Phylogenetic analysis of *Iridaceae* with parsimony and distance methods using the plastid gene *rps4*

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**Abstract:** A molecular phylogeny of the family *Iridaceae* based on the plastid gene *rps4* was obtained using both parsimony and distance methods. Thirty-four species were examined together with eight outgroup species. Results show that the *Iridaceae* are monophyletic, and that *Isophysis* is likely to be the earliest emerging genus. Subfamily *Ixioidae* plus the genera *Aristea* and *Nivenia* form a strongly supported clade. Within subfam. *Iridoideae,* the tribe *Irideae* includes the genus *Bobartia* (of disputed position), and the tribe *Mariceae* includes *Cypella.* The division of *Iridoideae* into tribes is consistent with their geographical distribution.


The plastid gene most commonly used for phylogenetic analyses in plants is *rbcL,* which encodes the large subunit of ribulose biphosphate carboxylase/oxygenase. More than 500 complete *rbcL* sequences are now available for land plants (*Chase* et al. 1993). Other chloroplast genes have also been used, such as *matK* (*Johnson & Solitis* 1994, *Steele & Vidalyn* 1994, *Johnson & Solitis* 1995), *atpB* (*Hoot* et al. 1995), *ndhF* (*Olmscheid & Reeves* 1995) and *rps* 4 (*Nadot* et al. 1994, 1995). Some non-coding regions of chloroplast DNA, such as the *atpB-rbcL* intergenic region (*Manen* et al. 1994), the *trnL* intron and the *trnL-trnf* spacer (*Taberlet* et al. 1991, *Gielly & Taberlet* 1994, *Ham* et al. 1994, *Mes & Hart* 1994) have been successfully used for evolutionary studies of closely related taxa. For the present work on *Iridaceae,* we chose to use the *rps* 4 gene (encoding protein 4 of the small chloroplastic ribosomal subunit) for its relatively small size (generally...
600 bp), its good sequence variation and its previously successful use in a phylogenetic reconstruction in the Poaceae (NADOT & al. 1994).

The Iridaceae are a medium-sized family of plants including 77 genera and 1750 extant species (GOLDBLATT, pers. comm.), mostly found in the Southern Hemisphere. Africa is the centre of diversity of the family, and the majority of species are concentrated in the temperate and Mediterranean regions in the Southern part of the continent (GOLDBLATT & al. 1995). The tropical and subtropical America is also a centre of Iridaceae. Some genera may have a markedly disjunct distribution in Oceania. Many genera of Iridaceae are economically important because of their ornamental value. DAHLGREN & al. (1985) classified Iridaceae into 5 subfamilies (Isophyoideae, Aristeoideae, Sisyrinchioideae, Iridoideae, and Ixioideae). The recent classification of Iridaceae by GOLDBLATT (1990) divides the family into only four different subfamilies (Isophsyoideae, Nivenioideae, Iridoideae, and Ixioideae). These classifications differ mainly in the position of Patersonia (subfam. Nivenioideae according to GOLDBLATT) and subfam. Sisyrinchioideae sensu DAHLGREN & al. (1985), and in the inclusion or not of the genus Geosiris in the family Iridaceae.

Some aspects of the phylogeny of Iridaceae are still disputed, such as the position of the genera Isophysis (DAHLGREN & al. 1985, GOLDBLATT 1990, RUDALL 1994, CHASE & al. 1995), Geosiris (CRONQUIST 1981, DAHLGREN & al. 1985, GOLDBLATT 1990) and Bobartia (GOLDBLATT & RUDALL 1992), the delimitation of the genera within the Iridoideae tribes Mariceae, Tigridieae, and Irideae (GOLDBLATT 1991) and the position of the subfam. Ixioideae within the Iridaceae (GOLDBLATT 1990, RUDALL 1994). Our goal was to contribute to the solution of some of these problems using the molecular information provided by the rps 4 sequences. The results are presented in this paper. In order to add information to the reconstructions obtained by a parsimony program (PAUP), we also used the new distance method Anataxis (BITTAR 1995), that we have already applied to a phylogeny of monocots (NADOT & al. 1995). In the discussion, the phylogenetic relationships in Iridaceae provided by this molecular approach are compared to other phylogenetic and biogeographic data on this family.

Material and methods

Plant material. Fresh leaves were collected from 34 species of Iridaceae listed in Table 1. In order to select the best set of outgroup species to reconstruct phylogenies, comparative tests were carried out using plants belonging to the Liliales sensu DAHLGREN (in which Iridaceae are included) and to the Asparagales, based on RUDALL’S (1994) suggestion that Iridaceae are closer to Asparagales than to Liliales. Representatives from all Liliales families except Iridaceae were included in this study. Asparagales encompass 33 families; samples from 12 of them were assayed. Comparative trials showed that much better resolution and robustness of trees was obtained with Asparagales than with Liliales, thus confirming suggestion by RUDALL (1994). Using this information, eight Asparagales species, listed in Table 1, were finally retained to constitute the outgroup species.

DNA isolation, amplification and sequencing. Total DNA was extracted by the CTAB method modified by DOYLE & DOYLE (1987). A fragment of approximately 800 bp including rps 4, a non-coding region and the trnS gene was amplified by PCR (Fig. 1). PCR primers (see below) were selected at the 5’ end of the rps 4 and at the at 5’ end of trnS conserved regions. The selection of the primer trnS was based on the comparison of the