Pl. Syst. Evol. 206: 393–410 (1997)

Plant Systematics and Evolution

Chloroplast *rps*16 intron phylogeny of the tribe *Sileneae* (*Caryophyllaceae*)*

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Received April 16, 1996; in revised version December 2, 1996

Key words: *Caryophyllaceae*, *Sileneae*, *Silene*, *Lychnis*, *Cucubalus*, *Petrocoptis*, *Agrostemma*, *Eudianthe*, *Melandrium*. – *rps*16 intron sequences, multiple sequence alignment.

Abstract: Intron sequences of the chloroplast gene *rps*16 from 46 species were used to examine phylogenetic relationships indicated by nrDNA ITS sequence variation in the tribe *Sileneae* (*Caryophyllacaeae, Caryophylloideae*). This region has previously not been utilized for phylogenetic purposes but the results presented here suggest that it is a consistent and valuable complement to the ITS sequences. The *rps*16 intron trees are largely congruent with the ITS trees. All the major hypotheses suggested by the ITS data are supported, often at similar bootstrap levels. The joint usage of *rps*16 intron and ITS sequences provides a powerful tool for resolving many of the difficult taxonomic issues in the tribe *Sileneae*.

The development of molecular methods has greatly increased the data available for phylogenetic inference. Restriction site analyses, particularly of the chloroplast genome, have dominated studies at lower taxonomic levels (SOLTIS & al. 1992), but recent advances in PCR and DNA sequencing technologies have made DNA sequencing advantageous in several respects. It is comparatively cost-effective and requires much less in terms of amount and preservation state of the plant material (BÖHLE & al. 1994), especially when the target region is within the abundant chloroplast genome or the tandemly repeated ribosomal DNA of the nuclear genome. Consequently, plant systematists have recently paid more attention to comparative sequencing studies. In particular, the *rbc*L gene of the chloroplast genome has been utilized for higher taxonomic levels (SOLTIS & SOLTIS 1995), whereas the internal transcribed spacer (ITS) sequences of the nuclear ribosomal DNA are extensively used at lower taxonomic levels (e.g., BALDWIN & al. 1995). However, too much reliance on results from ITS sequences alone may be dangerous for several reasons. First, drawing conclusions from one sequence

* Dedicated to emer. Univ.-Prof. Dr FRIEDRICH EHRENDORFER on the occasion of his 70th birthday

region only suffers from the same risks as do other 'one-character phylogenies' (DOYLE 1992). Different genes (or genomes) in the same organism may have different histories due to for example introgression (RIESEBERG & SOLTIS 1991) or lineage sorting (PAMILO & NEI 1988). Second, functional dependencies among nucleotide sites may inflate misleading signals (see BALDWIN & al. 1995). Third, bidirectional interlocus concerted evolution of rDNA paralogues may lead to erroneous conclusions (WENDEL & al. 1995).

Recently, the focus of plant molecular systematists has shifted toward more rapidly evolving chloroplast DNA (cpDNA) loci. These include the genes *mat*K (e.g., JOHNSON & SOLTIS 1995) and *ndh*F (e.g., OLMSTEAD & REEVES 1995). Non-coding sequences include the intergenic spacers between *rbc*L and *atp*B (e.g., EHRENDORFER & al. 1994), between *trn*L (UAA) and *trn*F (GAA) (e.g., BÖHLE & al. 1994), and between *trn*T (UGU) and *trn*L (UAA) (BÖHLE & al. 1994). Intron studies include *trn*L (UAA) (e.g., BÖHLE & al. 1994, GIELLY & TABERLET 1994) and *rpl*16 (JORDAN & al. 1996). In this study, we use the intron sequence of the ribosomal protein gene *rps*16 to examine the results of a previous ITS sequence study of the tribe *Sileneae* (*Caryophyllaceae*, OXELMAN & LIDÉN 1995).

The *rps*16 exons are separated by a group II (or group III, NEUHAUS & al. 1989) intron. Sequencing studies have revealed that the gene is entirely absent from the chloroplast genomes of *Marchantia polymorpha* L. (OHYAMA & al. 1986), *Pinus thunbergii* PARL. (TZUDSUKI & al. 1992), *Pisum sativum* L. (NAGANO & al. 1991), and the parasitic *Epifagus virginiana* (L.) BART. (WOLFE & al. 1992). Hybridization studies have further suggested the absence of the entire gene or parts of it in some other *Leguminosae* and a few other taxa (DOWNIE & PALMER 1992, DOYLE & al. 1995). In the six Genbank accessions available in spring 1995, the length of the *rps*16 intron varies from 790 bp (*Hordeum vulgare* L., SEXTON & al. 1990) to 887 bp (*Sinapis alba* L., NEUHAUS & al. 1989). The two *Solanaceae* sequences (*Nicotiana tabacum* L. and *Solanum tuberosum* L.) are identical in 95% of the aligned positions, whereas the *Poaceae* sequences (*Zea mays* L., *Hordeum vulgare*, *Oryza sativa* L.) have pairwise similarity scores of 85–86%. These scores suggested that this region could be valuable for phylogenetic studies at family level and below. The possibility to construct 'universal' primers in the flanking exons or in the conserved 5′ and 3′ extremes of the intron, the possibility to cover the whole intron with one PCR reaction and two overlapping sequencing reactions using optimized automated sequencing protocols (e.g., OXELMAN 1996) made us believe that the *rps*16 intron might be an ideal complement to our previous ITS study on inter/intrageneric relationships in the tribe *Sileneae*.

Our ITS study (OXELMAN & LIDÉN 1995) resulted in five main hypotheses, some of which had not been suggested from morphological data. (1) *Agrostemma* (AGR) is sister-group to the rest of the tribe. (2) *Eudianthe* (EUD), *Petrocoptis* (PET), *Heliosperma*, *Steris* ADANS. (= *Viscaria* RÖHL.), *Silene* sect. *Rupifraga*, and *Silene* sect. *Compactae* reside outside of the core of *Silene*. (3) The latter four taxa unexpectedly form a strongly supported clade (in the following denoted STE). (4) *Lychnis* (incl. *Coronaria*, *Uebelinia* and *Coccyganthe*, excl. *Polyschemone* and *Steris*) form a well supported clade (LYC). (5) *Silene* forms a weakly supported clade with three main subgroups: (i) LYC, (ii) a well supported clade approximately corresponding to ROHRBACH'S (1869) sects. *Botryosilene* and

Cincinnosilene (SIL-SIL), and (iii) a weakly supported clade (SIL-OBE) containing *Cucubalus*, *Pleconax*, *Oberna*, *Melandrium* s. l. and several other taxa.

The close relationships indicated by the ITS sequences between sect. *Behenantha* and subg. *Behen* (= genus *Oberna* ADANS.) is not reflected in previous classifications (but see GREUTER 1995). We suspected that this might be due to heterogeneity of sect. *Behenantha* and we therefore included the New World cleistogamous *S. anthirrina* L., assumed to be similar to the rest of the section due to convergence (GREUTER 1995), and the two Mediterranean species *S. cretica* L. and *S. muscipula* L. in the present study. *Silene littorea* BROT. resides at an isolated position in the ITS tree and preliminary analyses of the *rps*16 sequences also showed much deviation. Therefore, *S. pendula* L., classified in the same section as *S. littorea* (TALAVERA 1979), was included in order to reduce the risk of long branches (FELSENSTEIN 1978). *Silene aprica* TURCZ. (far East), *Lychnis sibirica* L., and *L. lagrangei* Coss. are controversial taxa that have recently become available to us. Despite these additions, the 81-taxon matrix from the ITS study has been reduced to a total of 46 *rps*16 sequences, which is sufficient for the questions raised in this paper. A forthcoming paper will deal more in detail with the congruence between the ITS and *rps*16 intron results, as well as with morphological character evolution within the group.

The aims of this study were: to test the conclusions from the nrDNA ITS *Sileneae* phylogeny; to evaluate the usefulness of the *rps*16 intron as a complement to nrDNA ITS; to infer the position of some controversial taxa for which molecular data have previously been missing.

Material and methods

Plant material, DNA extraction, amplification and sequencing. Data on vouchers and origins for plants and their affiliation in the ITS tree are presented in Table 1. Total genomic DNA from taxa not present in OXELMAN & LIDÉN (1995) was extracted as described in that paper, with the exception that lysis was performed at 74 °C in a buffer consisting of 2% CTAB, 1% PEG 6000, 1.4 M NaCl, 10 mM Tris-HCl, 20 mM EDTA. For DNA from herbarium specimens, additional purification was made with GeneClean. The *rps*16 intron was amplified with the PCR primers rpsF (GTGGTAGAAAGCAACGTGCGACTT) and rpsR2 (TCGGGATCGAACATCAATTGCAAC). The 3′ end of rpsF is located eleven positions inside the intron, whereas the 3′ end of rpsR2 is located eighteen positions downstream from the 5′ end of exon 2. These oligos were designed using the six EMBL/GenBank angiosperm accessions which are almost invariable for these regions, the exception being the fourth position from 5′ on rpsR2, where the grasses have an A instead of a G. Usually, one 50 µl reaction per template was run on a Perkin-Elmer Cetus 480 thermal cycler, each reaction containing 2.5 units Taq polymerase (Advanced Biotechnologies), reaction buffer IV supplied by the manufacturer, 10 µM tetramethyl ammonium chloride (TMACl), 0.1 mM of each dNTP, 3–10 pmol of each primer, 1–50 ng template DNA and 1 mM $MgCl_2$. Denaturation (2 min at 95 °C) was followed by 33 cycles of (95 °C 30 s, 57–60 °C 1 min, 72 °C 2 min), ending with 7 min at 72 °C. Presence of fragments was checked on 1% SeaKem agarose gels, and amplification products were purified with Qiaquick (Qiagen) spin columns according to the manufacturer's instructions.

Four different sequencing strategies were used: (1) a cycle sequencing protocol with initial 3′-labelling with ^{35}S described in OXELMAN & LIDÉN (1995), (2) a ^{35}S-based

Sequenase (version 2, Amersham) protocol modified after THEIN (1990) and HOOT & al. (1995), 3) solid-phase sequencing with a Cy5-labelled rpsR2 primer (a biotinylated rpsF primer in the PCR reaction) using the AutoLoad kit (Pharmacia Biotech) according to the manufacturers instructions, and (4) cycle sequencing with Thermosequenase (Amersham) flourescent labelled primer cycle sequencing kit with 7-deaza-dGTP. For the latter protocol, 25–100 ng of Qiaquick-purified template DNA and 2.5 pmol Cy5-labelled primer were used per reaction. The Thermosequenase sequencing reactions were performed on a Perkin-Elmer Cetus 480 thermal cycler programmed for 2 min at 96 °C followed by 18 cycles of 30 s at 95 °C and 40 s at 60 °C. The program ended with a 5 min step at 60 °C.

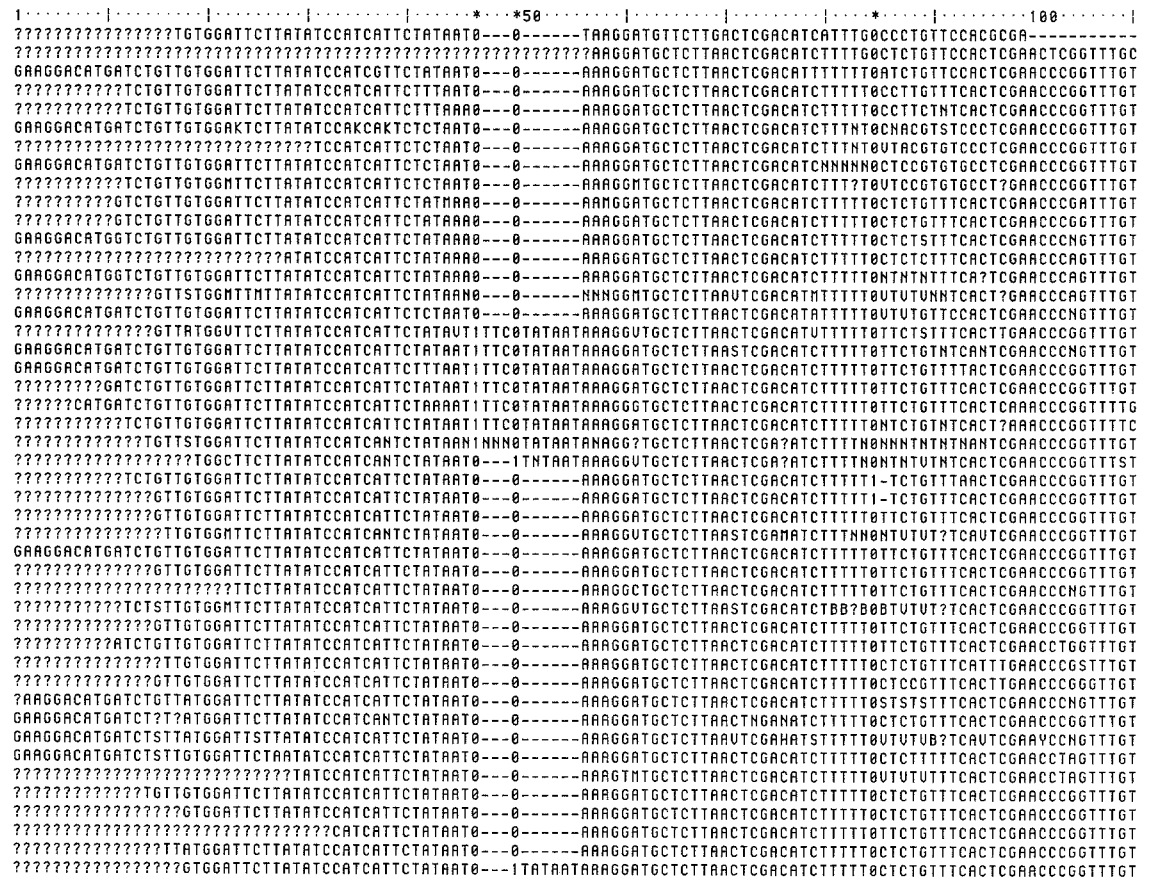
^{35}S-labelled fragments were separated on polyacrylamide gels (35 × 45 cm) using the salt gradient method of SHEEN & SEED (1988). Before loading, the samples were denatured at 90 °C for 2 min and then put on ice. With this procedure, 500–600 bases/reaction could often be read manually when the Sequenase protocol was used. The cycle sequencing reactions tended to generate more background and more ambiguities.

Cy5-labelled fragments were separated on 0.5 mm LongRanger Hydrolink (FMC Bioproducts) gels on ALFExpress (Pharmacia Biotech) automated sequencers with the following electrophoresis conditions preset: 1500 V, 60 mA, 25 W, 55 °C, 2 s sampling interval, 800 min run time. With the primers rpsF and rpsR2 about 600 bases (rarely up to 800) long sequences were generated, which, by careful comparison with known sequences, gave sufficient overlap for unambiguous base determination in most cases. The initial manual protocols demanded, however, the designation of two internal sequencing primers: rpsMF (GTGCGGAAATCCCTCGTTCATATGA) and rpsMR2 (GGTTTAGACAT-TACTTCGTTGA).

Multiple sequence alignment and gap coding. Multiple sequence alignment and gap coding were made by hand. The following alignment and mutational interpretation criteria were used (slightly modified from GOLENBERG & al. 1993). (1) Indels are placed so as to keep the number of substitutions within an aligned region to a minimum. (2) If gaps of equal length occur in more than one sequence, they are coded as the same character state if they can not be interpreted as different duplication or insertion events. For example, at position 273 (Fig. 1). *Silene latifolia* and *Lychnis sibirica* share an extra base (T and C, respectively). (3) When two or more gaps are not identical but overlapping, overlapping portions were considered shared events only when the region could be partitioned into informative insertion/duplication regions on at least one side. Thus, the gap from position 670 to position 681 in *Silene bergiana* was considered as two separate characters (lack of duplicated ATAAATG and CTAAA), whereas the gap from 668 to 683 in *S. samia* was considered a single event with the intervening characters coded as missing. (4) In cases where indels were interpreted as duplications of adjacent sequences, the character was coded as unordered multistate if duplications could be regarded as additive. For example, the T run in region 1091–1097 was recoded as unordered multistate whereas the region 47–55 was coded as two separate binary characters: duplication of TATAAT and duplication of TTCTATAAT. (5) Indels and substitutions were given equal weights.

Application of the above criteria was particularly problematic for gap-coding of the region 697–711 and the substitutions in positions 664 and 791. Consequently, these were excluded from the phylogenetic analysis. Some sequences deviated much from the others for short motifs in otherwise very conserved locations without correlated length variation. These were interpreted as independent events and the base calls were not included in analysis (e.g., *S. sordida* and *S. cryptoneura*, position 676–681).

We believe that the criteria outlined above are conservative and reliable. Our purpose is to exclude (or in other ways render phylogenetically uninformative) parts where conflicting



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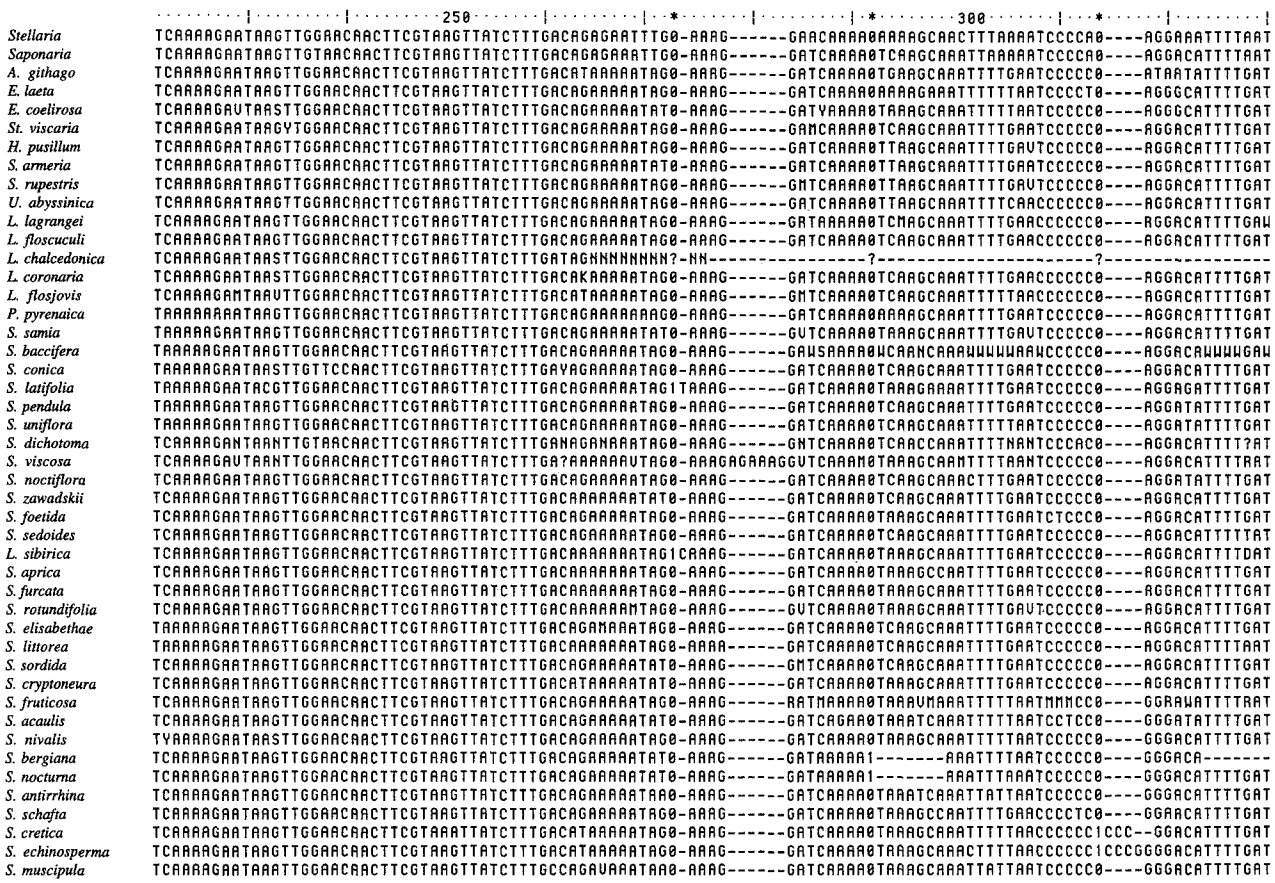

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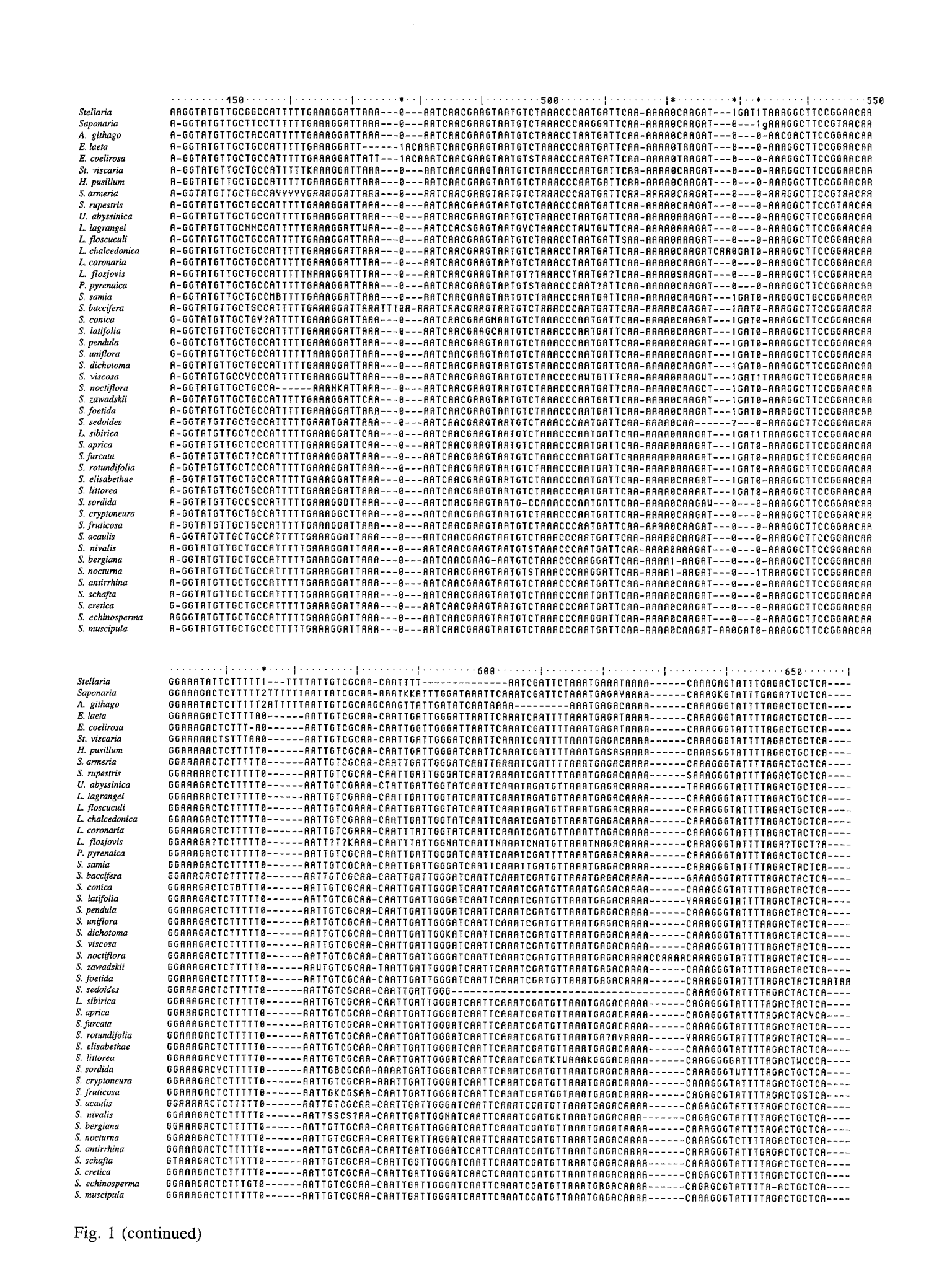

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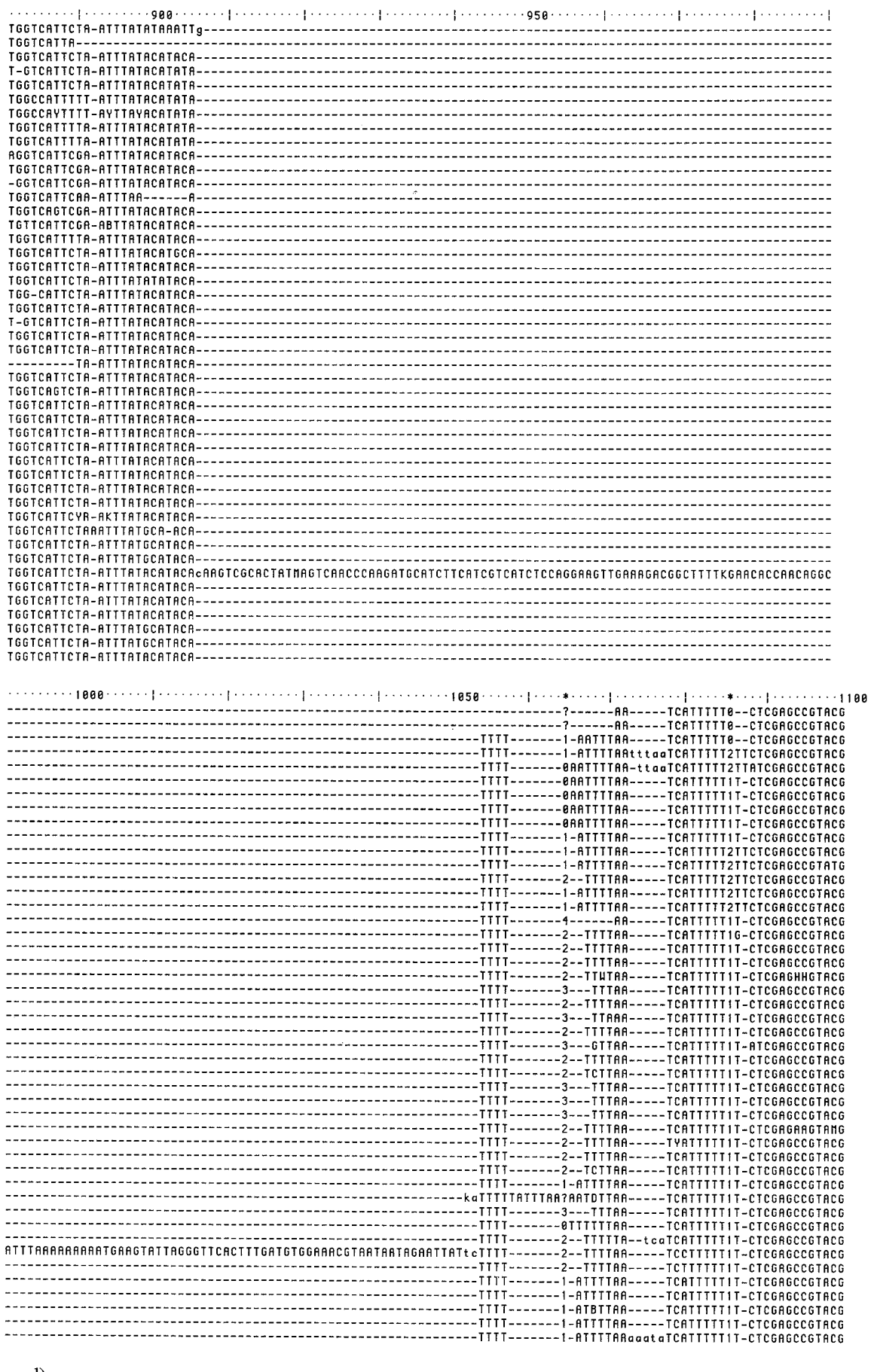

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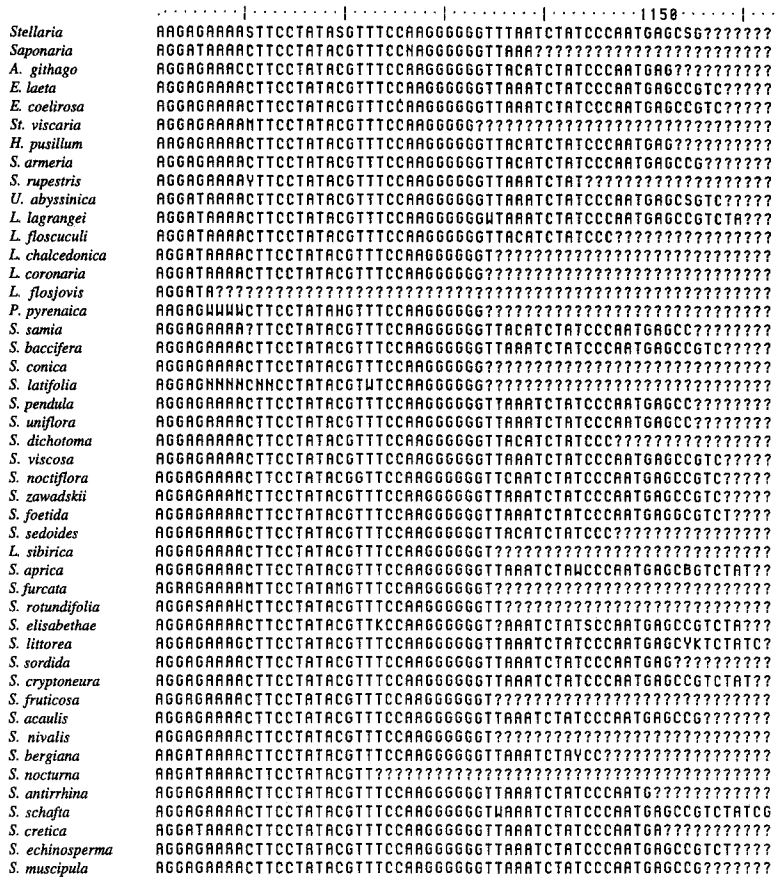

Fig. 1. Aligned *rps*16 intron sequences. Gaps are indicated by -. Unsequenced parts are indicated by "?". Uncertain base calls are indicated by the following symbols: R=G or A; Y=C or T; M=A or C; K=G or T; S=C or G; W=A or T; B=not A; H=not G; D=not C; V=not T; N=A, C, G, or T. Lowercase letters indicate positions that have been coded as missing in the phylogenetic analysis due to uncertain alignment. Position 1 in the alignment corresponds to the eleventh base in the intron. Informative gap codes have been inserted at relevant positions with the different states as numbers

phylogenetic signal would result from alternative alignments. Of course, many alternative alignments are possible by altering gap penalty/substitution costs, but we do not see any objective way to choose among these.

MacClade version 3.05 (MADDISON & MADDISON 1992) was used for calculation of transitions and transversions on the resulting trees (see below). Polytomies were arbitrarily resolved and ambiguous base calls were ignored.

Cladistics. The resulting alignment and the gap codes were analysed using PAUP version 3.1.1 (SWOFFORD 1993). The most parsimonious solutions were searched for heuristically with 50 replicates of random sequence addition, TBR swapping, MULPARS on, and uninformative characters ignored. An initial run, with all 46 sequences included, hit the absolute limit for MAXTREES, that is 32768. In order to reduce computation time, seven sequences that were closely similar to others were removed from the parsimony analysis. As measures of the amount of homoplasy in the characters, consistency index (CI) and the rescaled consistency index (RC) are presented (FARRIS 1989, SWOFFORD 1993) with

autapomorphies excluded. Clade robustness to character perturbations was analysed with 400 bootstrap replicates, each with 15 random sequence addition replicates, NNI swapping, and MULPARS off. One thousand invariable characters were added to the matrix prior to the run in order to avoid the effect of irrelevant characters on the bootstrap values

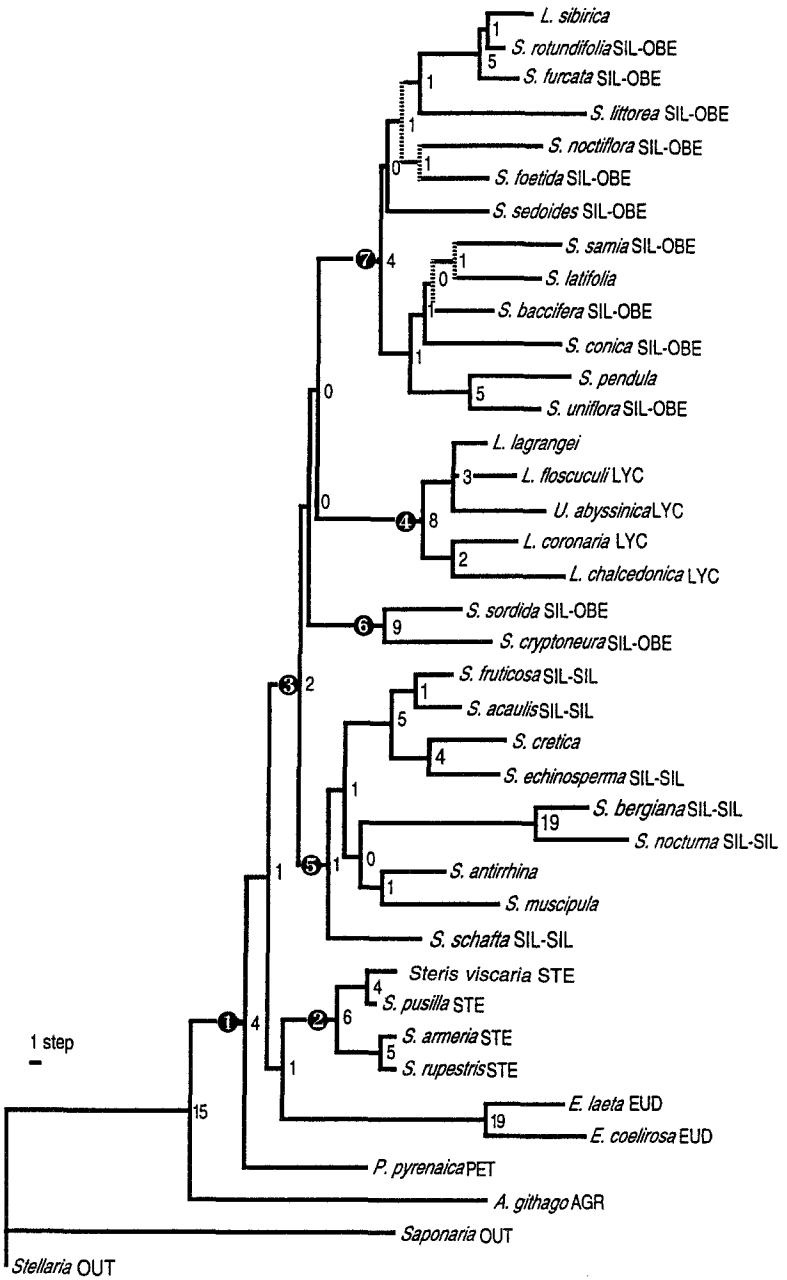

Fig. 2. Phylogram of one of the 179 most parsimonious trees found for the reduced taxon matrix. Numbers to the right of branching represent estimated Bremer support (i.e. the number of extra steps required to collapse that clade). Dashed lines indicate clades that were not found after successive character reweighting according to maximum RC values (Farris 1989, Swofford 1993). Numbers in dots refer to specific clades in the discussion

(HARSHMAN 1994). Random sequence addition guarantees that the order of the taxa in the matrix does not bias the results, but the heuristic tree-building algorithm may lead to underestimation of the support for internal branches (OXELMAN, unpubl.). This effect can be expected to be inversely correlated with the ability of the tree-building method to find the optimal solution. Preliminary searches indicated that 15 random sequence addition replicates were enough for this matrix, as no significant increase in bootstrap values could be observed with higher numbers of replicates. Bremer support (BREMER 1988) were calculated with the aid of the AutoDecay program (v. 3.03, ERIKSSON & WIKSTRÖM 1995). The PAUP settings used for finding the reversely constrained trees were: ten replicates of random sequence addition, TBR swapping, MULPARS on, and uninformative character ignored.

Results

Figure 1 displays the aligned 46 *rps*16 intron sequences with the recoding of informative gaps included. 498 of the aligned positions include substitutions and 205 of these are cladistically informative. The length of the *rps*16 intron varies from 707 bases (*Lychnis chalcedonica*) to 951 (*Silene bergiana*). These two sequences are characterised by one large deletion (119 bases) and insertion (148 bases), respectively. A total of 109 gaps had to be inserted. Fourteen of the gaps were interpreted as duplication events and 22 were runs of single bases. Twenty-nine gaps are cladistically informative. The AT-content ranges from 66.5% in *S. bergiana* to 70.1% in *Lychnis flos-jovis*.

The PAUP search on the reduced matrix resulted in 179 most parsimonous trees, each requiring 707 steps (CI excluding autapomorphies = 0.60, RC = 0.40). 149–150 of these are indels (CI = 0.60 – 0.59, RC = 0.44 – 0.43). One of the most parsimonous trees is shown in Fig. 2 with branch lengths and Bremer support values indicated. Successive character reweighting according to maximum RC values (FARRIS 1989, SWOFFORD 1993) did not converge to a smaller subset of these trees. Instead, 442 trees with slight incongruencies relative to the original trees (indicated by dashed lines in Fig. 2) were found. The strict consensus tree resulting from the 32700 trees identical using the unreduced matrix is shown with bootstrap values plotted in Fig. 3. The length (including autapomorphies) of the most parsimonious trees is 753 steps (CI excluding autapomorphies = 0.59, RC= 0.41).

The inferred number of transitions in the 179 trees of the reduced matrix varies from 233 to 245, whereas the number of transversions are 312–325.

Discussion

At levels of divergence comparable to that in *Sileneae*, the *rps*16 intron has several properties making it attractive for comparative sequencing studies of angiosperms. It is easily amplified compared to rDNA. Multiple sequence alignment is mostly straightforward and the variability is rather uniformly distributed over the whole intron. The more conserved parts correspond more or less to stem regions in the inferred secondary structure of *Sinapis alba* (NEUHAUS & al. 1989), but there is weak, if any, correlation between substitutions. The most AT-rich parts tend to pose alignment problems, and may also introduce problems in the sequencing reactions,

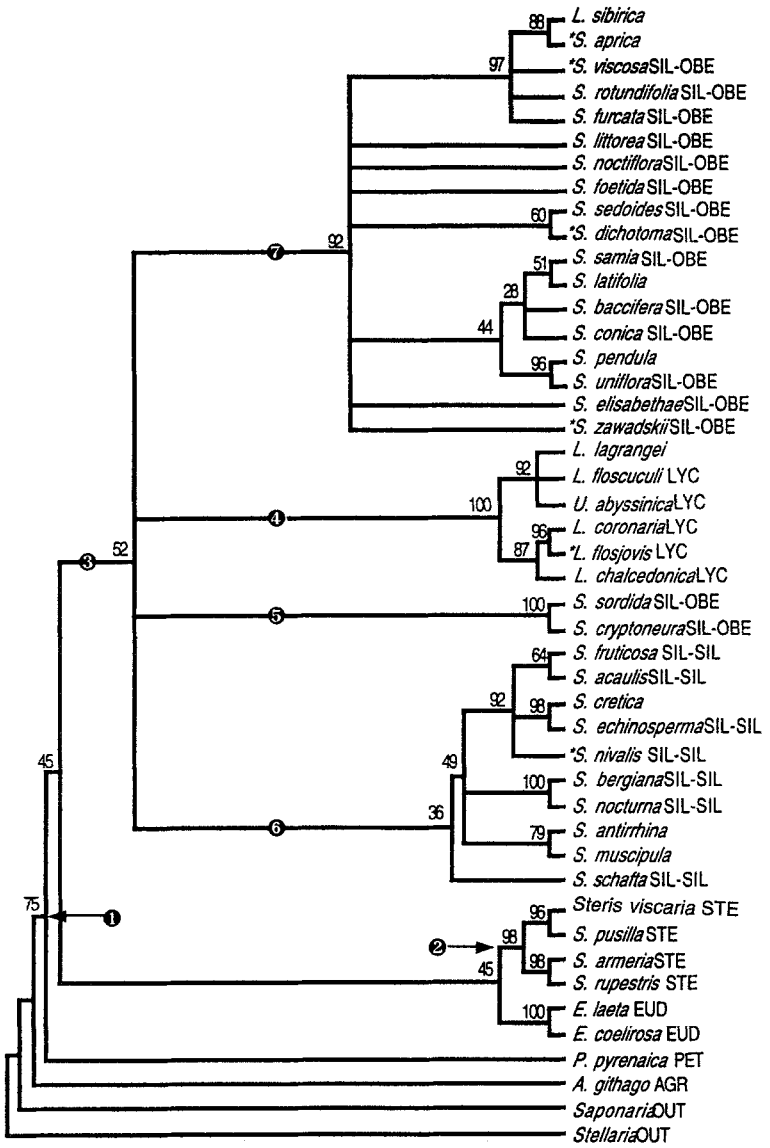

Fig. 3. Strict consensus tree of the 32700 most parsimonious trees found for the unreduced taxon matrix. Numbers above branches indicate bootstrap percentages from 400 replicates, * indicates that the taxon is excluded in Fig. 2

because DNA polymerases sometimes fail to incorporate a base in long A or T runs, making the sequences unreadable farther away. This problem was resolved with the introduction of TMACl in the PCR reaction.

The cladistic results of this study are largely congruent with the ITS study of Oxelman & Lidén (1995). The sister-group relation (node 1, Figs. 2, 3) between *Agrostemma* and the rest of the tribe is further corroborated. Unfortunately, the basal relationships in the remaining clade are still vague. *Petrocoptis*, *Eudianthe*, and the *Steris* clade (2) reside outside of the core *Silene* (3) with moderate support,

which is consistent with the ITS results, but the interrelationships among these three taxa/clades are poorly resolved. The strong support for the *Steris* clade (2) makes it hard to dismiss from taxonomical considerations. Within the core *Silene* (3), four clades can be identified with no resolution among them. *Lychnis*, incl. *Uebelinia* (4) receives strong support with *L. lagrangei* clearly nested. The SIL-SIL clade (5) is weakly supported in this study. The ITS study found two strongly supported subgroups in this group: one, represented here by *Silene bergiana* and *S. nocturna*, corresponding to Rohrbach's (1869) section *Cincinnosilene* (with the addition of sect. *Rubellae*), and one corresponding approximately to his sect. *Botryosilene* (with the addition of *Polyschemone* and sect. *Rigidulae*). These groups are found also in this investigation with strong support (100% and 92% bootstrap values, respectively), but the inclusive clade receives only weak support. Notable is that *S. schafta*, which could not be assigned to any of the two subgroups, has an ambiguous position also in this study. Surprising is the strong alliance between *S. cretica* (sect. *Behenantha*) and *S. echinosperma* (sect. *Rigidulae*), previously never classified in the same section. They share, however, several characters, i.e. glabrous calyx, viscid stem internodes, and similar seed morphology. *Silene antirrhina* and *S. muscipula* are two other species previously classified in sect. *Behenantha*. They group strongly together but their relationship to others is unclear from the *rps*16 sequences alone. Greuter (1995) suggested that *S. fabaria* and sect. *Behenantha* are more related to each other than *S. fabaria* to the *S. vulgaris* group (to which *S. uniflora* belongs). The ITS data are in agreement with this hypothesis, but the section was then only represented by *S. reinholdii*. It is clear from the present study and accompanying ITS studies (Oxelman & Lidén, unpubl.), that the sect. *Behenantha*, as traditionally circumscribed, is polyphyletic.

Silene sordida and *S. cryptoneura* are two SW Anatolian species that previously have been classified closely together in sect. *Atocion* subsect. *Delicatulae* nom. illeg. (Chowdhuri 1957). The aberrant morphology of *S. sordida* made Oxelman (1995) suspect that this classification could be erroneous, and this was also indicated by the ITS data. However, the two species form a strongly supported *rps*16 intron clade (6). In the ITS study, both species resided in the weakly supported SIL-OBE clade. When clade (6) is excluded, SIL-OBE (7) receives substantial support from *rps*16 sequences, whereas the ITS sequences only gave weak support. As with the ITS sequences, there is not much resolution within this clade. The most strongly supported group includes representatives from sect. *Occidentalis* (*S. rotundifolia*), sect. *Physolychnis* (*S. furcata*), sect. *Chloranthe* (*S. viscosa*), sect. *Lasiostemones* (*S. aprica*) and *Lychnis sibirica*. In the ITS tree, also the sect. *Odontopetalae* (*Silene zawadskii*, *S. oblanceolata*) was grouped here with high support. Unexpectedly, *S. uniflora* and *S. pendula* form a strongly supported *rps*16 intron clade. This was double-checked with independent DNA preparations. The clade is very difficult to identify by morphological characters, but is an agreement with ITS data (unpublished results).

Conclusion

The *rps*16 intron sequences provide valuable additional information to the ITS sequences for resolving relationships within the tribe *Sileneae*. The strong

Table 1. Acronyms and geographical origins of plant materials used for cpDNA sequence comparisons in this study. Subdivisions here listed are those given in the ITS trees (OXELMAN & LIDÉN 1995). Section designations in square brackets are those used in *Silene* by CHOWDHURI (1957). Species not treated by CHOWDHURI are tentatively assigned to his sections. Voucher specimens are deposited at GB unless otherwise stated *Ox* BENGT OXELMAN, *KGB* Kunming/Göteborg Botanical Expedition to Yunnan 1993; herbarium abbreviations according to HOLMGREN & al. 1990). See text for further explanation. *E. Eudianthe, L. Lychnis, S. Silene, H. Heliosperma, U. Uebelinia, P. Petrocoptis*

ITS group/species	Voucher; origin (EMBL accession number)
OUT (Outgroup)	
Stellaria media (L.) VILL.	Ox 2231; Sweden, Göteborg (Z83152)
Saponaria sicula RAFIN.	Ox 2298; Garden (Z83153)
AGR (*Agrostemma* L.)	
A. githago L.	Ox ITS-AGR30616; Garden (Z83154)
PET (*Petrocoptis* ENDL.)	
P. pyrenaica (J. P. BERGERET) A. BR.	Ox 2276; Garden (Z83167)
EUD [*Eudianthe* (RCHB.) RCHB.]	
E. laeta (AIT.) RCHB.	Ox 1876; Algeria, Berrahal (Z83155)
E. coeli-rosa (L.) ENDL.	Ox 2285; Garden (Z83156)
LYC (*Lychnis* L., incl. *Uebelinia* HOCHST.)	
L. chalcedonica L.	Ox 2277; Garden (Z83164)
L. flos-cuculi L.	Ox 2200; Sweden, Hisingen (Z83163)
L. coronaria (L.) DESR.	Ox 2278; Garden (Z83165)
L. flos-jovis (L.) DESV.	Ox ITS-FLO30610; Garden (Z83166)
U. abyssinica HOCHST.	GILBERT & FRIES 8418 (UPS); Ethiopia, Illubabor (Z83161)
STE (*Steris* ADANS, s. l., see text)	
Steris viscaria (L.) RAFIN.	Ox 2199; Sweden, Hisingen (Z83157)
H. pusillum (WALDST. & KIT.) VIS.	Ox 2281; Austria, Hochschwab (Z83158)
S. armeria L. [sect. *Compactae*]	Ox ITS-ARM30611; Garden (Z83159)
S. rupestris L. [sect. *Rupifraga*]	Ox 2198; Sweden, Hisingen (Z83160)
SIL-OBE	
S. baccifera (L.) ROTH	Ox 2287; Italy, Torino (Z83169)
S. furcata RAFIN. [sect. *Physolychnis*)	Ox 2282; Sweden, Abisko (Z83182)
S. noctiflora L. [sect. *Elisanthe*]	Ox 2230; Sweden, Göteborg (Z83176)
S. rotundifolia NUTT. [sect. *Occidentales*]	Ox 2231; Garden (Z83183)
S. elisabethae JAN [sect. *Odontopetalae*]	Ox 2261; Garden (Z83184)
S. uniflora ROTH [sect. *Behen*]	Ox 2197; Sweden, Hisingen (Z83173)
S. zawadskii HERBICH [sect. *Odontopetalae*]	Ox 2241; Garden (Z83177)
S. conica L. [sect. *Conoimorpha*]	Ox 1944; Greece, Sterea Ellas (Z83170)
S. viscosa (L.) PERS. [sect. *Chloranthae*]	Ox 2288; Garden (Z83175)
S. dichotoma EHRH. [sect. *Dichotomae*]	Ox 2221; Greece, Amorgos (Z83174)
S. littorea BROT. [sect. *Succulentae*]	Ox ITS-LIT20604; Spain, Punta Paloma (Z83185)
S. samia MELZH. & CHRISTOD. [sect. *Atocion* e descr.]	Ox 2208; Greece, Samos (Z83168)
S. cryptoneura STAPF [sect. *Atocion*]	Ox 1691; Turkey, Antalya (Z83187)
S. sedoides POIR. [sect. *Atocion*]	Ox 1195; Greece, Cape Maleas (Z83179)
S. sordida HUB.-MOR. [sect. *Atocion*]	Ox 2206; Turkey, Marmaris (Z83186)
S. foetida LINK [sect. *Cordifoliae*]	Ox ?; Portugal, Castelo de Vide (Z83178)
SIL-SIL	
S. fruticosa L. [sect. *Siphonomorpha* s. l.]	Ox 934; Greece, Peloponnisos (Z83188)
S. nivalis (KIT.) ROHRB. [*Lychnis*]	Ox 2255; Romania, Rodna (Z83190)
S. acaulis (L.) JACQ. [sect. *Nanosilenae*]	Ox 2243; Sweden, Härjedalen (Z83189)
S. echinosperma BOISS. & HELDR. [sect. *Rigidulae*]	Ox 2227; Greece, Peloponnisos (Z83196)
S. nocturna L. [sect. *Scorpioideae*]	Ox 654; Greece, Delfi (Z83192)
S. bergiana LINDM. [sect. *Atocion*]	HOLMDAHL 1182; Spain, Monda (Z83191)
S. schafta G. GMEL. [sect. *Cucubaloideae*]	Ox 2264; Garden (Z83194)
Not included in the ITS study	
L. sibirica L.	GUBANOV 143 (MV); Mongolia, Bogd Uul (Z83180)
L. lagrangei COSS.	HUBER-MORATH 3408 (LD); Morocco, Tetouan (Z83162)
S. latifolia POIR. [sect. *Elisanthe*]	Ox 2310; Garden (Z83171)
S. aprica TURCZ. [sect. *Lasiostemiones*]	WU & CZHUANG 7294; China, Yunnan (Z83181)
S. cretica L. [sect. *Behenantha*]	Ox 1324; Greece, Peloponnisos (Z83195)
S. muscipula L. [sect. *Behenantha*]	Ox 1780; Morocco, Djebel Azrou Achkar (Z83197)
S. antirrhina L. [sect. *Behenantha*]	VINCENT & LAMMERS 3137; USA, Ohio (Z83193)
S. pendula L. [sect. *Erecto-refractae*]	Ox 2291; Garden (Z83172)

correlation between the results suggests that the combination of the two regions will provide a valuable framework for more detailed future studies. Several of the molecular results are unexpected and have not been previously hypothesized, which does not necessarily mean that they are in conflict with morphology. Examples are the *Steris* clade, the disassemblage of several sections (i.e. *Atocion*, *Behenantha*, and *Erectorefractae*), the inclusion of *Uebelinia* in *Lychnis*, and the strong relationship of *Lychnis sibirica* with several *Silene/Melandrium* taxa. Given that, although sampling is representative of hypothesized diversity, only c. 10% of the species in the tribe have yet been studied with molecular data, it is likely that there is more to find, especially concerning lower level classification.

The ease with which sequences are obtained, even from old herbarium material (see LIDÉN & al. 1997, this volume), as well as the relatively straightforward alignment of sequences makes the *rps*16 intron attractive for taxonomic problems below the family level. Sequence divergence is two to three times lower than in nrDNA ITS (OXELMAL & LIDÉN, unpubl.) and similar to that of other chloroplast introns (GIELLY & al. 1996, DOWNIE & al. 1996), suggesting that these two regions complement each other.

Financial support for this study was obtained from the Swedish Natural Science Research Council and Carl Skottsbergsfonden. We are grateful to JEFF DOYLE and an anonymous reviewer for their helpful and constructive criticism.

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Accepted December 3, 1996 by M. HESSE and I. KRISAI-GREILHUBER