

Recent advances in the systematics and phylogeny of Mediterranean groups of Boraginaceae

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Introduction - Taxonomic problems in family Boraginaceae s.l. range from the level of order to that of species and below, mainly due to the still largely incomplete knowledge of many groups and to the difficult interpretation of shared characters as phylogenetically informative homologies or rather as simple analogies.

Based on recent molecular phylogenetic analyses, there is evidence that Boraginaceae, as traditionally treated, is a paraphyletic group also including Hydrophyllaceae and Lennoaceae. To avoid paraphyly, it either has to include these two well-established families at subfamily rank or, alternatively, the traditional subfamilies Cordioideae, Ehretioideae, Heliotropioideae and Boraginoideae must be elevated to family rank. Such a second taxonomic option is preferable, in order to have a monophyletic order Boraginales consisting of six natural families: Cordiaceae, Ehretiaceae, Heliotropiaceae, Hydrophyllaceae, Lennoaceae and Boraginaceae s.str. Position of Boraginales is within the Euasterid-I clade of the APG, and possibly sister to Solanales, Gentianales and Lamiales.

Intended in a narrow sense, Boraginaceae consists of at least five tribes, Lithospermeae, Boragineae, Cynoglosseae, Eritrichieae and Echiochileae, plus two still ill-defined groups, Trigonotideae and Trichodesmeae.

The spine of the family phylogeny as resulting from the few molecular studies published to date suggest that Boragineae, Lithospermeae and Echiochileae are monophyletic groups, while the genera of Cynoglosseae and Eritrichieae seem to form a broad paraphyletic complex in which distinction of the two tribes as established on a traditional morphological basis can not be clearly recognized. The focus of our research in the last years has been the systematics and phylogeny of tribes Boragineae and Lithospermeae. Taxonomic problems in these groups are historically at the genus level.

Boragineae - Boragineae are exclusive to the Old-World and comprise 15 genera some of which of difficult circumscription. A molecular phylogenetic study using the plastid *trnL*_(UAA) intron and the nuclear ITS1 region has supported the monophyly of the tribe and an early split into two main clades, the sister of which is the western-european monotypic genus *Pentaglottis*. The first clade consists of the three lineages *Pulmonaria-Nonea*, *Borago-Symphytum* and *Brunnera*.

Nonea-Pulmonaria - We have recently investigated in detail the relationships in the critical *Pulmonaria-Nonea* lineage, in order to arrive to satisfactory generic delimitations. Our phylogeny shows that: 1) the eastern Mediterranean species *Nonea obtusifolia* is sister to the rest of the ingroup and outside either *Nonea* or *Pulmonaria*; 2) *Nonea/Elizaldia* and *Paraskevia/Pulmonaria* are sister groups; 3) *Nonea* as currently circumscribed is paraphyletic because *Elizaldia* is firmly nested inside it; 4) the critical monotypic genus *Paraskevia* is closely related to *Pulmonaria*. Combined with morphological, karyological and chorological aspects, there is strong support for: (i) the institution of a new monotypic genus (*Melanortocarya*) for *N. obtusifolia*, (ii) the inclusion of *Elizaldia* within *Nonea*, and (iii) the inclusion of *Paraskevia* in *Pulmonaria*.

Borago - In the *Borago-Symphytum* clade we have investigated the phylogeny of *Borago* in relation to karyotype variation and evolution and cytogeography of endemic subgenus *Buglossites* in the Corso-Sardinian microplate.

Chromosome variation in *Borago* is wider than previously known, with four base numbers, $x = 6, 8, 9$ and 15 , and three ploidy levels based on $x = 8$. In subg. *Buglossites* the Sardinian endemic *B. morisiana* is diploid with $2n = 18$, while *B. pygmaea* includes three allopatric cytotypes with $2n = 30$ (Sardinia), $2n = 32$ (southern Corsica) and $2n = 48$ (central northern Corsica and Capraia). In subgenus *Borago*, the Moroccan endemic *B. trabutii* and the

widespread *B. officinalis* have $2n = 12$ and $2n = 16$, respectively. Two base numbers may represent the ancestral condition in this small genus, $x = 6$ or $x = 8$. Increase in chromosome number and karyotype asymmetry, a decrease in chromosome size and heterochromatin content, and the appearance of polyploidy are the most significant karyological changes associated with the divergence of *Buglossites*. High ITS1 variation in the tetra- and hypotetraploid races of *B. pygmaea* suggests a multiple origin, while the lower polymorphism of the hexaploid race and its allopatric distribution in northern Corsica and Capraia is better explained with a single origin via union of unreduced and reduced gametes.

Anchusa s.l. - The second main clade of the Boragineae corresponds to the well-supported group of *Anchusa* s.l., with monotypic genus *Trachystemon* as sister taxon. *Anchusa* s.l. is highly paraphyletic to its small, segregate genera *Phyllocara*, *Hormuzakia*, *Gastrocotyle*, *Cynoglottis*, *Lycopsis* and *Anchusella*. These morphologically well-characterized, segregate genera are all supported by DNA sequences and should be definitively accepted. Also the subgenera of *Anchusa* s.s. (*Buglossum*, *Buglossoides*, *Buglossellum*) are all supported as monophyletic groups by DNA data, in spite of their weak morphological characterization with respect to *Anchusa* s.str. The only exception is *Anchusa* subgenus *Limbata* which is nested in *A.* subgenus *Anchusa* despite marked divergence in floral morphology. A more natural classification will be required in the future if other markers will confirm such a paraphyly of *Anchusa* s.str. Species relationships within *Anchusa* s.str. are poorly resolved due to the low rate of molecular variation. This appears in contrast with both the morphological differentiation between species and with the infraspecific phenotypic polymorphism of several taxa, suggesting a recent history of rapid and partly adaptive radiation in the Mediterranean and continental Europe. The only exception is given by the endemic Sardinian taxa, which show a weak divergence with respect to the rest of *Anchusa*. We wanted therefore to investigate in greater detail the origin and the relationships of the six endemovariant taxa of the Corso-Sardinian microplate, adding AFLP fingerprinting to the ITS phylogeny. AFLP provide a finer level of resolution, as well as reliable estimates of within-population genetic diversity and among population genetic distances; these data are also important for planning conservation actions of these extremely vulnerable island endemics. ITS phylogeny of the whole group suggests that the three orophytic taxa *A. formosa*, *A. capellii* and *A. montelinasana* form a clade sister to the psammophytic coastal endemics *A. crispa*, *A. littorea* and *A. sardoa*, but preliminary AFLP data fit only in part in this picture.

Lithospermeae - This group is represented by ca. 24 genera in both the Old and New world, 18 of which are represented in the Euro+Med region. In the total lack of modern phylogenetic studies, relationships and generic limits are in some cases still tentative. We are using morphology and karyology combined with nuclear ITS and plastid *matK* DNA sequences to open a window on the phylogeny of the old-world genera of the tribe. At this moment, the obtained trees (16 genera sampled) form a grade in which most of the currently accepted genera seem to be resolved. Of particular interest is the position of *Halacsya* and *Paramoltkia*, two remarkable monotypic genera endemic to the Balkan serpentine outcrops. Also in view of their edaphic specialisation (obligate serpentine endemics), they have been viewed as isolated, relict taxa of very ancient origin without clear relationships within extant *Lithospermeae*. According to some other authors (e.g. Johnston), however, they are both close to *Moltkia*, a small genus of six species with some morphological affinity. This second hypothesis does not receive support from our preliminar trees, which bring to the light a considerable distance of the two genera with respect to *Moltkia*. Shared characters in, e.g., fruit seem to be homoplastic. *Halacsya* and *Paramoltkia* are instead sistergroups in a well supported clade, in spite of their considerable morphological "distance". This finds support in pollen morphology, confirming the strong phylogenetic signal of palynological characters in the systematics of Boraginaceae. In a phylogenetic perspective, the obligated

serpentinophytism of *Halacsya sendtneri* and *Paramoltkia doerfleri* may represent an ecological symplesiomorphism due to common ancestry, rather than an ecological homoplasy originated independently in two otherwise unrelated lineages. The clade *Halacsya/Paramoltkia* belongs to a larger group also including the monotypic genera *Mairetis*, *Molkiopsis* and *Neatostema*, but not *Moltkia*. The latter has possibly closer relationships with the *Lithospermum/Buglossides* clade and contains two well distinct subclades corresponding to the West Asiatic subgenus *Moltkia* (*M. aurea* and *M. caerulea*) and the South European subgenus *Echianthus* (*M. petraea* and *M. suffruticosa*).

Cerinthe - At the species level, we are finally investigating the systematics and phylogeny of genus *Cerinthe*. Preliminary trees show that the genus is split in two well supported lineages, one of species with $2n = 18$, including all species of subg. *Cerantho*, and one of taxa with $2n = 16$, mainly corresponding to the *C. major* s.l. group (subg. *Cerinthe*). The ancestral forms of the the genus are possibly found in subgenus *Cerantho*, and possibly within the perennial group of *C. glabra* distributed in south European mountains. The rare Corsican endemic *C. tenuiflora*, doubtless a good species, is sister to the all the other taxa and is well distinct from the continental *C. glabra*, of which it is currently considered a subspecies. The annual group of *C. major* has instead derived characters. Descending aneuploidy ($2n = 18 \rightarrow 2n = 16$) paralleled by a shortening to the life-cycle (perennial \rightarrow annual) as adaptive response during radiation in dry habitats, is known to occur elsewhere in Boraginaceae (e.g. *Nonea*). Psammophytic *Cerinthe oranensis* (N Africa) is sister to *C. major*, which includes the variant subsp. *gymnandra*. Work on this genus is still in progress, with the analysis of *C. smithiae*, *C. retorta*, *C. tristis* and *C. palaestina*.

Conclusion: recommendation of the genera of Boraginaceae to be recognised in the forthcoming Euro+Med project.