

## **Supplementary Material (online)**

associated with

### **Internally symmetrical stwintrons and related canonical introns in Hypoxylaceae species**

Erzsébet Fekete<sup>1\*</sup>, Fruzsina Péntes<sup>1,2</sup>, Norbert Ág<sup>1</sup>, Claudio Scazzocchio<sup>3,4</sup>,  
Michel Flipphi<sup>1</sup> and Levente Karaffa<sup>1</sup>

<sup>1</sup> Dept. of Biochemical Engineering, Faculty of Science, University of Debrecen, 4032, Hungary.

<sup>2</sup> Juhász-Nagy Pál Doctoral School of Biology and Environmental Sciences, University of Debrecen, 4032, Hungary.

<sup>3</sup> Section of Microbiology, Dept. of Infectious Diseases, Imperial College London, SW7 2AZ, UK

<sup>4</sup> Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA & CNRS Unité Mixte de Recherche UMR 9198, Gif-sur-Yvette, 91190, France

\* Author for correspondence: kicsizsoka@yahoo.com

**Supplementary text document S1: Fastas sister stwintrons CO-27-5**

**Supplementary text document S2: Fastas derived canonical introns CO-27-5**

**Supplementary text document S3: Fastas sister stwintrons E7406B**

**Supplementary text document S4: Fastas sister stwintrons *H.rubiginosum*-Daldinia**

**Supplementary figure S1: Alignment Type-1 cropped sister introns**

**Supplementary figure S2: Alignment HCOc066A–HECc034A**

**Supplementary figure S3: Generation type-2 cropped sister intron**

**Supplementary figure S4: Alignment HECc217A–HECc217B–HCOc004A**

**Supplementary figure S5: Phylogeny mitochondrial carrier protein**

**Supplementary figure S6: Base pairing TIRs double-stranded RNA molecules**

**Supplementary figure S7: Alignment *Daldinia childiae* Dchc003A–Dchc003S**

**Supplementary table S2: Oligonucleotide primers used**

**Supplementary table S3: SRA identifiers**

Supplementary table S1 is provided separately as an Excel file (Supplementary\_Table\_S1.xlsx): Identifiers, SRA reads, GenBank accession numbers and more information sister (stw)introns

Supplementary text document S1.

Fastas sister stwintrons and sheared sister stwintrons in *Hypoxylon* sp. CO27-5.

(ordered per sequence contig)

Full sister stwintrons (23)

>HCOc002A  
GAATATGGACTCTATggtatgttcagggtactcagtggtctagaaagacctcaaaatttctaaaaataggaacaagctaacatcataaccagtaag  
taagaatttctaataatcgccgtgtccagattaaaaaggcgatacttggtagatattgaaaacaaaagggtcaagatgctgacacgacatggtgc  
gatacactagACCACGTTTCTGGT

>HCOc004A  
ACTGGGGTGACTACAggtgagtatcaaaacacgtcgtcctggcatttttagtccttaatttctaaagaaaaaaaaaaccctaaatttctacaacat  
aggaataaactaacattatgaatagtaagttaaccggttcctatttctgttggcatcggggttaagagaaatgtctagtagacattccagaaaccagg  
gataaaaatgctgacacgacatgtcttaatacactagTCGAGGTGAAAGTCA

>HCOc017A [mitochondrial carrier gene]  
GTTTCTGGATTACGggtgagtataaaaaacatgtcgtttctggcatttttagatattgatttctagaaaaaacccccgtttctacaatatatgaa  
tcagctaacgttataaccagtaagttaagcgttcttatttctgtatcggttagaagagcagtagctggtatgttctaaaaacgaaggata  
aaaatgctgacgacatgttttaatacatcagAGTTACCCAAGCGAC

>HCOc017B [other gene]  
AGAGCGGGATTCTCTggttaagtgaaaaaatctgcggttttgggtgttttatacttcaatttcttgaaagatcaccaaattcgctaacggttatgaca  
gtaagtcaagtgttctgtttctgcgcacatcggggttaagaagcaatatctggtatattctagaaacgcggtataaagtactgacacgacgtg  
ttttaatagATGTTGGAGATGAAC

>HCOc021A  
TTTCTAAAGATGTTcgtacctaagtattaagatatatcatgtttaggatttcggcctttgatttctagaagaaaccctatgacatagaaaactag  
ctaacgttatagcagtgagtaaaatcttttttttttttttttcaatactatgaaacttaaaaaggcaatttcggcgctactataagaacgaag  
gtaaaaatagcactgacataatatgtttcaacacactagGGTCTATATGAAGCT

>HCOc047A  
GCCTTGGGTTGCAATggtacgtacaaaaacttctcgtgtggagggttttaaaccttgacgtctgaaagaatgtcgaataataactaattttataac  
agtgagtataatgttctcgttctcctctttttaaagcgttcggttagcctggaagagaaaggtaagaaaaaaacgaaggagagaaaaagctga  
cacgatatgttttaataccttagGTTACAGCAAGTTGT

>HCOc052A  
CCATCTGGCTACTCGggtgagtattaaaaacatatcgttctggcatttttagaccttgatttctagaagaactcccatatttccagtgatatg  
aattagctaacggttatgacagtaagttaagcgttcttattctcgtcgtatcggggttagaaggtagtagtctggtatattctagaaatgaagga  
taaaaatgctaatacgcacatgttttaatacattagACTATCGCGCGCAGC

>HCOc058A  
CCGTACACGAACAATggtatgtagaagacataccgctcttggcgtatttagaccttgatttctagaagaaaccctgaggttttcacaatatagggga  
ttaagctaacggttaggacagtaagttaagcgttctcgttctcgtcattcagggttaaaaaggaaatgtctggtatattccggaagcaatggtcg  
aaaaacgctgacacgcacatattttaatacaccagATCATTTGGGTCCAG

>HCOc061A  
GCTATTGGTTCGGATggtgagcacaaaaacatgtcgtttctgacattttataccttaatttctacaaaccccctatatattttacaacatataaa  
ttaagctaacggttatgatagtaagttatcggttcttatttcttactatcgaggttcagaaggtagtagtatttagtatattttagacacgaaggata  
aaaatgctaacacgcataatgttttaatacaccagGGCAGTACTGGTGGG

>HCOc066A  
TATGCTCTGGTGTGAggtgagtataaaaaacatgtcgtttctggcatttttaaacctaaattttctagaaaatctcccatgtttttacaatatatga  
attagctaacattataacagtaagttaagcgttcttatttctcgtcgtatcggttagaagagcagtagctggtatattctagaaacgaaggat  
gaaaatgctgacacgcacatgttctaatacattagCCTGCGCCTGTCTAC

>HCOc070A  
CTTTGTTGCTCTGGGggttaagtataaaaaatgtcgtttccagcatttttagaccttgatttctagaaaacccccctatttctacgatatatgaatt  
agctaacattatgatagtaagctaagcgttcttatttctcgtcgtatccgggttaaggagggcagtagctggtatattctacaaacgaaggatgaa  
aatgctaacacggaatgttttaatacaccagCCGGGGTGGCTTTGA

>HCOc076A  
ACGCTAGTGAAGCCCggtgagtataaaaaacatgtcgtttctagcatttttagaccttgatttctagagaaaccctatatctttacaatatatgaa  
ttaagctaacggttataatagtaagttaagcgttcttatttctcgtcgtatcggttagaagagcagtagctggtatattccagaaacgaaggata  
aaaatgctaacacgcacatgttttaatacaccagTGCTGCAGAAGGTGG

>HCOc102A  
CATTTAATAGTCAGTggtgagtataaaaaacatgtcatttctggcatttttaggccttgtttctagaaaacccctcatatttctacaatatatgaa  
taagctaacggttatgatagtaagctaagcgttcttatttctcgttctcgtatcagggttagggaggaaggataaaaatgctgacacgcacatgtttta  
acaccagTTCAAGAAGCTACAA

>HCOc164A

TAAGGCAACTACAACggtatgtattaggtagatatatggtgtggtgggattccggcccttgcttcctagaaaagccttcatttctctatgatgc  
aggaaccaagctaatttttatgacagtgaagtaaaacacttttattttgtcgcgctgaacttgtgaagagaatatcaagta~~tatcctagaaa~~tg  
aagactatagatattgaactacatgttttaacacttagGGAAGGGAGCGATGT

>HCOc178A  
CCTAGACCACCTTCggtgagtataaaaacatgtcattctggcatttttaaacattga~~tttctagaaa~~cccccatatttctacgatatatgaa  
ttagctgacggttgtagtagtaagttaagcggttcttatttctcgtacgtacccgggttaagaaggcggtatctgggtat~~attctagaac~~gaaggataaa  
aatgctgacacgacattttttaatacac~~cag~~GGATATCAGCGAGCAT

>HCOc236A  
CGCGCTTAGCATCTTggtgagtatacgaatatgtcgtttttgtcacttttagaccttga~~tttctagaaa~~gatccctcaaagtgttcaatgttagga  
attagctaacgctcgtgacagtaagtgaagcggttcttatttctcgccatcggtgttaagaaaacaatgctaggta~~tatcttagaaa~~cgaagtgt  
taaaatgctaaccagttatgtttaattacac~~cag~~CGCGCTTAGCATCTT

>HCOc224-179 [split over contigs 224 and 179]  
[GenBank Accession number MW477887]

ACGGACAGCGCCTCTggtgagtataaaaacatgtcgtttctggcatttttagaccttga~~tttctagaag~~accccgaccccccccccaacatatatt  
ctacaagatatgaatttagctaaggttatggttagtaagttaaacggttcttattctcgctcgtatcggggttaagaaggcagtatctcttat~~attct~~  
~~agaaa~~cgaaggataaaaaatgctaaccacgacatgttttaatacat~~cag~~TCTGTCCAACATGCG

>HCOc252A  
AGGGTTGGCGTCCCAGgtacgtataaacatatgccattttcggcatttttagatccctgggtccct~~tacctagagg~~tttagctaaaggtaagacagtaag  
tgatcgtttttatttctgttattgtcaggttaacaaggaaatgtttgggtt~~attctagaat~~gaagtattgaaaaggctaaccacgatattgtgt  
aatatcc~~tag~~GGATGGAGGGATGCAA

>HCOc271A  
ATTGGATACGTAATTggtgagtataaaaactatgtcgtttttggcgttttagacatttgg~~tttctggaaa~~gaccatcaaattctcaatataaaaatc  
agctaaggttatgacagtgagtaaatgttcttatttctcgctcgtatcggggttaagaaggcaatgcctggtata~~attctagaaa~~cgcacaggtaa  
aatgctgacacgacgcttttaacacgttagTTATATGCCGATTG

>HCOc332A  
GGTGGATGCTACAGTggtatgtataaaaatatgtcgttttggcatcttggagactgg~~ttttcataaaa~~atatatatgaattagctaaccactgggac  
agtaagtaaacggttctcatttctgttctatcgagtccaagaaggcaatgtctagtat~~attctagaag~~caaaggccaagaatgctgacacgaca  
tgttctaatacac~~cag~~AATCGAACTGAGAAT

>HCOc378A  
GAAAGGGGTCATATTggtgagtacaaaagcatgtcattctggcatttttagaccttga~~tctctaa~~gaaaaccactatatttctataatatatta  
ttggctaaccggttatgacagtaagttaagcggttcttatttctcgctcgtatcggggttaagaagtagtggttggtat~~tattctagaaa~~cgaaggata  
aaatgctgacacgacatgttttaatacac~~cag~~TCTTTACCGAACTGA

>HCOc406A  
TGGCGCAAGACTGTTggtgagtataaaaagacgtcatttttggcatttccataccagat~~ttcccaca~~aatataggaatttcgctaaccactatagaact  
aagttgagcggttttatctcgtcgtcgcgtcgggttaagaaggcaatgcctacctagtat~~attctagaaa~~tgatgggttaacaatgctgacacgac  
atgctttaacacac~~tag~~TTACATGCCATCGAC

>HCOc522A  
CGACGCCCCGCAGATggtatgtataaaaacatgttgcccttggatttccaagcatttga~~tctctg~~gaaagactcgtaaatcccatagtttaggaat  
tagctaaccggttgggtagtgagtaagaattatcattttatcgccatcgagtttaagagcgcgacgacctggtaa~~agctctagaaa~~cagaagtca  
aaattgctgacaggatacattttaatacac~~tag~~AAAAGGTGGCATGAC

## sheared sister stwintrons (12)

patches of homology with full sister stwintron HCOc017A (yellow and underlined)

>HCOc002B  
AGTGGCAGATTAATCggtacgtataaaaacggcaagagatattcatatttcagtcctccagattttcgaacatccttcaaattttcacaacgcaa  
tagctaaccatttgtaaagtgaagt~~agaaatatcgtttgccttactatcaaatttgcgagggaacgatctg~~ttccctccatgatgagaattttacaa  
tgctaaccatgacatattttgatacat~~cag~~CTTCTCCTGGAAGTA

>HCOc004B  
TTTGAGATGCCGATTggtatgtaggaagacgctctcgtcgttattctaaactccgactgtcaaatgacttctaaatttctgtatagtagta~~act~~  
aaccttctagtagtgagtcgaatatcctcgttttatctccccaaac~~ttaagatggaaattcccagtatattctagaaa~~tagatgtttataat~~ac~~  
tgatatgacgcggttccatacac~~cag~~CTTCTAGGGAAAGTG

>HCOc016A  
TCGGAAGCTTAATATggttaagtatccctacataacctgtcctgtttctcagcttatagttottgatcccttctgttcagtagtcaatcaaactg  
gtagctaaccattcacttagtagctacaggatcttcttctcttcaatcattttctataacaattaaatgcctaggagacacctcaaactggtcca  
aggtaaacggttgttagattactaacgcgacatgatgtaata~~tga~~~~cag~~ATCGATACACTGGCG

>HCOc016B [other gene]  
AGAGTATATCCGTGAggtgctgttctattctttatctccattcatccttgcatacttctatttat~~atgcatgcatacgcctaaccgataggacag~~  
~~aagt~~taaccaagatttcttaacctcatcttcaggttagatctccttctcggagaaacctacgagaggctccttt~~tctaag~~cttata~~aag~~ATAAT  
TGTACAGTCG

>HCOc020A  
TCCTGCAAGTCATCCggtgCGtgaagaccatctcgatctttaattcttgccttgactatTTtaataattttaagattcgtataggatagtaac  
taacTatacggtagtgagtgaaacatttgtttattaacactgaatctagaaaagaaaagcaacttctagtgataggaaaggaggttagaagtact  
gacacgggtatgtttcaataaaaaCagGGGTGAAGATTCCACA

>HCOc024B  
ATCTCTTTTCAGCTCggtatgtagtagatggcctcaatttcggtattccataccccctttttatctcttcggttcactcttcgtactaacttgttg  
a tagtgagtgaactaaccaccttctacctattctacggattaagggtcaatgaacgatttgagatactaacgtattgcagAAATCTTCAAATG  
GG

>HCOc046A  
GGAGTGACCGATTGTggtatgtacgaaaacatctcgtttgagcatTTcaaagccctttttotagaataatcttcgaacttctcgtaacatccatt  
gctaactccccatagtgagttataaacactctaagacgttcgagatgatggtgatataatgctgacacaagatgttttcctccacCagACGACAA  
CGTACTAA

>HCOc091A  
ATGGATTGAGTCATCggttaagtattagagcaagggatcatatttctgaacccttcagccccatttcatatttgctaccccgattgactgtcgg  
taggtaattagctaaccctcctcatagtatgtgaactatttcttcggttcaccgttctattactatagtgagctatgaaataagttataagatt  
actgacgaacaattttatccatgttagAATATTCGACGCCAG

>HCOc091B [other gene]  
GCTGGGCGAACACTTggtacgtagtgctggtgcgagatttcaagagctatctttcgaacggtctccaattctctttggtatagataaaagctaa  
cttctctggcagtgagtaaaatgacctattccatttgcacgatatttattcggtagtgactaaaagtaaatgacgagatactgaggtgcataa  
tatactagATGATTCGCTTGAAT

>HCOc159A  
TCTGGTTTATGACTTggtacgtatgaagatatgccatgttacagtttgaaaccttagcccccttgaagatctcctagtttattcaatacttCag  
ctaacgttacggcagtaagtgaacacattcatttctcgcgtgctcaatttagaacagcaacgtctagcgttttagagaaaagagatatggcat  
actgacatgatatactctggtattaaGGTTAACCCGACGG

>HCOc263A  
TCCTGCCACCCACCCggtacgtctaattcccatatcggtgtgatatcgaatgatctccgtgttcctataatacagtactaacgctcagacagtgag  
taacatcgagttcttttatttcacctgtcgacctgtcgcgtgggccagcgtttccaagttaggaggaaatgtggaatggtcaccctaaaacatgc  
ctaaaactcccgaacaccttgaaaaaatcatCactaaccttctctaCagCGTCCCAGTCCCTCA

>HCOc304A  
AACCTTTGGCCAAATCggtacgtatagatgcatttctagagtatctttcaattcttctcacacgcaaacgctaacatttcaCagtaagttgaata  
attctgctatgccccaaagcttgctacggatcccttagatgcaactcgagctagggaatatgatggttagccctcatggctagcgtgattgctga  
cacaacacgcctttaataacaatagTTTACTCTGCCAAT

## half-sister stwintron (1)

>HCOc103A  
TGTGAGGAGATGCTTggtatgtatcaaaaatgtgtgattcaggatTTtgaaatttggtttttacaagaatgtttttgaaatttggtttttacaa  
gaatgtttttgaaatttggtttttacaagaattttcgaatagctataatatagtggaagactaacggttagaatagtatgtcaaacacccttatt  
tcgttgcTaccagcctagaaagcaccgcctggtatattctagaacacgaatcttaaaattaggtactaatactatagttgtgtctaaaaaaaa  
aCagACAGACCAGAGCGGG

**Supplementary text document S2. Fastas cropped sister introns in *Hypoxylon* sp. C027-5.**

HCoc047B (1) and HCoc229A (1) are the two type-1 cropped sister introns.

Clade of related type-2 cropped sister stwintron with the extra G near 3' marked magenta: The marked T is A in all other sister (stw)introns.  
(ordered per sequence contig)

```
>HCoc024A (2)
TGC GGACCATGCTCTgtcagtaataaaaaacatgtcggttctggcatttttagacctaatttctagaaacgaaaaataaaaatgctaacacgacatg
ttttaatagGGGCCGCAATGCAGG
```

```
>HCoc047B (1)
AGTATTCGAGGTTTgtgagtaataaaaaacatgtcggttctggcatttttagacctaatttctagaaaaatcggcgtatttctattaaaaaatat
gaattaactaacgttataacagGTTGCATAGGGGCAT
```

```
>HCoc096A (2)
CGATGATGTCCTAATgtgagtaataaaaaacatgtcggttcttagcatttttagacctaatttctagaaacgaaagataaaaatgctgacatgatatg
ttttaatacactagCGCGCTATCAGACA
```

```
>HCoc100A (2)
CAGGAGAGTTTTAACgtgagtaataaaaaacatgtcggttctggcatttttagacctaatttctagaaacgaaagtataaaaatgctgacacgatatg
ttttaatacactagTCGAACGGAAACCGAG
```

```
>HCoc105A (2)
CGAGACGATGCAGTgtgagtaataaaaaacatgtcggttctggcatttttagacctgttttctagaaacgaaagataaaaatgctgacgcgacacg
ttttatatacacgcagAACTAGACCGCGAG
```

```
>HCoc121A (2)
TCGCATCAGCTACTGgtgagtaataaaaaacatgtcattctggcatatttagaccttagtttctagaaacgaaagataaaaatgctgacacgacatg
ttttatataccgtagACCAAGCCTACGCAC
```

```
>HCoc147A (2)
CACCTCGCCTTCCGTgtgagtaataaaaaacatgtcattaaggaatttttagacctaatttctagaaacgaaagataaaaatgctgacacgacata
ttttatatacacgcagACCACGGACGCGTAC
```

```
>HCoc153A (2)
ACATGCCAATTGAAGgtgagtaataaaaaacatgtcggttctggcatttttagacctaatttctagaaacgaaagataaaaatgctgacacgacatg
ttttatatacacgcagCTCTACGACGAAGCC
```

```
>HCoc164B (2)
GAGACACGTCCTATCgtgagtaataaaaaacatgtcggttctggcatttttagacctaatctctagaaacgaaagataaacaggctgacacgacatg
ttttaatacactagCACTAGGACCGTGTT
```

```
>HCoc171A (2)
CTCGGCTATATATTgtgagtaataaaaaacatgtcggttcggcatcttagacctaatctctagaaacgaaagataaaaatgctgacacgacatg
ttttatatacacgcagGGACACTCCAATCGC
```

```
>HCoc229A (1)
TGGATATTGCCTTgtgagtaataaaaaacatgtgacatttcgacatttagatttgaaatctgaaagatccttaaaagtctattataccagacctag
ctaacgttatgacagACGTCCTTTCGCTAG
```

```
>HCoc343A (2)
TGTTTTCGGCCGTTgtgagtaataaaaaacatgtcggttctggcatttttagactttaatttctagaaacgaaagataaaaatgctgacacgacatg
ttttatatacacgagATGATCGTGGTACG
```

**Supplementary text document S3.**

**Fastas sister introns and the type-2 cropped sister intron in *Hypoxylon* sp. E7406B.**

includes one sister stwintron unique to *H. pulicicidum* (i.e., absent from E7406B)  
(ordered per sequence contig)

```
>HE7c016A
ATCTGTGTGATGACCggtacgtatgaaaatattcccagatttagataatgtagacgtcagctaaccatttaagcagtaagtaaagcatttttcatt
tcgtctccatcgagcttaaagaagcaacgtccggtatgttctagaaacgaaggcctaaaatgctgacacgaaacgttttaatgcatagATAAC
CCACAAGACG

>HE7c026A
GCCAAGTCTCGATTGgtacgtataaaaacgtgtcgtcccgccatttttagccctgatttctagagatacccctatacatacctacctccatg
tatttgctaaccattaaacagtaagttagagcattctcattccgtcgccaccgagcctaaagaagcaacgtccagtagcttctcgaaacgaaaccc
ccaaaatgctgaacatggcatttttcaatacatagGCCGGTACTCCTTGG

>HE7c026B
GAGATGGCATCAGGAggtacgtgtgaaacatgccgtgccggtatttttagcccttgatttctagaaatatacccggatttgtataacgttaggcat
tatctaacatcaaaatagtaagtgaagcattttcattcgatcgctatcgagcttagagaagaatgttcgatataattctagaaacgaaggccga
aaatgctaacatgacacgttttaatacacagCCAGGGTGGTTTGAA

>HE7c035A
CCCCAAATACATATggtatgtacaacattatgccgtgttgccacttttggtccttgtgtgttagttaagtcctaaaatttctataatgatgaa
ttaagctaaccgttatgacagtaagcagagcaaccttatttttcattatcgagcttaagaagaccataaccgatataattatggaagcgtaggtacc
tagagagttaaaagcgtgacacgatatcttttaatatgctagAACACTCCCGCAA

>HE7c050A
TCGGCATCATCACTTggtatgtataagatcaagtcgttccggcatttagccctgatttccatggtatagacattagctaaccattgtggcagta
agcaaaacacttttattccgttgccatcgagcttaagaaggcatatcccagaaactgaggtaaaaatgctaaccgcgacgtgcttttagATAATT
GGGTGAAGC

>HE7c129A
AGGTGGCAGCATTTATggtacgtataaagatatgctgtcctggcatttttaaccctgattttagaagtatccctacatttagatattatagggat
tagctaaccattaaacagtaagttagagcattctcattccgcccgcacgtacttaagggacacgtccggtatgctcttgaaacgaagggttaa
aatgttgacgcgacgtgttttaatacatagTCCGGTACATTAT

>HE7c137A [unique to E7406B]
CCGTAAC TGAGATCGgtacgtataaaaatgtgttgccccggcatttttaaccctgatttctggaatatcattatgtttacgtagtgtaaagca
tcgggctaaccgttaaacagtaagttagagcattctgttcggtcaccgcccgtatctaaagaacaacgtccggtagcttctagaaataaagggttaa
aaatgctgacacgacgtgttttaatacacatagTTGGGTAGTAGGGT

>HE7c276A
CTACCCGATTATGCGgtatgtatggaaaatgtgtcgtcccgccatttcagccctttattttttattttttatttttttagaaataccccaggt
ttattatataaacgttactgacattttaaacagtaagttaaccatttcggttcggtcgccatcgagcttaaacagtaacgttttggtatattct
aaaaacgaaggctgaagatgctgacacgacatgttctaaccatcagCTATGACTTTTCCGG

>HE7c301A
CAGTCAGGGTCGCGTggtacgtatggaaaagtgccttccggcatttttaacctctgatatccagaagtaaccctagatttatatagtacgggca
ttagctaaccattaaacagtaagttagagcattctcattttgtcgcgtcaagcttaaaggagcaaggtccagagtgttttgaaaaacgaagctag
agattctgacacgatatgtcttaatacacatagCTACATTACAGCAGA
```

**Type-2 cropped sister intron *Hypoxylon* sp. E7406B & *H. pulicicidum* MF5954**

```
>HE7c057A
CTAGTTGGTTCAATGgtacgtataagaatgtgtcattccaggcatttttagcccctaaatctctagaaatatccctaaaattatataatgttaggcat
tagctaaccattaaacagATGATGTCGGCTGTG
```

***Hypoxylon pulicicidum* MF5954 [unique]**

```
>Hpuc023A
CAGCGTGATCATTACggtacgtataaaaatgtgtcgtcccgccattttcaaccaccgatttctagaaatataccctatgtttacgtagtacaggca
ttagctaaccattaaatagtaagttagagcattctcgttcggtaccaccgagcttaaaagaagcaatgtccggtatgttctagaatcaaagggttaa
aaatgctgacacggcatgttttaatacacatagGATATTCTAGCTAAG
```

**Supplementary text document S4. Fastas sister stwintrons in *Hypoxylon rubiginosum* and the *Daldinia* genus.**

[*Daldinia childiae*, *D. concentrica*, *Daldinia* sp. EC12 and *Daldinia eschscholzii*]  
(ordered per sequence contig)

***Hypoxylon rubiginosum* MUCL52887 (5)**

>Hruc29A  
TAACCTTCATGTATTTGgtacgt aataccttgttgtaacttaaagattatcacatctccccgacctccaagaatggtcagggtaaagctatctc  
gaagattcttagataaacctgagaagaattaaaaggggtaaaagaagaagttttctgcctagaagctacttgcactttctcgaatactaaa aaaaa  
tgtcgacgtgggatgtttttatatacgtacgtacgtatgaaaacatgttggtgcagcattttttattgccccctttctagaataatctcgagtttat  
atgatataatagtaatttgcctaacttctaacagtaagtattctagtttcttcgctgtcgagttgataagagcagcgtctggtagtttcgagaa  
acctgcttaaaaaatgctaacacgacatgttttctgtacacttagACACCGGCCAAGGGT

>Hruc31A  
GTGCGCTGGGGCCATggtatgtatgaaaacatgtcgtgtcggcattttttatcgtccctttctagatataatctcgagcatatataataggtaat  
ttcgctaactattctaacagtaagtattctagtttctccgctgttgagttgaagagagcaacgtctggtatgttcgaaaacctgcttaaaaaatg  
ctaactatgacatgttttctgtacactcagATACAACCTCGAATG

>Hruc55A  
TGTTTTTTTATTTCGggtatgtatgaaaacatgtcgtgtcggcattttttatcgtccctttctagatataatagtaatttgcctaacttctaa  
cagtaagtattctagtttcttcgctgttgagttgaagagagcaacgtctggtatgttcgagaaacctgcttaaaaaatgctaacacgacatgtt  
ttcgtacacttagGAACGACCATCTTCC

>Hruc56A  
TGGAACCGAGTCGTCggtatgtatgaaaacatgtcgtgtcggcattttttatagccccctttctagaataatctcgagcttatataatagtaat  
ttcgctaactattctaacagtaagtattctagtttcttcgctgtcgagtttgagagagcagcgtctggtatactcgagaaacctgctgtaa  
aaattgctaacacgacatgttttctgtaacacagGAGCAGTACTCGCAG

>Hruc59A  
AGACGACGCGGTACTggtatgtatgaaaacatgtcgtgtcggcattttttatcaccctttctagaataacctcgagcttatataatattttt  
gctaactattctaacagtaagtaattctagtttcttcgctgtcggctgtcggtatatttcgagaaaccaacgttgaaaaatgtgctaacacgac  
atcttttgcgaataaaaagGGGATTGCTGTAAGC

***Daldinia childiae* JS-1345 (18)**

>Dchc001A  
TTGAAGGAGACGCGTggtatgtatgatatacgtttctgtagtattattttccaaccatcccttttctaaaaatagttctcaaactctgccttttaggaggt  
aacagtagctaaccctcggtagtaagtgtaaaagcctatcatttccactcattcgaaaaaggacgatggagattctaaagattctaagattgcaa  
agctgacttggaatattttcatacatagATGGATATGTGGTCC

>Dchc001B [same gene]  
CTGGACCTCAATAAggtatgtacctaataaaaacattccgttttctgctgttttcgaaccttctttttagaattgtcccgagttatagttgctaa  
cgatcaatagtaagtgtagcagacctataatttcgactcgcccaaggttatgattctataatcgtagtactacaaaactaatacaaaaatgttcc  
caaatagTGGAGGGGTAGGTTC

>Dchc001C [same gene]  
GGAGCTAGCCTTCGTgtaagtacaatgaactccttttctaaaaattgttttcgaaatatagttgctaacagtcagtagtaagtgtagcagatcctat  
aattctgacctactagaatacagatgaagattctaggatcattggttatagagctgacatgtaatatatttcacatatagTCTGATGGCGCGCCT

>Dchc001D  
AACCATCTCAATATggtatgtatgaaaacatccctttttatagagtggttttcgaaattatcttgataagataacctatacagtggtgacgctca  
gpagtaagtgtagcgcctatcatttgcacctaatcgaacctttctctctctctcccccccccccccccccccccggtgaagattc  
tggaagtgtagtatcgcaaaagctgacgtaaaatgttttcatacatcagAGCTCAGTTCCAGGA

>Dchc002A [half sister stwintron - external intron]  
CCAAGGTGGATATGTggtatgtataaagacgagacatgttacccttgcccttcttcaacctacctctctgttctttcatgtatagttgctaatt  
aattagtagtaagtagaagatccctgttctcccatcatctggcaaaagaacattcaaacctctgtaaaggtaggatcgcgaaactaacactagat  
tttctcatgcattagCAACACGCCGGGAGG

>Dchc003A [see Fig.4]  
ATCATGCCTAGCAATggtatgtatgaaaacatcccggtgttattcttttgaacccccctttctagaacgggttttcggaaccacctaggtatacagtt  
agctaacgctcagtagtaagtgtagtgccctatcatttgcactaaataaaaaaagaacagtgaaagattctagaattgtagtaaatgcaaaagctg  
atcagagatgttttcatacattagGTGATCCAGCGACC

>Dchc003B  
TAGAGAAACCACTCCggtatgtatgaaaacatcccggtgttattttcaagccccctttctagaagggtctcgaaactatcctcgtaaggta  
tacagtgctaacgctcagtagtaagtagtagccctgccatttcgacccaatcgaaaaataacgatgaagattttagaattgtagtagttgca  
aagctaacgcaaaatgttttcatacattagCAATCGGCTTCAGAG

>Dchc003S [paralog 1: lacks internal PB-A]  
CGACTCATTCATCTGgtatgtatgaaaggatcctgtgtgtatttttcgagcccttttccatggcagtcctcgaaactaccttcgtaaggtata  
tagtggtatcagtagtggtatggccctatcattttcgacgaaatcgaaaaattatggtggaaattctaaaaattgtaatatgtcaaaagtgcacac  
taaatgttttcgtacatgcagGTATGGTCCACGATG

>Dchc003T [paralog 2: lacks internal PB-A]  
ACTTCCAAGAGACTGgtatgttcgattcgtacactatgtatcaggaggcgttgaagcagaggaagcggagaaagtgagagtgaagtggtacac  
cgatcagtagtatgtatacatatccagagcattggagctcaagtcaactaacctattcatcaagcagTCCGCCATATGTTTC

>Dchc004A  
GGTGCGGTCTCCGTCggtatgtatcaaaaaatactctatgcttgttattttgaaacccctttctataatagtctcgaaaccgccttcgtaaggt  
atacagtggttaacgttttagtagtaagtgtgatagccctatcattttcaacccaatcaaaaaaaaaataacgccaaagattctagaattgttagta  
tcgtaaaagtgcatacgaaatgtttgcatacattagATTGATACTTCGGAT

>Dchc004B  
AACACGCATGAATCCggtacgtgtaaaaatactctgtcgtttgctttcaacctccctattatagaatgacacctacactacctcctttgggtaca  
cagtgactgacgtttaatagtagtaagtataaccttatcattttgacttaaaaggacaggaagatcctataacataggattgtaaagcgtgata  
cagaatgatttatatatcagAAATATCTGGTGCGA

>Dchc004C  
AACCTGGCTACGCTTggtatgtattaaaatgtcccatatccttattttgttagccctattccaaaattattccaaactgcattcattaggcata  
caatgactaacgctcagtagtaagtccgacaatccctattattttgactcgtttgtaaaggagtcgtgggtatcctagaacataggattgtaga  
atctaatgtggaatgtttcatatattagATACCATTGACGTTG

>Dchc005A  
AGACACAAATCCATTggtatgtatgaaacatccctgttgttatttttggaactctctctctataatggtcttgaatatagtagctaacctctc  
agttagtaagtgtgatgaccccccctcacttagagtcactcgaaatggtggaagactctagaatttgcagggttgaaggtgacacgaaatgttt  
tcatacattagCAATGGATGGGCGAA

>Dchc005B  
GTAGCGGTAAACCGggtatgtattgacaattatattgtaatatccaaatctcctttcctatatcgtttcaaacatttatttcatctacat  
cggctaacgaatagtagtgagtaggattgtcctattatttcgacttactcaagaaataagtttgaatcttctagaattataagacctcaagcgt  
aacacaagatctttcgtttgcttagAAAGACGTGAAGGAA

>Dchc007A  
CTGGAGCTCCTCTTggtacgtacgaaaccatcccggtgttattttgaaacttctctctagagtagtctcaaaattgccttcgtagagtatac  
agtggctaacgttttagtagtaagtgtaacgaccatattcgcactcaatcgaaagataatggtgaggattcttagagttgtagggttaaaagc  
tgacacgaaatgtttcataccttagATGCGAAGCATGGGT

>Dchc007B  
ACATGGGCTTGGTACggtacgtattaaagcatctcatgttgttattttcgactctcctttttagaatgatctcgaatacaatggttaattg  
tagtagtaagtgcgatggtcctatcacctggattctctcgaaacgcgatgatcctagaattgtagggttacgaggttaacacgatttcataaattag  
GCGTACCGTGGGTAT

>Dchc011A  
CGGGATTATTTTATggtatgtatgaaaacgccccacgttgttacttttcgagccattctttatagaatggcctcgaaactacttccctaaggta  
tacagtggttaacgctcagtagtaagtgtgatgacgacctatcatttcgaccaatcgaaaaaaacacacgggtgaagattctggaatc  
atagtattgcaaaagttaacatgaaatgcttcacgcatttagACGGTGCTGCAGCCA

>Dchc013A  
GGTGACAGTTTTTACggtataaaattaattttgaaccccttttagaatgtatgcaatcgctaacgttcagtagtaagtcattgatgatcttatca  
tttcgactccctcgaaaagaaaacggtaaaagattctggaattacaagaatgtaaaagtgcacaaaagatatattcgtatatatagATGTCGTGCT  
ACCAA

***Daldinia concentrica* CBS 113277 (9)**

>Dcoc02A  
CTGGAGCTCCCTTTggtacgtatgaaaacatcccggtgttattttcgaaatccctttctagaatgggtctcgaaaccaccttcttaaggtat  
acagtggttaacgctcacttagtaagtgtgatggtcctatcatttcgactcaatcgaaaaataacgggtcaagattctagaattgtagtagttgcaa  
agctaacacgaaatgtttcatacattagATGCGAAGCATGGGT

>Dcoc02B  
TATTAATTTAATCACggtatgtataacccccctacctcctagaatgggtttccaagtatacagtaactaacactcagtagtaagttgacgacgac  
ctatcatttcgactcactcgaaaaagaacagtaaaaaattccggaattgtaagggtataaaagttaacacaggatgttttcatcagTTATTATGCA  
CCAGT

>Dcoc03A  
GACACTAGAAATATggtatgtatgaaaacatcccggtgttattattcttagaaatatctttcgagaatagtctcggaatcatcctcgtagggtat  
acagtagctaacgctcagtagtaagtttgattatcatttcgactcaatcgaaaaatattggcgaagattctacaattatagcattgcaaaactg  
acacaactgacacaatgttttcataacttagGTATGGTCCCCGTAC



>Dcoc05A  
GTAGCAGTAACCCGggtatgtataaataattatgtattgtgaacctccaaaactccctttatcggatagtttcttagattttcttttatgtacaa  
tggtctaattgcatagcagtgagtgagatgatcctattatttcgacttaagaaataattttgaaaacttcttagaattggttaggatttctaagctaac  
caagattttctcggttgtagAAAGACGTGAAGGAA

>Dcoc06A  
TTTGATGGGACTATTggtatgtataaaaaagcatttcgtagattgttttcgaaatgtttgacttgacagtagctaatagttaaagtaagtggg  
ataaactctgtcatttcgactctctcaagagaatgtcgaagtatatatacgtacaacggtaggggtgcaagctaatacaagatgcttcgatat  
actagATGGCATCCTCACAC

>Dcoc11A  
CCGGAATTAACCTCAGgtatgtataaaaatgacccggttttattacttcccaacccctattccttcgtaagggtatacagttgctaacatttagta  
gtaagtgccatcacttaataaaaaaagaacgatgaagattttagaattataaaactgacacagaatgttttcacgcattagTTTCTACTTCG  
GAA

>Dcoc20A  
GGCGCACAAATTCTACggtatacaattaacccattctacttttaacccccccttttaggaggtttacaaccgctaagcccaatagtaagtggatg  
gacccatcatttcgactcgctcgaaaaagaaaagaacattaaggttctagaattacaggattataagctgacgcaagatgtattcgtataat  
tagATGTCGTGCTACCAG

>Dcoc38A  
TCAAAGGAGACCCATggtacgtctgatatgcgtcccatgtttgttattttcaatcatcccttcttaagtgtatctcgaatccgctttataaggt  
atatagtggttaacgctcagtagtaagtgtagaaagtcctatcatttcggtcattcgaaaaaggattgtgaatattttaagacgctaggattgt  
gaagctgacacagaatgttttcatacataagATGGCTATGTGGTCC

>Dcoc41A  
AGCCAGGTACGCTTggtacgtatgaaaacgctcccatgctgtttattttcaaatccaaattctagaacagctctcgaactgcattcattaggtttac  
agtagctaacgctcagtagtaagtgcgatgacctatgatttcgactcgctgaaaaggaccttgggtattctacaactagaggggttttaagt  
ctaatacggacgctttcatacatcagATACCATTGATATTG

*Daldinia* sp. EC12 (7: two are identical)

>DECc253A  
TCCCATCATGCTGGGggtacgtattaaaacatccttctttgttattttccatatcgccattatatactgctttagaaactgcctttataagatgt  
gcaatagctaactttcagcagtgagtcacgatgaagtcgtatatgacactcgataaggaaccgtgaaggtttcagagtagtaaaattgcaag  
ctgacacgagatgtttttcttatattagATGGAAAGAACCTCG

>DECc262A  
GGACACAAACCCCTTggtacgtatggaaacgctctcatattgtttatatttaaatcatcattcctaaataatcaagcataaataagctaactttcag  
tagtaagtgcggtggtatctattaaaccacttttaaaaaggatggataaggttctagcgatgtaggattgcaaaactaatacgagatgtttacat  
atactagCAACGGCTGGGCAA

>DECc304A [identical gene: twice in the contigs]  
CTGGAGTCTCTTTggtacgtacaaaattgtcctgtattgttattttcagaattgtattctagaatggtattaaatagctaacgttttagtagt  
aagtttactagccctatcaactgatccactcgacaaggaacggtaaagattttggagttataggattgtaagactaacatagattcataat  
tagACGCGAAGTATGGGT

>DECc498A [identical gene: twice in the contigs]  
CTGGAGTCTCTTTggtacgtacaaaattgtcctgtattgttattttcagaattgtattctagaatggtattaaatagctaacgttttagtagt  
aagtttactagccctatcaactgatccactcgacaaggaacggtaaagattttggagttataggattgtaagactaacatagattcataat  
tagACGCGAAGTATGGGT

>DECc320A  
GCGGCAAATTCACCTggtatgtatacaaatgtcattctagaataactctttaaactgtccttgcaagatatacagtagctaacatttagtagta  
agtgtgataaccttatatacgaactgcccattgaagaacgatgaaaatttcagaatagtagattgtgaaagctgacacgatatgttttaacacat  
tagACTGGGAAAAAGCTG

>DECc413A  
GTTAGCACACGATATggtacgtagaagaagaaaaaaaccatccagcgtgctattttctatgcccttttccataatcatctcgaaattgtcctttg  
gtatacagtggttaacgtttcagtagtaagtttcgacttaatcgacaaagaaccgtggagattctagattttagcaacacgaaactgacgatga  
aatttttacttatcagAACCAACCAAGTATT

>DECc501A  
ATTGAGGAGGCTTTTggtatgtataaacacgtcccgcatgtttatcttcgtaacctaaatttcagaatggtttctaagctgcttttatataacta  
agcttttacgattgctgaccttcagtagtaagtgcatcgctcgtctatttgacggactcaaaaaggagaaatgatagagctacaagagtgcac  
cagctgacttgggatgttttcatacttagACGAGAAAGGTTATT

***Daldinia eschscholzii* IFB-TL01 (6)**

>Desc187A [= same location as DECC501A]

ATCGAGGAGGCTTTTggtacgtataaacacgtccgcattggtgtttttcgtaacctaattctagaatggttctaaagctgcttttatataact  
aagctttacgattgctgaccttcagtagtaagtgtcatcgctcgtcctattgacggactcaaaaagggaatacगतगctacaagagtgc  
acagctgactttggagtgttttcatataccagAGCGAGAAGGTTATT

>Desc198A [= same location as DECC253A]

TTCCATCATGCTGGGggtacgtatgaaaacatcctgctttgtttattttcttatcgccattctatactgctttacaaactgcctttataagatgt  
gcaatagctaacgttttaatagttaagttacgatgacgtcgtatatattgactctcgataaggaaacggtgaaggtattagagtagtaagattgcaaa  
ctgacacgagatgtttctcttatactagATGGAAAGAACCTCG

>Desc274 [= same location as DECC304A & DECC498A]

CTGGAGCTCCTCTTTggtacgtacaaaattgtcctgtgtgtttattttcagaatttcattctagaatggtctcaaatagctaacgttttagtagt  
aagtttaccaaccctattcactaattttatttcgagaacggtaagactctattgtaagactaacatagattcgtatattagACGCGAAGTATG  
GGT

>Desc420A [= same location as DECC262A]

GGACACAAACCCCTTggtacgtatggaacacgtctcgtattattatattttaaatacatcattcctaataatacaagcattaatagctaacctttcag  
tagtaagttacagtगतatctattaaaccacttaaaaagggatgggtaatgttctagcgatgtaggattgcaaaagctaataggggggtgtctcata  
tactagCAACGGCTGGGCGAA

>Desc618A [= same location as DECC320A]

GCGGCGAATTACCTTggtatgtataaaaatgtcattctagaatacgtttgaaaatgcccttacaagatatacgatagctaacgttttagtagta  
agtgcgatgatcttगतatcgactgccgataaggaaacggtaagattttggaatagcatgattgcaaaagctgacacgatatgttttaacacct  
tagACCGGGAAGCTG

>Desc640A [= same location as DECC413A]

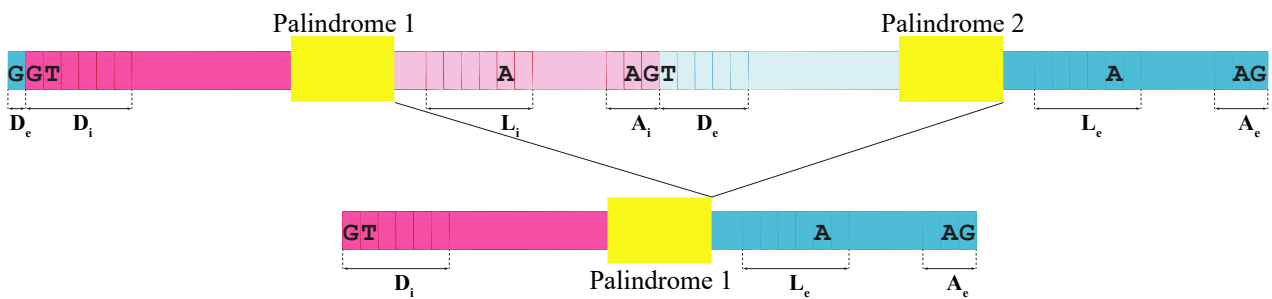
GTTAGCACACGATATggtatgtataaaaatgtccttggtattgttatttccatgcccccttttcataatcatctcgaaaattgtctttatgatgta  
tacagtggttaattgttcagtagtaagttttgacttagtcgacaaagaacggtagattctagattttgtaggaacacgaagctgacggtgaaat  
gtttacttatcagAACCAACCAAGTATT

HCOc004A [Int] GTGAGTATCAAAACACGTGCGTCTGGCATTTTGTCTCTTAA-TTTCTAGAAAAAACCTTAAATTTCTACAACATAGGAATAAACTAACATTATGATAG  
 HCOc017A [Int] GTGAGTATAAAAACATGTCGTTCTGGCATTTTGTATTGA-TTTCTAGAAAAAACCCCGCTTTCTACAATATATGAATCAGCTAACGTTATAACAG  
 HCOc076A [Int] GTGAGTATAAAAACATGTCGTTCTAGCATTTTGTACCTTGA-TTTCTAGAGAAACCCCTATATCTTTACAATATATGAATTAGCTAACGTTATAATAG  
 HCOc102A [Int] GTGAGTATAAAAACATGTCATTCTGGCATTTTGTGCGCTTG-TTTCTAGAAAAACCCCTCATATTTCTACAATATATGAATTAGCTAACGTTATGATAG  
 HCOc178A [Int] GTGAGTATAAAAACATGTCATTCTGGCATTTTAAACATTGA-TTTCTAGAAAAACCCCATATTTCTACCGTATATGAATTAGCTGACGTTGTGATAG  
 HCOc061A [Int] GTGAGCACAAAACATGTCGTTCTGTACATTTTATACCTTAA-TTTCTACAAACCCCTTATATTTTACAAACATATAAATTAGCTAACGTTATGATAG  
 HCOc070A [Int] GTAGTATAAAAATATGTCGTTCTCAGCATTTTGTACCTTGA-TTTCTAGAAAAACCCCT--TATTTCTACGATATATGAATTAGCTAACATTATGATAG  
 HCOc378A [Int] GTGAGTACAAAACCATGTCATTCTGGCATTTTGTACCTTGA-TCTCTAAGAAAACCACTATATTTCTATAATATATTAATTGGCTAACGTTATGACAG  
 HCOc066A [Int] GTGAGTATAAAAACATGTCGTTCTGGCATTTTAAACCTAAATTTTCTAGAAAATCTCCCATGTTTGTACAATATATGAATTAGCTAACATTATAACAG  
 HCOc224-c179 [Int] GTGAGTATAAAAACATGTCGTTCTGGCATTTTGTACCTTGA-TTTCTAGAAGACCCCCCATATTTCTACAAGATATGAATTAGCTAACGTTATGCTAG  
 HCOc047B (1) GTGAGTATAAAAACATGTCGTTCTGGCATTTTGTGACCTCAA-TTTCTAGAAAATCGCGGTATTTCTATTAAATATGAATTAACCTAACGTTATAACAG  
 HCOc052A [Int] GTGAGTATAAAAACATATCGTTCTGGCATTTTGTACCTTTGATTTCTAGAAGACTCCCATATTTCCAGTGATATGAATTAGCTAACGTTATGACAG  
 HCOc058A [Int] GTATGTATGAAGACATACCGTCTTGGCGTATTAGACCTTGA-TTTCTAGAAGACCCCTGAGTTTTCACAATATAGGGATTAGCTAACGTTAGGACAG  
 HCOc236A [Int] GTGAGTATACGAATATGTCGTTTGTGCACTTTAGACCTTGA-TTTCTAGAAGATCCTCAAATGTCTTCAATGTAGGAATTAGCTAACGTCGTGACAG  
 HCOc271A [Int] GTGAGTATAAAACTATGTCGTTTGTGCGTTTTAGACATTGTTGTTTCTGGAAAGACATCAAATCTC--AATATAAAAATCAGCTAACGTTATGACAG  
 HCOc229B (1) GTATGTATGAAAACGTGACATTTCGACATTT-AGA--TTTG-AACTGTGAAAAGATCCTTAAAGTTCTATTATACCAGACCTAGCTAACGTTATGACAG  
 HCOc164A [Int] GTATGTATAGATATATGTTGTTGGTGGGATTCCGGCCCTTTC-TTCTAGAAAAGCCTTCATTCTCTATGATGCAGGAACCAGCTAATTTTATGACAG  
 consensus GtgaGtAtaaaaacatgtcgtTtctggcatttttagaccTtra-tttCTagaaarnycccyawattTctayaatatadgaattagCTaAcgTtatgaYAG

**Supplementary figure S1. Alignment of type-1 cropped sister introns with the internal introns of sister stwintrons.** The type-1 cropped sister introns in *Hypoxylon* sp. CO27-5, HCOc047B (1) and HCOc229B (1), are shown in red. The sequences were aligned by MAFFT using the G-INS-i iterative refinement module with the PAM20 scoring matrix. Aligned positions that are only occupied in one or two of the 17 aligned U2 introns were removed and the manually trimmed alignment (98 informative nt) was shaded. 5'-Donor-, (predicted) BP- and 3' acceptor sequences are highlighted in magenta. The centrally located 10-nt palindrome is highlighted in yellow in the consensus line.

[illegible]

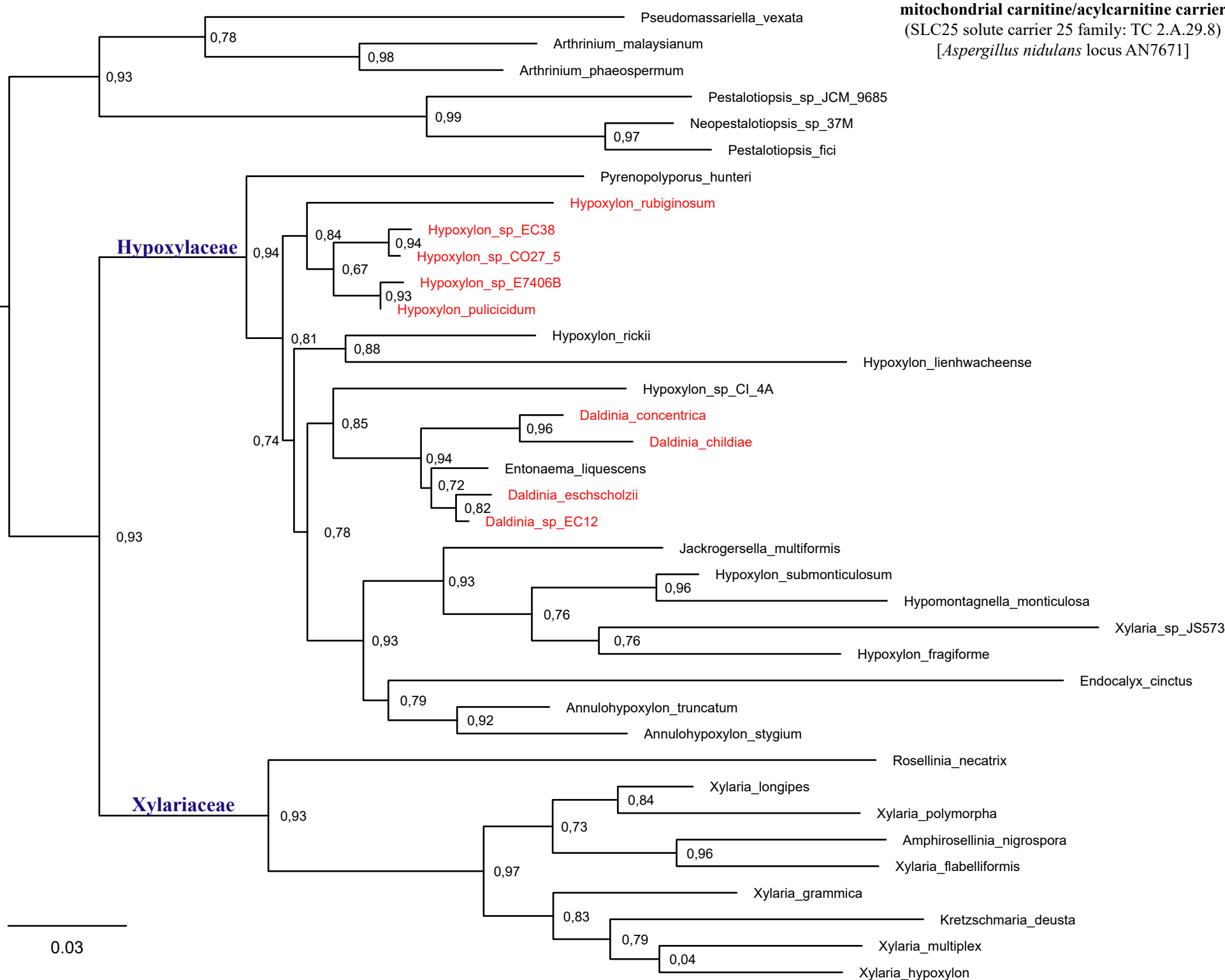
**Supplementary Figure S2. Alignment of sister stwintron HC0c066A (CO27-5) and the corresponding type-2 cropped sister intron HECc034A (EC38).** The alignment illustrates the deletion associated with the derivation of a type-2 cropped sister intron in strain EC38 from a sister stwintron ancestor (similar to the one in CO27-5) at the same intron position in a gene encoding a putative integral membrane protein of 413 amino acids. The last uninterrupted codon of the upstream exon (Val) and the first uninterrupted codon of the downstream exon (Cys) are underlined. 5'-Donor-, (predicted) BP- and 3' acceptor sequences are highlighted in magenta or turquoise, respectively. The 10-nt palindrome is highlighted in yellow; The 5' copy in the stwintron and the single copy in the type-2 cropped sister intron are both perfect (i.e., 5'-TTCTAGAAA). Note the change in intron phase from phase one (stwintron) to phase two (canonical intron).



**Supplementary Figure S3. Generation of a type-2 cropped sister intron from a [D1,2] sister stwintron during double-stranded DNA break (DSB) repair by microhomology-mediated end-joining (MMEJ).** A double-stranded break occurs between the two copies of the 10-nt palindrome (yellow box) in the parent sister stwintron, where the crucial microhomology is (part of) that palindrome. Since the retained palindrome is perfect (5' W=T) we presume the imperfect 3' copy from the parent stwintron to be deleted.

[illegible]

**Supplementary Figure S4. Alignment of three sister stwintrons in a multicopper oxidase gene in *Hypoxylon* sp. CO27-5 and EC38.** Strain EC38 harbours a sister stwintron absent in CO27-5. The additional sister stwintron (HECc217A: phase two) is located 71 nt upstream of the sister stwintron (HECc217B: phase one), which corresponds to CO27-5 sister stwintron HCOc004A (~ 84.4 % identity). Identities between the top and the bottom sequences but not occurring in the EC38 3' stwintron, conserved in both fungi (middle sequence), are indicated with the colon signs. 5'-Donor-, (predicted) BP- and 3' acceptor sequences are highlighted in magenta or turquoise, and the 10-nt palindromes in yellow. The paralogue sister stwintrons in the EC38 gene are ~ 85.0 % identical.



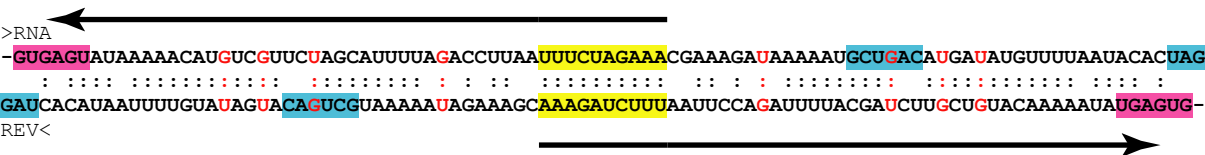
**Supplementary Figure S5. Presence and absence of CO27-5-like sister stwintrons of high sequence similarity among Hypoxylaceae species.** The names of species with recognisable sister stwintrons are printed in red lettering in taxon annotation of a maximum likelihood tree inferred for the well-conserved mitochondrial carrier protein (329 amino acids in *Hypoxylon* sp. CO27-5; cDNA: GenBank MW498246), encoded by the gene that carries sister stwintron HCOc017A. NCBI's WGS database was TBLASTN screened for the orthologue genes in another 36 Xylariales. Intron-exon structures were deduced guided by comparison of coding information. The orthologue proteins were aligned with MAFFT using G-INS-i iterative refinement and the BLOSUM 62 scoring matrix. This alignment was trimmed to 316 informative residues with Block Mapping and Gathering using Entropy (BMGE), using the BLOSUM 62 similarity matrix and block size 4. A maximum likelihood tree was then inferred with SMS-PhyML employing the LG + G + I + F substitution model. Branch support was estimated by Shimodaira-Hasegawa-like approximate likelihood-ratio tests (SH-like aLRT) (Anisimova and Gascuel 2006). The tree was rooted on the branch with the six species not assigned to the Hypoxylaceae or the Xylariaceae. The scale bar corresponds to an evolutionary distance of 0.03 substitutions per residue. The tree should not be considered authoritative molecular taxonomy for Hypoxylaceae.

**Reference (aLRT node statistics):**

Anisimova, M.; Gascuel, O. Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Syst. Biol.* **2006**, 55, 539–552.

*Hypoxylon* sp. C027-5 cropped sister intron HC0c096A

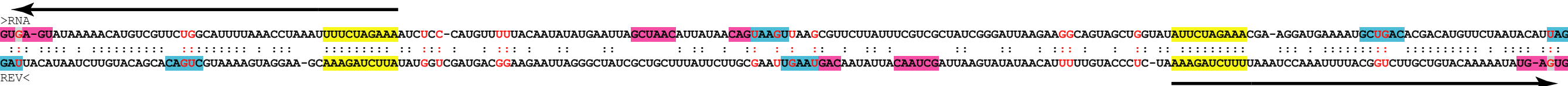
base pairing without GU = ~ 70.5 %; with GU = ~ 79.5 %



*Hypoxylon* sp. C027-5 sister stwintron HCOc066A

base pairing in the TIRs (only):

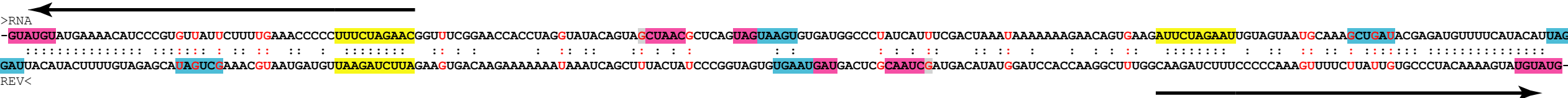
without GU ~ 72.2 %; with GU = ~ 77.8 %



*Daldinia childiae* sister stwintron Dchc003A

base pairing in the TIRs (only):

without GU = ~ 68.7 %; with GU = ~ 79.2 %



**Supplementary Figure S6. Base pairing in double-stranded RNA of two linear molecules of the same single-stranded sister (stw)intron RNA.** The three example sister (stw)introns are the same as those in Figure 4a. Watson-Crick base pairing is indicated with the colon sign. Non-canonical GU base pairing is highlighted in red. The terminal inverted repeats (TIRs) are indicated by horizontal arrows. Percentages of base paired nucleotides are given with and without GU pairing (TIRs only).



ggtatgtatgaaaacatcccgtgttattcttttgaaaccccc**tttctagaac**gggtttcggaaaccacct-----aggtatacagtag**gctaac**gctcag**tagtaagt**gtgatggccctatcatttcgactaaataaaaaaagaacagtgaag**attctagaat**tgtagtaatgcaaa**gctgata**cggagatgttttcatacat-**tag** 197 nt  
|||||  
Ggtatgtatgaaaggatcctgtgttggtatttttcgagccccct**tttcca**tggcagtcctcgaaactaccttcgtaagggtatatagtggtta---tcagtag---tgtgatggccctatcatttcgacgaaatcgaaaaattatggtggaa**attctaaaat**tgtaatatgcaaa**gctgac**actaaatgttttcgtacatg**cag** 195 nt

**Supplementary Figure S7. Alignment of the *Daldinia childiae* sister stwintron Dchc003A and the long sister intron Dchc003S.** The alignment shows the divergence of a long canonical sister intron from a sister stwintron of very similar length. Both intervening sequences are in sequence contig VYXO01000003 (see Supplementary Table S1, sheet Daldinia). Sister stwintron Dchc003A is at the seventh intron position in the gene for a GTP-binding protein of the dynamin family. The canonical long sister intron Dchc003S is at the first intron position in the gene for an arylsulfatase. Donor-, BP- and 3' acceptor sequences are colour-coded. The internal splice sites of the stwintron (top) are absent from the canonical intron (bottom), seemingly the result of two small deletions. Note that the 5' G in the lower sequence (capital letter) is exonic.

**Supplementary Table S2. Oligonucleotide primers used in this study.**

Oligonucleotide primers for cDNA and/or gDNA sequencing.

Gene Hypoxylon sp. CO27-5	Name of oligo	Sequence (5'-)
c047B (I)	c047B_seq_F	ACCGGCGCTAATTCCTAAT
c047B (I)	c047B_seq_R	GCATCGTATCCCACGCTATT
c153A (II)	c153A_seq_F	GCTAGCAACAGAAAAGCAAC
c153A (II)	c153A_seq_R	AGTTGCATGTGTGGTTATGA
c229A (I)	c229A_seq_F	TGACCGAACAACAGCAACAG
c229A (I)	c229A_seq_R	GTCTATCGGCATACCTCGCT
c105A (II)	c105A_seq_F	TTACTAGCCGGCGGATAAGG
c105A (II)	c105A_seq_R	ATTACATTGCCCCGGATCCA
c164B (II)	c164B_seq_F	TATGCCCAGAGAGCCATTGT
c164B (II)	c164B_seq_R	TCACCCGAATTGTCCTCTTGA
c343A (II)	c343A_seq_F	AGAGGGGCAAACGTCATCTA
c343A (II)	c343A_seq_R	CGCTGAGTACATGCCTACCT
c024A (II)	c024A_seq_F	TCGAGAGTCGCGCCGGAATC
c024A (II)	c024A_seq_R	TAAGTCTGCCAGGAGATCGC
c147A (II)	c147A_seq_F	ATCCTCAAGCCGCCTCATAA
c147A (II)	c147A_seq_R	GCATCACAACATAATTGCACGA
c121A (II)	c121A_seq_F	AAGGAGAAGGAACCAGAGCC
c121A (II)	c121A_seq_R	CGCAACGCCTCTCATCTAAC
c004A	c004A_seq_F	CATCGTTCCTATGTAATTAT
c004A	c004A_seq_R	AGAGCTGTAATAGCTATCCT
c017A	c017A_seq_F	GTACCCCAATGTAGTATACG
c017A	c017A_seq_R	CAGATCGCATAACTCGATGA
c017B	c017B_seq_F	GAACAGTGTCTTCGTCAATCT
c017B	c017B_seq_R	GTTACCGCAAGATTGTTAGAC
c021A	c021A_seq_F	ATGATACAGAATCTCTAGTCG
c021A	c021A_seq_R	ACATGTTGCTTCCACTGTTTC
c052A	c052A_seq_F	CATTCCTACCCTTGGAGAAG
c052A	c052A_seq_R	AGGCCATCATACTCATCCATC
c061A	c061A_seq_F	GACGTCGAGGTTTACCTAAC
c061A	c061A_seq_R	ATTTACGGTATATACCATTC
c066A	c066A_seq_F	CTTGCTAGAGACACTCTCTA
c066A	c066A_seq_R	TCCCTAGGTTCCAAGGAGAC
c070A	c070A_seq_F	GATGAGGTGGTGCAGTTGCG
c070A	c070A_seq_R	TCAGAACTCATCGACTACAG
c076A	c076A_seq_F	CGACGTCAGCATCCCTACTA
c076A	c076A_seq_R	AGGTTTCCCACGTCAAACAG
c102A	c102A_seq_F	CCTAGGTATTTACACACCATG
c102A	c102A_seq_R	TCCCTCTCATGTAGCTCAAG
c164A	c164A_seq_F	CAATTCTTTACGATATAGATA
c164A	c164A_seq_R	CATTATTTAGACATTGCAGCAG
c178A	c178A_seq_F	ACAGTCAACTTATAAAAAGCTC
c178A	c178A_seq_R	CTAACTGTAACCTATCGG
c236A	c236A_seq_F	GCTCTCCACAGTCGAGTCTCA
c236A	c236A_seq_R	CTAGCGACGTAATATAACCGA
duf636	duf636_seq_F	GACTTACTACTCGGAACCTCC

duf636	duf636_seq_R	GTACTTGGATAAAACCTTT
c252A	c252A_seq_F	CAGGTCAAGAGGCTTCATCAC
c252A	c252A_seq_R	TGCTCCAGCGTATAATAGATG
c271A	c271A_seq_F	GCTCTGCGTATGTTGTGGAA
c271A	c271A_seq_R	AGAGAATGGGGAGAAGCACA
c332A	c332A_seq_F	GCATATTTTGCGCCACTTCT
c332A	c332A_seq_R	TTCTAACACAGCGGGAGTCA
c378A	c378A_seq_F	AGGTTTGCTGTAGATGAGAG
c378A	c378A_seq_R	AAGTACCACACAGAGATATT
c406A	c406A_seq_F	GAGATTGCACCGGCTCTCAC
c406A	c406A_seq_R	CTTGCCGCAAAACTACCAAC
c522A	c522A_seq_F	GAAGACGTTCCCTAGTGCTA
c522A	c522A_seq_R	GACATATCTCTCAGGACGGA
c004B	c004B_seq_F	AAGGACTGTCGCCCATGAT
c004B	c004B_seq_R	CAAGAACTCCTAGGCCATCG
c016A	c016A_seq_F	CATCGGATAAAGCTCGAGGCCT
c016A	c016A_seq_R	CACGGGCAGCACCCAATTTAAC
c016B	c016B_seq_F	ACTTGCTATGTCCAAGCTTGT
c016B	c016B_seq_R	TACATTCTAGATTACGTATC
c020A	c020A_seq_F	TCCAACCTCCACCTCGTCTTC
c020A	c020A_seq_R	AATGTTGGTAGCGATGAGGC
c024B	c024B_seq_F	TCATTGATCTTCGCTCCGTA
c024B	c024B_seq_R	TAGATGTATCTCTAGATCTA
c046A	c046A_seq_F	CAGAAGCATTCCAAAAAGGAAC
c046A	c046A_seq_R	CTGGACAAAACACCATTCGTC
c091A	c091A_seq_F	GTAAACATCTTGTGTCAAAAC
c091A	c091A_seq_R	TAGGTACGTTAATTATATCAT
c103A	c103A_seq_F	GCATGACTGAAAGCCTCACA
c103A	c103A_seq_R	CGCAGGCAGAAAATGGACTA
c159A	c159A_seq_F	GTTCGTCTTTCGATAGTTCGT
c159A	c159A_seq_R	GTGCAAGGTGAACACTTGAAC
c263A	c263A_seq_F	CAACGGTTCTAGCCTGAGGA
c263A	c263A_seq_R	ATTCGCCCTTTTGAGATGAA
c304A	c304A_seq_F	GAGAACTTAAATAGTTCGATAT
c304A	c304A_seq_R	CGACGTATCTCTAGAATATTTC
helQ	helQ_seq_F	GCATCTTGCCAGCGTCTATC
helQ	helQ_seq_R	CACAAGGCAAGGTACAGAAG

Oligonucleotide primers for RT-PCR verification of stwintron splicing intermediates.

Gene Hypoxylon sp. CO27-5	Name of oligo	Sequence (5'-)
c004A	c004A_splinter_F	ATGGCCAAAACGAACAATCC
c004A	c004A_splinter_R	GTGTATTAAGACATGTCGTG
c017A	c017A_splinter_F	GACTGCAGATTTCAGTACTCG
c017A	c017A_splinter_R	AGTATTAAAACATGTCGCGTC
c017B	c017B_splinter_F	GTACTTGGGGATATCAGGATC
c017B	c017B_splinter_R	TAAAACACGTCGTGTCAGTAC
c052A	c052A_splinter_F	CATTTCCTACCCTTGGAGAAG
c052A	c052A_splinter_R	TAAAACATGTCGTATTAGCAT

c061A	c061A_splinter_F	GACGTCGAGGTTTACCTAAC
c061A	c061A_splinter_R	GTATTAAAACATATCGTGTTA
c043A	c043A_splinter_F	GAAATATTGCGACAGACATC
c043A	c043A_splinter_R	GTATTAGAACATGTGCGTGTC
c070A	c070A_splinter_F	CAATACCATTATGTGGCGATG
c070A	c070A_splinter_R	GTGTTAGCATTTTCATCCTTC
c076A	c076A_splinter_F	GCATGGTCGGATAATGAAAAA
c076A	c076A_splinter_R	CCTAATCCCGATAACGACGA
c102A	c102A_splinter_F	ATGGTATTTACTGTAATATAT
c102A	c102A_splinter_R	GTGTATTAAAACATGTGCGTGTC
c164A	c164A_splinter_F	GTTCACTACATCGAAGACCAG
c164A	c164A_splinter_R	GTAGTGTCAATATCTATAGTC
c178A	c178A_splinter_F	AAAAACATGTCATTCTGGCATT
c178A	c178A_splinter_R	TCGTCCCAACCTAAACATCC
duf636	duf636_splinter_F	CCTAGGTAGCTATATGCTGT
duf636	duf636_splinter_R	GCATGTTGGACAGACTGATG
c236A	c236A_splinter_F	GCTCTCCACAGTCGAGTCTCA
c236A	c236A_splinter_R	TAACTTGTTAGCATTTTAAACAC
c252A	c252A_splinter_F	CAGGTCAAGAGGCTTCATCAC
c252A	c252A_splinter_R	AGGATATTACAACATATCGTG
c271A	c271A_splinter_F	GCACTCGTACGGTCTTCTCC
c271A	c271A_splinter_R	GCGTCGTGTCAGCATTTTTA
c332A	c332A_splinter_F	GCATATTTTGCGCCACTTCT
c332A	c332A_splinter_R	CATTCTTGGCCTTTGCTTCT
c378A	c378A_splinter_F	AGGTTTGCTGTAGATGAGAG
c378A	c378A_splinter_R	ATTAAAACATGTCGTGTCAG
c406A	c406A_splinter_F	ATCCTCAATGCGACGAGCGG
c406A	c406A_splinter_R	GCATGTCGTGTCAGCATTGT
c522A	c522A_splinter_F	ATGAAGTGGTATACTCTACTT
c522A	c522A_splinter_R	TCCTGTCAGCAATTTTGACTTC
c004B	c004B_splinter_F	AAGGACTGTCGCCCCATGAT
c004B	c004B_splinter_R	TGTATGAAACGCGTCATATCAGT
c016A	c016A_splinter_F	GAGACCTGCCGGAACGTTGT
c016A	c016A_splinter_R	GTTTACCTTGGACCGTTTGAG
c016B	c016B_splinter_F	GTCATTGATCTGACCTCCAT
c016B	c016B_splinter_R	TGTTAGCTCTCTCCAGCTGA
c020A	c020A_splinter_F	GATCCCATGACACGAACAGG
c020A	c020A_splinter_R	CCGTGTCAGTACTTCTAACTCC
c024B	c024B_splinter_F	GTCGCGATTAAGCTGCAGAAG
c024B	c024B_splinter_R	TCATTGAGCCCTTAATCCGTA
c046A	c046A_splinter_F	CAGAAGCATTCCAAAAAGGAAC
c046A	c046A_splinter_R	GGAAAACATCTTGTGTCAGC
c091A	c091A_splinter_F2	CGCCTCAGCGGAGCAGATCTA
c091A	c091A_splinter_R2	GTTCGTCAGTAATCTTATAAC
c091A	c091A_splinter_F1	ACGGCGAGCATTCCATCGTTC
c091A	c091A_splinter_R1	AGGTATGTATTAGTGTCGGTG
c103A	c103A_splinter_F	ATCCTGCGGATGCATCTAAC
c103A	c103A_splinter_R	TATACCAGGCGGTGCTTTCT

c159A	c159A_splinter_F	CGATACTAGGCTTCATAAATAC
c159A	c159A_splinter_R	ATACCAGAGTATATCATGTCA
c263A	c263A_splinter_F	TCGCTTTCCCGAGACTAAGA
c263A	c263A_splinter_R	TAGGGGTGACCATTCCACAT
c304A	c304A_splinter_F	GAGATACATTGTCCCACCAAC
c304A	c304A_splinter_R	CAGCAATCAGCCTAGCCATG
helQ	helQ_splinter_F	GCACATCTATAGCACATTCT
helQ	helQ_splinter_R	CTAAATGTTAGCTTACCTGA

**Supplementary Table S3.** List of RNA SRAs that illustrate alternative excision of sister stwintrons in one U2 reaction with the retention the 5'-G<sub>1</sub> of the external intron

CO27-5 [D1,2] sister stwintron	SRA Alternative splicing event
HCOc271A	SRR1801290.13818186
HCOc004A (alternative 3' splice)	SRR1801288.17896446
HCOc017A (alternative 3' splice)	SRR1801291.21659993
HCOc236A (alternative 3' splice)	SRR1801290.14509435
HCOc378A (alternative 3' splice)	SRR1801290.31839927
HCOc004B (alternative 3' splice)	SRR1801290.29352535
EC38 [D1,2] sister stwintron	SRA Alternative splicing event
HECc145A	SRR1798129.1669524
HECc326A	SRR1798132.10935711
HECc010A (alternative 3' splice)	SRR1798131.9126740
HECc011A (alternative 3' splice)	SRR1798129.17227791
HECc140A (alternative 3' splice)	SRR1798130.18795761
HECc217B (alternative 3' splice)	SRR1798131.15498780
HECc296A (alternative 3' splice)	SRR1798128.3418214

In all cases but HCOc004B, HECc296A and HECc140A the alternative splicing reaction leads to premature translation termination codons and a C-terminally truncated peptide product, ordinarily provoking nonsense-mediated mRNA decay of the product mRNA.