

A molecular reappraisal of *Scabiosa* L. and allied genera (Dipsacaceae)

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Riassunto. Un riesame molecolare di *Scabiosa* L. e dei generi affini (Dipsacaceae)

È stata intrapresa un'analisi filogenetica di vari generi di Dipsacaceae, con l'obiettivo di verificare le relazioni tra i generi inclusi in passato in *Scabiosa* L. sensu lato. Gli introni del *trnL*^(UAA) plastidiale sono stati sequenziati per le seguenti specie: *Cephalaria syriaca*, *Dipsacus sylvestris*, *Knautia arvensis*, *Lomelosia cretica*, *Pseudoscabiosa limonifolia*, *Pterocephalus perennis*, *Pycnocomon rutifolium*, *Scabiosa uniseta* e *Sixalix atropurpurea* subsp. *maritima*.

L'analisi cladistica dell'introne del *trnL*^(UAA) ha prodotto un singolo cladogramma a massima parsimonia, che mostra due cladi principali, uno che comprende *Pseudoscabiosa*, *Succisa*, *Knautia*, *Cephalaria* e *Dipsacus*, e l'altro con *Scabiosa*, *Lomelosia*, *Pycnocomon*, *Pterocephalus*, e *Sixalix*. All'interno di quest'ultimo, si notano relazioni di sister group tra *Scabiosa* e *Sixalix*, e tra *Lomelosia* e *Pycnocomon*, con *Pterocephalus* sister group agli ultimi due taxa.

Il cladogramma ottenuto è diverso da quelli presenti in letteratura basati sulla morfologia dell'epicalice, ma è congruente con dati recenti sul differenziamento del frutto e con la palinologia. L'albero filogenetico ottenuto indica che l'adattamento delle diaspore è stato il fattore più rilevante nell'evoluzione delle Dipsacaceae.

Key words: Chloroplast DNA, Dipsacaceae, Molecular phylogeny, *Scabiosa*, *trnL*^(UAA).

INTRODUCTION

Dipsacaceae Juss. (Dipsacales Lindley) is a small family (12-13 genera, 250-350 species), mainly distributed around the Mediterranean basin, with outliers in the Middle and Far East and in Central to Southern Africa. The family is characterized by the presence of an epicalyx, or involucl, which encases the fruit and whose shape, symmetry and ornamentations are extremely relevant for the systematic subdivisions of the family. Dipsacaceae are customarily divided in three tribes (*Dipsaceae*, *Knautieae*, and *Scabioseae*). The tribal subdivisions date back to the beginning of the last century (COULTER, 1823) and are based on the shape and symmetry of the epicalyx and the relative length of the involuclal and receptacular bracts of the capitulum.

Regardless, the tribes are circumscribed in such a way that not all genera can be easily placed in one of them. In fact, only the monotypic *Knautieae* are unequivocally defined as Dipsacaceae with dorsiventrally flattened involuclals [genus *Knautia* (L.) Coulter]. The other two tribes are more loose in definition, and the taxa regarded as archaic in each tribe (members of *Dipsacus* L. sect. *Sphaerodipsacus* Lange, as well as the genera *Succisa* and *Succisella*) are difficult to place with certainty in either one. However, for the past years, *Dipsaceae* were regarded as including *Cephalaria* Schrad. and *Dipsacus* L., whereas *Scabioseae* included *Pterocephalus* (Vaill.) Adans., *Pycnocomon* Hoffmans. & Link, *Scabiosa* L. sensu latissimo, *Succisa* Necker, and *Succisella* Beck. In the last twenty years, the studies by VERLÁQUE (1977a,b; 1984a,b; 1985a,b; 1986a,b) brought about a better understanding of the biology of the family, with special reference to involucl morphology, palynology and karyology. As a consequence, GREUTER & BURDET (1985) splitted genus *Scabiosa* in several segregates: *Lomelosia* Raf. (= *Scabiosa* sect. *Trochocephalus* Mert. & Koch), *Sixalix* Raf. (= *Scabiosa* sect. *Cyrtostemma* Mert. & Kock), and *Scabiosa* L. s.s. (= *Scabiosa* sect. *Scabiosa*). At the same time, DEVESA (1984) segregated sect. *Asterothrix* Font Quer of genus *Scabiosa* into *Pseudoscabiosa* Devesa and LÓPEZ GONZÁLES (1987) segregated into *Pterocephalidium* G. López two species, namely *P. diandrum* (Lag.) G.

López and *P. centennii* Cannon & Cannon, formerly belonging to genus *Pterocephalus*.

In spite of these nomenclatural changes, there has been little doubt on the fact that *Scabioseae* constitute a closely knit unit. In addition, a phylogenetic analysis by CAPUTO & COZZOLINO (1994) based on morphology showed that *Scabioseae*, as circumscribed above, appeared monophyletic. Regardless, the work of HILGER & HOPPE (1984) on the anatomy of the diaphragm in the epicalyx and, later, the papers by MAYER & EHRENDORFER (1999; 2000) showed that a monophyletic circumscription of *Scabioseae* should include less taxa than previously known, also indicating that the shape of the epicalyx, with special reference to its distal part (the corona, which is a membranous expansion in various taxa), has been modified more than once in the family as a response to adaptative pressures.

This paper aims at a molecular verification of the circumscription of *Scabioseae*, in the light of the aforementioned uncertainties. To this purpose, chloroplast *trnL*^(UAA) intron sequences were obtained for a choice of dipsacaceous taxa. These sequences were chosen as they are regarded to have a mutation rate which makes them suitable for studies of relationships below the family level (GIELLY & TABERLET, 1996).

MATERIAL AND METHODS

The taxa employed in this study are listed in Tab. 1. All specimens were in cultivation at the Botanical Garden of Naples, Italy. Leaves were collected at flowering time. Voucher specimens of the examined plants are deposited at NAP.

Leaves (0.5 g per sample) were ground on liquid nitrogen and total DNA was extracted following either the procedure described in CAPUTO *et al.* (1991) or that by DOYLE & DOYLE (1990).

Chloroplast *trnL*^(UAA) intron was amplified using the two primers reported by TABERLET *et al.* (1991) and according to ACETO *et al.* (1999). PCR fragments were then purified by using Microcon 100 microconcentrators (Amicon) and double-strand sequenced in both

directions by using a modification of the Sanger dideoxy method (SANGER *et al.*, 1977) as implemented in a double strand DNA cycle sequencing system with fluorescent dyes. Sequence reactions were then loaded into a 373A Applied Biosystems Automated DNA sequencer (Applied Biosystems). Various sequencing experiments were repeated to solve all uncertainties.

Tab. 1 - Species and chloroplast *trnL*^(UAA) intron length for the taxa in study.

Species	Length (bp)
<i>Cephalaria syriaca</i> (L.) Roem. & Schult.	512
<i>Dipsacus sylvestris</i> Huds.	513
<i>Knautia arvensis</i> (L.) Coult.	526
<i>Lomelosia cretica</i> (L.) Greuter et Burdet	516
<i>Pseudoscabosa limonifolia</i> (Vahl) Devesa	510
<i>Pterocephalus perennis</i> Coult.	547
<i>Pycnocomon rutifolium</i> (Vahl) Hoffmans. & Link	537
<i>Scabiosa uniseta</i> Savi	537
<i>Sixalix atropurpurea</i> (L.) Greuter et Burdet	
subsp. <i>maritima</i> (L.) Greuter et Burdet	541
<i>Succisa pratensis</i> Moench	512

Sequences were then reduced to chloroplast *trnL*^(UAA) intron only by aligning them with various sequences available in the literature.

The sequence alignment was accomplished by using Clustal W ver. 1.6 (THOMPSON *et al.*, 1994) with default gap opening and extension settings, but with transitions and transversions equally weighted, and the parameter MAXDIV (which controls the delay of the alignment for the most divergent sequences) set to 85%. Various alignment experiments were carried out by varying the gap open and extension costs from 4 to 15, with unit increments, in order to verify the effect of the gap distribution on the resulting phylogenies.

The resulting matrix was analysed by using the cladistic software environment Winclada (NIXON, 1999), running Nona (GOLOBOFF, 1993-99) as a daughter process, with the following parameters: hold 100000; hold/100; mult*100; max*, and treating insertions/deletions (indels) as missing characters. The resulting cladograms were investi-

gated with Winclada, which was also used to calculate bootstrap percentages. As an outgroup, the most inclusive monophylum containing *Dipsacus* and *Cephalaria* was used.

RESULTS

Chloroplast *trnL*^(UAA) intron lengths for all the investigated taxa are reported in Tab. 1. The parsimony analysis of the resulting matrix (consensus length 570, informative characters 3%) yielded one single most parsimonious trees (length = 47, Consistency Index = 0.95, Retention Index = 0.93), shown in Fig. 1. The topology shows the ingroup divided in two clades, one including *Lomelosia* and *Pycnocomon* and the other including *Pterocephalus*, sister group to a group containing *Scabiosa* and *Sixalis*. The outgroup clade shows *Dipsacus* in a basal collapse, and then two clades including *Pseudoscabiosa* and *Succisa*, *Knautia* and *Cephalaria*, respectively.

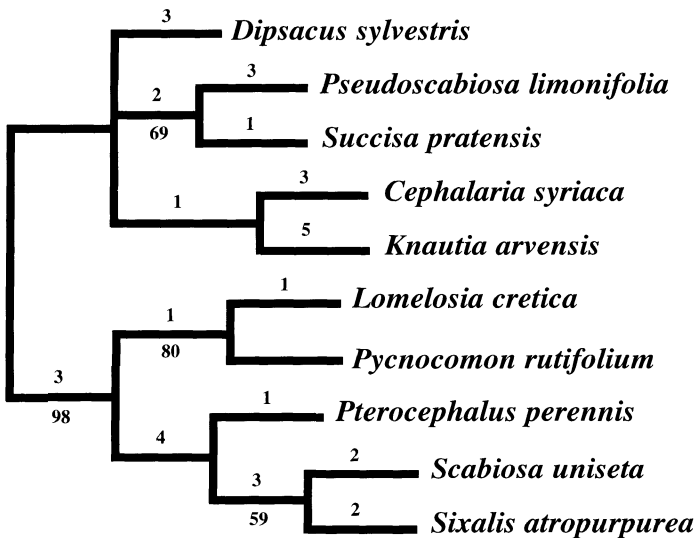


Fig. 1 - Single most parsimonious cladograms (length = 47, consistency index = 0.95, retention index = 0.93). Numbers above internodes represent apomorphies. Numbers below internodes are bootstrap percentages (only those >50% are shown).

Bootstrap percentages out of 1000 replicas (Fig. 1) indicate that the major groups of taxa are statistically supported.

The attempts to vary the gap open and extension costs during the alignments did not yield any difference in topology.

DISCUSSION

The results obtained here are remarkably different from those by VERLÁQUE (e.g., 1986b) and by CAPUTO & COZZOLINO (1994). In fact, the cladogram in Fig. 1 shows that *Pseudoscabiosa*, *Succisa* and *Knautia* are more closely related to *Dipsacus* and *Cephalaria* (i.e., to Dipsacaceae) than to the *Scabiosa*-like members of Dipsacaceae. Such a finding, on the contrary, is broadly congruent with the hypothesis by MAYER & EHRENDORFER (1999, 2000) mainly based on the patterns of fruit differentiation. According to the just mentioned authors, in fact, a renewed concept of *Scabioseae* should include only those members of Dipsacaceae which have been regarded for the past as belonging to *Scabiosa* s.l. (i.e., *Lomelosia*, *Pycnocomon*, *Scabiosa* and *Sixalix*), as well as the majority of the species of genus *Pterocephalus* (MAYER & EHRENDORFER, 1999; 2000). In detail, they suggest that *Lomelosia* and *Pycnocomon* are sister groups, sharing triporate pollen, deep pits in the epicalyx and supplemental sclerenchima strands, that *Sixalix* and *Scabiosa* are sister group to each other, mainly sharing an apomorphic base chromosome number $x = 8$, and that *Pterocephalus* (or, at least those species not segregated into the new genus *Pterocephalodes* V. Mayer & Ehrend.) is either sister group to all the above mentioned genera (MAYER & EHRENDORFER, 1999) or sister group to *Scabiosa* and *Sixalix* (MAYER & EHRENDORFER, 2000). The same authors (1999; 2000) suggest that *Pseudoscabiosa* (as well as other genera not considered here) should be excluded by the *Scabioseae*, on the grounds of its lack of diaphragm, pollen with caveae and, overall, the presence of “surprising similarities with the characteristic epicalyx anatomy otherwise found only in *Cephalaria*” (MAYER & EHRENDORFER, 1999).

Our results are in the same line, in that they support such renewed concept of *Scabioseae* as well as the placement of *Pseudoscabiosa* outside the tribe. Our results, furthermore, would better concur with the hypothesis of sister group relationship between *Pterocephalus* and *Scabiosa/Sixalix* (MAYER & EHRENDORFER, 2000) than with that of a sister relationship between *Pterocephalus* and the rest of *Scabioseae* (MAYER & EHRENDORFER, 1999). Overall, the phylogenetic hypothesis shown here (Fig. 1) would indicate that the fruit differentiation has been the strongest driving force in Dipsacaceae evolution. Similar selective pressures have caused the selection of similar fruit shapes and dispersal modes in a parallel fashion in various taxa of the family. In fact, as MAYER & EHRENDORFER (1999) clearly showed, various epicalyx adaptations in Dipsacaceae developed more than once. *Pycnocomon rutifolium* and *Sixalix*, for example, which developed a woody epicalyx and have been regarded for the past as closely related, for this and other features (VERLÁQUE, 1986a,b; CAPUTO & COZZOLINO, 1994), developed independently this feature, probably as a response to settlement in environments in which diaspores are dispersed by rolling on the ground (VERLÁQUE, 1986a). By the same token, the pitted epicalyx and the membranous corona of *Lomelosia* developed independently from the same, homoplastic characteristics of *Pseudoscabiosa limonifolia*. The cladogram in Fig. 1, however, would indicate that *Pterocephalus* is more closely related to *Sixalix* than to *Scabiosa sensu stricto*. In spite of the fact that the feather-like multiplied calyx bristles which are characteristic of *Pterocephalus* independently developed in *Pterocephalodes*, *Pterocephalidium*, some species of *Pseudoscabiosa* and one species of *Pycnocomon* (MAYER & EHRENDORFER, 1999; 2000), and are therefore homoplastic within the family, we prefer not to comment upon the topology of the outgroup clade, awaiting for further study, with more taxa, including members of the related Valerianaceae, and more characters, so as to exclude paucity of sampling.

In conclusion, it is presumable that the plesiomorphic bodyplan in Dipsacaceae is represented by harmless plants with globose capitula without radiant flowers, radially symmetrical involucrel with an undifferentiated corona and no diaphragm, resembling a present-day

Succisa or *Pseudoscabiosa*, and that the gross morphology of the involucre has been so much shaped by selection to be deceptive in inferring relationships. Ongoing studies, widening the choice of taxa and adding other DNA fragments to the analysis, will provide further evidence to test our phylogenetic hypothesis and will elucidate the position of other genera not yet investigated here.

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Abstract

A phylogenetic study was undertaken on various genera of Dipsacaceae, aiming at verifying the circumscription of the group of genera formerly included in *Scabiosa* L. sensu lato. Chloroplast *trnL*^(UAA) intron sequences were obtained from the following species: *Cephalaria syriaca*, *Dipsacus sylvestris*, *Knautia arvensis*, *Lomelosia cretica*, *Pseudoscabiosa limonifolia*, *Pterocephalus perennis*, *Pycnocomon rutifolium*, *Scabiosa uniseta* and *Sixalix atropurpurea* subsp. *maritima*.

The cladistic analysis of the *trnL* intron dataset yielded a single most parsimonious cladogram, showing two clades, one including *Pseudoscabiosa*, *Succisa*, *Knautia*, *Cephalaria* and *Dipsacus*, and the other including *Scabiosa*, *Lomelosia*, *Pycnocomon*, *Pterocephalus*, and *Sixalix*. Within the latter, sister group relationships are shown for *Scabiosa* and *Sixalix*, and for *Lomelosia* and *Pycnocomon*, with *Pterocephalus* sister group to the former two taxa.

The cladogram obtained is different from previous ones based on classical epicalyx morphology, but are congruent with recent findings on fruit differentiation and palynology. The phylogenetic hypothesis depicted here would indicate that the fruit adaptation has been the strongest driving force in Dipsacaceae evolution.