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Phylogenetic relationships of *Discyphus scopulariae* (Orchidaceae, Cranichideae) inferred from plastid and nuclear DNA sequences: evidence supporting recognition of a new subtribe, Discyphinae

GERARDO A. SALAZAR[1], CÁSSIO VAN DEN BERG[2] & ALEX POPOVKIN[3]

[1]*Departamento de Botánica, Instituto de Biología, Universidad Nacional Autónoma de México, Apartado Postal 70-367, 04510 México, Distrito Federal, México; E-mail: gasc@ib.unam.mx*

[2]*Universidade Estadual de Feira de Santana, Departamento de Ciências Biológicas, Av. Transnordestina s.n., 44036-900, Feira de Santana, Bahia, Brazil*

[3]*Fazenda Rio do Negro, Entre Rios, Bahia, Brazil*

Abstract

The monospecific genus *Discyphus*, previously considered a member of Spiranthinae (Orchidoideae: Cranichideae), displays both vegetative and floral morphological peculiarities that are out of place in that subtribe. These include a single, sessile, cordate leaf that clasps the base of the inflorescence and lies flat on the substrate, petals that are long-decurrent on the column, labellum margins free from sides of the column and a column provided with two separate, cup-shaped stigmatic areas. Because of its morphological uniqueness, the phylogenetic relationships of *Discyphus* have been considered obscure. In this study, we analyse nucleotide sequences of plastid and nuclear DNA under maximum parsimony and maximum likelihood criteria with the aim of clarifying its systematic position and discussing its peculiar morphology in an explicit phylogenetic context. Our analyses failed to support inclusion of *Discyphus* in Spiranthinae, signifying instead that this genus represents an additional isolated lineage of "core spiranthids." The notable morphological disparity among such major lineages, as compared with the short internal branches subtending them in the molecular trees, would support the hypothesis that *Discyphus* represents a relict from an early radiation that also gave rise to Cranichidinae and Spiranthinae, putatively driven by adaptation to different pollinators given the morphological differences in floral morphology among these taxa.

Key words: ITS nrDNA, *matK-trnK*, Neotropical orchid phylogenetics, Orchidoiodeae, *rbcL*, Spiranthinae, *trnL-trnF*

Introduction

The genus *Discyphus* Schlechter (1919: 417) was created to include a single species formerly described as *Spiranthes scopulariae* Reichenbach (1854a: 11) and included in subtribe Spiranthinae Lindley (1840: 441) by Schlechter (1920), Balogh (1982), Garay (1982), Szlachetko (1992, 1995), Chase *et al.* (2003) and Salazar (2003b). However, *Discyphus* displays several vegetative and floral morphological peculiarities that cast doubts on its subtribal placement. *Discyphus scopulariae* (Reichenbach 1854a: 11) Schlechter (1919: 417) stands out vegetatively because of its single, sessile, cordate leaf that clasps the base of the inflorescence and lies flat on the substrate (Fig. 1A, B, D). In contrast, most genera of Spiranthinae bear one or more leaves that are petiolate or at least attenuate towards the base, thus never clasping the base of the scape. The only other genus of Spiranthinae with similar leaves is *Nothostele* Garay (1982: 339), which includes two species restricted to the Brazilian Plateau. In the latter, plants produce one or two sessile, round, cordate leaves that lie on the substrate, but the inflorescence and leaves are produced at different times of the year; therefore, a leaf base clasping the scape is impossible (Batista *et al.* 2011). Furthermore, in *Discyphus* the petals are free from the dorsal sepal, but their proximal half is decurrent on the column; in addition, the margins of the labellum are free, whereas in the typical Spiranthinae the petals are adherent to the dorsal sepal, their bases are free from the column and the margins of the labellum are fused to the sides of the column to form a tube leading to the nectary (Garay 1982; Salazar 2003b). Moreover, the column of *Discyphus* has two separate, cup-shaped stigmatic areas (Figs. 1E, 2H), a condition unique among Spiranthinae or of any other genus in Cranichideae Pfeiffer (1874: 901). In Cranichideae, the stigma is usually entire, or, in the few instances when it has two receptive areas, these are never

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Taxonomy

Discyphinae Salazar & van den Berg, *subtribus nova.*

Type: *Discyphus scopulariae* (Reichenbach 1854a: 11) Schlechter (1919: 417)

Acaulescent, deciduous geophytes with fasciculate roots; a single orbicular, cordate leaf lying on the substrate and clasping the base of the scape; inflorescence densely glandular-pubescent; flowers campanulate, resupinate, petals free from the dorsal sepal but adnate to the proximal half of the column, labellum free (i.e., its margins not adhering to the sides of the column); column provided with two separate receptive areas, those concave and with raised margins ("cup-shaped").

This subtribe consists of a monospecific genus distributed from Panama, northern Venezuela and Trinidad to eastern Brazil (Szlachetko 1992, Salazar 2003b).

Discyphus Schlechter (1919: 417). Type species: *Discyphus scopulariae* (Rchb.f.) Schltr.
Dikylikostigma Kraënzlin (1919: 321). Type species: *Dikylikostigma preussii* Kraenzlin (1919: 321)

Discyphus scopulariae (Rchb.f.) Schltr..
Basionym: *Spiranthes scopulariae* Rchb.f.. Type: VENEZUELA. Caripe, *Moritz 626* (holotype W-R!).
Homotypic synonym: *Gyrostachys scopulariae* (Rchb.f.) Kuntze (1891: 664).

Heterotypic synonyms: *Dikylikostigma preussii* Kraenzl. Type: VENEZUELA. La Victoria, *Preuss 1626* (B, destroyed); *Spiranthes rotundifolia* Cogniaux (1906: 542). Type: BRAZIL. Bahia, *Salzmann 538* (G); *Cyclopogon rotundifolius* (Cogn.) Schlechter (1920: 394)

Discyphus scopulariae is terrestrial in savannas and riparian forests from near sea level to about 800 m. Full descriptions and additional information on *Discyphus* can be found in Foldats (1969), Garay (1982), Szlachetko (1992) and Salazar (2003b).

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