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RESEARCH ARTICLE

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Multiple group I introns detected in the nuclear small subunit rDNA of the autosporic green alga *Selenastrum capricornutum*

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Abstract A phylogenetic investigation of the autosporic chlorophycean alga species *Selenastrum capricornutum* using the small subunit (SSU) rRNA gene revealed the unusual presence of six group IC1 introns. Previous studies showed that numerous green algal taxa contain group IC1 introns. However, whereas some algal species harbor multiple introns in a single ribosomal gene, none have contained as many as *S. capricornutum*. Three of the *S. capricornutum* introns are located at conserved algal intron sites and the remaining three are located at novel eukaryotic positions. The SSU rRNA genes and their introns have been sequenced and putative secondary structures are proposed for the introns. Also, their similarity to other group IC1 introns from algal, fungal, and viral sources is investigated. Results suggest the initial presence of introns at conserved locations, followed by duplication and insertion to novel positions within the SSU rRNA gene.

Keywords Algae · Group I intron · *Selenastrum* · SSU rDNA

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Introduction

Group I introns have been found in a wide variety of organisms, from eubacterial genomes to the organellar and ribosomal RNA genes of eukaryotes. These introns are characterized by a conserved secondary structure that is involved in excision of the intron following transcription (Burke and RajBhandary 1982; Michel et al. 1982; Cech 1988; Lambowitz and Belfort 1993 and references therein). The observed covariation of substitutions in these introns and the presence of short conserved regions of primary sequence suggested a proposed secondary structure. Based on the expanding database of sequenced introns (Damberger and Gutell 1994) tertiary structures for these molecules were proposed (Cech et al. 1994). Also of great interest was the discovery that some group I introns are unique in that they may act as ribozymes to catalyze their own splicing reactions, which are less or non-dependent on *trans*-factors for excision (Cech 1986, 1988, 1990). In eukaryotes, group I introns were reported in the rRNA genes of amoebae, fungi, acellular slime molds, and algae (Cech 1988; Kuhsel et al. 1990; Wilcox et al. 1992; Belfort 1993; DeJonckheere 1994; Gast et al. 1994; Bhattacharya et al. 1996a; Schroeder-Diedrich et al. 1998; Müller et al. 2001). The insertion positions of these introns within the small subunit ribosomal (SSU rRNA) gene appeared to be conserved in those organisms that contained them (Bhattacharya et al. 1996b). However, as more introns were discovered, novel insertion locations were reported (Gargas et al. 1995; Müller et al. 2001). The reconstruction of the evolutionary history of these group I introns is complicated by the apparent ability of some to transpose themselves by horizontal or vertical transmission (Sogin et al. 1986; Van Oppen et al. 1993; Lonergran and Gray 1994; Vaughn et al. 1995; Haugen et al. 1999; Bhattacharya et al. 2001; Nikoh and Fukatsu 2001; Tanabe et al. 2002). Here, we report the discovery of six group I introns (which lack open reading frames) located in the nuclear SSU rRNA gene of

the autosporic green alga *Selenastrum capricornutum*. Three of these introns are located in previously reported intron positions, whereas three are in locations novel among eukaryotes within the gene.

Materials and methods

Taxa studied

S. capricornutum (UTEX 1648), *S. bibraianum* (UTEX 324), and *S. minutum* (UTEX 326) utilized in this study were obtained from the University of Texas, Austin (UTEX) algal culture collection.

DNA extraction

An aliquot of ca. 25 µl of an alga culture was concentrated by full-speed centrifugation in a table top centrifuge for 15 min and re-suspended in 50 µl of lysis buffer (50 mM Tris-HCl, pH 7.2, 50 mM EDTA, 3% SDS, 1% beta-mercaptoethanol). Samples were then microwaved (Sears Kenmore model 747.9987821) on the high setting (45 s, 3×). Following microwave treatment, an additional 350 µl of lysis buffer was added and samples were incubated at 80°C for 1 h. DNA was then extracted using standard phenol/chloroform procedures. It was precipitated using 250 µl of isoproponol and 10 µl of 3 M NaOAc. Samples were washed in 70% EtOH and the DNA was re-suspended in 30 µl of Tris-EDTA buffer.

PCR amplification and sequencing

SSU rDNA gene amplifications that included these introns were generated using 3 µl of re-suspended genomic DNA as a template in 100-µl PCR reactions. These PCR reactions used 5 µl (10 µM stock solution) of each amplification primer, 2.5 mM $MgCl_2$, 1.5 mM dNTPs and 1.0–2.5 units of Taq DNA polymerase (Gibco BRL, Gaithersburg, Md.). SSU rDNA genes were amplified in two overlapping halves. The 5′ portion was amplified using primer pair CRN5 and 1137 (Table 1) and the 3′ half was amplified using primer pair 892C and SSU2. Amplifications were replicated in multiple tubes. Three microliters of pooled PCR product was sequenced directly by double-stranded cycle sequencing, using a commercial kit (dsDNA cycle sequencing system; Gibco BRL). Sequencing was done using SSU rDNA primers and primers made specifically within the various introns (Table 1).

Table 1 Sequencing and amplification primers for *S. capricornutum* SSU rRNA gene. Primers shown are those designed in our laboratory for algal amplification and sequencing. Numbered primers indicate the approximate location of the primer in the SSU rRNA gene. Primers followed by a capital *C* are forward primers (5′ → 3′). Those without a C designation are reverse primers (3′ → 5′). All primer sequences are presented 5′ → 3′

Primer	Sequence	Orientation
CRN5	tggttgatcctgccagtag	Forward
18S59	tgaaactgcgaatggctc	Forward
In174.1[a]	cttcccacagacccacattgc	Reverse
In174.2[a]	tccgggagcagacggtcagac	Reverse
170	gcatgtattagctctaga	Reverse
373	aggctccctctccggaatc	Reverse
373C	gattccggagagggagcct	Forward
570	gcattggagctggaattac	Reverse
570C	gtaattccagctccaatagc	Forward
892	ccaagaatttcacctctgac	Reverse
892C	gtcaaggtgaaattcttgg	Forward
In943.1C[a]	agaacagggatgatgggcagc	Forward
1137	gtgcccttccgtcaat	Reverse
1137C	aattgacggaagggcaccacc	Forward
PCR2	gaaacttaaaggaattga	Forward
In1120.1[a]	cgacaaaagtcctctcacacg	Reverse
In1120.2C[a]	ttgcaaccggctggcgacacc	Forward
1315	ccggaaatcaacctgacaaggc	Reverse
1262	gaacggccatgcaccac	Reverse
1262C	gtggtgcatggccgttctta	Forward
1200	gggcatcacagacctg	Reverse
1200C	caggtctgtgatgccc	Forward
In1512.1C[a]	gggtacggtaataatgcaggtgg	Forward
1/F	cacaccgcccgtcg	Forward
SSU2	ccgcggccgcggatcctgatccctccgcaggttcac	Reverse

[a]These primers are located within an intron; and the number designation given for the primer corresponds to the intron inserted at that location

Phylogenetic reconstruction and data analysis

The primary sequences of the group I introns studied here were aligned with other group I intron sequences, using the ESEE (Eyeball sequence editor) sequence alignment program for the personal computer (Cabot and Beckenbach 1989). Phenetic analyses were carried out using the MEGA2 (Molecular evolutionary genetic analysis) phylogenetic reconstruction programs (Kumar et al. 2001). Distances were calculated using a Kimura 2 parameter model and phylogenetic gene tree reconstruction was performed using the neighbor-joining algorithm method in MEGA. Proposed secondary structures of *S. capricornutum* introns were produced by comparison with the proposed secondary structure of group I introns of Cech et al. (1994). Intron name designations follow the nomenclature proposed by Johansen and Haugen (2001).

Results

The PCR amplicons for the SSU rRNA gene of *S. capricornutum* were significantly larger than those of algae that lacked introns in the SSU rRNA gene (Fig. 1). This was observed in multiple PCR amplifications from repeated extractions and also from separate culture orders from UTEX. The two other *Selenastrum* species did not produce PCR products of anomalous size; and they were not characterized further for the present study. Sequencing of the larger PCR product of

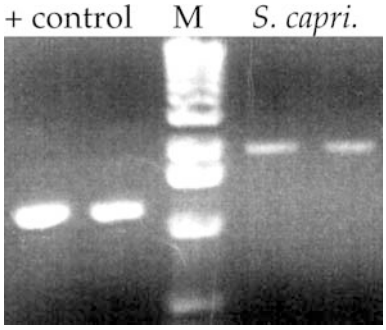


Fig. 1 PCR products produced using primers CRN5 and 1137, which amplify the 5′ half of the SSU rDNA gene. *Lanes 1, 2, left* Products produced using control algal DNA, representing the expected size of the product (1,140 bp). *Lane 3, M* 1 kb marker. *Lanes 4, 5, right* PCR products produced using the same primers in *S. capricornutum* (ca. 1,950 bp)

S. capricornutum produced seven SSU rDNA primary exon regions and six intervening sequences (introns). Comparison of the intervening sequences with other introns in GenBank suggested that these were all group IC1 introns. The subset IC1 is a subdivision of the group I introns that is based on intron primary sequence and secondary structure characteristics. The seven SSU exon sequences and the six intron primary sequences were submitted to GenBank under accession number AF169628.

These introns are located at the following positions in the SSU gene (all positions given are relative to the primary sequence of the SSU ribosomal gene of *Escherichia coli*). These introns are designated: Scap.S165, Scap.S174 (both of which are close to the 5′ end of the gene), Scap.S943, Scap.S1046, Scap.S1120, and Scap.S1512 the last of which is close to the 3′ end of the gene. Three of these positions (Scap.S943, Scap.S1046, Scap.S1512) are positions where group I introns have been found in other algal taxa. The remaining three introns (Scap.S165, Scap.S174, Scap.S1120) are located at novel SSU rDNA intron locations among eukaryotes, including algae.

The proposed secondary structures of the six introns of *S. capricornutum* are presented in Fig. 2. All of the introns have the characteristic stem and loop structures of other group IC1 introns. Differences in the length of stems P5 and P9 are the major sources of variation among the introns. The common GAAA tetraloop in the P5b loop is observed in all six introns (Pley et al. 1994).

Primary sequence homology between introns that are found at different insertion locations in various taxa is limited. However, certain regions are relatively highly conserved in all group I introns. These conserved sequence regions are referred to as P, Q, R, and S (Cech 1988). They act to maintain the base pairing required to produce a conserved tertiary structure needed for efficient intron excision. Therefore, in order to examine the phylogenetic relationships of the six introns found here to other group I introns, we aligned their P, Q, R, and S regions with the same regions from a number of other

Fig. 2 *S. capricornutum* group I intron proposed secondary structure. Exon sequences are in *lowercase*. Putative intron stems are identified, e.g., P6a

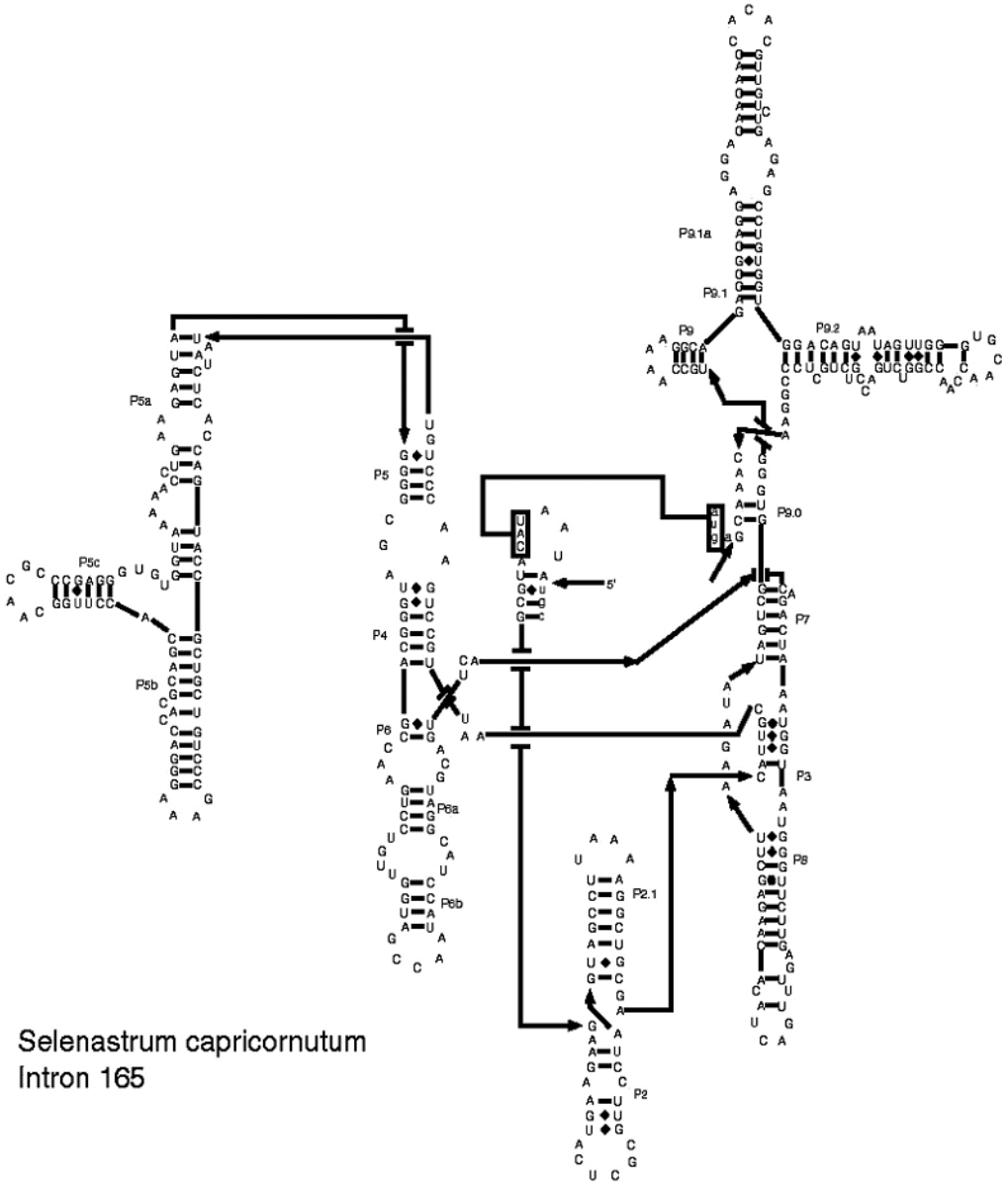
introns from SSU and large subunit ribosomal genes. In addition, P, Q, R, and S conserved regions from group I introns located in the $tRNA^{LEU}$ of chloroplast and eubacterial genomes were included as an outgroup. The alignment resulted in a data matrix of 51 conserved sites.

Phenetic analysis of this conserved region sequence data using the neighbor-joining reconstruction algorithm produced the intron gene tree presented in Fig. 3. Results of this analysis show that the *S. capricornutum* intron located at position 1,046 falls into a clade with introns from other taxa that are inserted at this position. However, introns at the other two previously reported positions (Scap.S943, Scap.S1512) do not group with other introns sharing the same insertion location. In addition, the introns located at the novel positions of Scap.S165, Scap.S174, and Scap.S1120 are similar to the *S. capricornutum* Scap.S943 intron.

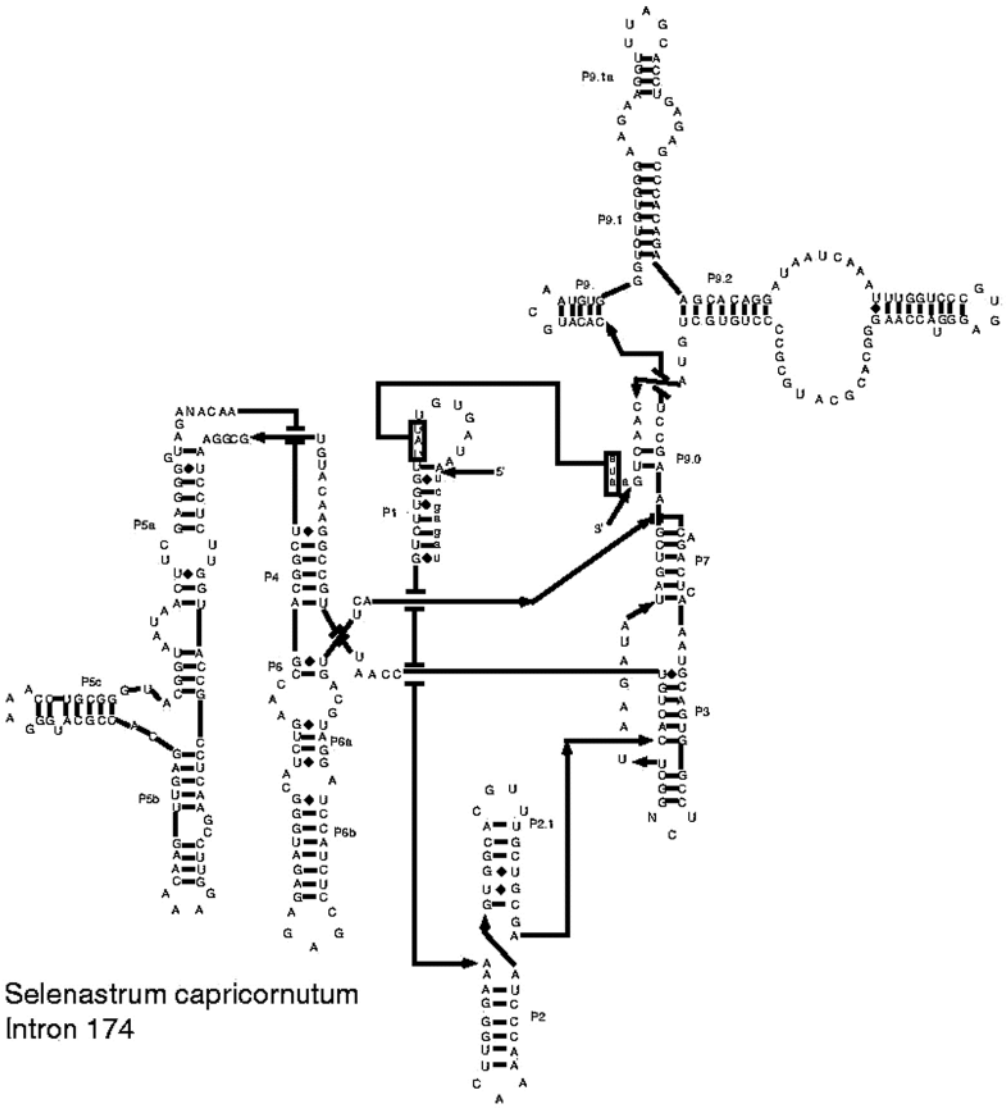
Discussion

Group I introns have a wide phylogenetic distribution. They are found in fungi, algae, amoebae, and in plant mitochondrial and chloroplast genes. They have also been observed in eubacteria and bacteriophage genomes. As demonstrated here, nuclear introns can be found in some of the normally intron-lacking SSU and LSU rRNA genes. The number of introns observed in a particular ribosomal RNA gene has been found to vary from as few as one in various taxa, to as many as nine, in some fungal taxa. In this study, we report the unusual occurrence of a large number on introns in the nuclear rRNA gene. The lack of significant phenetic clustering of introns from the same position within the SSU rRNA gene due to the small number of sites examined using conserved sequences of P, Q, R, and S, does not allow for a statistical evaluation of the gene tree produced. Nonetheless, some of the introns from various taxa (for example, the introns located at position 1,046) do cluster together even with this limited data set. Also, introns from *Acanthamoeba lenticulata* at position 943 also cluster together.

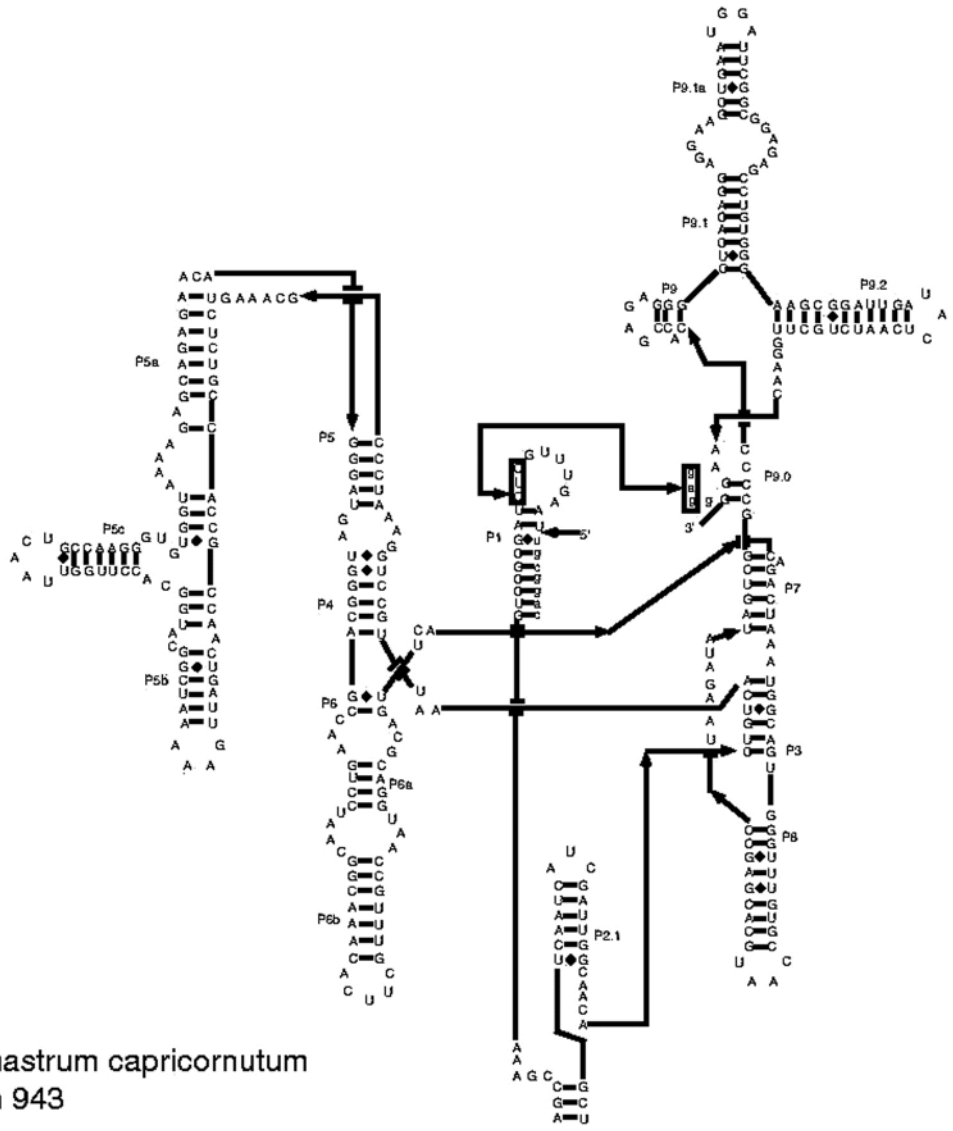
Two sets of the *S. capricornutum* introns (Scap.S174/Scap.S1120, Scap.S165/Scap.S943) are most closely related to each other in these analyses. Three of these positions, the locations of Scap.S174, Scap.S165, and Scap.S1120, are novel whereas the location of Scap.S943 is observed as an intron insertion site in many other taxa. These novel intron insertion locations, coupled with their phenetic similarity to the intron found at location 943, may suggest an ancestral presence of an intron at the 943 position, followed by duplication to the susceptible novel locations. However, as mentioned above, no introns are found in two other species of *Selenastrum* examined, suggesting that the introns were not



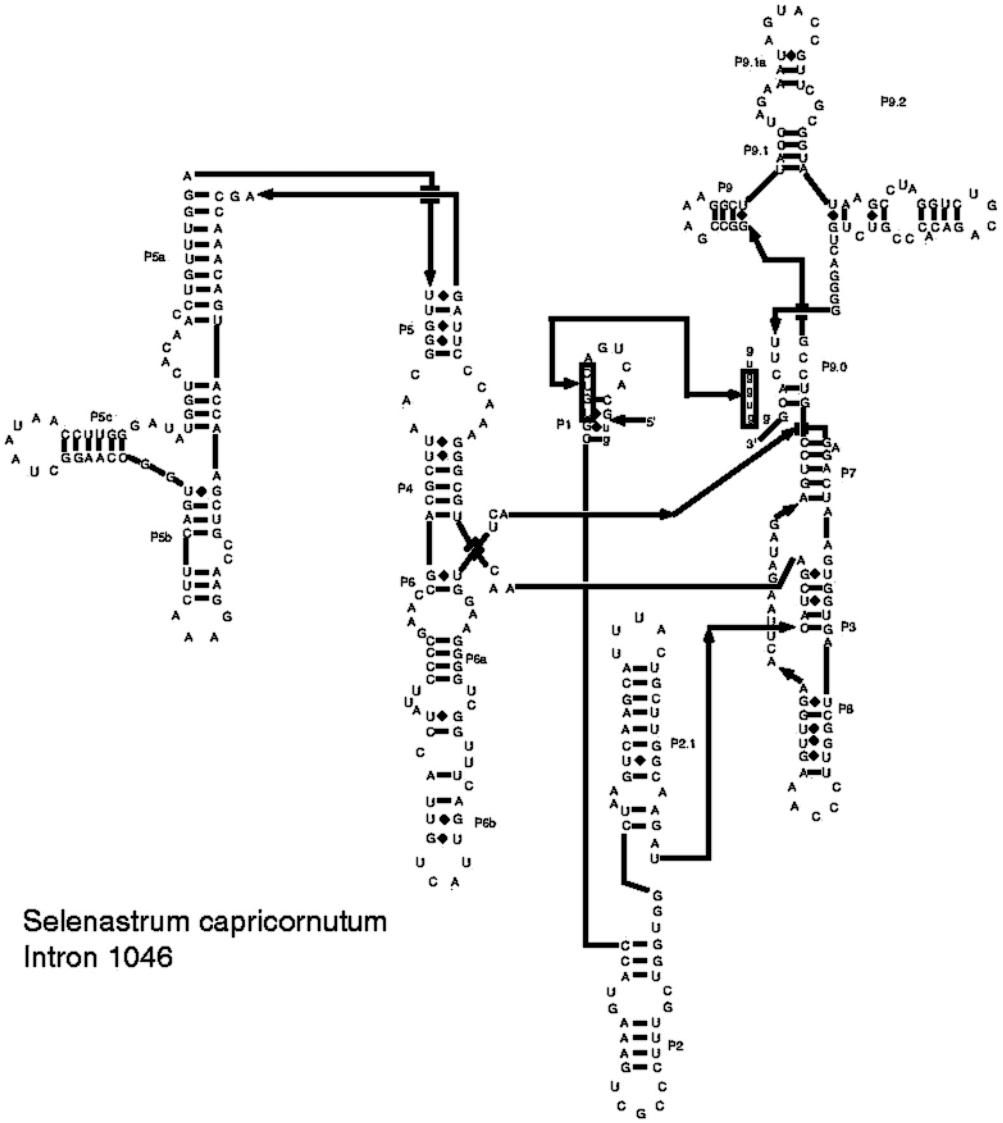
Selenastrum capricornutum
Intron 165



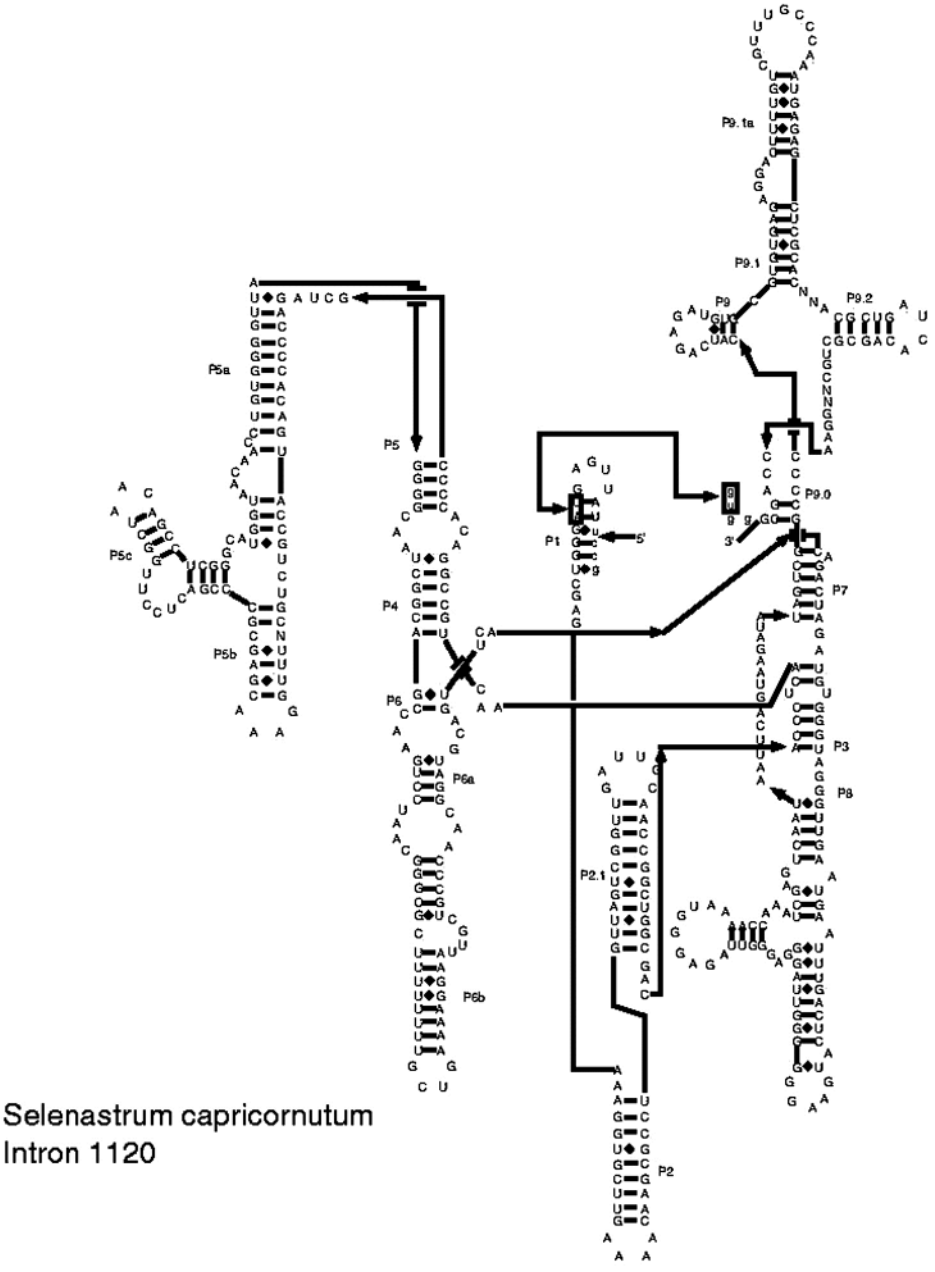
Selenastrum capricornutum
Intron 174



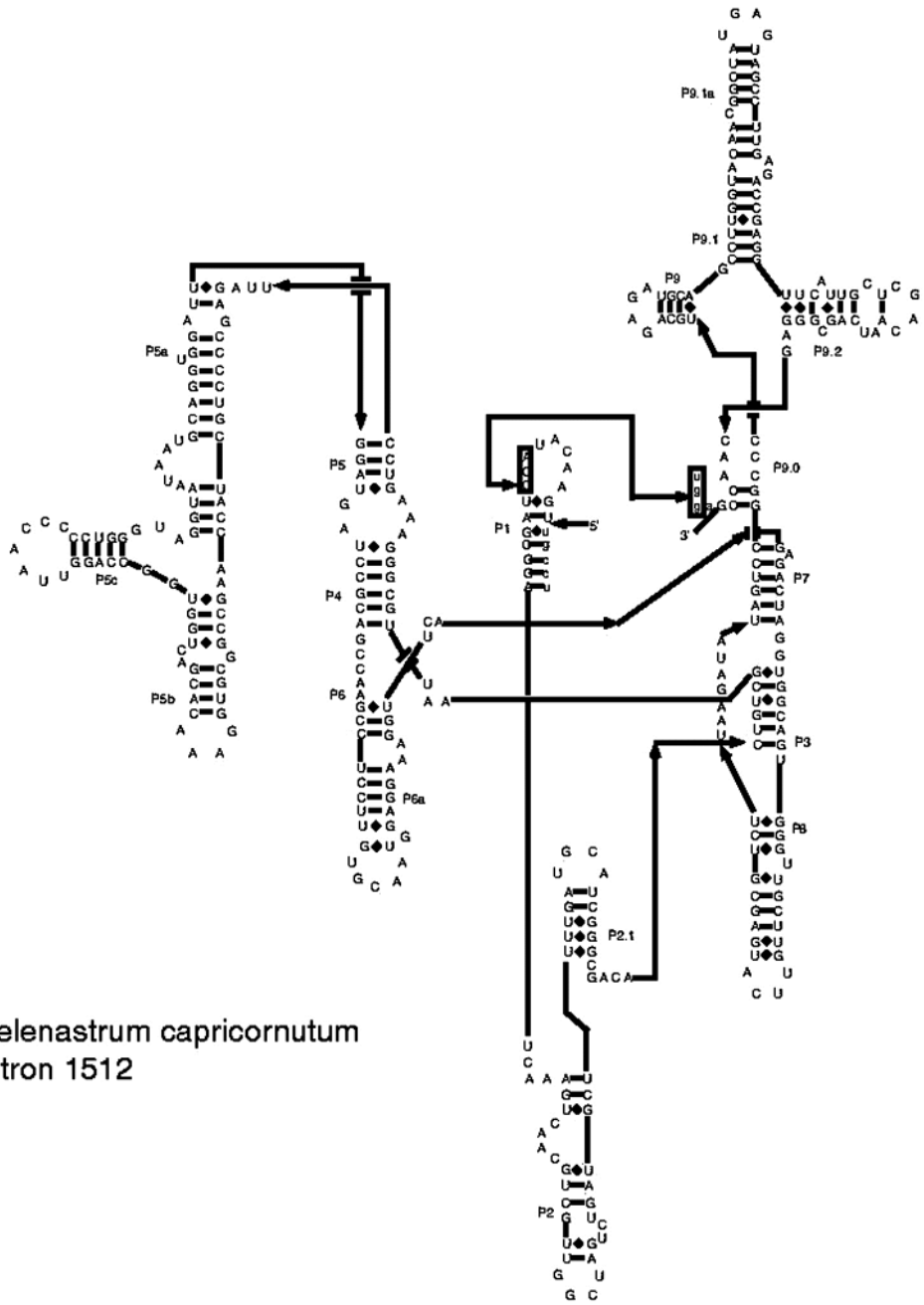
Selenastrum capricornutum
Intron 943



Selenastrum capricornutum
Intron 1046



Selenastrum capricornutum
Intron 1120



Selenastrum capricornutum
Intron 1512

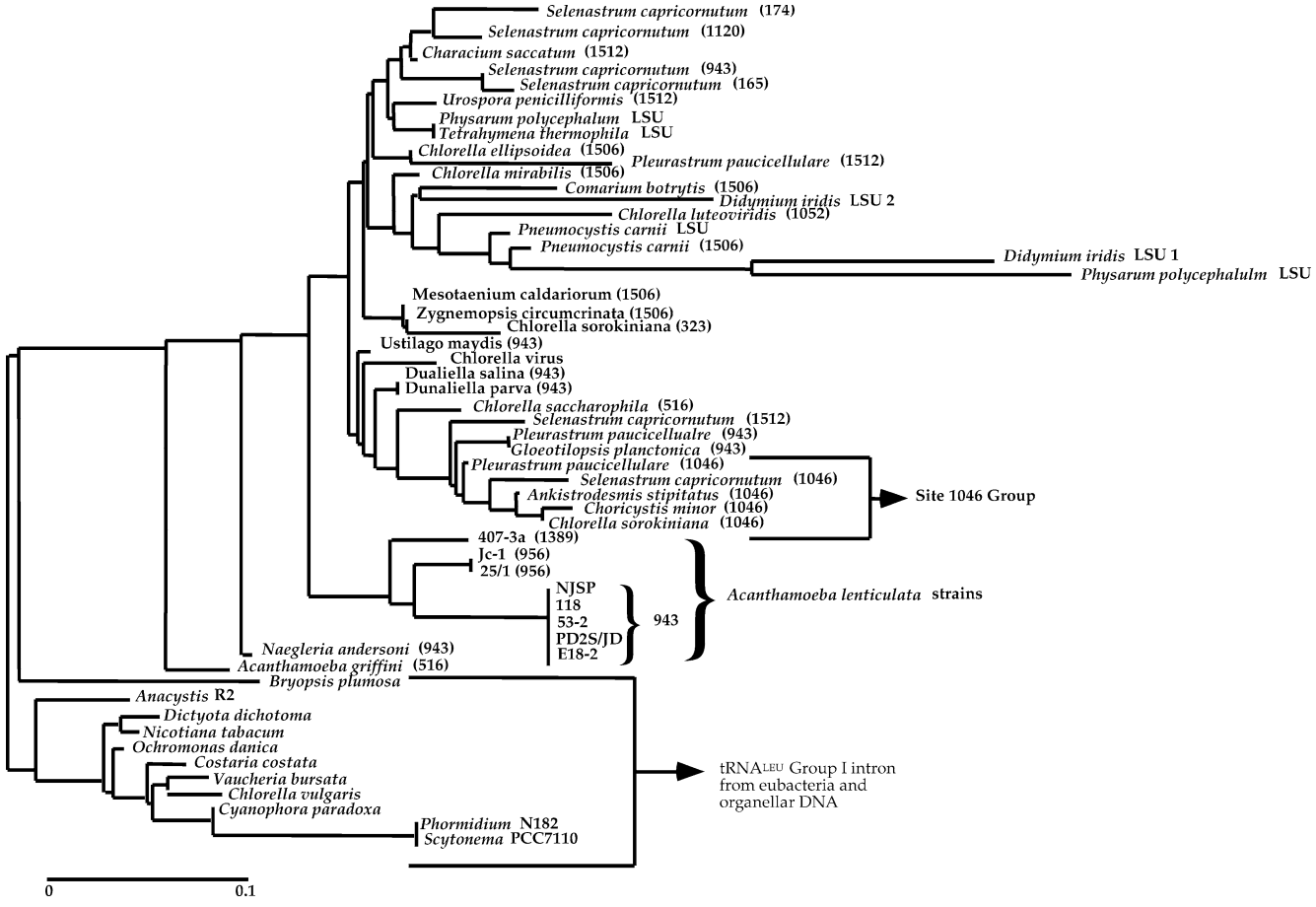


Fig. 3 Group I intron phylogenetic tree. Conserved P, Q, R, and S regions of group I introns were aligned and distances were calculated using a Kimura 2 parameter model. Phylogenetic gene tree reconstruction was performed using the neighbor joining algorithm method in MEGA. Intron insertion locations are shown in *parentheses* following the species name. *LSU* Large subunit, *LSU1* large subunit intron 1, *LSU2* large subunit intron 2. Percent dissimilarity is shown on the scale bar below figure. Additional introns found in the SSU and LSU ribosomal genes used in the tree reconstruction (intron position, GenBank accession number listed after the species name, in *parentheses*) are: *Characium saccatum* (1512, M84319), *Dunaliella salina* (943, M84320), *D. parva* (943, M62998; Wilcox et al. 1992) *Urospora pencilliformis* (1512, AB049417; Van Oppen et al. 1993) *Physarum polycephalum* LSU1 (L03183), *P. polycephalum* LSU2 (L03183; Ruoff et al. 1992), *Tetrahymena thermophila* LSU (X03107; Nielsen and Engberg 1985), *Chlorella ellipsoidea* (1506, X63520; Krienitz et al. 1996), *Pleurastrum paucicellulare* (1512, Z47997), *P. paucicellulare* (943, Z47997), *P. paucicellulare* (1046, Z47997; Bhattacharya et al. 1996b), *C. mirabilis* (1506, X74000), *C. leutoviridis* (1053, X73998), *C. sorokiniana* (323, X73993), *C. saccharohipla* (516, X73991), *C. sorokiniana* (1046, X73993; Huss et al. 1999), *Cosmarium botrytis* (1506, X77453), *Mesotaenium caldariorum* (1506, X75763), *Zygnemopsis circumcrinata* (1506, X79495; Bhattacharya et al. 1994), *Pnemocystis carnii* LSU (M86760), *P. carnii* (1506, M86760; Sogin and Edman 1989), *Didymium iridis* LSU1 (X60210), *D. iridis* LSU2 (X60210; Johansen et al. 1992), *Ustilago maydis* (943, X62396; DeWachter et al. 1992), *Chlorella virus* (D29631; Yamada et al. 1994), *Gloeotilopsis planctonica* (943, Z28970; Friedl and Zeltner 1994), *Ankistrodesmis stipitatus* (1046, X56100; Davila-Aponte et al. 1991), *Chorocystis minor* (1046, X89012; Krienitz et al. 1996), *Acanthamoeba lenticulata* strains 407-3A (1389, U94734), JC1 (956, U94739), 25-1 (956, U94740), NJSP (943, U94738), 118 (943, U94736), 53-2 (943, U94737), PD2S/JD (943, U94741), and E18-2 (943, U94735; Schroeder-Diedrich et al. 1998), *Naegleria andersoni* (943, Z16417; DeJonckeere 1994), *A. griffini* (516, U02540; Gast et al. 1994). $tRNA^{LEU}$ introns used in alignment (with GenBank accession number following species name, in *parentheses*): *Bryopsis plumosa* (M61159), *Anacystis* R2 (M61158), *Dictyota dichomata* (M61161), *Ochromonas danica* (M61162), *Costaria costata* (M55288), *Vaucheria bursata* (M61165), *Chlorella vulgaris* (M61160), *Phormidium* N182 (M61163), *Syctonema* PCC7110 (M61164; Kushel et al. 1990), *Nicotiana tabacum* (M16898; Yamada et al. 1986), *Cyanphora paradoxa* (M22563; Evrard et al. 1988)

present in the common ancestor of *Selenastrum* taxa and were acquired following the divergence of *Selenastrum* taxa. Alternatively, the introns were present in the ancestral taxa and have subsequently been lost in the other two *Selenastrum* taxa examined. An examination of this question will require the analysis of more *Selenastrum* isolates and other closely related taxa (Krienitz et al. 2001).

The remaining two introns are located at the previously reported insertion positions of 1,046 and 1,512. While insertions at both positions have been reported, introns found at 1,046 are more common. These two introns are phylogenetically relatively closely related to one another in the current study. In the case of these two introns, it is possible that they also represent an ancestral intron insertion followed by duplication to a sus-

ceptible position, or perhaps both were acquired independently. Again, the lack of introns in the other *Selenastrum* taxa examined currently prevents further resolution of this question.

As the ribosomal DNA genes of more taxa are examined, more group I introns have been found. The accumulation of these data helps to elucidate the nature of these genetic elements and allows us to begin to piece together their evolutionary history. In this study we have revealed the occurrence of an unusually large number of group I introns found in the SSU ribosomal DNA gene of the autosporic green algae *S. capricornutum*. The origin and maintenance of these introns is not yet clear, but as more data are obtained from other taxa, these questions can be addressed.

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References

Belfort M (1993) An expanding universe of introns. Science 262:1009–1010

Bhattacharya D, Surek B, Ruesing M, Damberger S, Melkonian M (1994) Group I introns are inherited through common ancestry in the nuclear-encoded rRNA of Zygnematales (Charophyceae). Proc Natl Acad Sci USA 91:9916–9920

Bhattacharya D, Damberger S, Surek B, Melkonian M (1996a) Primary and secondary structure analyses of the rDNA group-I introns of the Zygnematales (Charophyta). Curr Genet 29:282–286

Bhattacharya D, Friedl T, Damberger S (1996b) Nuclear-encoded rDNA group I introns: origin and evolutionary relationships of insertion site lineages in the green algae. Mol Biol Evol 13:978–989

Bhattacharya D, Cannone JJ, Gutell RR (2001) Group I intron lateral transfer between red and brown algal ribosomal RNA. Curr Genet 40:82–90

Burke JM, RajBhandary UL (1982) Intron within the large rRNA gene of *N. crassa* mitochondria: a long open reading frame and a consensus sequence possibly important in splicing. Cell 31:509–520

Cabot EL, Beckenbach AT (1989) Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. Comput Appl Biosci 5:233–234

Cech TR (1986) A model for the RNA-catalyzed replication of RNA. Proc Natl Acad Sci USA 83:4360–4363

Cech TR (1988) Conserved sequences and structures of group I introns: building an active site for RNA catalysis—a review. Gene 73:259–271

Cech TR (1990) Self splicing of group I introns. Annu Rev Biochem 59:543–568

Cech TR, Damberger SH, Gutell RR (1994) Representation of the secondary and tertiary structure of group I introns. Nat Struct Biol 1:273–280

Damberger SH, Gutell RR (1994) A comparative database of group I intron structures. Nucleic Acids Res 22:3508–3510

Davila-Aponte JA, Huss VA, Sogin ML, Cech TR (1991) A self-splicing intron in the nuclear pre-rRNA of the green alga, *Ankistrodesmus stipitatus*. Nucleic Acids Res 19:4429–4436

DeJonckheere JF (1994) Evidence for the ancestral origin of group I introns in the SSU rDNA of *Naegleria* spp. J Eukaryot Microbiol 41:457–463

DeWachter R, Neefs JM, Goris A, Van de Peer Y (1992) The gene coding for small ribosomal subunit RNA in the basidiomycete *Ustilago maydis* contains a group I intron. Nucleic Acids Res 20:1251–1257

Evrard JL, Kuntz M, Straus NA, Weil JH (1988) A class-I intron in a cyanelle tRNA gene from *Cyanphora paradoxa*: phylogenetic relationship between cyanelles and plant chloroplasts. Gene 71:115–122

Friedl T, Zeltner C (1994) Assessing the relationship of some coccoid green lichen algae and the Microthamniales (Chlorophyta) with 18S ribosomal RNA gene sequence comparisons. J Phycol 30:500–506

Gargas A, DePriest PT, Taylor JW (1995) Positions of multiple insertions in SSU rDNA of lichen-forming fungi. Mol Biol Evol 12:208–212

Gast RJ, Fuerst PA, Byers TJ (1994) Discovery of group I introns in the nuclear small subunit ribosomal RNA genes of *Acanthamoeba*. Nucleic Acids Res 22:592–596

Haugen P, Huss VAR, Nielsen H (1999) Complex group-I introns in nuclear SSU rDNA of red and green algae: evidence of homing-endonuclease pseudogenes in the Bangiophyceae. Curr Genet 36:345–353

Huss VAR, Frank C, Hartmann EC, Hirmer M, Kloboucek A, Seidel BM, Wenzeler P, Kessler E (1999) Biochemical taxonomy and phylogeny of the genus *Chlorella* sensu lato (Chlorophyta). J Phycol 35:587–598

Johansen S, Haugen P (2001) A new nomenclature of group I introns in ribosomal DNA. RNA 7:935–936

Johansen S, Johansen T, Haugli F (1992) Structure and evolution of myxomycete nuclear group I introns: a model for horizontal transfer by intron homing. Curr Genet 22:297–304

Krienitz L, Huss VAR, Huemmer C (1996) Picoplanktonic Choricystis-species (Chlorococcales, Chlorophyta) and the misconception of '*Nanochloris*-like algae'. Phycologia 35:332–341

Krienitz L, Ustinova I, Friedl T, Huss V (2001) Traditional generic concepts versus 18S rRNA gene phylogeny in the green algal family Selenastraceae (Chlorophyceae, Chlorophyta). J Phycol 37:852–865

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA2: molecular evolutionary genetic analysis software. Bioinformatics 17:1244–1245

Kushel MG, Strickland R, Palmer JD (1990) An ancient group I intron shared by eubacteria and chloroplasts. Science 250:1570–1572

Lambowitz AM, Belfort M (1993) Introns as mobile genetic elements. Annu Rev Biochem 62:587–622

Lonergan KM, Gray MW (1994) The ribosomal RNA gene region in *Acanthamoeba castellaniii* mitochondrial DNA: a case of evolutionary transfer of introns between mitochondria and plastids? J Mol Biol 239:476–499

Michel F, Jacquier A, Dujon B (1982) Comparison of fungal mitochondrial introns reveals extensive homologies in RNA secondary structure. Biochimie 64:867–881

Müller KM, Cannone JJ, Gutell RR, Sheath RG (2001) A structural and phylogenetic analysis of the group IC1 introns in the order bangiales (Rhodophyta). Mol Biol Evol 18:1654–1667

Nielsen H, Engberg J (1985) Sequence comparison of the rDNA introns from six different species of *Tetrahymena*. Nucleic Acids Res 13:7445–7455

Nikoh N, Fukatsu T (2001) Evolutionary dynamics of multiple group I introns in nuclear ribosomal RNA genes of endoparasitic fungi of the genus *Cordyceps*. Mol Biol Evol 18:1631–1642

Pley HW, Flaherty KM, McKay DB (1994) Model for an RNA tertiary interaction from the structure of an intermolecular complex between a GAAA tetraloop and an RNA helix. Nature 372:111–113

Ruoff BM, Johansen S, Vogt VM (1992) Characterization of the self-splicing products of a mobile intron from the nuclear rDNA of *Physarum polycephalum*. Nucleic Acids Res 20:5899–5906

Schroeder-Diedrich JM, Fuerst PA, Byers TJ (1998) Group I introns with unusual sequences occur at three sites in nuclear 18S rRNA genes of *Acanthamoeba lenticulata*. Curr Genet 34:71–78

Sogin ML, Edman JC (1989) A self-splicing intron in the small subunit rRNA gene of *Pneumocystis carinii*. Nucleic Acids Res 17:5349–5359

Sogin ML, Ingold A, Karlok M, Nielson H, Engberg J (1986) Phylogenetic evidence for the acquisition of ribosomal RNA introns subsequent to the divergence of some of the major *Tetrahymena* groups. Eur Mol Biol Organ 5:3625–3630

Tanabe Y, Yokota A, Sugiyama J (2002) Group I introns from Zygomycota: evolutionary implications for the fungal IC1 intron subgroup. J Mol Evol 54:692–702

Van Oppen MJH, Olsen JL, Stam WT (1993) Evidence for independent acquisition of group I introns in green algae. Mol Biol Evol 10:1317–1326

Vaughn JC, Mason MT, Sper-Whitis GL, Kuhlman P, Palmer JD (1995) Fungal origin by horizontal transfer of a plant mitochondrial group I intron in the chimeric *CoxI* gene of *Peperomia*. J Mol Evol 41:563–572

Wilcox LW, Lewis LA, Fuerst PA, Floyd GL (1992) Group I introns within the nuclear-encoded small-subunit rRNA gene of three green algae. Mol Biol Evol 9:1103–1118

Yamada K, Shinozaki K, Sugiura M (1986) DNA sequences of tobacco chloroplast genes for tRNA-Ser (GGA), tRNA-Thr (UGU), tRNA-Leu (UAA), tRNA-Phe (GAA): the tRNA-Leu gene contains a 503 bp intron. Plant Mol Biol 6:193–199

Yamada T, Tamura K, Aimi T, Songsri P (1994) Self-splicing group I introns in eukaryotic viruses. Nucleic Acids Res 22:2532–2537