FLORAL ANATOMY AND SYSTEMATIC POSITION OF DIPLOARRHENA (IRIDACEAE): A NEW TRIBE DIPLOARRHENAE

P. J. RUDALL* and P. GOLDBLATT**

*Royal Botanic Gardens, Kew, Richmond, Surrey. TW9 3AB. UK
** B. A. Krukoff Curator of African Botany, Missouri Botanical Garden, P. O. Box 299, ST. LOUIS, MISSOURI 63166 U.S.A.

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 Ixioideae sister to Diplarrhena plus Iridoideae.
Figure 1 - *Diplarrhena latifolia*, flower sections (scale bars: 100μm). 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 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 stigmas 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: 100μm). 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 (Ixicoideae), TS ovary, with septal nectaries arrowed.
Figure 3 - Pollen (scale bars: in A, B, D = 10μm, in C = 100μm). 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 Aristeia) 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, Aristeia 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 Niveniid 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 sepal 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**


