septal nectaries in *Diplarrhena* suggests a close connection of *Diplarrhena* with *Nivenioideae*/*Ixioideae* and argues against its inclusion in *Iridoideae*. The latter subfamily either has perigonal nectaries or glandular, oil-secreting trichomes on the filaments and/or tepals. If, however, the presence of septal nectaries in *Iridaceae* is plesiomorphic, then the septal nectaries of *Diplarrhena* are phylogenetically uninformative.

Inaperturate pollen has been recorded in several other genera of *Iridaceae*, in *Ixioideae* (*Syringodea* and some *Crocus* species) and *Nivenioideae* (*Patersonia*), but rarely in *Iridoideae*, although possibly in *Iris*, which has diverse pollen morphology (Schulze, 1971; Goldblatt and Le Thomas, 1992a; Furness and Rudall, in press). Exine patterning in *Diplarrhena* differs from that of *Iridoideae* (Goldblatt and Le Thomas, 1992a), and somewhat resembles that of the Australian Nivenioid genus *Patersonia* (Figs. 2B, D). *Diplarrhena* is unusual in having orbicules (or Ubisch bodies) associated with pollen grains in the anther locule (Figs. 2A, C); these are composed of sporopollenin and derived from the tapetum; their production is normally associated with secretory tapeta (Huysmans *et al.*, 1998). Furness and Rudall (1998) recorded orbicules in a few other 'lower' asparagoids (*Lanaria*, *Cyanastrum* and *Dianella*) and some lilioids (*Tricyrtis* and *Lapageria*), but there is little information on this for other *Iridaceae*.

Despite these differences with *Iridoideae* and similarities with *Nivenioideae*, *Diplarrhena* does not match *Nivenioideae* in some other respects, notably inflorescence morphology. *Nivenioideae* always have binate rhipidia (monochasial cymes in two series, enclosed by the outer spathe of each unit) (Goldblatt, 1990). *Diplarrhena* also lacks the repeated codon of the *rps4* gene (Sousa-Chies *et al.*, 1997), found in the Nivenioid-Ixioid clade. The leaf margin anatomy of both *Diplarrhena* and *Libertia* (*Iridoideae* - *Sisyrinchieae*) consists of marginal veins rather than marginal sclerenchyma, and both genera have sclerenchyma girders at most veins, although in general *Diplarrhena* has much thicker, less ridged leaves than *Libertia*. This differs

from the typical condition in many other *Iridoideae* and *Nivenioideae* which have a strand of subepidermal sclerenchyma at each margin (Rudall, 1986; 1994; 1995). *Diplarrhena* also differs from *Nivenioideae* in that the root metaxylem vessels in all Nivenioid genera have only scalariform perforation plates, whereas in most other *Iridaceae*, including *Diplarrhena*, they are simple (Cheadle, 1963).

These differences make infrafamilial classification of *Diplarrhena* uncertain. The genus clearly has several morphological similarities with *Nivenioideae* but available molecular data exclude it from the subfamily, placing it at the base of the Iridoid clade with which it appears to share no apomorphic features, and differing especially in the style branches which are flat lobes, unlike the tubular style branches (sometimes with apical appendages) and terminal or subterminal stigma lobes typical of *Iridoideae*.

SYSTEMATICS

The taxonomic isolation of *Diplarrhena* and the uncertainty of its subfamilial position make it impossible to assign the genus to any of the tribes of the *Iridaceae* that are currently recognized. We propose describing a new tribe *Diplarrheneae* for the genus.

Diplarrheneae Goldblatt, tribus nov. Type: *Diplarrhena latifolia* Labill.

Herba rhizomatosa, sempervirens, foliis unifacialibus ensiformibus, caule tereti, floribus in rhipidio terminali fugacibus, tepalis inaequalibus liberis, staminibus fertilibus duobus, ovario infero, ramulis styli inaequalibus.

Evergreen herb with the rootstock and rhizome. Leaves unifacial and oriented edgewise to the stem, sword-shaped, forming a distichous fan, the blade margins not thickened with subepidermal sclerenchyma strands. Flowering stem aerial, more or less terete, usually branched, with inflorescences terminal. Inflorescence an umbellate, monochasial cyme (rhipidium) enclosed in a pair of leathery spathe-like bracts. Flowers fugaceous, several per rhipidium, borne sequentially from the spathes on slender pedicels, each flower except the first produced subtended by a 2-keeled, membranous, bifurcate bract; perianth of 3 tepals in 2 whorls, the tepals basally united with the filaments; stamens 3, free, the anterior a short, sterile staminode, the posterior 2 with unequal filaments and unequal, obliquely fixed, extrorse anthers. Ovary 3-locular, with axile placentation, with septal nectaries present in the upper half; style slender, dividing shortly beyond the anther apices, stigma lobes 2, unequal, broad and flat, ciliate, the margins lightly serrate. Seeds numerous, discoid. Basic chromosome number $x = 16$ or 8 ($2n = 32$) (Kenton and Heywood, 1984).

Comprising only *Diplarrhena*, with 2 species, restricted to SE Australia and Tasmania.

REFERENCES

- CHEADLE V.I., 1963 – *Vessels in Iridaceae*. *Phytomorphology* **13**: 245–248.
- COOKE D. 1986 – *Iridaceae*. In: *Flora of Australia* 46:1–66. Australian Government Publishing Service, Canberra.
- DIELS D., 1930 – *Iridaceae*. In: ENGLER A. and PRANTL K. (eds.). *Die Naturlichen Pflanzenfamilien*. 2nd ed. Pt. 15a. Engelmann, Leipzig. pp. 469–505.

- FURNESS C.A. and RUDALL, P.J., 1998 – *The tapetum in monocotyledons: structure and systematics*. Bot. Rev. **64**: 201–239.
- FURNESS C.A. and RUDALL P.J., 1999 – *Inaperturate pollen in monocotyledons*. Int. J. Pl. Sci.: in press.
- GOLDBLATT P., 1979 – *Preliminary cytology of Australasian Iridaceae*. Ann. Missouri Bot. Gard. **66**: 851–855.
- GOLDBLATT P., 1990 – *Phylogeny and systematics of Iridaceae*. Ann. Missouri Bot. Gard. **77**: 607–627.
- GOLDBLATT P., 2000 – *Phylogeny of the Iridaceae and the relationships of Iris*. Irises and Iridaceae: biodiversity & systematics. Proceedings of the International Iridaceae Conference (Rome, Italy, 1998) Ann. Bot. (Roma) **n. s. 58**:13–28.
- GOLDBLATT P. and LE THOMAS A., 1992a – *Pollen apertures, exine sculpturing and phylogeny in Iridaceae subfamily Iridoideae*. Rev. Palaeobot. Palynol. **75**: 301–15.
- GOLDBLATT P. and LE THOMAS A., 1992b – *Pollen morphology of Madagascan Aristeia and Geosiris (Iridaceae-Nivenioideae) in relation to systematics and phylogeny*. Adansonia **14**: 223–233.
- GOLDBLATT P., 1998 – *Iridaceae*. In: KUBITZKI K. (ed.). The families and genera of vascular plants. III. Flowering plants - Monocotyledons - *Liliana*. Springer-Verlag. pp. 295–333.
- HUTCHINSON J., 1934 – The families of flowering plants. Vol. 2. Oxford University Press, Oxford.
- HUYSMANS S., EL-GHAZALY G. and SMETS E., 1998 – *Orbicules in angiosperms: Morphology, function, distribution and relation with tapetum types*. Bot. Rev. **64**: 240–272.
- KENTON A. and HEYWOOD C., 1984. *Citological studies in South American Iridaceae*. Plant Syst. Evol. **146**:87-104.
- PAX F., 1888 – *Iridaceae*. In: ENGLER A. and PRANTL K. (eds.). Die Natürlichen Pflanzenfamilien. **2**: 137–157.
- REEVES G., CHASE M.W., DE CHIES T., COX A.V., GOLDBLATT P., LEJEUNE B., RUDALL P.J. and FAY M.F., 1997 – *Molecular systematics of Iridaceae: a combined analysis of three plastid DNA sequence matrices*. Amer. J. Bot. **84**: 225. [Abstract]
- REEVES G., GOLDBLATT P., RUDALL P.J. and CHASE M.W. 2000 – *Molecular systematics of Iridaceae: a combined analysis of four plastid DNA sequence matrices*. Irises and Iridaceae: biodiversity & systematics. Proceedings of the International Iridaceae Conference (Rome, Italy, 1998) Ann. Bot. (Roma) **n. s. 58**: 29-42.
- RUDALL P.J., 1986 – *Taxonomic significance of leaf anatomy in Australasian Iridaceae*. Nord. J. Bot. **6**: 277–289.
- RUDALL P.J., 1994 – *Anatomy and systematics of Iridaceae*. Bot. J. Linn. Soc. **114**: 1–21.
- RUDALL P.J., 1995 – Anatomy of the Monocotyledons. VIII. *Iridaceae*. Oxford University Press.
- SCHULZE W., 1971 – *Beiträge zur Pollenmorphologie der Iridaceae und ihre Bedeutung für die Taxonomie*. Feddes Repertorium **82**: 101–124.
- SIMPSON M.G. and RUDALL P.J., 1998 – *Tecophilaeaceae*. In: KUBITZKI K. (ed.). The families and genera of vascular plants. III. Flowering plants - Monocotyledons - *Liliana*. Springer-Verlag. pp. 429–436.
- SOUSA-CHIES T.T., BITTAR G., NADOT S., CARTER L., BESIN E. and LEJEUNE B., 1997 — *Phylogenetic analysis of Iridaceae with parsimony and distance methods using the plastid gene rps4*. Pl. Syst. Evol. **204**: 109–123.
- WEIMARCK H., 1940 – *Monograph of the genus Aristeia*. Lund. Univ. Arsskrift (N.F.) Avd. 2, **36**: 1.
- WILLIAMS C.A., HARBORNE J.B. and GOLDBLATT P., 1986 – *Correlations between phenolic patterns and tribal classification in the family Iridaceae*. Phytochemistry **25**: 2135–2154.