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FATIMA SALES

IAN C. HEDGE

WILLIAM EDDIE

JILL PRESTON

MICHAEL MOELLER

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Jasione L. taxonomy and phylogeny

Fátima SALES*, Ian C. HEDGE
Royal Botanic Garden Edinburgh, EH3 5LR, Scotland

William EDDIE
Office of Lifelong Learning, The University of Edinburgh, Edinburgh EH8 9LW, Scotland

Jill PRESTON, Michael MOELLER
Royal Botanic Garden Edinburgh, EH3 5LR, Scotland

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Abstract: *Jasione* L., a genus with small flowers in compact ebracteolate heads subtended by involucre bracts, is very isolated in the *Campanulaceae*. It is mainly a Mediterranean-European genus with c. 15, mostly polymorphic, species, which show evidence of recent speciation, possibly as recent as the last glaciation. In the west of its distributional range, where the greatest morphological variation occurs, there has been much recent investigation on morphology, anatomy, phenetics, molecular evidence and nomenclature. This research has provided considerable evidence for a reduction in the number of taxa that should be recognised. On-going research is focused on more detailed molecular studies and incorporating the East Mediterranean taxa within the framework established for those of the West. In Flora of Turkey (6: 86, 1978), 4 mostly localised species were recognised in one of which, *J. supina* Sieber, 4 subspecies are accepted. Further investigation of these Turkish taxa is a prerequisite towards a fuller understanding of the taxonomy and phylogeny of this interesting genus.

Key Words: *Jasione*, *Campanulaceae*, ITS sequencing, molecular phylogenetic analysis

Introduction

Ever since Alphonse de Candolle's monograph of 1830, *Jasione* L. has been associated with the wahlenbergioid taxa on account of the apical valvate dehiscence of the capsule. This was accepted by numerous authors, from Endlicher (1836-1840) and Schönland (1889-1894) onwards, although the relative distinctness of *Jasione*, even within this association, was often recognised. Fedorov (1957) treated it as a separate tribe, the *Jasioneae* Fed. within Schönland's subfamily *Campanuloideae*, and a similar view was adopted in the provisional classification of Eddie (unpublished PhD thesis, 1997).

Cosner (1993), using rearrangements of the chloroplast genome, showed that *Jasione* is, on balance, closer to the campanuloids. It shares with both wahlenbergioid and campanuloid taxa a drastic contraction of the inverted repeat (IR) at its large single copy (LSC) end, plus 6 inversions and 2 putative

transpositions into the IR. It forms a clade with *Adenophora* Fisch., *Campanula* L., *Edraianthus* A.DC., *Musschia* Dumort., *Symphyandra* A.DC. and *Trachelium* L., but it has the most highly rearranged chloroplast genome in the family. It has at least 8 more inversions and a clearly derived IR expansion into the small single copy (SSC) region.

Jasione appears to have more morphological similarities to campanuloids (e.g. coloured pollen and *Adenophora*-like stigmatic lobes) than to wahlenbergioids, and its Mediterranean distribution is typically campanuloid. It bears some resemblance to *Feeria* Buser, with which it shares a similar mode of capsule dehiscence, but it has a chromosome number of $2n = 12$ ($x = 6, 9, 18$) versus $2n = 34$ for *Feeria* (Eddie et al., in press). The position of *Jasione* is probably somewhat intermediate between wahlenbergioid and campanuloid taxa, and therefore it is likely to be relatively basal within the complex of northern hemisphere genera, although its exact relationships remain unclear. Eddie et

* Departamento Botânica, Universidade Coimbra, 3000 Coimbra, Portugal (fsales@ci.uc.pt)

al. (in press) have hypothesised that many oligotypic genera of the *Campanulaceae* centred around the Mediterranean region are relatively primitive within the family, and represent initial waves of campanuloid evolution from essentially southern hemisphere wahlenbergioid ancestors.

Most recent work on *Jasione* has concentrated on taxa in the West Mediterranean, where the most morphological variation occurs. Now, however, studies are under way to understand better the relationships of the East Mediterranean taxa with those in the West.

In Turkey, 4 species and a total of 8 taxa are recognised (Damboldt, 1976): *J. montana* L., *J. heldreichii* Boiss. & Orph. vars *heldreichii* & *papillosa* J.Parnell (belonging to the "*J. montana* species-group"); *J. idaea* Stoj., *J. supina* subsp. *supina*, *pontica* (Boiss.) Damboldt and *akmanii* Damboldt & *tmolea* (Stoj.) Damboldt (belonging to the "*J. sessiliflora* Boiss. & Reut. species-group").

With the exception of the West Anatolian *J. heldreichii* and the North Anatolian *J. supina* subsp. *pontica*, these taxa are only known from few or solitary gatherings. Clearly, more gatherings and information about them are essential, and in this work local botanists can make a significant contribution. Their involvement in future research would be much welcomed by the authors of this short note. Judging by the results of studies in the Iberian peninsula, it is likely that new studies in Turkey will demonstrate that the current total of 8 taxa should be reduced.

In this study, we used a molecular approach, using sequence data from the internal transcribed spacer (ITS) of nuclear ribosomal DNA (nrDNA). We present here our initial results of molecular phylogenetic analyses on taxa from the 2 "species-groups" mentioned above.

Materials and Methods

Outgroup taxa: Recently available results of a molecular analysis using ITS nrDNA sequences on the *Campanulaceae* (Eddie et al., in press) suggested that *Jasione* had no closer relative amongst the taxa sampled, and resided on a polytomy with several well-supported clades (Figure 1). Thus, ITS sequences for 4 potential outgroup taxa were retrieved from GenBank: *Campanula glomerata* L. (GenBank no. AH006455), *Edraianthus*

graminifolius A.DC. (AH008215), *Asyneuma japonica* (Miq.) Briq. (AH008214) and *Adenophora remotiflora* Miq. (AH008212). The sequences of these outgroup taxa were reasonably alignable to the ingroup taxa and were used to root the resulting phylogenetic trees.

Ingroup taxa: Twelve samples of *Jasione*, comprising 5 species [*J. crispata* (Pourr.) Samp., *J. laevis* Lam., *J. maritima* (Duby) Merino, *J. Montana* and *J. sessiliflora* Boiss. & Reut.] and 3 varieties (*J. montana* var. *gracilis* Lange, *J. montana* var. *latifolia* Pugsley and *J. maritima* var. *sabularia* (Cout.) Sales & Hedge), were selected to represent genus diversity. Duplicate samples of *J. montana* var. *montana*, *J. montana* var. *gracilis*, *J. montana* var. *latifolia* and *J. maritima* var. *sabularia* were included to represent geographical diversity (Table 1). Voucher specimens for all *Jasione* samples are deposited in the herbarium of the Royal Botanic Garden Edinburgh (E).

DNA extraction, PCR and sequencing: DNA was extracted from silica gel dried leaf material according to a modified CTAB procedure (Doyle & Doyle, 1987). PCR and sequencing strategies basically followed Möller & Cronk (1997). The complete ITS region was amplified using primers ITS5P and ITS4. For sequencing additional primers ITS3P and ITS2G were employed.

Phylogenetic analysis: Sequence boundaries of both internal transcribed spacers were determined by comparison to *Gesneriaceae* sequences. Thirty-two base pairs (bp) at the 3' end of ITS1 were missing for *Jasione* sequences. The remaining characters were analysed unordered and unweighted by maximum parsimony using PAUP 4.0b8a (Swofford, 1998). With a view to the low number of taxa, a branch-and-bound search using the factory settings was performed (except for collapse option = max.). Branch support was provided as bootstrap values of 10,000 replicates using the factory settings in PAUP.

Results of molecular investigations

Matrix characteristics: ITS1 and ITS2 were on average 498 bp long. Alignment of the outgroup taxa required the inclusion of 34 indels (insertion or deletion events). The outgroup taxa were very diverse with a divergence in pair-wise comparisons between 6.7% and 23.3%. Between ingroup and outgroup taxa the divergence was between 88 (21.3%) and 131 (26.%) character state

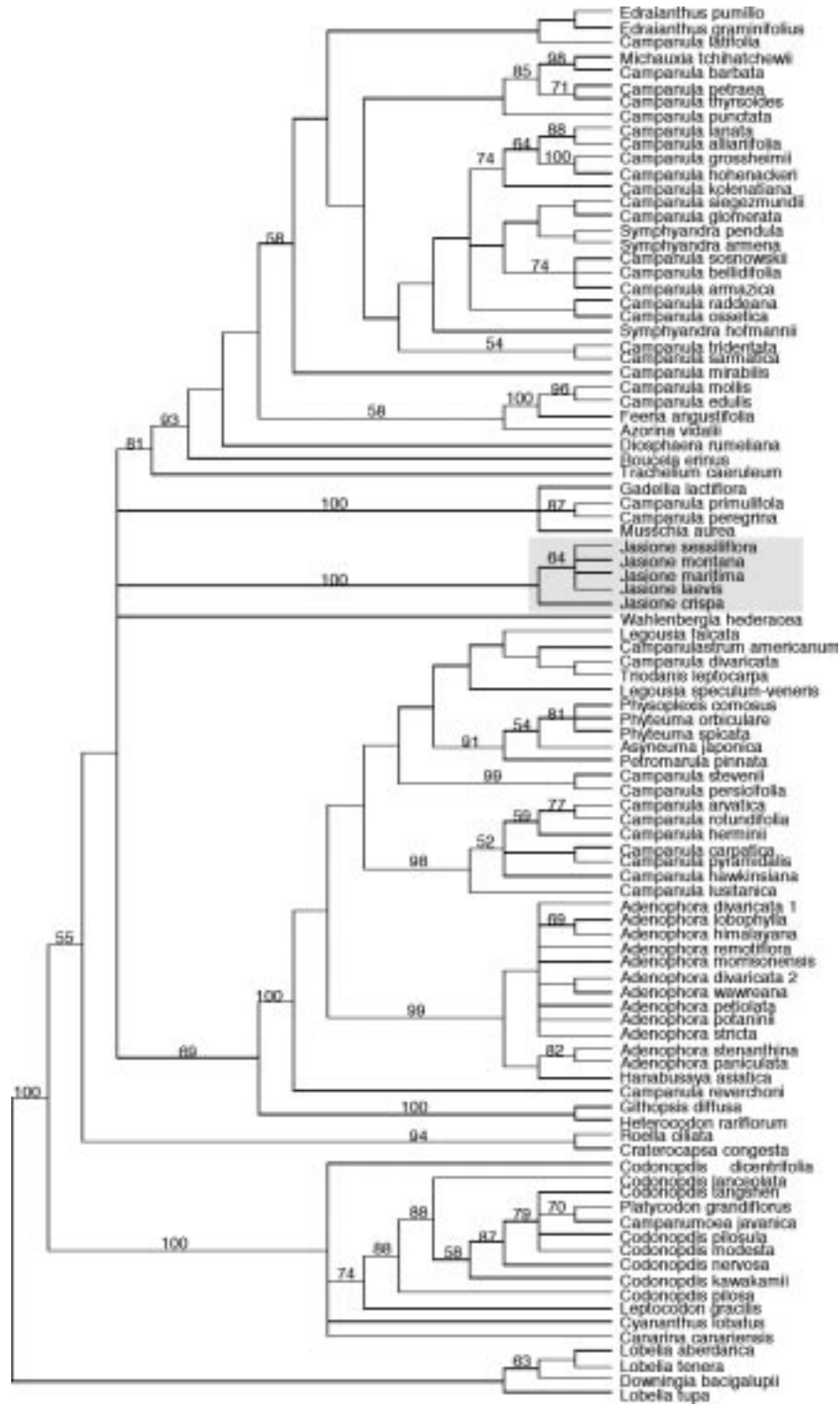


Figure 1. Strict consensus of 2629 most parsimonious trees of 2130 steps length of 93 ingroup taxa of Campanulaceae and 4 outgroup taxa of Lobeliaceae based on internal transcribed spacer (ITS) sequences of ribosomal DNA (rDNA) with (bootstrap values above branches). (CI: 0.3703, RI: 0.7583). Source: Eddie WMM *et al.* (2002).

Table 1. List of *Jasione* and outgroup samples included in the ITS phylogeny and their localities.

TAXON	DNA NO.	LOCALITY
<i>J. montana</i> var. <i>gracilis</i>	DNA02	Portugal: Bragança
<i>J. montana</i> var. <i>gracilis</i>	DNA38	Spain: La Coruña, near Muros
<i>J. montana</i> var. <i>latifolia</i>	DNA28	Spain: Asturias, Cabos de Peñas
<i>J. montana</i> var. <i>latifolia</i>	DNA98	Spain: Pontevedra, near Noia
<i>J. montana</i> var. <i>montana</i>	DNA25	Spain: Cantabria, Picos de Europa
<i>J. montana</i> var. <i>montana</i>	DNA73	Scotland: Kintyre, Carradale
<i>J. maritima</i> var. <i>maritima</i>	DNA49	Spain: La Coruña, near Muros
<i>J. maritima</i> var. <i>sabularia</i>	DNA79	Portugal: Douro Litoral, Vila do Conde
<i>J. maritima</i> var. <i>sabularia</i>	DNA89	Portugal: Douro Litoral, Furadouro
<i>J. laevis</i>	DNA18	Spain: Cantabria, Picos de Europa
<i>J. crispa</i>	DNA (WE)	Spain: uncertain provenance
<i>J. sessiliflora</i>	DNA13	Spain: Leon, Sierra del Teleno
<i>Adenophora remotiflora</i>	AH008212*	
<i>Asyneuma japonica</i>	AH008214*	
<i>Campanula glomerata</i>	AH006455*	
<i>Edraianthus graminifolius</i>	AH008215*	

Note: * = GenBank accession numbers.

changes, suggesting a significant phylogenetic distance. The sequence divergence within *Jasione* was very low. Six taxa had identical sequences irrespective of their taxonomy (*J. montana* var. *montana* DNA25, *J. montana* var. *latifolia* DNA28 and DNA98, *J. montana* var. *gracilis* DNA38, *J. maritima* var. *maritima* DNA49 and *J. maritima* var. *sabularia* DNA89). Three out of the 4 duplicate samples showed sequence variation (*J. montana* var. *montana*, *J. montana* var. *gracilis* and *J. maritima* var. *sabularia*) with a maximum of 2 character state changes (0.4%). The maximum divergence within *Jasione* was 23 character state changes (5.3%) between *J. crispa* and *J. sessiliflora*.

Phylogenetic trees: A single most parsimonious tree of 270 steps was recovered (Figure 2) with a consistency index of 0.933, a retention index of 0.923 (inclusive uninformative characters) and an average of 0.5 steps per character, indicating a high fit of the data to the phylogenetic tree and a low saturation of changes across the matrix.

Among the outgroup taxa, high bootstrap support (100%) was also evident for the sister relationships of *Campanula* and *Edraianthus* on the one hand, and *Asyneuma* Griseb. & Schenk and *Adenophora* on the

other. The monophyly of *Jasione* was supported by 100% and 60 character state changes. Little resolution was observed within the ingroup taxa. Only *J. sessiliflora* was resolved as sister to the rest of the taxa (bootstrap 82%) which resided in a hard polytomy. Within this polytomy, *J. crispa* had the longest terminal branch with 11 steps).

Discussion

Molecular analysis: *Jasione* is phylogenetically surprisingly isolated within *Campanulaceae* (Figure 1; Eddie et al., 2002). In the strict consensus trees of a maximum parsimony analysis the genus resides unresolved on a polytomy with *Wahlenbergia* Schrad. ex Roth and larger clades, all including a highly paraphyletic genus *Campanula*. None of the other *Campanulaceae* genera included in the analysis are suggested as being more closely associated with *Jasione*. This is somewhat problematic in choosing appropriate outgroup taxa for a detailed phylogenetic analysis on the genus alone. We tried including diverse representatives from the various major *Campanulaceae* clades in order to root our *Jasione* ITS analysis and eventually included 4 taxa representing the major clades on the polytomy with *Jasione* clade

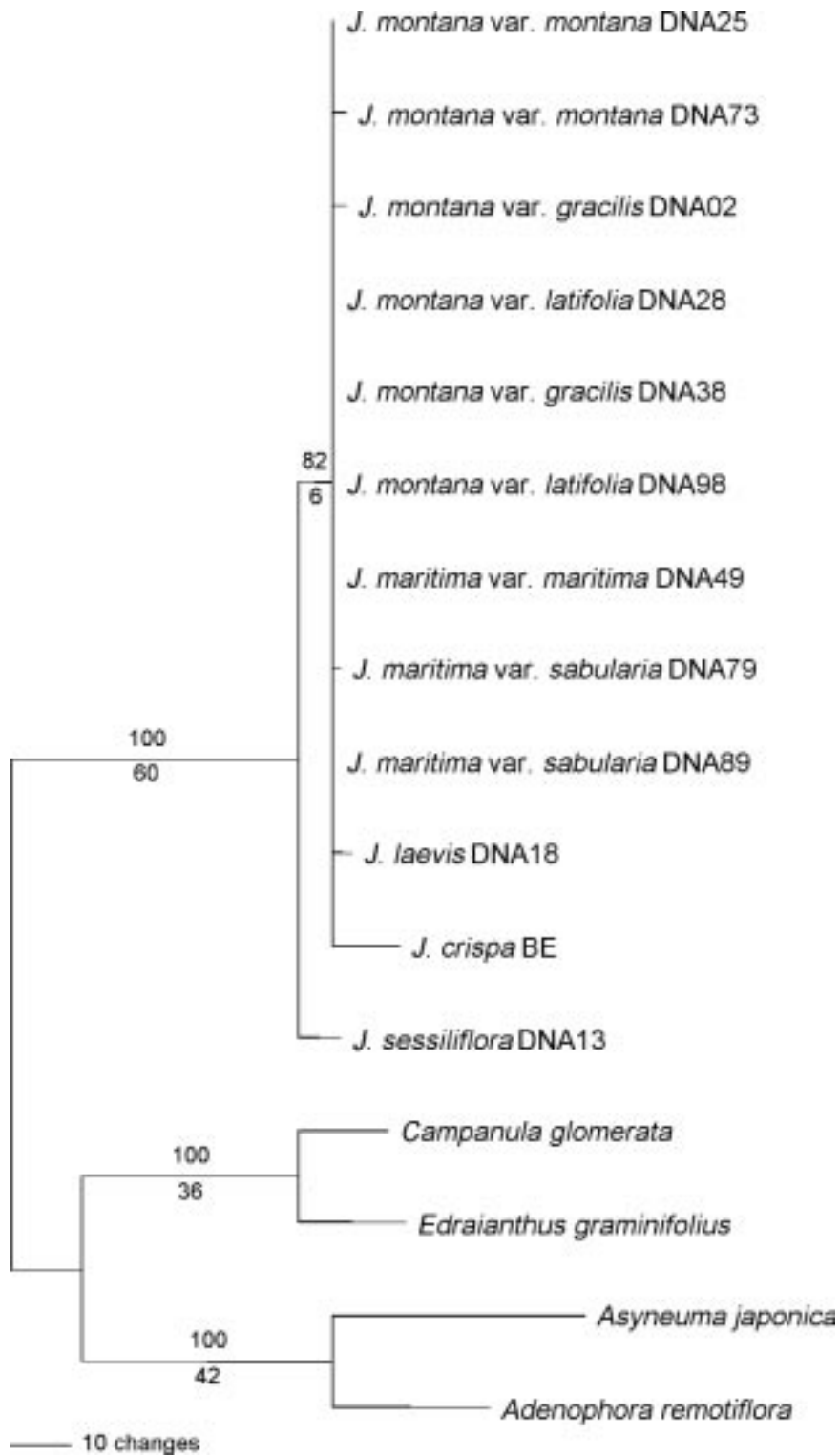


Figure 2. Single most parsimonious tree of 270 steps length of 12 *Jasione* accessions and four outgroup taxa based on internal transcribed spacer (ITS) sequences of ribosomal DNA (rDNA) with bootstrap values above and branch lengths below branches.

(*Adenophora remotiflora*, *Asyneuma japonica*, *Campanula glomerata* and *Edraianthus graminifolius*) (Figure 1). The isolation of *Jasione* from other *Campanulaceae* genera is also reflected in our present analysis, as divergences between 21.3% and 26.7% were present.

We obtained ITS sequences, sometimes of multiple accessions, from 5 species of *Jasione* (*J. crispa*, *J. laevis*, *J. maritima*, *J. montana* and *J. sessiliflora*), including 3 subspecies of *J. montana* and 2 of *J. maritima*, but found no significant sequence variation among the *J. montana* and *J. maritima* samples. The maximum divergence within *Jasione* was 5.3% between *J. crispa* and *J. sessiliflora*; this is relatively low compared with divergences of ITS sequences calculated for intrageneric analyses in other families, e.g. in *Gesneriaceae*, where *Streptocarpus* Lindl. shows a maximum intrageneric ITS variation of >20%. Even within subgenus *Streptocarpella*, the morphologically more homogeneous subgenus, a higher maximum divergence is present (10.8%) (Möller & Cronk, 2001) author.

The fact that 7 taxa, representing 4 species, fell on a hard polytomy, and that of those 6 had identical sequences, may indicate relative recent speciation events within *Jasione*. Considered from a biogeographical angle, it is interesting to note that all but 1 taxon with an identical sequence came from Spain. This indicates that geography is more important in phylogenetic terms than morphology or taxonomy. Thus, we appear to have obtained a gene tree, not a species tree.

We have only included 5 out of 12 species, 8 out of c. 23 taxa currently recognised, and only 1 representative

of the Turkish taxa. To properly analyse the species and variety relationships, more taxa need to be included for analysis, particularly from Turkey, but also further samples of the *J. sessiliflora* "species-group" - e.g. from North-west Africa. Furthermore, with a view to the low sequence divergence across the samples included, an alternative molecular strategy should be employed, such as population-based methods such as RAPDs, AFLPs or microsatellites.

Conclusions

Although there is a substantial *Jasione* literature dealing with its taxonomy (Tutin, 1976; Parnell, 1987; Sales & Hedge, 2001b), anatomy (Bokhari & Sales, 2001) and nomenclature (Sales & Hedge, 2001a), new approaches and new data are surely the way forward.

Our preliminary molecular data suggest that although *Jasione* is a relatively old genus with no close ally (based on its isolated position in the family phylogeny), speciation within the crown group may have occurred very recently, possibly linked to the last glaciation cycle in Europe. The ITS sequences were not evolving sufficiently fast to illustrate individual species relationships, but show a group of taxa with identical, or near identical, base composition.

Future work will focus on the inclusion of all species and subspecies in the analyses and additional approaches employing alternative methods, population-genetics-based, to clarify partition of genetic diversity within and between populations and species relationships.

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