



Structural features and evolutionary considerations of group IB introns in SSU rDNA of the lichen fungus *Teloschistes*

María P. Martín,^a Dag H. Coucheron,^b and Steinar Johansen^{b,*}

^a Real Jardín Botánico, CSIC, Plaza de Murillo 2, 28014 Madrid, Spain

^b Department of Molecular Biotechnology, RNA Research Group, Institute of Medical Biology, University of Tromsø, NO-9037 Tromsø, Norway

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Abstract

Different species of the lichen-forming ascomycete fungus *Teloschistes* were found to contain group IB introns at position S1506 in the small subunit ribosomal RNA gene. We have characterized the structural organization and phylogeny of the *Teloschistes* introns Tco.S1506, Tla.S1506, and Tvi.S1506. Common features to all the introns are a small size, a compact RNA structure, and an atypical catalytic ribozyme core sequence motif. Variations in intron sizes, due to sequence extensions in the P1 and P8 loop segments, were observed in different species and isolates. Phylogenetic analyses based on the ITS1-5.8S-ITS2 region as well as the introns show that the *Teloschistes* S1506 introns represent a distinct evolutionary isolated cluster among the nuclear group I introns. Furthermore, introns from different lineages of *Teloschistes villosus* appear not strictly vertically inherited probably due to horizontal transfer in one of the lineages.

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1. Introduction

A significant fraction of fungi and protists contain self-splicing group I introns within the highly conserved nuclear ribosomal RNA (rRNA) genes (see Jackson et al., 2002; Johansen and Haugen, 2001; Johansen et al., 1996). Group I introns are considered as mobile genetic elements both at the DNA and RNA levels (Lambowitz and Belfort, 1993; Roman et al., 1999). The efficient homing mobility at the DNA level is dependent on the intron-encoded homing endonucleases (Belfort and Roberts, 1997), but most nuclear group I introns known lack homing endonuclease genes (HEGs). Loss or gain of HEGs appear common in group I intron evolution (Cho et al., 1998; Foley et al., 2000; Goddard and Burt, 1999; Haugen et al., 1999). Mobility at the RNA level is probably less efficient than homing at the DNA level. RNA mobility (reverse splicing) appears to contribute significantly to the widespread, but sporadic, distribution pat-

tern of nuclear group I introns, as well as to the incongruent pattern of inheritance observed when intron and host phylogenies are compared.

Both intron splicing and RNA mobility are dependent on the intron-encoded group I ribozymes (Cech and Herschlag, 1996; Roman and Woodson, 1995). These large ribozymes possess a well-defined three-dimensional structure (Golden et al., 1998; Lehnert et al., 1996) usually consisting of 10 paired segments named P1–P10, and organized into three functional domains. Here, the substrate domain (P1, P2, and P10) binds exon sequences and coordinates the splice sites, the folding domain (P4, P5, and P6) initiates RNA folding of the ribozyme, and the catalytic domain (P3, P7, P8, and P9) creates the catalytic site of the ribozyme.

Lichenization may promote transfer of intron sequences into the ribosomal DNA (rDNA). Group I introns have been reported in lichen-forming algae (Friedl et al., 2000), and are frequently observed in lichen-forming ascomycetes at a number of insertion sites in the small subunit (SSU) and large subunit (LSU) rRNA genes (e.g., Bhattacharya et al., 2002; DePriest and

* Corresponding author. Fax: +47-77-64-53-50.

E-mail address: steinarj@fagmed.uit.no (S. Johansen).

Been, 1992; Gargas et al., 1995; Grube et al., 1999). Furthermore, small spleisosomal-like introns in lichen-forming fungi have also been described in nuclear rDNA (Bhattacharya et al., 2000; Cubero et al., 2000; Stenroos and De Priest, 1998). A common feature among group I introns in lichen-forming fungi is the small size (150–300 bp) that reflects a compact structural organization of the group I ribozyme. Here we report new optional group I introns present in six of seven analysed species/strains of the lichen-forming fungus *Teloschistes* Norman. These small group IB introns are located at position 1506 (S1506) in the SSU rRNA gene, an rDNA site commonly known to harbour intron insertions in algae and free-living ascomycetes.

2. Materials and methods

2.1. DNA extractions

Herbarium specimens of *Teloschistes conthortuplicatus* (Ach.) Clauz. Et Rondon (MA-Lichen 12757) and *Teloschistes villosus* (Ach.) Norman (MA-Lichen 12759) were visually examined, and portions of the specimens that appeared to be in good condition (less than 0.01 g) were used for the DNA extractions. DNA was extracted using an E.Z.N.A. Fungi DNA miniprep kit (Omega Biotek) that combines the reversible nucleic acid-binding properties of a HiBind matrix with the speed and versatility of spin columns to eliminate polysaccharides, polyphenols, and other enzyme inhibitors from lysed fungal hyphae as described by Martín et al. (2000).

2.2. PCR amplification

The primer pairs ITS1F and ITS4 were used to amplify the internal transcribed spacer 1 (ITS1), the 5.8S rRNA gene, and the internal transcribed spacer 2 (ITS2) of the rRNA gene cluster, as described by White et al. (1990) and Gardes and Bruns (1993). Amplification reactions were done using Ready-To-Go PCR Beads (Amersham-Pharmacia Biotech), using cycling parameters as described in Martín and Winka (2000). Each 25 µl reaction included 10 pmol/µl of each primer. The PCR amplifications were performed in a Perkin-Elmer Cetus DNA Thermal cyler (GeneAmp 2400). Results from the amplifications were monitored by electrophoresis of 5 µl aliquots in a 1% Seakem Agarose gel (FMC Bioproducts).

2.3. DNA sequencing

Amplification products were cleaned using the E.Z.N.A. Clean kit (Omega Biotek). Both strands were sequenced separately using the primers mentioned above. Sequence reactions were performed with the ABI

Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit and AmpliTaq DNA Polymerase (Perkin-Elmer Applied Biosystem), and analysed on ABI Prism 310 Genetic Analyzer. Sequence Navigator Sequence Comparison software (Perkin-Elmer) was used to identify the consensus sequence from the two strands of each isolate. The new sequences have been lodged in the EMBL Nucleotide Sequence Database with the Accession Nos. AJ421258 (*T. conthortuplicatus*) and AJ421263 (*T. villosus*).

2.4. Sequence alignment and phylogenetic analysis

Sequences obtained in this study were compared with homologous sequences of *Teloschistes* retrieved from EMBL/Genbank (see Tables 1 and 2), and reported in a previous work by Martín and Winka (2000). Computer analysis of nucleic acid sequences was performed using the software package program GeneCompare from the Applied Maths (Version 2.0; Kortrijk, Belgium). Multiple alignment of sequences were performed by using ClustalX (version 1.81) (Thompson et al., 1997) and manual refinements. Phylogenetic analyses were conducted using MEGA version 2.1 (Kumar et al., 2001), PAUP* (version 4.0b10) (Swofford, 2002), and MrBayes (version 2.01) (Huelsenbeck and Ronquist, 2001). Trees were built with the methods of neighbor-joining (NJ) using different distance matrixes, maximum parsimony (MP) with the branch and bound search method, as well as maximum likelihood (ML) and Bayesian analysis (BAY) using different evolutionary models. The reliability of the tree topologies was evaluated by bootstrapping (NJ, MP, and ML), and posterior probability (BAY).

3. Results and discussion

3.1. SSU rDNA group I intron insertions in *Teloschistes*

Insertions at the SSU rDNA position 1506 (relative to the *Escherichia coli* numbering) were found in isolates of *T. conthortuplicatus*, *Teloschistes lacunosus*, and *T. villosus*, but not in *Teloschistes chrysophthalmus*. An alignment of the insertions and flanking SSU rRNA sequences is presented in Fig. 1. The insertions, all representing group I introns, are clearly homologous in sequence but vary in size from 250 to 330 nt (see Table 1). S1506 is the most frequent group I intron insertion site in nuclear SSU rDNA with more than 210 intron entries in the database (<http://www.rna.icmb.utexas.edu>), and the majority of these introns are found in ascomycete fungi and in algae (representative examples are listed in Table 1). However, S1506 group I introns in lichen-forming ascomycetes are rare with only few examples reported (e.g., Bhattacharya et al., 2002).

Table 1
Representative nuclear rDNA group I introns

Host taxon	Intron name ^a	Size ^b	Class ^c	Accession No. ^d
FUNGI—Ascomycetes				
<i>Acanthostigmella brevispina</i>	Abr.S1506	366	C1	L35291
<i>Bulbithecium hyalosporum</i>	Bhy.S516	294	E	AF096172
<i>Cenococcum geophilum</i>	Cge.S1506	469	C1	Z11998
<i>Cryphonectra parasitica</i>	Cpa.S1199	547	E	L42441
<i>Cyclaneusma niveum</i>	Cni.S1507	193	B	AF013223
<i>Exophiala dermatitidis</i>	Ede.S516	542	E	Z75303
<i>Encoelia fascicularis</i>	Efa.S788	309	C1	AJ226080
<i>Fusicladium effusum</i>	Fef.S1506	360	C1	U63629
<i>Geniculifera cystosporia</i>	Gcy.S1506	400	C1	U51966
<i>Isaria japonica</i>	Ija.S943	381	C1	AB016607
<i>Monacrosporium bembicodes</i>	Mbe.S1506	387	C1	U51976
<i>Myriosclerotinia demmisi</i>	Mde.S788	294	C1	AJ226072
<i>Nectria galligena</i>	Nga.S1199	363	E	Y16422
<i>Ophiosphaerella narmari</i>	Ona.S1506	425	C1	AF102179
<i>Symbiotaphrina buchneri</i>	Sbu.S1506	232	B	D49657
<i>Teloschistes conthortuplicatus</i>	L ^e Tco.S1506	284	B	AJ421258
<i>Teloschistes lacunosus</i>	L Tla.S1506-1	250	B	AJ421259
<i>Teloschistes lacunosus</i>	L Tla.S1506-2	250	B	AJ421260
<i>Teloschistes villosus</i>	L Tvi.S1506-1	275	B	AJ421261
<i>Teloschistes villosus</i>	L Tvi.S1506-2	330	B	AJ421262
<i>Teloschistes villosus</i>	L Tvi.S1506-3	273	B	AJ421263
<i>Venturia inaequalis</i>	Vin.S1506	386	C1	U63624
PROTOCTISTA				
<i>Acanthamoeba griffini</i> (amoeba)	Agr.S516	519	C1	U02540
<i>Bangia atropurpurea</i> (red alga)	Bat.S1506	1038	C1	L36066
<i>Naegleria jamiesoni</i> (amoebae-flagellate)	Nja.S516	1308	C1	U80250
<i>Physarum polycephalum</i> (myxomycete)	Ppo.L1925	944	C1	L03183
<i>Porphyra spiralis</i> (red alga)	Psp.S1506	1057	C1	L26177
<i>Porphyra tenera</i> (red alga)	Pte.S516	972	C1	AB013175
<i>Scottilopsis terrestris</i> (green alga)	Ste.S943	486	C1	AB012847
<i>Tetrahymena thermophila</i> (ciliate)	Tth.L1925	413	C1	V01416
<i>Trebouxia usneae</i> (green alga)	L Tus.S1512	440	C1	AJ249573

^a Named according to the new nomenclature system of group I intron in rDNA reflecting both the intron host and intron insertion site (Johansen and Haugen, 2001).

^b Size in base pair of intron elements.

^c Subclass of group I introns (Michel and Westhof, 1990; Shu et al., 1999).

^d EMBL/GenBank Accession numbers.

^e L, lichen-forming.

Table 2
Species and isolates used in the ITS-sequence based phylogeny

Taxon	Source of rDNA	ITS Accession No. ^a
<i>Teloschistes chrysophthalmus</i>	(Tch) BCC-Lich 13258	AF098409
<i>Teloschistes conthortuplicatus</i>	(Tco) MA-Lichen 12757	AJ421258
<i>Teloschistes lacunosus</i>	(Tla1) BCC-Lich 13173	AF098405
<i>Teloschistes lacunosus</i>	(Tla2) BCC-Lich 13205	AF098406
<i>Teloschistes villosus</i>	(Tvi1) BCC-Lich 13174	AF098408
<i>Teloschistes villosus</i>	(Tvi2) BCC-Lich 13203	AF098407
<i>Teloschistes villosus</i>	(Tvi3) MA-Lichen 12759	AJ421263
<i>Diploschistes ocellatus</i>	(Doc) Isolate 107346	AF228316

^a EMBL/GenBank Accession numbers of sequences including the rDNA internal transcribed spacer 1 (ITS1), the 5.8S rRNA gene segment, and ITS2.

3.2. Structural characteristics of the *Teloschistes* group IB introns

A secondary structure model of the *Teloschistes* intron, represented by Tco.S1506 from *T. conthortuplica-*

tus, is presented in Fig. 2A. This structure is based upon known general features among the group I introns (Cech et al., 1994; Golden et al., 1998; Lehnert et al., 1996; Michel and Westhof, 1990) and shows that the *Teloschistes* introns are typical members of the group IB

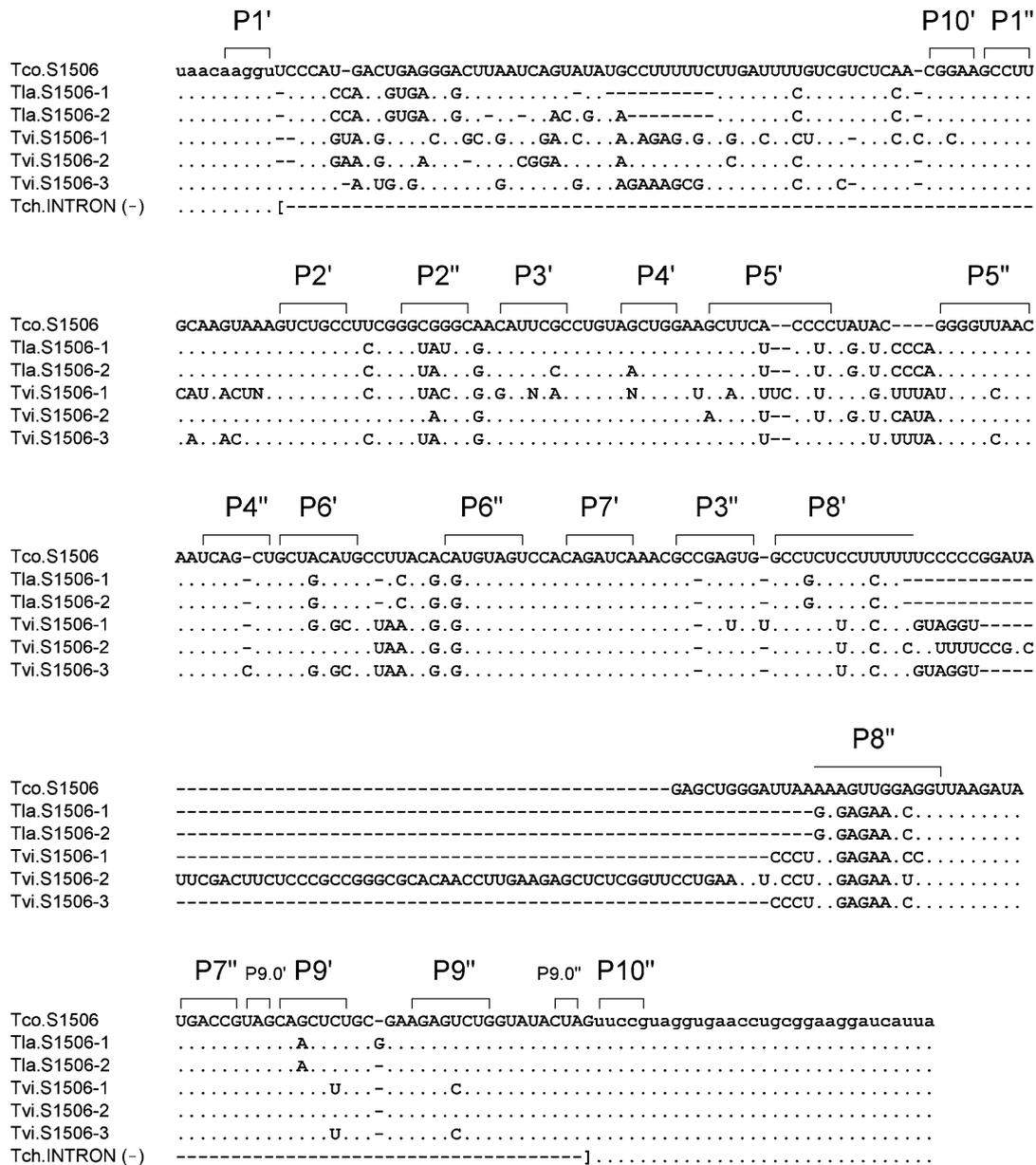


Fig. 1. Secondary structure alignment and sequence variability of different *Teloschistes* S1506 group I intron RNAs. Identical sequence positions to the *T. conthortuplicatus* intron Tco.S1506 (AJ421258) are indicated by dots and deletions by dashes. Pn (boxed) indicates paired segments, and Pn' designates the 5' branch of a pairing and Pn'' its 3' branch. Intron sequences are shown in uppercase and SSU rRNA sequences in lowercase, and unresolved nucleotides are indicated with 'N'. The additional introns are Tla.S1506-1 (AJ421259) and Tla.S1506-2 (AJ421260) from *T. lacunosus*, and Tvi.S1506-1 (AJ421261), Tvi.S1506-2 (AJ421262), and Tvi.S1506-3 (AJ421263) from *T. villosus*. Tch.INTRON (-), SSU rRNA sequence (AJ421257) from *T. chrysophthalmus* lacking S1506 intron. The introns are named according to Johansen and Haugen (2001).

subclass. The presence of group IB introns in nuclear rDNA is highly unusual, but commonly found within protein coding genes in fungal mitochondria or algae chloroplasts. Group IB-like rDNA introns have been reported in some lichen-forming ascomycetes, but at different sites compared those of *Teloschistes* (DePriest and Been, 1992). A group IB intron is recognized by a small size, as well as a compact structure with minimal sequences located in the P2, P5, and P9 segments (Jaeger et al., 1996; Michel and Westhof, 1990).

We note several interesting structural features of the *Teloschistes* intron. The observed size variations among introns are mainly due to two segments, the P1 and P8. All introns have an extended P1 of about 40–50 nt (see Fig. 1). Sequence extensions in P1 are known to occur in a fraction of the S1506 introns and include HEG-like sequences as well as structural segments (e.g., Haugen et al., 1999; Holst-Jensen et al., 1999; Müller et al., 2001). However, the *Teloschistes* P1-extensions appear too short to harbor recognizable HEGs. The P8

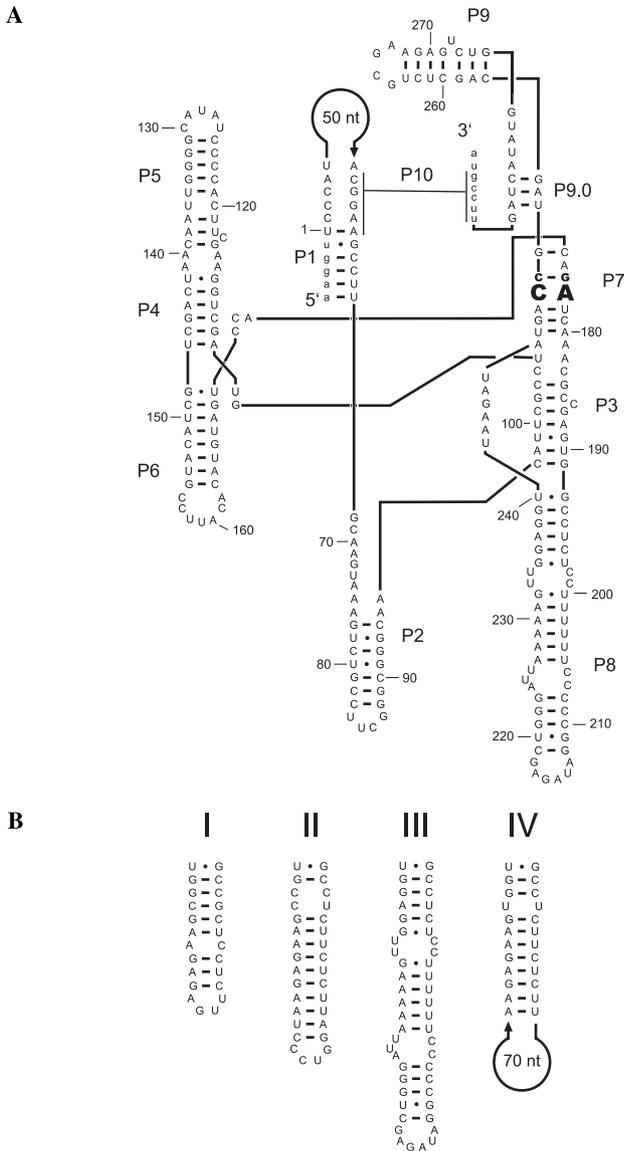


Fig. 2. (A) Secondary structure model of Tco.S1506 representing the *Teloschistes* S1506 group I introns. Intron sequences are shown in uppercase and SSU rRNA sequences in lowercase. The atypical A:C pair in P7 is indicated. (B) Secondary structure of P8-extensions among the *Teloschistes* S1506 group I introns. Type I, Tla.S1506-1 and Tla.S1506-2; Type II, Tvi.S1506-1 and Tvi.S1506-3; Type III, Tco.S1506; and Type IV, Tvi.S1506-2.

segment is the most variable region of the introns (Fig. 2B). Whereas P8 in Tla.S1506 is 24-nt, Tvi.S1506-2 has an extended P8 segment of 94-nt. Interestingly, large size differences in P8 are seen between different isolates of the same species (*T. villosus*) probably due to a recent loss, or gain, of extension sequences. A similar variability to that observed in P8 of the *Teloschistes* introns has been reported in red alga group I introns of *Porphyra* and *Bangia* (Haugen et al., 1999; Müller et al., 2001; Oliveira and Ragan, 1994). However, the variability of these introns was located to the

P1 segment. Large extension sequences in P8 are rare among nuclear group I introns, but introns from ericoid mycelia fungi contain HEG sequence hallmarks within P8 (Perotto et al., 2000).

The catalytically important guanosine binding site within the P7 segment is unusual in the *Teloschistes* introns. The P7 segment of a self-splicing group I intron contains a highly conserved guanosine binding site consisting of a universally conserved G:C pair followed by an A:U pair (Michel and Westhof, 1990). The corresponding guanosine binding site of the *Teloschistes* introns contains a G:C pair followed by an unconventional A:C pair (indicated by bold characters in Fig. 2A), a sequential feature that probably affects catalysis. An identical P7 sequence feature to that observed in the *Teloschistes* introns is also seen in the two related group IB introns Sbu.S1506 and Cni.S1507 (Fig. 3) located in SSU rDNA of the ascomycetes *Symbiotaphrina buchneri* and *Cyclaneusma niveum*, respectively. The atypical P7 feature present in the *Teloschistes*, *Symbiotaphrina*, and *Cyclaneusma* introns may imply that in vivo splicing of these introns depends on cellular host factors.

3.3. The *Teloschistes* S1506 introns are only distantly related to free-living ascomycete introns at the same site in ribosomal DNA

Fig. 4 presents a NJ tree that include the various *Teloschistes* introns as well as other representative nuclear group I introns (see Table 1). The intron phylogeny was based on 100 sequence positions within the catalytic core of corresponding ribozymes, strictly aligned according to the secondary structure. The group IB and IE intron (Shu et al., 1999) clusters are distinct (bootstrap values of 98–100%) from the C1-subclass of nuclear group I introns (Fig. 4). Furthermore, the NJ-tree presented supports a common trend in group I intron phylogeny that introns at the same rDNA location are the most similar ones (e.g. Bhattacharya et al., 1994; Jackson et al., 2002; Nishida et al., 1998; Shinohara et al., 1996; Shu et al., 1999). Interestingly, the *Teloschistes*, *Symbiotaphrina*, and *Cyclaneusma* introns form a separate cluster, the group IB intron subclass, which is clearly distinct from other known S1506 introns of ascomycete fungi and algae (boxed in Fig. 4). These results strongly suggest that the *Teloschistes* introns and most introns at the same rDNA site in free-living ascomycetes have independent evolutionary histories, and were gained from distantly related group I intron ancestors.

Only two related introns to those in *Teloschistes* were identified in the database (<http://www.rna.icmb.utexas.edu>). One of the introns is observed in the yeast-like symbiont (YLS) *S. buchneri* (Noda and Kodama, 1996). This group IB intron is located at the exact

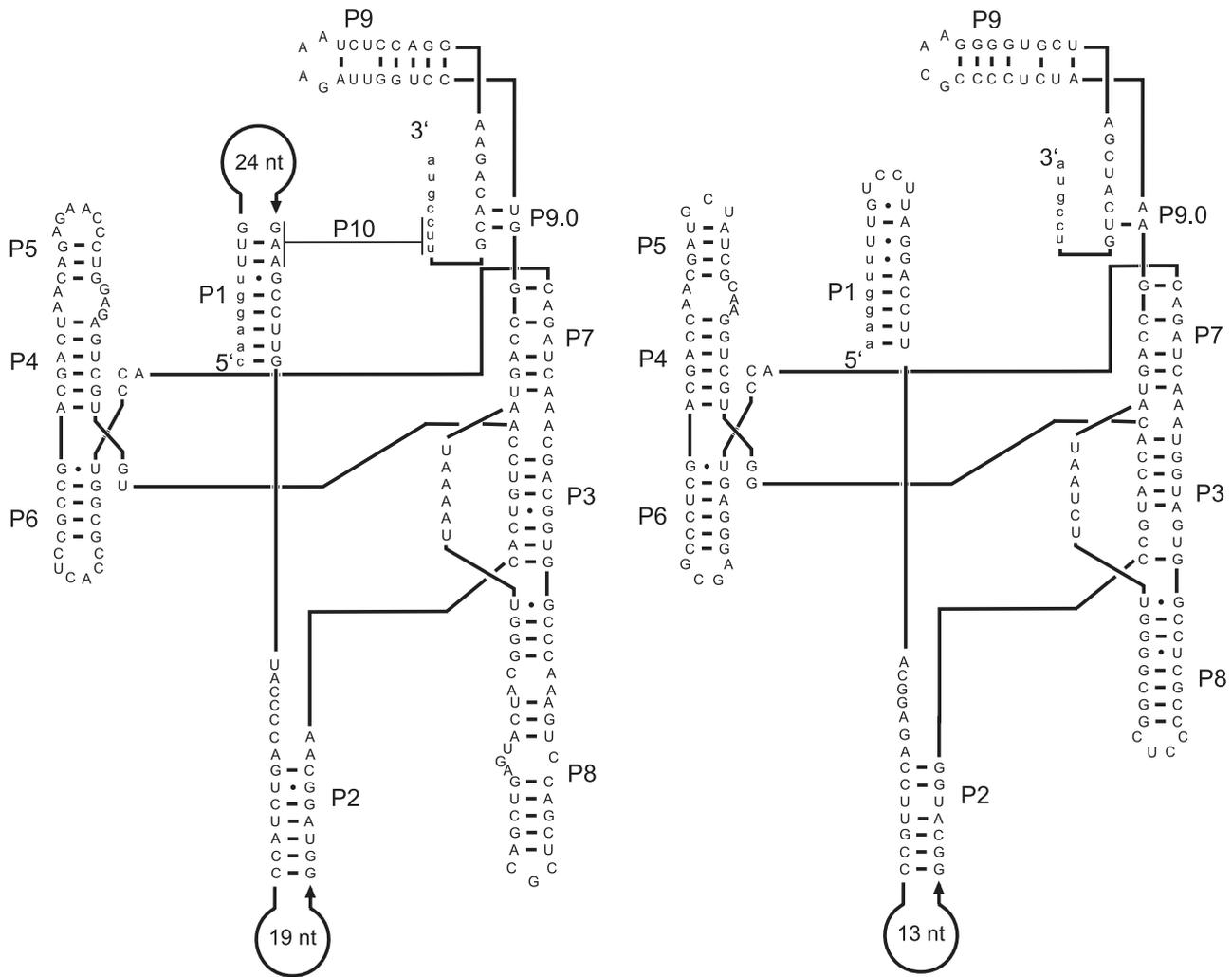


Fig. 3. Secondary structure models of fungal group I introns Sbu.S1506 (D49657) from *S. buchneri* and Cni.S1507 (AF013223) from *C. niveum*.

same site in the SSU rDNA (S1506) with a structural organization resembling the *Teloschistes* introns (Figs. 2A and 3). Similarly, an intron from the pine-needle associated fungus *C. niveum* is closely related to the *Teloschistes* but located one position off (S1507) that of the *Symbiotaphrina* and *Teloschistes* introns (Fig. 3). The probability appears low that both the *Teloschistes*, *Symbiotaphrina*, and *Cyclaneusma* introns all originated from a single, vertically inherited fungal group I intron since most ascomycetes lack group IB introns and these fungal hosts are only distantly related. Thus, transfer of introns between the fungal genera during evolution is more plausible. It is interesting to note that both the YLS and lichen-forming fungi are involved in symbiotic relationships with beetles and algae, respectively (Noda and Kodama, 1996; Shu et al., 2001). Symbiosis is a biological state proposed to stimulate horizontal transfer of group I introns (e.g., Einvik et al., 1998; Friedl et al., 2000; Holst-Jensen et al., 1999; Nikoh and Fukatsu, 2001), and numerous examples of

rDNA introns in lichen-forming fungi are a further support of this notion. Many species of *Trebouxia* genus, the phycobiont genus in *Teloschistes* lichens, are known to harbor group I introns in nuclear SSU rDNA (Friedl et al., 2000). However, these introns, which are inserted at position S1516 and belong to the group IC1 subclass, are clearly different from the group IB introns in *Teloschistes* (see Fig. 4). The observed accumulation of group I introns in nuclear rDNA in both the symbiotic partners makes lichens a very interesting biological system for studying horizontal intron transfer and evolution.

3.4. Indication for horizontal transfer of intron within *Teloschistes*

Phylogenetic reconstruction of the *Teloschistes* species was based on sequences obtained from the ITS1-5.8S-ITS2 rDNA region. Different methods based on MP, ML, BAY, and NJ were used to build phylogenetic

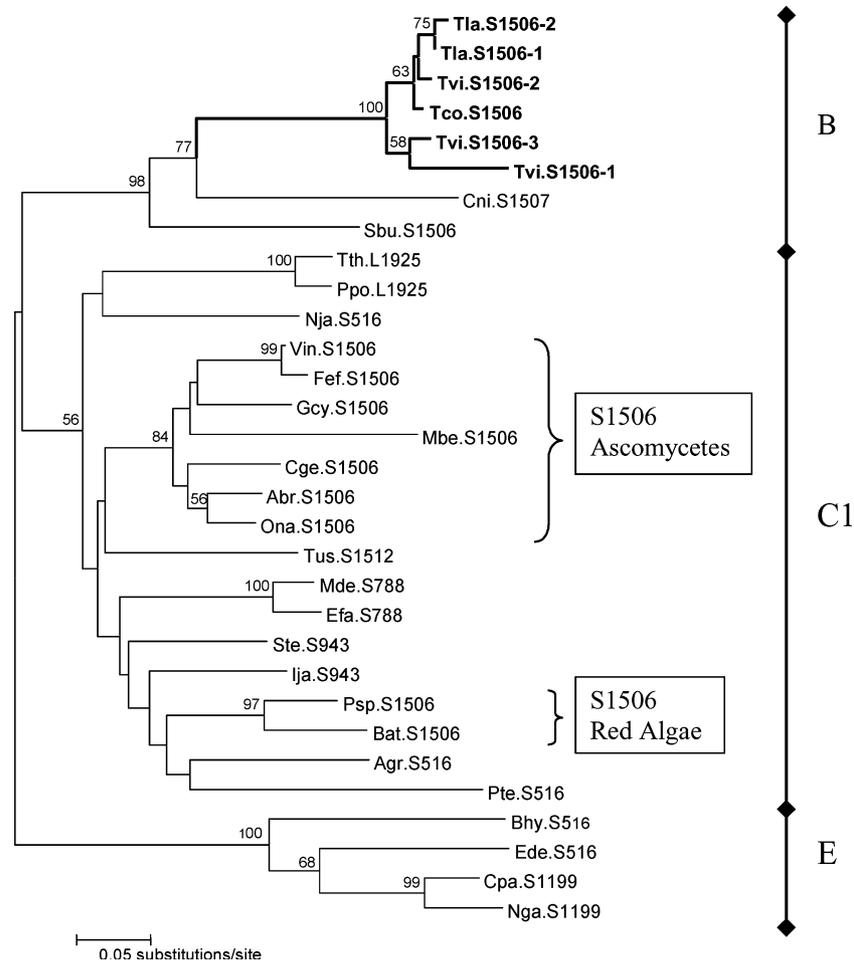


Fig. 4. Neighbor-joining (NJ) tree of the *Teloschistes* S1506 introns and other representative nuclear group I introns based on distance matrix generated with the uncorrected pairwise distance method. The intron phylogenetic analysis was based on 100 sequence positions within the catalytic core strictly aligned according to the secondary structure. Bootstrap values (10,000 replications) above 50% are shown at the branches. The *Teloschistes* S1506 introns are presented in bold, and the S1506 introns from ascomycetes and red algae are boxed. The three main subclasses of nuclear group I introns (B, C1, and E) are indicated. The EMBL/GenBank Accession numbers, host species, and key features of nuclear introns included in the analysis are given in Table 1.

trees. All trees were essentially identical in topology, and a representative MP tree is presented in Fig. 5A. The results are in accordance with current taxonomy of *Teloschistes* with *T. conhortuplicatus* clearly distinct (bootstraps between 89 and 100% in different methods) from *T. lacunosus* and *T. villosus*.

Introns from *T. conhortuplicatus* (Tco.S1506), *T. lacunosus* (Tla.S1506-1) and *T. villosus* (Tvi.S1506-3) share 84–89% identical positions. However, introns from different isolates of *T. villosus* (Tvi.S1506-1, -2, and -3) are less similar and found to share only 81–88% identical positions. Thus, the conserved catalytic core (137 sequence positions) from all five *Teloschistes* introns was used to construct phylogenetic trees based on MP, ML, BAY, and NJ methods. The different trees generated were similar in topology, and a representative NJ tree is shown in Fig. 5B. Tco.S1506 and Tvi.S1506-2 appear to cluster together, a feature sup-

ported by high bootstrap values. Furthermore, a synapomorphy was identified in Tco.S1506 and Tvi.S1506-2 (10-nt sequence located within the P1-loop; Fig. 1) representing an additional support of the clustering. Even if we cannot rigorously exclude processes like rare gene duplication events, internal transfer from organelle genomes, or different mutation rates among the individual *Teloschistes* introns, a likely explanation to the observation is intron gain (Tvi.S1506-2) by horizontal transfer in one of the *T. villosus* lineages. Data supporting horizontal transfer of group I introns between closely related species and strains have been reported in yeast (Goddard and Burt, 1999), and suggested in several other systems (e.g., Cho et al., 1998). Thus, to address questions concerning the more detailed molecular evolution of the *Teloschistes* introns, additional analyses including more species and introns have to be performed.

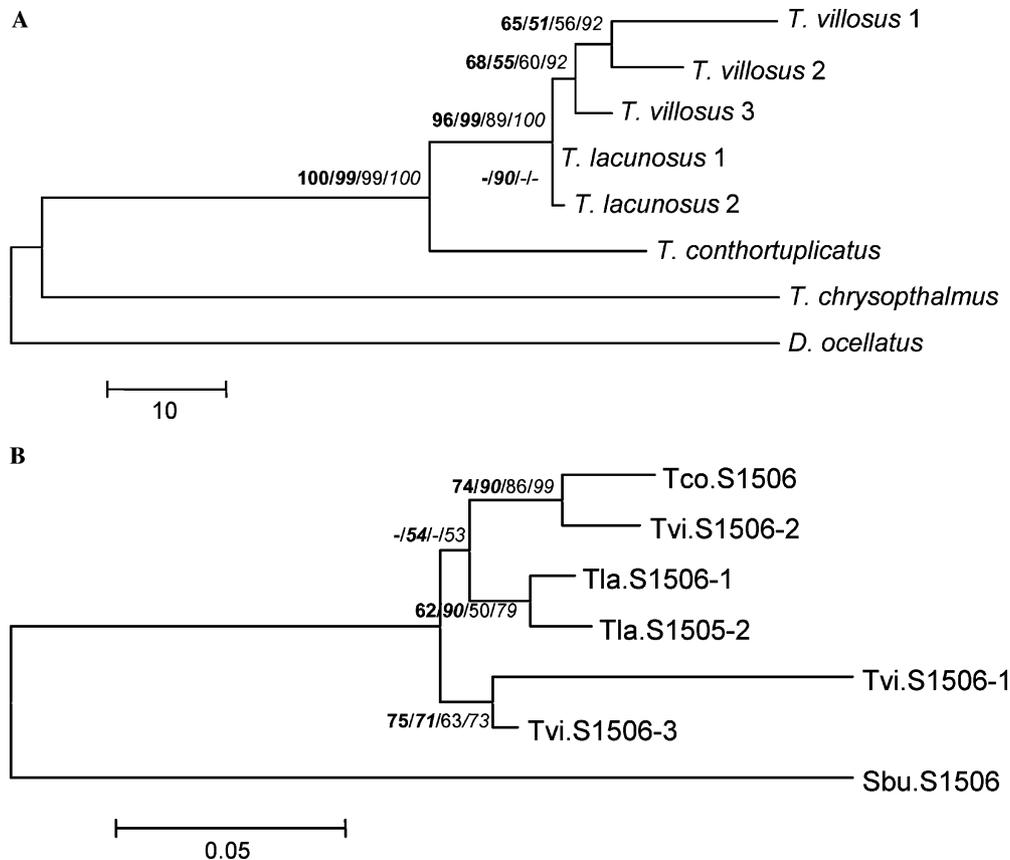


Fig. 5. Phylogenetic analyses of intron-host relationships in *Teloschistes*. Bootstrap values from 1000 replicates and Bayesian posterior probability values, all over 50%, are shown at the branches. The values are from, in order, maximum parsimony, neighbor joining, maximum likelihood, and Bayesian analysis. Jukes and Cantor substitution models were used except in parsimony and for neighbor-joining bootstrapping in (B) where uncorrected pairwise distances were used. (A) Maximum parsimony tree of the host *Teloschistes* based on internal transcribed spacer 1 (ITS1), 5.8 S rDNA, and ITS2 sequences (Table 2), constructed by the branch and bound algorithm. The tree is based on alignment of 443 positions. The trees were rooted by including *T. chrysophthalmus* (see also the legend to Fig. 1) and *D. ocellatus* as outgroups. (B) Neighbor-joining tree of the *Teloschistes* S1506 group I introns. The analyses are based on 137 sequence positions within the catalytic core strictly aligned according to the secondary structure. Trees were rooted by including Sbu.S1506 as an outgroup.

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