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Molecular approach to the phylogeny and systematics of *Cytisus* (Leguminosae) and related genera based on nucleotide sequences of nrDNA (ITS region) and cpDNA (*trn*L-*trn*F intergenic spacer)

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Abstract. Phylogenetic relationships of *Cytisus* and allied genera (*Argyrocytisus*, *Calicotome*, *Chamaecytisus*, *Cytisophyllum*, and *Spartocytisus*) were assessed by analysis of sequences of the nrDNA internal transcribed spacer (ITS) and the cpDNA *trn*L-*trn*F intergenic spacer. Genera of the *Genista*-group (*Chamaespartium*, *Echinospartum*, *Genista*, *Pterospartum*, *Spartium, Teline* and *Ulex*) were included to check the position of *Cytisus* species transferred to *Teline*. The tree obtained by combining both sets of data indicates that the *Genista* and *Cytisus* groups form two separate clades. *Cytisus heterochrous* and *C. tribracteolatus* are more closely related to the *Cytisus*-group, thus their transfer to *Teline* is not supported by molecular data. *Cytisus fontanesii* (syn. *Chronanthos biflorus*) groups with *Cytisophyllum sessilifolium* and *Cytisus heterochrous* within the *Cytisus*-group. Similarly, *Argyrocytisus battandieri* falls within the *Cytisus*-group as a well differentiated taxon. All these taxa seem to have early diverged from the *Cytisus*-group. Their taxonomic rank should be reconsidered to better reflect their phylogenetic separation from *Cytisus*. On the contrary, *Chamaecytisus proliferus* and *Spartocytisus supranubius* enter in the main core of *Cytisus*, and they should better be included in sections of *Cytisus* (sect. *Tubocytisus* and *Oreosparton*, respectively). Sect. *Spartopsis* is not monophyletic and the position of several species, currently included in this section, deserves reevaluation: *C. arboreus* aggregate is closely related to *C. villosus* (sect. *Cytisus*) and to *Calicotome*; *C. striatus* is closely related to *Cytisus* sect. *Alburnoides*; and the position of *C. commutatus* (incl. *C. ingramii*) remains unclear. The relationships and positioning of several minor taxa (*C. transiens*, *C. megalanthus*, and *C. maurus*) are also discussed.

Key words: *Cytisus*, *Cytisus*-group, Genisteae, ITS region, *trn*L-*trn*F intergenic spacer, phylogeny, systematics.

Cytisus Desf. is a large genus distributed in Europe, northern Africa and the Canary Islands, extending also into Asia. Within the tribe Genisteae, *Cytisus* is the central genus of what has been termed the *Cytisus*-group (Polhill 1976, Bisby 1981). However, delimitation of *Cytisus* is still controversial, with taxa that have been alternatively merged into the genus, or segregated into several closely allied genera (e.g. *Argyrocytisus*, *Spartocytisus, Chronanthos, Chamaecytisus*). Different views on the status of these genera have been made by Frodin and Heywood (1968), Tutin et al. (1968), Polhill (1976), Cristofolini (1997) and Talavera (1999), among others. The infrageneric limits at the

sectional and specific levels, critical for the systematic framework of *Cytisus*, have been also subject different proposals, with species moved from one section to another, and sections included in or excluded from *Cytisus*. Examples of these taxonomic problems, reflecting the different views concerning the phylogenetic relationships within the *Cytisus*-group are: (1) The monospecific genus *Argyrocytisus* merged into *Adenocarpus* (*Adenocarpus battandieri;* Talavera and Salgueiro 1999); (2) *Spartocytisus* considered a separate section of *Cytisus* (sect. *Oreosparton*), or included in an expanded *Cytisus* sect. *Alburnoides* (Talavera and Salgueiro 1999); (3) *Chamaecytisus* species forming a well-differentiated section within *Cytisus* (sect. *Tubocytisus*; Polhill 1976, Cristofolini 1991); (4) *C. fontanesii* placed into a separate genus *Chronanthos* (*C. biflorus*; Heywood 1968), included in *Cytisus* sect. *Chronanthus* (together with *C. orientalis* and *C. heterochrous*; Polhill 1976), or included in a monospecific section of *Cytisus* (sect. *Heterocytisus*; Talavera 1999); (5) *Cytisus tribracteolatus*, considered as a member of the relatively primitive *Cytisus* sect. *Cytisus* (syn. sect. *Trianthocytisus*) together with *C. villosus* and *C. aeolicus* (Polhill 1976), placed in sect. *Spartopsis* (Frodin and Heywood 1968), or transferred to *Teline* (Talavera and Salgueiro 1999); (6) *Cytisus heterochrous* (syn. *C. patens* auct.) included in sect. *Chronanthus* (Polhill 1976), in sect. *Spartopsis* (Frodin and Heywood 1968), or transferred to *Teline* sect. *Chronanthus* (Talavera and Salgueiro 1999); (7) *Cytisus arboreus*, *C. malacitanus* and *C. transiens* removed from sect. *Spartopsis* to a separate group (sect. *Verzinum*; Talavera and Salgueiro 1999); and (8) *Cytisus commutatus* (including *C. ingramii*) transferred from sect. *Spartopsis* to sect. *Corothamnus* (Talavera 1999).

Uncertainty and so instability at the infraspecific level have been also common, and there are still several entities which have alternatively been considered as varieties, subspecies, or even separate species (e.g. *C. maurus*, *C. transiens*, *C. ingramii*, *C. valdesii*, *C. scoparius* subsp. *reverchonii*).

Molecular data from DNA studies on Genisteae have been included in broader studies on the whole phylogeny of Papilionoideae, based on *rbc*L gene sequences and ITS region sequences (Käss and Wink 1995, 1997a). There is also useful information in studies on *Lupinus*, which are based on ITS sequences (Käss and Wink 1997b, Aïnouche and Bayer 1999). However, specific molecular data on *Cytisus* are still scarce. In this study we use molecular data from two different DNA regions: the nuclear ITS region, and the chloroplast non-coding *trn*L-*trn*F intergenic spacer. To address some of the controversies on the generic and sectional limits, and to evaluate the phylogenetic distances between minor entities with molecular data, we have included samples ascribed to different genera, sections and infraspecific taxa. The aims of this study are to: (1) evaluate the level at which molecular data from these two regions provide phylogenetic information; (2) establish whether the combination of the ITS region and the *trn*L-*trn*F intergenic spacer (IGS) sequences reflects organismal evolution, and provides valuable data for elucidating taxonomic and phylogenetic relationships within the *Cytisus*-group; and (3) compare how these relationships agree with the pattern of evolution supported by morphological, serological and other systematic analyses.

Materials and methods

Plant material. Fifty seven samples representing 38 species of Genisteae from the Iberian Peninsula, France, Canary Islands and Morocco were analysed. Plants were collected on the field, either frozen or in silica gel. Alternatively, seeds from wild plants were grown and material was extracted from young seedlings. A list of the samples studied is given in Table 1, which includes voucher information and accession number for GenBank sequences. Several samples of each species were analysed when possible to evaluate infraspecific variation.

DNA isolation and PCR amplification procedure. Total DNA was extracted from leaves of an individual plant for each sample using

Table 1. Accession data for the Genisteae taxa sampled for phylogenetic analyses for the ITS region of nuclear ribosomal DNA and *trn*L-*trn*F intergenic spacer of chloroplast DNA

Taxon	Sample	Origin, Voucher	GenBank no.	
			ITS region	*trnL-trnF* IGS
Adenocarpus argyrophyllus (Rivas Goday) Caball.	325	Spain, Cáceres, Parque Natural de Montfragüe, Torrejón el Rubio, MAF159911	AF443627	AF443652
Adenocarpus complicatus (L.) J. Gay	231	Spain, Orense, San Cibrao de las Viñas, MAF148747	AF351085	AF352181
	326	Spain, Cáceres, Parque Natural de Montfragüe, Torrejón el Rubio, MAF159914	AF443628	AF443653
Adenocarpus hispanicus (Lam.) DC.	224	Spain, Segovia, Puerto de la Quesera, 1710 m, MAF159059	AF351086	AF352182
Argyrocytisus battandieri (Maire) Raynaud	195	Morocco, Jbel Tizirene, RAB62163	AF351099	AF352195
Calicotome intermedia C. Presl	358	Spain, Murcia, El Gorguel, 130 m, MAF159917	AF443634	AF443659
Calicotome spinosa (L.) Link	356	Spain, Castellón, Desierto de la Palmas, MAF159888	AF443635	AF443660
Calicotome villosa (Poir.) Link	32	Spain, Cádiz, Puerto Galis a Puerto Algarrobo, MAF143861	AF351089	AF352185
	284	Spain, Málaga, Coin-Mijas, MAF153711	AF443633	AF443658
Chamaecytisus proliferus (L.f.) Link subsp. *proliferus*	244	Spain, Canarias, La Palma, cruce al Pico de la Nieve, cumbres de Santa Cruz de la Palma, 1800 m, ORT	AF351101	AF352197
Chamaespartium sagittale (L.) P. E. Gibbs	436	Spain, Soria, Puerto de Oncala, 1454 m, MAF160403	AF443630	AF443655
Cytisophyllum sessilifolium (L.) O. Lang	52	Spain, Jardin Botánico de Madrid 684	AF351104	AF352200
Cytisus arboreus (Desf.) DC. subsp. *catalaunicus* (Webb) Maire	98	Morocco, Taza, Dayat Chiker, RAB62138	AF351124	AF352220
Cytisus commutatus (Willk.) Briq.	413	Spain, Santander, Castro-Urdiales, Coterillo, pr. Cerdigo, MAF160437	AF443648	AF443673
	414	Spain, Santander, Fumaril, pr. Cueto, MAF160436	AF443649	AF443674
Cytisus decumbens (Durande) Spach	433	Spain, Soria, Puerto de Oncala, 1454 m, MAF160401	AF443650	AF443675
Cytisus fontanesii Spach subsp. *plumosus* (Boiss.) Nyman	221	Morocco, Imouzzer du Kandar – Ifrane, 1500 m, RAB62146	AF351103	AF352199
Cytisus grandiflorus (Brot.) DC. subsp. *haplophyllus* (Maire & Sennen) Maire	223	Morocco, Gourougou, RAB62126	AF351116	AF352212

Table 1 (continued)

Taxon	Sample	Origin, Voucher	GenBank no.	
			ITS region	*trnL-trnF* IGS
Cytisus heterochrous Colmeiro	332	Spain, Jaén, subida al Yelmo desde Hornos, MAF159227	AF443636	AF443661
	395	Spain, Castellón, Cervera del Maestrat, 250 m, MAF160144	AF443637	AF443662
Cytisus ingramii Blakelock	341	Spain, Coruña, Monfero, cerrado de Cerqueiras, LOU24689	AF443646	AF443671
	342	Spain, Coruña, cerrado de Cerqueiras, LOU24690	AF443647	AF443672
Cytisus malacitanus Boiss.	367	Spain, Granada, subida a la Sierra de Lújar, desde Orgiva, 900 m, MAF159925	AF443644	AF443669
Cytisus maurus Humbert & Maire	218	Morocco, jbel Tazekka, RAB62129	AF351117	AF352213
	236	Morocco, jbel Tazekka, RAB62129	AF351118	AF352214
Cytisus megalanthus (Pau & Font Quer) Font Quer	302	Morocco, 5 km before Issaguène to Bad Berred, 1450 m, RAB62118	AF443643	AF443668
Cytisus multiflorus (L'Hér.) Sweet	71/73	Spain, Avila, Puerto Mijares a Mijares, 1300m, MAF156721	AF351107	AF352203
	89	Spain, Avila, Puerto del Pico, 1420 m, MAF148132	AF351106	AF352202
	131	Spain, Avila, Puerto del Pico a Cuevas, 1210 m, MAF147903	AF351105	AF352201
	324	Spain, Cáceres, Parque Natural de Montfragüe, MAF159912	AF443640	AF443665
Cytisus oromediterraneus Rivas Mart. et al.	48	Spain, Avila, Cepeda La Mora, La Serrota, 2240 m, MAF148721	AF351110	AF352206
	67	Spain, Avila, Puerto Mijares a Mijares, 1300 m, MAF156723	AF351108	AF352204
	81	Spain, Avila, Puerto del Pico, 1420 m, MAF148133	AF351109	AF352205
Cytisus ×*praecox* Beauverd	44/43	Spain, Jardín Botánico de Madrid 689	AF351113	AF352209
	59/60	Spain, Avila, Puerto Mijares a Mijares, 1300 m, MAF156718	AF351112	AF352208
	93	Spain, Avila, Puerto del Pico, 1420 m, MAF156719	AF351111	AF352207
Cytisus scoparius (L.) Link subsp. *reverchonii* (Degen & Hervier) Rivas Goday & Rivas Mart.	22	Spain, Jaén, Siles a Orcera, 700 m, MAF148149	AF351122	AF352218

Table 1 (continued)

Taxon	Sample	Origin, Voucher	GenBank no.	
			ITS region	*trnL-trnF* IGS
Cytisus scoparius (L.) Link subsp. *scoparius*	27	Spain, Avila, Puerto de Casillas, 900 m, MAF148134	AF351119	AF352215
	178	Portugal, de Macedo de Cavaleira a Mogadouro, 230 m, MAF148178	AF351121	AF352217
	232	France, Bretagne, Carnac, MAF159290	AF351120	AF352216
Cytisus striatus (Hill) Rothm. subsp. *eriocarpus* (Boiss. & Reut.) Rivas Mart.	51	Spain, Avila, Puerto del Pico a Cuevas, 1210 m, MAF148141	AF351115	AF352211
Cytisus striatus (Hill) Rothm. subsp. *striatus*	283	Portugal, de Belmonte a Manteigas, MAF148183	AF443642	AF443667
Cytisus striatus (Hill) Rothm. × *C. multiflorus* (L'Hér.) Sweet	114	Spain, Avila, Puerto del Pico a Cuevas, 1210 m, MAF147902	AF351114	AF352210
Cytisus transiens (Maire) Talavera	282	Morocco, Ezzhiliga, jbel Tirmah, RAB62171	AF443645	AF443670
Cytisus tribracteolatus Webb	430	Spain, Cadiz, San Roque, Los Charcones, MAF139998	AF443638	AF443663
Cytisus valdesii Talavera & P. E. Gibbs	300	Morocco, jbel Oukaimeden, 2650 m, RAB62147	AF443641	AF443666
Cytisus villosus Pourr.	321	Morocco, Bab Taza-Bab Berred, RAB62157	AF443639	AF443664
Echinospartum barnadesii (Graells) Rothm.	208	Spain, Avila, Plataforma Circo, Prado de las Pozas, 1800 m, MAF159291	AF351087	AF352183
Genista anglica L.	207	Spain, Avila, subida al Prado de las Pozas, 1860 m, MAF159293	AF351090	AF352186
Pterospartum tridentatum (L.) Willk.	344	Spain, Pontevedra, Porriño, Gándaras de Budiño, LOU24694	AF443629	AF443654
Spartium junceum L.	247	Spain, Madrid, Dehesa de la Villa, MAF159908	AF351088	AF352184
Spartocytisus supranubius (L.f.) Webb & Berth.	242	Spain, Canarias, La Palma, Santa Cruz de la Palma, 1900 m, ORT	AF351102	AF352198
Teline canariensis (L.) Webb & Berth.	243	Spain, Canarias, Tenerife, cercanías del Bailadero, Anaga, 800 m, ORT	AF351098	AF352194
Teline linifolia (L.) Webb	258	Morocco, Mamora, RAB62170	AF443631	AF443656
Teline monspessulana (L.) K. Koch	259	Morocco, Larache, RAB62161	AF443632	AF443657
Teline pallida (Poir.) Kunkel	241	Spain, Canarias, Tenerife, Roque de Las Animas, Taganaga, Anaga, 350 m, ORT	AF351097	AF352193
Ulex parviflorus Pourr. subsp. *parviflorus*	257	Spain, Almería, Cabo de Gata, MAF159890	AF443626	AF443651

DNeasy Plant Mini Kit from Qiagen (Courtaboeuf, France) following the manufacturer's protocol, slightly modified to avoid viscous lisates. Double-stranded DNA amplifications were performed in 50 µl volume containing 2 mM $MgCl_2$, 200 µmol/l of each dNTP, 0.5 µmol/l of each primer, and 1U of DNA polymerase (Biotools), and 50 ng of DNA. The entire ITS region, comprising ITS1, 5.8S gene and ITS2, was amplified with external ITS5 and ITS4 universal primers (White et al. 1990). Standard PCR conditions were a preheat of 94 °C 5 min, 35 cycles of 30 s at 94 °C, 30 s at 54 °C, and 2 min at 72 °C, followed by 5 min at 72 °C. The *trn*L-*trn*F intergenic spacer of the chloroplast genome was amplified with primers e and f (Taberlet et al. 1991). Standard PCR conditions were a preheat of 94 °C 5 min, 35 cycles (1 min at 94 °C, 1 min at 48 °C, and 3 min at 72 °C), followed by 5 min at 72 °C. PCR products were purified on either Biotools Bioclean DNA purification columns or Qiaquick purification kit, according to manufacture's specifications. Sequencing was performed on both strands using the ABI PRISM^{TM} Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Biosystems), with the amplification primer. Sequencing reactions were electrophoresed on a ABI PRISM 377 DNA sequencer (Applied Biosystems) at the Centro de Secuenciación (Universidad Complutense, Madrid).

Phylogenetic analysis. Multiple alignment of the sequences was obtained using the Clustal program (Higgins et al. 1992). In the case of *trn*L-*trn*F IGS, the alignment was easily adjusted manually because of the existence of conserved regions and shared indels. Parsimony analyses were performed using PAUP version 4.0b4a for Macintosh (Swofford 2000) by heuristic searches on unweighted characters and characters states, excluding constant characters. Gaps were excluded in regions where the homology could not be ascertained, or mono- or dinucleotide repeats of different length occur. Indels of one or more base pairs were coded and considered as a single event, and the corresponding positions in the sequence alignment excluded from the analyses. We use starting trees obtained by simple stepwise addition sequences, with one tree held at each step and TBR branch swapping algorithm, MULTREES option in effect, accelerated transformation (ACCTRAN), branches of zero length collapsed and topological constraints not enforced. Several analyses were performed including or excluding the coded indels to check the degree of resolution provided by point substitutions alone and by the indels. Separate and combined analyses of the two nuclear and chloroplast sequences data sets were performed, and strict and 50% majority-rule consensus trees were generated. Due to the existences of polytomies the majority rule consensus trees reflect more accurately the morphological groupings. The bootstrap method (Felsenstein 1985) was used in order to estimate the robustness of the various clades revealed in the consensus tree. Bootstrap values were estimated from 1000 replicates of fast-heuristic searches using random addition sequence.

Outgroups and genera studied. *Adenocarpus* was used as an outgroup because it has been considered as an 'outlier' within the tribe (Bisby 1981, Cristofolini and Feoli Chiapella 1984), being a well-differentiated genus, standing apart either from the *Cytisus*-group and from the *Genista*-group. Analyses using as outgroup ITS sequences of more unrelated taxa (e.g. *Lupinus* and *Thermopsis*) obtained from the GenBank gave similar results. The ingroup includes most of the genera currently recognised in the *Cytisus*-group (*Argyrocytisus*, *Calicotome, Chamaecytisus*, *Cytisophyllum*, *Cytisus,* and *Spartocytisus*), and species of most of the sections of *Cytisus*, (Table 2). Species representing genera of the *Genista*-group (*Chamaespartium*, *Echinospartum*, *Genista*, *Pterospartum*, *Spartium, Teline* and *Ulex*) have been included to check the position of these genera in the tribe, and in particular, to establish the position of species of *Cytisus* that have been transferred to *Teline*. To facilitate the discussion, we present in Table 2 the positioning of the samples in two proposals for the systematic of *Cytisus* that have been used in the literature.

Results

The mean length of the ITS region is 595 bp, after excluding about 20 bp at the end of ITS2 to prevent ambiguous alignments. Mean base frequencies are $A = 0.20$, $C = 0.28$, $G = 0.31$ and $T = 0.21$. Multiple sequences alignment required the inclusion of several gapped positions. The aligned data matrix of the ITS region included a total of 635 sites (ITS1, 253 charac-

Table 2. Position of the studied taxa in previous classifications of the *Genista-Cytisus* complex

After Polhill (1976)	Species	After Talavera (1999)
Calicotome Link	*Calicotome villosa* (Poir.) Link	*Calicotome* Link
	Calicotome intermedia C. Presl.	
	Calicotome spinosa (L.) Link	
Cytisus sect. *Cytisus*	*Cytisophyllum sessilifolium* (L.) O. Lang	*Cytisophyllum* O. Lang
Cytisus sect. *Tubocytisus* DC.	*Chamaecytisus proliferus* (L.f.) Link	*Chamaecytisus* Link
Cytisus sect. *Petteria* (C. Presl) Polhill	*Argyrocytisus battandieri* (Maire) Raynaud	*Adenocarpus* DC.
Cytisus sect. *Chronanthus* DC.	*Cytisus fontanesii* Ball	*Cytisus* sect. *Heterocytisus* Briq.
	Cytisus heterochrous Colmeiro	*Teline* sect. *Chronanthus* (DC.) Talavera & P. E. Gibbs
Cytisus sect. *Trianthocytisus* Griseb.	*Cytisus tribracteolatus* Webb	
	Cytisus villosus Pourr.	*Cytisus* sect. *Cytisus*
Cytisus sect. *Corothamnus* (W.D.J. Koch) Nyman	*Cytisus decumbens* (Durande) Spach	*Cytisus* sect. *Corothamnus* (W.D.J. Koch) Nyman
Cytisus sect. *Spartopsis* Dumort.	*Cytisus commutatus* (Willk.) Briq.	
	Cytisus ingramii Blakelock	
	Cytisus transiens (Maire) Talavera	*Cytisus* sect. *Verzinum* (Raf.) Talavera
	Cytisus arboreus (Desf.) DC.	
	Cytisus malacitanus Boiss.	
	Cytisus grandiflorus (Brot.) DC.	*Cytisus* sect. *Spartopsis* Dumort.
	Cytisus maurus Humbert & Maire	
	Cytisus scoparius (L.) Link	
	Cytisus megalanthus (Pau & Font Quer) Font Quer	
	Cytisus striatus (Hill) Rothm.	
Cytisus sect. *Alburnoides* DC.	*Cytisus multiflorus* (L'Hér.) Sweet	*Cytisus* sect. *Alburnoides* DC.
	Cytisus oromediterraneus Rivas Mart. et al.	
	Cytisus × *praecox* Beauverd	
	Cytisus valdesii Talavera & P. E. Gibbs	
Cytisus sect. *Oreosparton* (Webb) Polhill	*Spartocytisus supranubius* (L.f.) Webb & Berth.	

ters; 5.8S, 165 characters; and ITS2, 217 characters). Several potentially informative indels are present (nine of 1bp and one of 5 bp; Table 3). The latter was coded as a single event, and the corresponding 5 sites were excluded. The data matrix has 171 variables sites (98 in ITS1, 5 in 5.8S and 68 in ITS2), 107 of them are potentially parsimony-informative.

Table 3. Distribution of parsimony-informative indels coded for the phylogenetic analyses

Region	Sequence	Position in the file	Taxa
ITS1	G/–	59	*Cytisus valdesii*, *C. ingramii*, *C. commutatus*, *C. oromediterraneus* 48 (G)/Others (–)
	G/T/–	73	*Teline canariensis*, *Chamaespartium sagittale* (T)/*Ulex*, *Pterospartum* (–)/Others (G)
	T/C/–	80	*Chamaecytisus*, *Cytisus valdesii*, *C. decumbens*, *C. transiens*, *C. malacitanus*, *C. arboreus*, *C. megalanthus* (T)/*C. scoparius scoparius* 178, *C. ×praecox* 93 (–)/Others (C)
	A/G/T/–	81	*Cytisophyllum* (A)/*Chamaespartium*, *Cytisus* × *praecox* 43, *C. oromediterraneus*, *C. striatus* × *C. multiflorus* (T)/*C. ×praecox* 93 (–)/Others (G)
	C/–	88	*Cytisus villosus*, *C. arboreus*, *C. maurus* (–)/Others (C)
	G/T/C/–	106	*Spartium junceum* (G)/*Echinospartum*, *Chamaespartium* (T)/*Adenocarpus*, *Ulex*, *Pterospartum*, *Teline*, *Genista*, *Cytisophyllum*, *Cytisus fontanesii*, *C. heterochro*us (C) / *Argyrocytisus*, *Chamaecytisus*, *Spartocytisus*, *Cytisus* (except *C. fontanesii and C. heterochrous*) (–)
	A/G/–	118	*Cytisus multiflorus*, *C. ×praecox* 59 (A) / *Argyrocytisus* (–)/Others (G)
	A/G/T/–	123	*Ulex*, *Genista* (T)/*Echinospartum*, *Spartocytisus* (–) / *Calicotome villosa*, *C. spinosa*, *Cytisus decumbens* (A)/Others (G)
ITS2	T/–	468	*Spartium*, *Calicotome* (T)/Others (–)
	GCAAG/–	454–458	*Cytisus striatus*, *C. megalanthus* (+)/Others (–)
trnL-trnF IGS	C/–	126	*Cytisus heterochrous* (–)/Others (C)
	TAATTATATG/–	155–164	*Adenocarpus*, *Genista*, *Ulex*, *Echinospartum*, *Pterospartum*, *Chamaespartium*, *Spartium*, *Teline*, *Cytisophyllum*, *Argyrocytisu*s, *Cytisus heterochrous*, *C. tribracteolatus* (–)/*Calicotome*, *Chamaecytisus*, *Spartocytisus*, *Cytisus* (except *C. heterochrous*, *C. tribracteolatus*) (+)
	ATTAAT/–	300–305	*Pterospartum*, *Cytisus fontanesii*, *C. heterochrous*, *C. tribracteolatus* (+)/Others (–)
	CAAA/–	354–358	*Calicotome* (–)/Others (+)
	ATT/–	363–365	Random
	TTATTTA/–	396–402	*Teline canariensis*, *T. monspessulana* (+)/Others (–)

The mean length of the *trn*L-*trn*F IGS region is 396 bp. This region has a high content of A and T (0.36 and 0.37 respectively) as compared to C and G (0.15 and 0.12). The aligned data matrix has 573 sites and required the inclusion of gapped positions. Indels of 1 to 10 bp length have been found. Most of them are constant at the specific level. Excluding the autapomorphous indels, 6 others have been coded as a single event and considered in the analyses (Table 3). The TAATTATATG indel is particularly informative (Table 3, Fig. 2), being absent in *Adenocarpus*, the *Genista*-group, *Cytisophyllum*, *Argyrocytisus* and

two *Cytisus* species (*C. heterochrous* and *C. tribracteolatus*). The 6 bp indel (ATTAAT) is present in *Pterospartum tridentatum*, *C. fontanesii*, *C. heterochrous* and *C. tribracteolatus*. A/T repeats and polyT sequences of variable length have been found. Following the proposal of Kelchner (2000), variable-length repeat strings have been removed from consideration in the analysis. In the data matrix, 38 out of 76 variables sites are potentially parsimony informative.

Phylogenetic results. Parsimony analysis of the ITS region recovered 10350 equally parsimonious trees. The 50% majority rule consensus tree (tree length 403, CI = 0.58, RI = 0.73, HI = 0.42, RC = 0.42) is shown in Fig. 1. Since several samples from the same species were included to check the consistency of sequences at the infraspecific levels, several polytomies are present as expected at the terminal branches.

The ITS tree (Fig. 1) reveals two major but weakly supported clades (bootstrap values ≤ 50%). These low values of statistical support have also been found in previous analyses of Genisteae (e.g. Käss and Wink 1997a). One clade is formed by all the genera usually included in the *Genista*-group (*Ulex*, *Pterospartum*, *Spartium*, *Genista*, *Echinospartum*, *Chamaespartium*, and *Teline* species). With the exception of *Teline*, only one species of each of those genera have been included, thus we do not draw conclusions regarding their generic relationships. Nevertheless, it must be mentioned that *Teline* does not form a monophyletic group*. The second group (*Cytisus*-group clade) includes all the *Cytisus* species and related genera (*Argyrocytisus*, *Spartocytisus*, *Chamaecytisus*, *Cytisophyllum*, and *Calicotome*).

In the *Cytisus*-group clade, the position of *Cytisophyllum sessilifolium*, *Cytisus fontanesii* and *C. heterochrous* is not resolved. The remaining species gather in a cluster where *Cytisus tribracteolatus* and *Spartocytisus supranubius* stand apart from the other species which form three main clades. In the first one, the position of *Chamaecytisus proliferus* and *Cytisus decumbens* is unresolved. *C. valdesii* forms a group with *C. ingramii* and *C. commutatus*. The latter two species have been a matter of taxonomic controversy, being merged or separated according to different authors. In our study, they form a monophyletic clade (100% bootstrap value). In the second clade, *C. villosus* is sister to the common ancestor of two well-supported subgroups: a first one is formed by *C. arboreus*, *C. malacitanus* and *C. transiens* (85% bootstrap value), and a second monophyletic group comprising the *Calicotome* species (100% bootstrap support). However, the sister relationship of *C. villosus* to these two groups has a low bootstrap support. In the third large clade (57% bootstrap support) *Argyrocytisus battandieri* is placed as sister to the clade containing the other taxa (*C. striatus*-*C. megalanthus*, *C. grandiflorus*-*C. maurus*, *C. scoparius*, *C. multiflorus*-*C. ×praecox*, and *C. oromediterraneus*-*C. ×praecox*).

The parsimony analysis of the *trn*L-*trn*F IGS region recovered 70 equally parsimonious trees. The 50% majority rule consensus tree (Tree length = 106, CI = 0.83, RI = 0.91, HI = 0.17, RC = 0.76, Fig. 2) shows: (1) *Argyrocytisus battandieri* at the base, its relationships unresolved; (2) a clade (73% bootstrap) including *Cytisophyllum sessilifolium* as sister to *Cytisus tribracteolatus* and *C. heterochrous*; (3) the *Genista*-group clade, in which, similarly to the result provided by the ITS region tree, *Teline* appears as a heterogeneous group; (4) a clade with the core of the *Cytisus*-group, supported by the presence of the 10 bp long indel (Fig. 2, Table 3). Thus, the *trn*L-*trn*F IGS tree separates the *Genista*-group as does the ITS tree. However, the *Cytisus*-group divides in two: a clade with *Cytisophyllum sessilifolium, Cytisus tribracteolatus* and *C. heterochrous* (73% bootstrap support); and

*__Note added in proof:__ Percy and Cronk (Amer. J. Bot 89(5): 854–864, 2002) has found a similar result. The genus *Teline* was polyphyletic with two groups (the "Teline monspessulana group" and the "Teline linifolia group") separately nested within *Genista*.

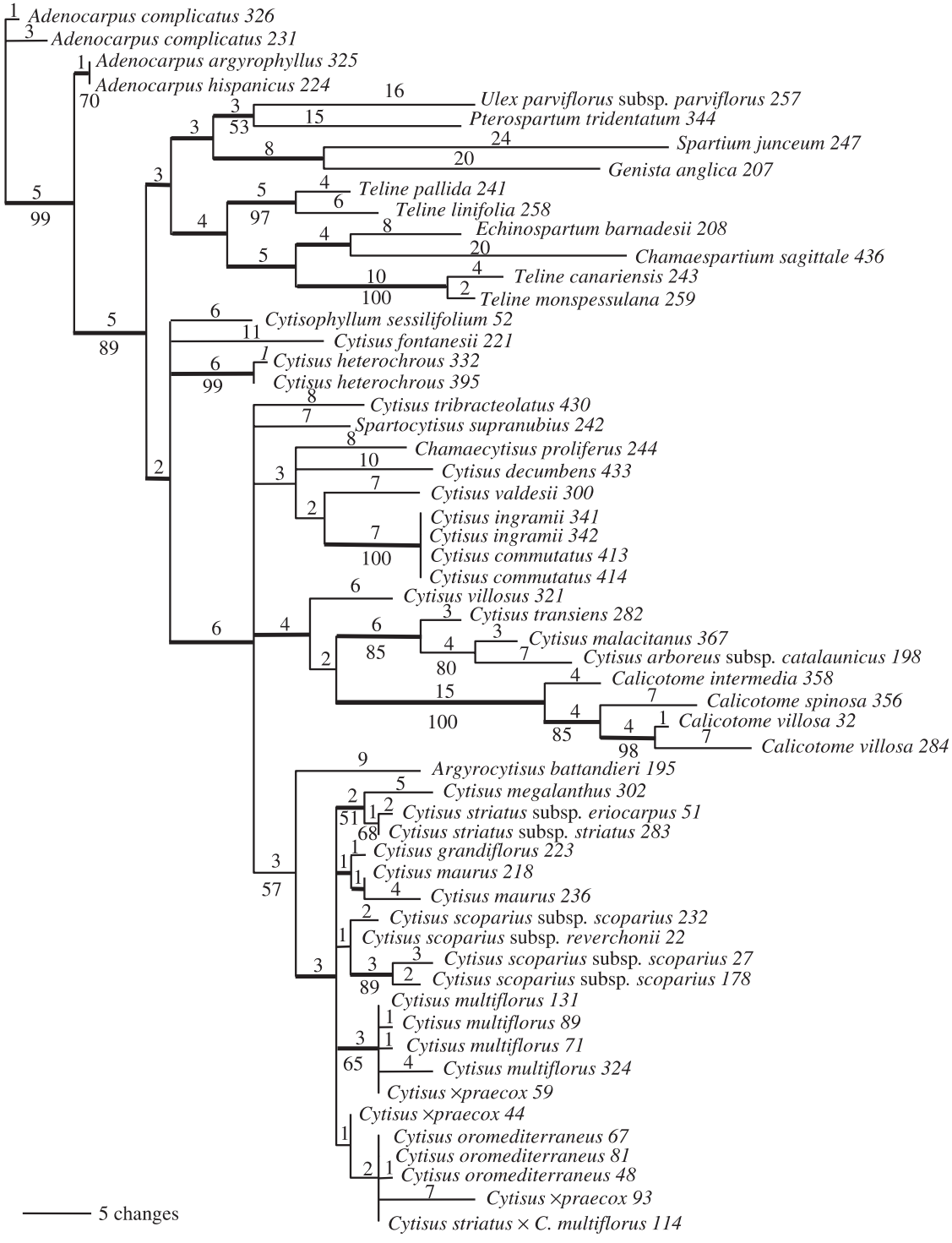

Fig. 1. 50% Majority rule consensus tree represented as phylogram from the ITS region data set. Numbers above the branches represent branch length, and numbers below indicate bootstrap values above 50%. Clades present in the strict consensus topology are indicated by bold lines

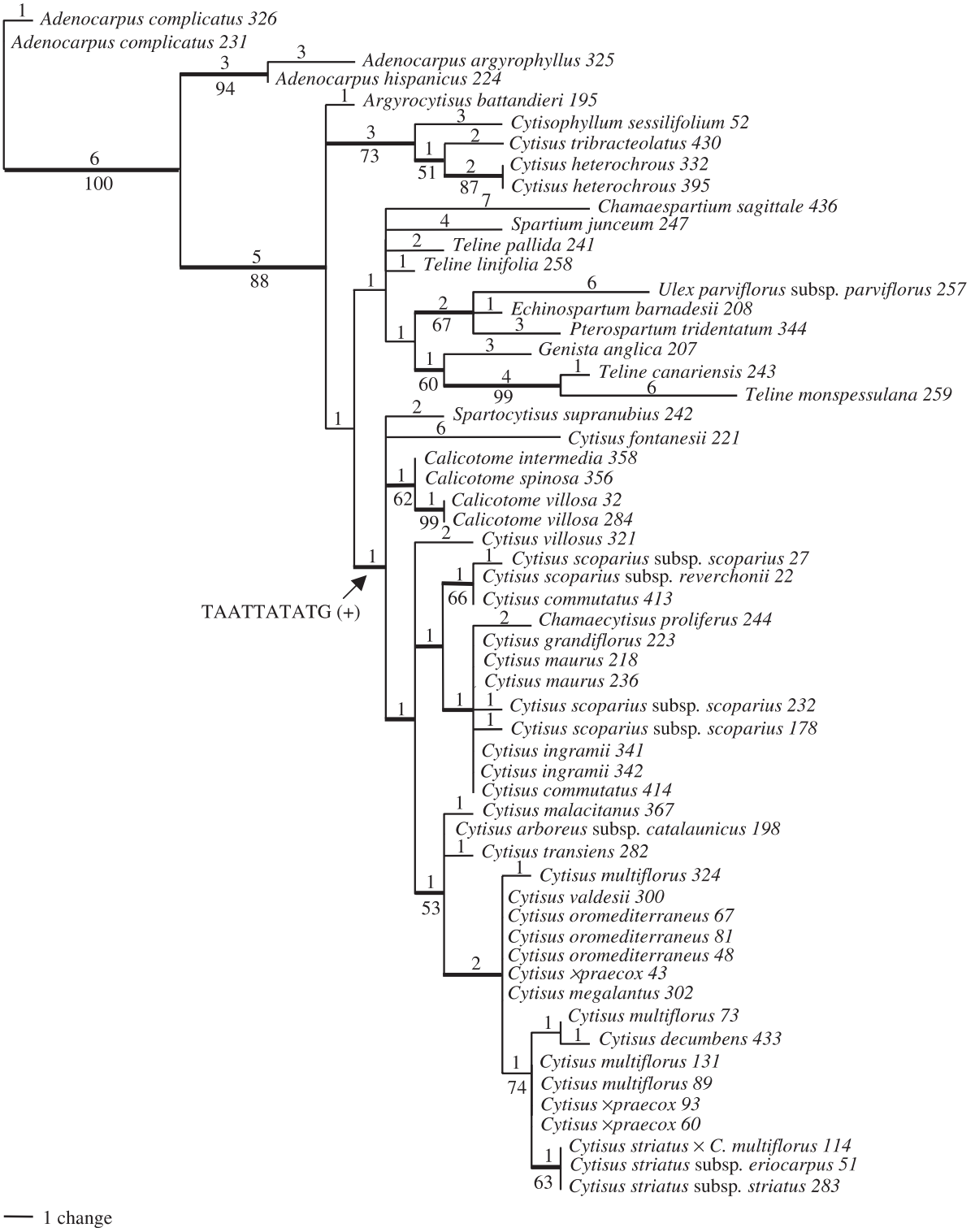

Fig. 2. 50% Majority rule consensus tree represented as phylogram from the *trn*L-*trn*F IGS data set. Numbers above the branches represent branch length, and numbers below indicate bootstrap values above 50%. The distribution of the most informative indel is indicated. Clades present in the strict consensus topology are indicated by bold lines

a second clade with the main core of the *Cytisus*-group, including most of the *Cytisus* species and related genera (*Spartocytisus*, *Calicotome* and *Chamaecytisus*).

Within this latter clade there is a basal polytomy in which: (1) the position of *Spartocytisus* and *Cytisus fontanesii* remain unresolved; (2) the species of C*alicotome* form a supported clade (62% bootstrap); (3) all the other taxa gather in a large clade, where *Cytisus villosus* stands apart, and two differentiated groups appear. In the first one, *Chamaecytisus proliferus*, *Cytisus scoparius*, *C. grandiflorus*, *C. ingramii*, *C. commutatus*, and related minor taxa gather. The second group (53% bootstrap) includes *C. arboreus*, *C. malacitanus*, *C. multiflorus*, *C. oromediterraneus*, *C. striatus*, *C. decumbens*, related taxa, and hybrids.

The parsimony analysis of the combined data matrix of ITS region and *trn*L-*trn*F IGS region (Fig. 3) recovered 11451 equally parsimonious trees (Tree length = 541, CI = 0.59, RI = 0.73, HI = 0.41, RC = 0.43). In this analysis the *Genista*-group and *Cytisus*-group are differentiated, and within the first one, *Teline* is not monophyletic. Within the *Cytisus*-group, a first clade separates and includes *Cytisus fontanesii* as sister to *Cytisophyllum sessilifolium* and *Cytisus heterochrous*. Secondly, *Argyrocytisus*, *Spartocytisus* and *Cytisus tribracteolatus* stand at the base of a cluster with the main core of the *Cytisus*-group. Within this cluster: (1) *Chamaecytisus proliferus, Cytisus valdesii* and *C. decumbens* are unresolved; (2) a well supported clade (98% bootstrap value) consists of *C. commutatus* and *C. ingramii*; (3) *C. villosus* is sister to the common ancestor of two well-supported groups: a first clade formed by *C. transiens*, *C. malacitanus* and *C. arboreus* (91% bootstrap value), and a second one with the *Calicotome* species (forming a monophyletic group with 100% bootstrap support); and (4) a clade with two separated groups, the first including *Cytisus grandiflorus*, *C. maurus* and *C. scoparius*, and a second one with *C. megalanthus*, *C. striatus*, *C. multiflorus*, *C. oromediterraneus* and hybrids.

Comparison of the trees. As has been found in many other groups of angiosperms (Soltis and Soltis 1998), the usefulness of both regions for phylogenetic reconstruction at the infraspecific level is small. However, at the specific, sectional and generic levels, the two regions provide important clues for the establishment of phylogenetic relationships. At generic and sectional ranks, a similar resolution is obtained in both trees. However, at the specific level, the ITS tree gives a more detailed picture of the genetic differentiation. The ITS tree forms separate groups with samples of each species and closely related taxa, whereas in many cases the *trn*L-*trn*F IGS does not. This agrees with the higher number of parsimony informative characters provided by the ITS region as compared to the *trn*L-*trn*F IGS sequences, and indicates that the interspecific variation of the ITS sequences is higher than that of the *trn*L-*trn*F IGS sequences (Fig. 3).

A comparison of the ITS and *trn*L-*trn*F IGS trees shows several differences: (1) *Argyrocytisus battandieri* is part of the main core of the *Cytisus*-group in the ITS tree, whereas in the *trn*L-*trn*F IGS tree its position is not defined. (2) The position of *Cytisus fontanesii*, *C. heterochrous* and *Cytisophyllum sessilifolium* is unresolved in the ITS tree, due to the many changes in the ITS sequences as compared to the main core of the *Cytisus*-group. In the *trn*L-*trn*F IGS tree, *Cytisus fontanesii* enters the *Cytisus*-group, and separates from *C. heterochrous* and *Cytisophyllum sessilifolium*. This is because *C. fontanesii* shares the 10 bp insertion characteristic of the *Cytisus*-group, which in turn is not present in *C. heterochrous* and *Cytisophyllum sessilifolium*. The combined tree joins the three taxa in a basal clade separated from the main core but within the *Cytisus*-group. (3) *Cytisus tribracteolatus* shares several *trn*L-*trn*F IGS characters with *C. fontanesii*, *C. heterochrous* and *Cytisophyllum sessilifolium*, and therefore enters the same clade as these taxa in the *trn*L-*trn*F IGS tree. However, the ITS sequence of *C. tribracteolatus* has more similarities to the main core of

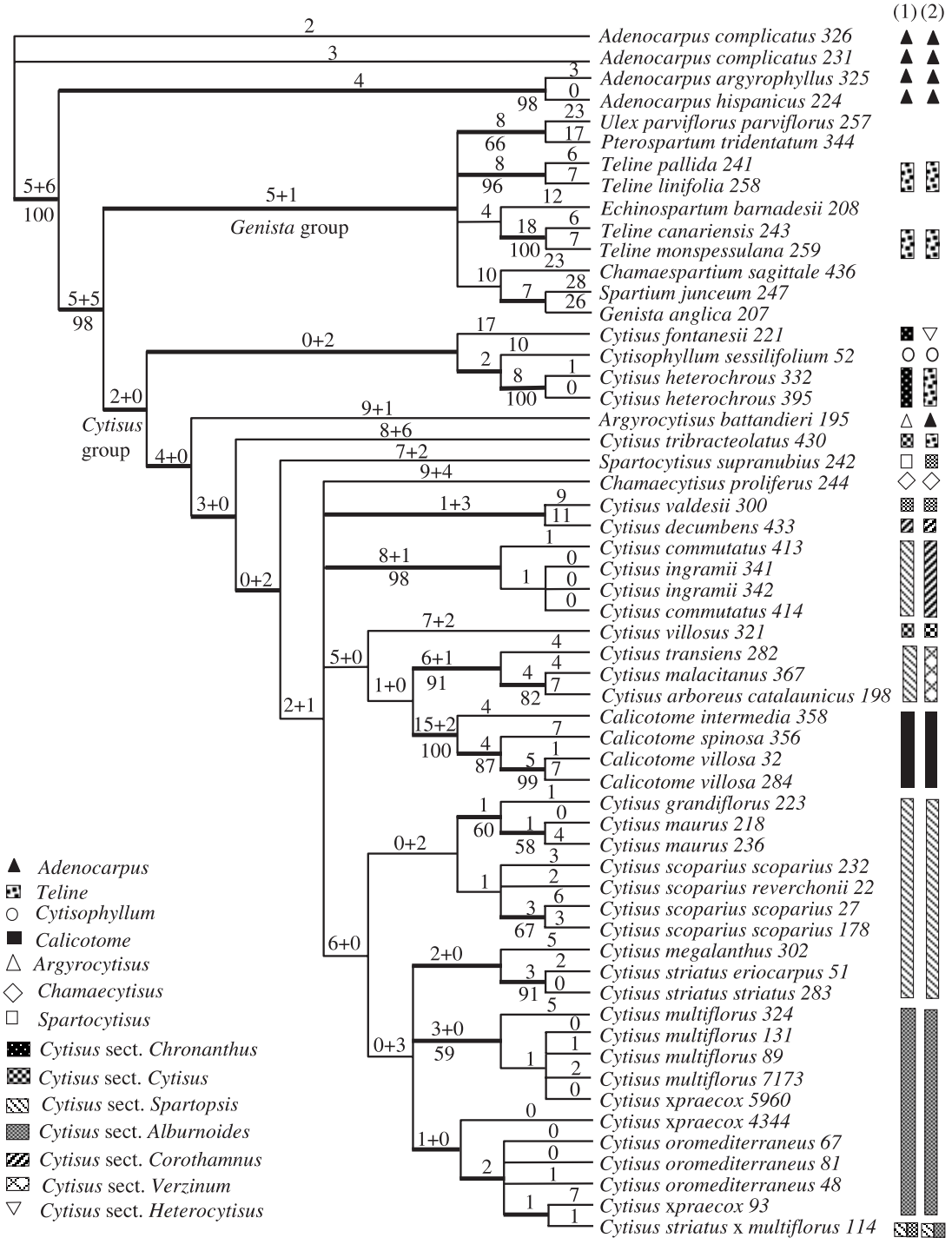

Fig. 3. 50% Majority rule cladogram from the combined data set. Numbers above the branches represent branch length. In the internal branches they are indicated as changes in ITS + *trn*L-*trn*F IGS sequences. Numbers below indicate bootstrap values above 50%. Generic and tribal affinities of taxa according to (1) Polhill (1976) and Polhill et al. (1978), and (2) Talavera (1999) are indicated by the bar patterns. Clades present in the strict consensus topology are indicated by bold line

Cytisus-group. The position of *C. tribracteolatus* in the combined tree reflects better the affinities to the main core of the *Cytisus*-group. (4) *Cytisus ingramii* and *C. commutatus* form a well-supported clade in the ITS tree, however, in the *trn*L-*trn*F IGS tree one of the samples of *C. commutatus* (413) stands apart of the others, and groups with *C. scoparius* (samples 22 and 27). Sample 413 shares one insertion (ATT) and one substitution with *C. scoparius* samples 22 and 27. Moreover, the group *C. commutatus-ingramii* separates from the other species of the sect. *Spartopsis* in ITS, whereas in the *trn*L-*trn*F IGS tree the group forms part of an unresolved clade. We suggest that this is mainly a consequence of the lower resolution provided by the *trn*L-*trn*F IGS sequences. (5) Similarly, the neat clade of *Cytisus arboreus* that forms in the ITS tree (with bootstrap support) is not seen in the *trn*L-*trn*F IGS tree. (6) The *Calicotome* clade is related to the *Cytisus arboreus* one in the ITS tree, whereas they separate in the *trn*L-*trn*F IGS tree due to one deletion of 4 bp, exclusive to *Calicotome* (Table 3). (7) *Cytisus megalanthus* groups with *C. striatus* in the ITS tree with only a weak support (51%), however, their relationships are unresolved in the *trn*L-*trn*F IGS tree.

Discussion

Essential tasks in the analysis of multiple data sets include the assessing of congruence between different phylogenetic trees and data sets, and ascertaining whether multiple data sets should be combined into a single data matrix prior to phylogenetic reconstruction (Johnson and Soltis 1998). In our study the differences observed in the topologies of the trees are mainly due to the different degree of resolution provided by both regions, i.e. the ITS region provides much more parsimony-informative characters (107) than the *trn*L-*trn*F IGS region (38). Following the definition given by Johnson and Soltis (1998; i.e. '*Two trees are congruent if one tree is a more resolved version of the second tree...*'), there are not significant incongruence between the ITS and *trn*L-*trn*F IGS trees. In our opinion, the combined tree reflects the relationships depicted in the ITS tree, incorporating also useful (but scarce) information provided by the *trn*L-*trn*F IGS tree, thus providing a more reliable picture of the phylogenetic relationships in the *Cytisus*-group. Nevertheless, it cannot be ruled out that some of the conflicting points found in the different trees are not reflective of some interesting evolutionary processes. A discussion of the phylogenetic results, based on the tree resulting from the combined data matrix follows.

***Genista*-group and *Cytisus*-group limits.** The position and taxonomic delimitation of *Teline* is crucial for the current distinction between the *Genista*-group and the *Cytisus*-group (e.g. Polhill 1976, Bisby 1981, among others). In our study the four species of *Teline* clearly belong to the *Genista*-group. Similar results have been obtained by Cristofolini and Feoli-Chiapella (1984) based on serological data from *T. monspessulana* and *T. linifolia*. Two other taxa that must be considered to address the separation of these groups are *Cytisus heterochrous* (*C. patens* auct.; López González 1985) and *C. tribracteolatus*. According to Polhill (1976) they are in different sections of *Cytisus* (Table 2). On the contrary, for other authors, both taxa conform a group included within *Cytisus* (Briquet 1894, Gibbs and Dingwall 1971), or within *Teline* (Talavera and Gibbs 1996, Talavera and Salgueiro 1999; Table 2). In our study, neither *C. heterochrous* nor *C. tribracteolatus* group with the *Teline* species, and not even enter in the *Genista* clade. Our data indicate that they are more closely related to the *Cytisus*-group.

The position of these taxa within the *Cytisus*-group is more controversial. In the combined and ITS trees, *Cytisus heterochrous* and *C. tribracteolatus* are clearly part of the *Cytisus*-group, however they appear in different clades. On the contrary, in the chloroplast based tree they form a clade with *Cytisophyllum sessilifolium* outside both the *Genista*-group and the *Cytisus*-group. This topology

reflects that although they are not part of the *Genista*-group, they share with this group the lack of the 10 bp insertion in the *trn*L-*trn*F IGS region. This insertion is present in all the other species of the *Cytisus*-group. This complexity stresses the importance of these taxa within the *Cytisus*-group.

Cytisus heterochrous has been included in the derived *Cytisus* sect. *Chronanthus* (Polhill 1976, Gibbs 1974), whereas *C. tribracteolatus* is a species whose systematic position is very uncertain. *Cytisus tribracteolatus* was first ranked under the relatively primitive sect. *Trianthocytisus*, together with *C. villosus* and *C. aeolicus*, by Polhill (1976), and later Frodin and Heywood (1968) put it at the end of sect. *Spartopsis*. Molecular data provide some support to *C. heterochrous* as one of the early diverged taxa in the *Cytisus*-group, and its close relationships to *Cytisophyllum*. The taxonomic rank to be granted to *C. heterochrous* requires the integration of all the available data. In the case of *C. tribracteolatus*, the molecular data shows that it is clearly integrated within the *Cytisus*-group, however, as an early diverged taxon, and not related to *C. villosus*.

In conclusion, our molecular data suggest the distinction between the *Genista*-group and the *Cytisus*-group. The first group includes *Genista*, *Chamaespartium*, *Pterospartum*, *Spartium*, *Echinospartum*, *Ulex* and also *Teline*. The latter genus is restricted to the limits given by Gibbs and Dingwall (1971). In the *Cytisus*-group enter *Cytisophyllum*, *Argyrocytisus, Spartocytisus, Chamaecytisus*, and *Cytisus*. Nevertheless, the unresolved position of *Argyrocytisus battandieri*, *Cytisophyllum sessilifolium*, *C. tribracteolatus* and *C. heterochrous* in the *trn*L-*trn*F IGS tree could be reflective of some real biological phenomena, e.g. some reticulate evolutionary process involving parental lineages originating from within (as supported by ITS data) and from outside of the core *Cytisus*-group (as shown by plastid sequences). This suggests interesting biological questions to be addressed by further studies also including other genera such as *Hesperolaburnum*, *Podocytisus*, etc.

Generic limits. Within the *Cytisus*-group several taxa have been alternatively merged into *Cytisus* or splitted into several close genera. Our study gives some information in this regard.

Cytisophyllum sessilifolium has been considered as a member of the *Cytisus*-group, well-differentiated from *Cytisus* on morphological characteristics (Frodin 1965, Polhill 1976, Bisby and Nicholls 1977, Polhill et al. 1978). Our data support its separation within the *Cytisus*-group, and its close position to other basal *Cytisus* species (e.g. *C. heterochrous* and *C. fontanesii)*. Serological reactivity also suggests that *Cytisophyllum* is a primitive genus within the tribe (Cristofolini and Feoli Chiapella 1977).

Argyrocytisus battandieri is unrelated to *Adenocarpus* species in all the trees. Thus, the transfer of *A. battandieri* to *Adenocarpus* as proposed by Talavera and Salgueiro (1999) lacks any support under the molecular point of view. Moreover, in the ITS and combined trees it clearly falls within the *Cytisus*-group as a well differentiated taxon. *Argyrocytisus battandieri* has retained several primitive morphological characters within the *Cytisus-Genista* complex (Gibbs 1974), and pollen morphology points to its separation from all the other *Cytisus* species (Pardo et al. 2000). Thus, its recognition as a separate genus (Heywood 1968, Raynaud, 1974, Greuter et al. 1989) seems justified.

Cytisus fontanesii has been considered either as a separate genus *Chronanthos* (*C. biflorus*; Heywood 1968), included in *Cytisus* sect. *Chronanthus* (together with *C. orientalis* and *C. heterochrous*; Polhill 1976), or as a monospecific section of *Cytisus* (sect. *Heterocytisus*, e.g. Rothmaler 1944, Talavera 1999). Morphological and serological data, and also pollen characteristics (Pardo et al. 2000) indicate that *C. fontanesii* is not closely related to the species of the main core of *Cytisus*. Our molecular data support this view. In fact, *C. fontanesii* joins a basal group formed by *C. heterochrous* and *Cytisophyllum sessilifolium* in the combined tree. Most probably the generic rank given by several authors

(e.g. *Chronanthos biflorus*; Heywood 1968) would better reflect its phylogenetic separation from the main core of *Cytisus*. As in the case of *C. heterochrous*, a definitive conclusion for this matter will also require the integration of all available data.

Calicotome species form a well supported clade within the main core of the *Cytisus*-group which agrees with the previous positioning of this genus on morphological grounds. Our molecular data, as well as those of Käss and Wink (1997a), indicate a close relationship between *Calicotome*, *Cytisus villosus* (sect. *Cytisus*) and other *Cytisus* species (Fig. 3). Similarly, based on serological reactivity data, Cristofolini and Feoli Chiapella (1984) also found these relationships, questioning the value of the genus *Calicotome*. However, the number of changes in the sequences between the *Calicotome* and *Cytisus* species also indicates that *Calicotome* has undergone an important process of genetic differentiation.

The relationships of *Chamaecytisus* (syn. *Cytisus* sect. *Tubocytisus*) cannot be unequivocally established based on the only species included in the analyses (*C. proliferus*). This genus is considered homogeneous with regard to the morphology (Bisby and Nicholls 1977), the secondary metabolites (Faugeras and Paris 1971) and serological reactivity of the seed proteins (Cristofolini and Feoli Chiapella 1977). However, this group has a widespread area of distribution and three main lines of diversification have been recognised on morphological grounds (Cristofolini 1991). In our study, *C. proliferus* enters in the main core of *Cytisus*. This agrees with the results of Käss and Wink (1997a) based on molecular data from *Chamaecytisus austriacus*, *C. supinus* and *C. purpureus*, indicating that *Chamaecytisus* is polyphyletic, and enters in different clades with species of *Cytisus*. Molecular data suggest that probably, if any, the sectional level would be more appropriate than the generic status.

Sectional boundaries. The molecular data from this study provide valuable information regarding the limits of widely accepted sections of *Cytisus*, particularly, sect. *Cytisus*, sect. *Spartopsis*, and sect. *Alburnoides*.

Cytisus sect. *Cytisus* is generally regarded as a relatively primitive, central section of the genus, characterised by a number of unspecialised features. There is general agreement to place *C. villosus* in this section. The vegetative and reproductive traits of *C. villosus* are mostly plesiomorphic (Feoli Chiapella and Cristofolini 1980), hence regarded as primitive in the genus. The molecular data show a close relationship to *Calicotome* and to the aggregate formed by *Cytisus arboreus*, *C. malacitanus* and *C. transiens*. The study also indicates that *C. tribracteolatus*, once included in this section (sect. *Trianthocytisus*; Polhill 1976), stands well apart of *C. villosus*. An expanded molecular study including other related species (e.g. *C. aeolicus*, and *C. emeriflorus*) is required before a definitive taxonomical proposal can be made about the delimitation of sect. *Cytisus*.

Cytisus sect. *Spartopsis* has been considered on morphological grounds as a well-differentiated group even deserving generic rank (*Sarothamnus*). However, our data indicate that sect. *Spartopsis* is not a monophyletic group. Consequently, the limits of this section must be reconsidered. First, *C. arboreus* and related taxa stand apart. The removal of these taxa to another section has already been proposed (sect. *Verzinum*; Talavera and Salgueiro 1999). However, as pointed out before, they are close to the primitive sect. *Cytisus* and to *Calicotome* species. Second, *C. commutatus* and *C. ingramii* form a well supported group, apart from sect. *Spartopsis*, where they have currently been included (Frodin and Heywood 1968, Polhill 1976). Their relationships to the others groups are not resolved yet. A proposal has been made to transfer *C. commutatus* (including *C. ingramii*) to sect. *Corothamnus* (Talavera 1999), however this change is not supported by our data, i.e. the study does not indicate a close relationships of this taxon to *C. decumbens*.

Cytisus sect. *Alburnoides* is another well-characterised section of the main core of

Cytisus. In our analyses, *C. multiflorus*, *C. oromediterraneus* and their hybrid (*C.* ×*praecox*) form a group. However, *C. striatus*, currently a member of sect. *Spartopsis*, also enters in this group. The molecular data suggest that *C. striatus* is more related to sect. *Alburnoides* than to sect. *Spartopsis*, where it is currently included. Further support for this relationship is provided by specimen 114, identified on morphological ground as the wild hybrid of *C. striatus* and *C. multiflorus*. This sample clearly joints the group formed by *C. multiflorus*, *C. oromediterraneus* and *C. striatus*.

A different matter are the relationships between sect. *Alburnoides* and *Spartocytisus supranubius*. This species was referred to *Cytisus*, either within sect. *Oreosparton* (Polhill 1976, Bisby 1981) or sect. *Alburnoides* (Talavera and Salgueiro 1999). Our study indicates a close relationships between *S. supranubius* and the main core of *Cytisus* (even sharing the 10 bp insertion in the *trn*L-*trn*F IGS region), although it stands in a basal position far from sect. *Alburnoides*. Thus, in our opinion this taxon should be better considered as a separate section within *Cytisus* (i.e. sect. *Oreosparton*).

Species and minor entities. The two DNA regions studied provide new information regarding several controversial taxa.

Cytisus transiens is a taxon undoubtfully close to *C. arboreus* and *C. malacitanus* but differs from these taxa in morphological characteristics (number of stem ribs, legume indument, etc.). It has been considered either as a variety, subspecies (*C. arboreus* subsp. *transiens*; Maire 1987), or as a separate species (Talavera and Salgueiro 1999). Our data indicate that *C. transiens* is sister to *C. arboreus* and *C. malacitanus*. This position, together with the morphological differences, supports its separation as an independent species, closely related to but separated from *C. arboreus* and *C. malacitanus*.

Cytisus ingramii is recognised as an independent species from *C. commutatus* based on morphological differences (Laínz Ribalaygua and Laínz 1958, Frodin and Heywood 1968) and chromosome number (Sañudo 1979). On the contrary, Horjales (1978), Greuter et al. (1989) and Talavera (1999) merge both taxa under the name *C. commutatus*, based on the existence of intermediate plants. Our data do not indicate any genetic difference between them. They form a single clade with a high bootstrap support.

Cytisus purgans auct. was first divided into two entities to differentiate the populations of north Africa from the European ones (López González and Jarvis 1984), and afterwards raised to the specific status (*C. balansae* and *C. oromediterraneus*; Rivas Martínez et al. 1984). Talavera and Gibbs (1997) split the group into four distinct species with separate areas of distribution: *C. oromediterraneus* and *C. galianoi* (Iberian Peninsula and France), and *C. valdesii* and *C. balansae* (Morocco and Argelia). The differentiation of these four entities is still a matter of controversy (López González 2000). At the molecular level there are strong differences between the central Spain (*C. oromediterraneus*) and the Atlas Mountain populations in Morocco (*C. valdesii*). However the relationships revealed by the ITS and *trn*L-*trn*F IGS regions are not congruent. Furthermore, the position of *C. valdesii* in the combined tree does not reflect its morphological characteristic. More samples covering the whole variation and geographical area is still needed before conclusions regarding the relationships between these taxa can be drawn.

Cytisus striatus is variable in the dimensions of leaves, flowers, indumentum, and the shape and size of the fruits. This variation has led to the recognition of two infraspecific taxa: *C. striatus* subsp. *eriocarpus,* endemic of the western of the Iberian Peninsula (Rivas Martínez 1978), and *C. megalanthus* restricted to the Rif (Morocco). Molecular data show that *C. megalanthus* has differentiated from *C. striatus*, which suggests that it could be recognised as a different taxon, nevertheless, closely related to *C. striatus*. On the contrary, the separation of *C. striatus* subsp. *eriocarpus* is not supported by our study.

Cytisus maurus is an endemic species of the northern Middle Atlas of Morocco. Talavera

and Salgueiro (1999) combine *C. maurus* into *C. scoparius* subsp. *maurus*, whereas Cubas et al. (2001) suggest that it is morphologically closer to *C. grandiflorus* than to *C. scoparius*. The combined tree supports this latter view. *Cytisus maurus* forms a clade with *C. grandiflorus* (62% bootstrap value). The taxonomic ranking, that deserves this taxon, should take into account the study of samples covering all the morphological and geographical variability of *C. grandiflorus*.

Cytisus scoparius is widely distributed in western, southern, and central Europe, extending northwards up to southern Sweden, and eastwards to central Ukraine. Additionally, this species is naturalised throughout the world. Several subspecies have been described based on morphological variation. Our study indicates that there is also some variability in the sequences, however, we have not found any correlation between this genetic differences and the morphological variation or geographical distribution. *C. scoparius* subsp. *reverchonii* does not separate from the other *C. scoparius* samples. More powerful DNA tools are needed to study the variability of this taxon.

Concluding remarks. The main difficulty in establishing the phylogenetic relationships within the *Cytisus*-group based on non-molecular data is that the pattern is complex, reticulate and weakly differentiated. At the molecular level, a comparison of the sequences shows a small degree of differentiation at the base of the Genisteae. However, data from the ITS region (nrDNA) and the *trn*L-*trn*F IGS (cpDNA) provide new clues for the establishment of phylogenetic relationships in the *Cytisus*-group at the specific, sectional and generic level. The ITS region yields more parsimony-informative characters than the non-coding chloroplast *trn*L-*trn*F IGS region. However, insertions and deletions in the latter have proved to be of considerable phylogenetic value, in particular, to characterise the group. Thus, the combination of the two DNA regions provides some data that help to clarify the long-standings doubts about the limits of *Cytisus* and related genera, and of the sectional groupings within *Cytisus*. The pattern depicted in this work reveals the separation of some taxa from the main core of *Cytisus* (i.e. *Cytisophyllum sessilifolium*, *Cytisus fontanesii, C. heterochrous* and *Argyrocytisus battandieri*). Within *Cytisus* the molecular tree shows new relationships between *C. villosus*, the *C. arboreus* aggregate and *Calicotome* species. The tree also indicates the proximity between *C. striatus* and sect. *Alburnoides*, and the polyphyletic condition of sect. *Spartopsis*. As earlier shown by Bisby (1981), the various sources of data (i.e. chromosome data, flavonoids and alkaloids, seed-protein data, etc.) yield variation patterns within the *Cytisus-Genista* complex that differ both from each other and for the most part from the morphological pattern. Molecular data provide a new tool to build up an accurate picture of the phylogenetic relationships within this complex group, bearing in mind, however, that a phylogenetic tree is a scientific hypothesis that should be subjected to attempts of falsification (Nei and Kumar 2000).

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