

Supplementary Materials: Analysis of the Mitochondrial Genome in *Hypomyces aurantius* Reveals a Novel Twintron Complex in Fungi

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1  CLUSTAL O(1.2.1) multiple sequence alignment
2
3
4  Hypomyces_aurantius_cox3      ATGACAACCTTAACTAGAAAGTAATTTCAAGATCATCCTTTCCATTTAGTGTACACCTTCT
5  Hypocrea_jecorina_cox3      ATGACAACCTTAACTAGAAAGTAATTTCAAGATCATCCTTTCCATTTAGTGTACACCTTCA
6  *****
7
8  Hypomyces_aurantius_cox3      CCTTGACCTTTATACACAAGTATATCTTTATTTACTTTAACAGTAAATGGAGCATTATCT
9  Hypocrea_jecorina_cox3      CCGTGACCTTTATACACAAGTATATCTTTATTTAATTTAACTGTAATGGAGCATTATCT
10 *****
11
12  Hypomyces_aurantius_cox3      ATGCATCTTTCAATAATAGCTATATATTTCTTTTATAGCTCTAGGTACAGTAGTAGCA
13  Hypocrea_jecorina_cox3      ATGCATCTTTCAATAATAGCTATATATTTCTTTTATAGCTTTAGCTACAGTAATTTCA
14  ***** * * * * *
15  Cox3-i1 insertion site
16  Hypomyces_aurantius_cox3      TCAATGTCTTTATGATTCAGAGATATTATCTCTGAAGGT↓ACATATTTAGGTAACCATACA
17  Hypocrea_jecorina_cox3      TCAATGGCTTTATGATTCAGAGACATAGTATCAGAAGGT ACATTTTTAGGTAATCACACT
18  ***** * * * * *
19
20  Hypomyces_aurantius_cox3      TTATCCGTTCAAAAAGGATTAAATTTAGGAGTTATATTTATATAGTATCTGAAGCTTTA
21  Hypocrea_jecorina_cox3      CTTGCTGTCAAAAAGGATTAAATTTAGGTGTAATATATTTATAGTATCTGAAGCTTTA
22  * * * * *
23
24  Hypomyces_aurantius_cox3      TTCTTCTTAGCTATATTTCTGAGCATCTTTTCATAGTGCTTTAACACCTACAGTTGAATTA
25  Hypocrea_jecorina_cox3      TTCTTCTTAGCTATATTTCTGAGCATCTTTCCATAGTGCTTTAACACCTACAGTTGAATTA
26  *****
27
28  Hypomyces_aurantius_cox3      GGTGCTCAATGACCACCTATGGGTATAGAACCTATAAATCCTTTCGAATTACCTTTATTA
29  Hypocrea_jecorina_cox3      GGTGCTCAATGACCACCTATGGGTATAGAACCTATAAATCCTTTCGAATTACCTTTATTA
30  *****
31
32  Hypomyces_aurantius_cox3      AACACAGTTATATGTTATCTAGTGGTGCTACAATTACTTATGCTCACCATTCTTTAATT
33  Hypocrea_jecorina_cox3      AACACAGTTATATATTTATCTAGTGGTGCTACAATTACTTTTCTCATCATAGTTAATA
34  ***** * * * * *
35
36  Hypomyces_aurantius_cox3      AAAGTGAAAGATCAGGAGCTTTATATGGTACTATTTTCGACAGTAGTATTAGCTTTAGTA
37  Hypocrea_jecorina_cox3      AAAGTGAAAGATCAGGAGCATTTATATGGAACTATTTTCACAGTAGTATTAGCTTTAATA
38  *****
39
40  Hypomyces_aurantius_cox3      TTTACTTTTATTTCAAGGAGTAGAATATAGCGTTTCTTCAATTTACAATAAGTGACGGGTGTA
41  Hypocrea_jecorina_cox3      TTTACTTTTATTTCAAGGAGTAGAATATAGCGTTTCTTCAATTTACAATAAGTGACGGGTGTA
42  *****
43  Cox3-i2 insertion site
44  Hypomyces_aurantius_cox3      TTTGGAACATGTTTCTTCTTGGAACAGGTTTTCATGGCT↓TCCATGTTATCATAGGTACA
45  Hypocrea_jecorina_cox3      TTTGGAACATGTTTCTTCTTGGAACAGGTTTCCACGGAT TCCACGTTATTTATAGGTACT
46  ***** * * * * *
47
48  Hypomyces_aurantius_cox3      ATCTTCTTATCTGTAGGATTATGAAGAATAATGGCATATCATTTAACAGATCATCCCAT
49  Hypocrea_jecorina_cox3      GTTTTCTTATCTGTGATTGTGAAGAATAATGGCATACCATTTAACAGATCATCATCAT
50  * * * * *
51
52  Hypomyces_aurantius_cox3      CTTGGTTATGAAGCTGGAATATTTACTGACATTTTGTAGATGTTGTTTGTATTCTTA
53  Hypocrea_jecorina_cox3      CTTGGTTACGAAGCTGGAATATTTACTGACATTTTGTAGATGTTGTTTGTATTCTTA

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Figure S1. Cox3 gene sequence alignment between *H. aurantius* and *H. jecorina*. Intron-less *cox3* gene sequence of *H. jecorina* was used to compare against that of *H. aurantius* to confirm locations of introns of *cox3* gene in *H. aurantius*. Results showed that the second intron located in position of 640 bp and was 2,877 bp in size. No exon sequences was found in the large intron. Consensus sequences were marked by asterisks. Arrows represent probable insertion sites. The alignment was performed using the program CLUSTAL O [27].

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1  Chaetomium_thermophilum_var._thermophilum_cox3-i2      TCAATTGTTATATTTTAAACAATGTAAT--TAATCGTTCTTCTTCTCTCAA
2  Hypomyces_aurantius_cox3-i2_intron_with_orf330          -----TTAGTTATTCTAACAAATGTTAATATTATTCTCACTTAAAACTAAA
3                                     * * * * *
4
5  Chaetomium_thermophilum_var._thermophilum_cox3-i2      GTAACAAAAGATTTTATTCTACTATGCTGTGTGAGCGTAAATACTATCTCCT
6  Hypomyces_aurantius_cox3-i2_intron_with_orf330          GCAATAAAGATTTCTATTCTACTATACCCGAGAGCGTAATACCTTTCTCCT
7                                     * * * * *
8
9  Chaetomium_thermophilum_var._thermophilum_cox3-i2      TAACAGGATTTGCGGATGCTGAATCAAGTTTGTAGTCTAAAGGTATCTAAAAA
10 Hypomyces_aurantius_cox3-i2_intron_with_orf330          TAACAGGATTTGCCGACGAGAGTCAAGTTTGTAGTCTAAAGGTATCTAAAAA
11                                     * * * * *
12
13 Chaetomium_thermophilum_var._thermophilum_cox3-i2      CTAGATCAGGATGACATATTATTCTGAATTTAGGATAGAGTTACATAATAGA
14 Hypomyces_aurantius_cox3-i2_intron_with_orf330          CTAAATCAGGGTGACACGTAATACCTGAATTTAGAATAGAGTTACACAAATAGA
15                                     * * * * *
16
17 Chaetomium_thermophilum_var._thermophilum_cox3-i2      ATCTATTAAAGAGAAATACATGCCTTTTTGGGGTAGGTATTATCAGAGATT
18 Hypomyces_aurantius_cox3-i2_intron_with_orf330          ACTTATTAAAGAGAAATACACGCCCTTTTTGGGGTAGGTATATTAGTGAGTAT
19                                     * * * * *
20
21 Chaetomium_thermophilum_var._thermophilum_cox3-i2      AAAATAAGGTAGTTTATAGTGTACAATCGCGTCGAGATTTAACTAATGCAATT
22 Hypomyces_aurantius_cox3-i2_intron_with_orf330          TAAATAAGGTAGTTTACAGTGTTCATCTCCTCGTGATTTAGCTAATGTAATT
23                                     * * * * *
24
25 Chaetomium_thermophilum_var._thermophilum_cox3-i2      ATTTTGATCAATATCCTTTAATAACTCAAAAAAGGGCGATTTACTTTTTATT
26 Hypomyces_aurantius_cox3-i2_intron_with_orf330          ATTTTGACAAATATCCTTTAATAACTCAAAAAATGCTGATTTACCTTTTATT
27                                     * * * * *
28
29 Chaetomium_thermophilum_var._thermophilum_cox3-i2      CTGTTGAGATGTTATGTTTAAATGCGCAATCAGATATTAGGGGAGTTCAAGT
30 Hypomyces_aurantius_cox3-i2_intron_with_orf330          CTATTGAGCTATTAATTTAAAGATACATTCAGACATTAACGGTTTCAAGGT
31                                     * * * * *
32
33 Chaetomium_thermophilum_var._thermophilum_cox3-i2      GTCTAAAAGCAGCAATGAATAAAGGATTTGCTGTAAATTAATTTCTGAGTT
34 Hypomyces_aurantius_cox3-i2_intron_with_orf330          GACTTAAAGCAGCAATGAACCTGAGGATTTGTCAGACAAATTAATGAATGAGTTC
35                                     * * * * *
36
37 Chaetomium_thermophilum_var._thermophilum_cox3-i2      ATGTTATCCCGCGAACCTCCTACAATTAATTTGAAAGAATACCTGATCCA
38 Hypomyces_aurantius_cox3-i2_intron_with_orf330          GTGTTATTTACAGCTATACGCTCCTGCAGTAAATTTTGAAGGTATACCCAATCCT
39                                     * * * * *
40
41 Chaetomium_thermophilum_var._thermophilum_cox3-i2      TAGCAGGTTTCGTGGACGGAGAGGTTGTTTCTATATTAACCAAAAAAGCT
42 Hypomyces_aurantius_cox3-i2_intron_with_orf330          TAGCAGGTTTCGTGGATGGAGAAGGTTGTTTATGTTAACACGAAAAAGCT
43                                     * * * * *
44
45 Chaetomium_thermophilum_var._thermophilum_cox3-i2      ATATAACTGGTTATCAGGTTATCATGACATTCTCTATATCTCAACATGTAAGA
46 Hypomyces_aurantius_cox3-i2_intron_with_orf330          ATTTAACAGGATATCAGGTTATTTATGCTCTCTATATGTCACACATGAAAA
47                                     * * * * *
48
49 Chaetomium_thermophilum_var._thermophilum_cox3-i2      TTTTATTAAGAAAGTTTATAGATTACTTAAATTTGTGTAATATAGAAAAAGTG
50 Hypomyces_aurantius_cox3-i2_intron_with_orf330          TTTTATTAATAAGTTTACACAGATTACTTAGGTTGTGGTAAATAGAAAAAGTT
51                                     * * * * *
52
53 Chaetomium_thermophilum_var._thermophilum_cox3-i2      GACCTGATGGAGTTAAATTTTATGATATAAATTTAGTGATATTAGAGACAAA
54 Hypomyces_aurantius_cox3-i2_intron_with_orf330          GACCTGATCAGGTAACATTTGTTACCTATAAATTTAGTGATATTCTGTAGATA
55                                     * * * * *
56
57 Chaetomium_thermophilum_var._thermophilum_cox3-i2      CTTTTTCCAAAATTTACCCCTTACATGGAATGAAGTCTAAGGATTTTAGAGAT
58 Hypomyces_aurantius_cox3-i2_intron_with_orf330          CTTTTTCCAAAATTTACCTTTACACGGGGTGAATCTAAGGATTACAGAGAT
59                                     * * * * *
60
61 Chaetomium_thermophilum_var._thermophilum_cox3-i2      AAATAGCTAAACTTATGAAAAACAATCACATTTAACTCTTGACGGTTTCAAG
62 Hypomyces_aurantius_cox3-i2_intron_with_orf330          AGATAGTTAACTTATGAAAAATAATCACATTTAACTCTTGACGGTTTCAAT
63                                     * * * * *
64
65 Chaetomium_thermophilum_var._thermophilum_cox3-i2      AATCTTAAATCTGGTATGAATAGAGGAAGAATGAGAATGCGCGATTAAAT
66 Hypomyces_aurantius_cox3-i2_intron_with_orf330          AATCATTAATCTGGAATGAATAGAGGTAGAATATACGAT-----
67                                     * * * * *
68
69 Chaetomium_thermophilum_var._thermophilum_cox3-i2      CGATTATGATGTGGAGGCCCTTTAATTATTAATTAATTAATTAATGTTAATAT
70 Hypomyces_aurantius_cox3-i2_intron_with_orf330          -----
71
72
73 Chaetomium_thermophilum_var._thermophilum_cox3-i2      TTAATAGCAATATTGGAACCTTTATAACGTGGAGGCCCTTTTATATGATTAT
74 Hypomyces_aurantius_cox3-i2_intron_with_orf330          -----TAATATTGGAACCTTTATAACGTGGAGGCCCTTTTATATGATTAT
75                                     * * * * *
76
77 Chaetomium_thermophilum_var._thermophilum_cox3-i2      TAAATATTAATTAATAATTAATAAGAAAACAAAGTAAATGCATAGAAGCTT
78 Hypomyces_aurantius_cox3-i2_intron_with_orf330          TAAATATTAATTAATAATTAATAAGAAAACAAAGTAAATGCATAGAAGCTT
79                                     * * * * *
80
81 Chaetomium_thermophilum_var._thermophilum_cox3-i2      -TAAGAGACAATATGCAGCCAAATATATTAGAACATTAATAATTAATTTCTAAT
82 Hypomyces_aurantius_cox3-i2_intron_with_orf330          TATTGAGATAATATGCAGCCAAACATATTAGAATATTAATTAATTTTAAAT
83                                     * * * * *
84
85 Chaetomium_thermophilum_var._thermophilum_cox3-i2      GTTCAACGACTAAAAAGATAGATTTCCTATCTTGCAAAATTTTGTGTATAATA
86 Hypomyces_aurantius_cox3-i2_intron_with_orf330          GTTCAACGACTAAAAAGATAGATCTTCTATCTTGCAAAATTTTGTGTATAGTA
87                                     * * * * *
88
89 Chaetomium_thermophilum_var._thermophilum_cox3-i2      TATTATACAAGTGACATAGTCTGAACAATAATGAAAATATTGAAGGATTGAT
90 Hypomyces_aurantius_cox3-i2_intron_with_orf330          TATTATATGGATGATATAGTCTGAACAATAATTAATTAATTTTGAAGGATTAA
91                                     * * * * *
92
93 Chaetomium_thermophilum_var._thermophilum_cox3-i2      TGCCGATTAATCCGTTAACAATTTTTTTTGG-----
94 Hypomyces_aurantius_cox3-i2_intron_with_orf330          ATCCGTTAACAATAAAAAAAGATTAGCCTTGTCTTATTAGCTTTCATGG
95                                     * * * * *

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Figure S2. Alignment between *cox3-i2* of *Chaetomium thermophilum* var. *thermophilum* and upstream intron in *cox3-i2* of *Hypomyces aurantius*. Among 1339 base pairs compared, 1069 were identified (79.8%). Consensus sequences were marked by asterisks. Gaps were indicated by dash lines. Comparison was performed by CLUSTAL O 1.2.1.

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1 H. aurantius cox3-i2 downstream intron TAACATATGGAGCCCTTTTACA-----TATATATGTTTAAATACAAAAAATCTTATG
2 F. solani cox3-i2 TAATTTTGGTAGCCCTTTTATATACATTAATATATATTTAATACAAAAAACAATATA
3 *****
4
5 H. aurantius cox3-i2 downstream intron TAAGTAACACTTCCACTTGTCTGTACACAGCAATTTCCCGATAAATGTTAATTAATT
6 F. solani cox3-i2 CAGTTAA-----GTCTTTAGCTCACAAATATACAGGGCATAGATAAATATATTAATCT
7 *****
8
9 H. aurantius cox3-i2 downstream intron TACCTAACTCTAATGATAGTTATTTTTTAGATAGACAATTTATAGAATGATTTATAGGGT
10 F. solani cox3-i2 TACCTGAATCTAAGATAATATTCTATAGATAAACAGTTTATAGAATGATTAGTAGGAT
11 *****
12
13 H. aurantius cox3-i2 downstream intron TTAGCGATGCGAGGGGATTTTAAACATTAACCTTACAGATTTTGAAGAAAATACATTTA
14 F. solani cox3-i2 TTACAGATGCGAGGGGTATTTTAACTTAACTTACAGATCTAAGAGATAATACGTTTA
15 *****
16
17 H. aurantius cox3-i2 downstream intron AATATGTTCAATTCACATCCAGATAGGCTTTCATGAAGATGACGTTTCATGTTTGAAT
18 F. solani cox3-i2 AATATGTTCAATATACATATCAAATATCTCTCATGAAGACGACATTTGAAGTATTAAGAT
19 *****
20
21 H. aurantius cox3-i2 downstream intron ATATTATGAATACATTAATAATGTTGACATATTTCTAGATCAAAGGATAGAGTTAATATT
22 F. solani cox3-i2 ATATTATGAATACCTTAAATGCGGCATATTTCCAGATCAAAGGTAAGCTAATTTACT
23 *****
24
25 H. aurantius cox3-i2 downstream intron TTGTTAATGATTTGAATCTTTACTTTATGTAATAATTCCTATATTTAATTTATGTTAATC
26 F. solani cox3-i2 TTGTTAATGATTTAAACTCTTTGTTATACATAAATAATTCCTATATTTAATACGTTAATC
27 *****
28
29 H. aurantius cox3-i2 downstream intron TTAATAGTTCAAATATCATCACTATAATTTGTTGCAAAGCAGTAGAATTAATAAAAA
30 F. solani cox3-i2 TTAATAGTTCAAAGTATCATATTTGTTGTTGCAAAGCAGTAGAATTAATAAAAA
31 *****
32
33 H. aurantius cox3-i2 downstream intron ATAATAAATAATATCTGATACAAATAAATTAGAAATATAAAACTTCAAAAAGAAATGC
34 F. solani cox3-i2 AAAATAAATAATATCAGATCTAAGAAATTAGAAATAAATAAACTTCAAAAAGAAATGC
35 *****
36
37 H. aurantius cox3-i2 downstream intron AAAATATGTCAGGTAATGAATACCTAATCTATAAATGATAAGATAAATAACTAAGT
38 F. solani cox3-i2 AAAATATGTCAGGTAATGAATACCTAATCTATAAATGATAAGATAAATAACTAAGT
39 *****
40
41 H. aurantius cox3-i2 downstream intron TTTGATTAGCAGGATTTATGACGGGGAAGCCACTTTTCACTAATAAGTATATACCCA
42 F. solani cox3-i2 TTTGATTAGCAGGATTTATGATGCGGAGCTACTTTTCTCAAAACAATAATATACCTTA
43 *****
44
45 H. aurantius cox3-i2 downstream intron GATTTAAATAGAAAATCATATAAAGAGTTGGAATATATAAAGATAAGAGATTTCT
46 F. solani cox3-i2 GATTTAAATAGAAAATATATAAAGAGTTGGAATATATAAAGATAAGAGATTTCT
47 *****
48
49 H. aurantius cox3-i2 downstream intron TAAGCACAGGTAGAGTCTATACACTTCTACTAGAGAAAATCGTAACCCCTACTGTAGTCT
50 F. solani cox3-i2 TAAGCACAGGTAGAGTCTATACACTTCTACTAGAGAAAATCGTAACCCCTACTGTAGTCT
51 *****
52
53 H. aurantius cox3-i2 downstream intron TAGAAATAAATAAATCCTGGAACATAAAGAAAATTAATTCCTTAATGTCATGATA
54 F. solani cox3-i2 TAGAAATAAATAAATCAGATTTAAAGGTAATTAATACCTTTAATGTCATGATA
55 *****
56
57 H. aurantius cox3-i2 downstream intron ATAGTGTATATTAATAAAGCTTAAATCTAAGATTCTTATTATGATTAAGACTAGTCG
58 F. solani cox3-i2 ATAGTGTATATTAATAAAGCTTAAATCTAAGATTCTTATTATGATTAAGACTAGTCG
59 *****
60
61 H. aurantius cox3-i2 downstream intron ATATATATTATAAAGGTTATCATACTACATTAAGAGGTAATATATATTCGATGCTATAA
62 F. solani cox3-i2 ATATATATTATAAAGGTTATCATACTACATTAAGAGGTAATATATATTCGATGCTATAA
63 *****
64
65 H. aurantius cox3-i2 downstream intron AATTGCATATGAATAAATATAGATTAACACTAATAGTAATTTACTTATCAATAAAGAAC
66 F. solani cox3-i2 AATTGCATATGAATAAATATAGATTAACACTAATAGTAATTTACTTATCAATAAAGAAC
67 *****
68
69 H. aurantius cox3-i2 downstream intron GTATTTCATAGATAAATAAGAGCCTTAATATCTGAGCCTTTATTTAAGCAAGCCCTT
70 F. solani cox3-i2 GTATTTCATAGATAAATAAGAGCCTTAATATCTGAGCCTTTATTTAAGCAAGCCCTT
71 *****
72
73 H. aurantius cox3-i2 downstream intron ACGAAATAAACAATAAATAAGATATATAGAAATACTGCTAAATAGTAAGTGAAGCAA
74 F. solani cox3-i2 ACGAAATAAACAATAAATAAGATATATAGAAATACTGATAAACTAGTAAGTGAAGCAA
75 *****
76
77 H. aurantius cox3-i2 downstream intron CTAAAATTGTAGCAATGAAAGATAATCATGTGAAAATATATGATAGTATATCTGAATGTG
78 F. solani cox3-i2 CTAAAATTGTAGCAATGAAAGATAATCATGTGAAAATATATGATAGTATATCTGAATGTG
79 *****
80
81 H. aurantius cox3-i2 downstream intron CAAAAGACCTTAATATTTCTAGAAAATGTAATTAAGGATGTATAGATTCAGGAAAATCAT
82 F. solani cox3-i2 CAAAAGATATATGATATATCTGTAATATATCAAGAAATGTTAATTTCAAGGTAATTTT
83 *****
84
85
86
87
88
89 Hypomyces_aurantius_cox3-i2_intron_with_orf327 AAAGCAAGAATA----TCTTACTAGACAACCTGCTGCAAGGTATATGTAATA
90 Fusarium_solani_cox3-i2 AAAGCAAGAAGGCTTTTATGAAAGAAAATTTGCTGCAAGGTATATGTAATA
91 *****
92
93 Hypomyces_aurantius_cox3-i2_intron_with_orf327 GTATACAAAGTTCAACGACTAGCTAATAAATATATGTTACTAAAATTAATTA
94 Fusarium_solani_cox3-i2 ATATATAGGTTCAACGACTAGCTAATAAATATATGTT-----ACTTAT
95 *****
96
97 Hypomyces_aurantius_cox3-i2_intron_with_orf327 ACTAAAAGAAATGGCATAAACTTACTTATTAATATCATTAGTTTCATAAGCAC
98 Fusarium_solani_cox3-i2 AGTAAAAGAAATGGCATAAACTTACTTATTAATATCATTAAATTTGATAAGAAC
99 *****
100
101 Hypomyces_aurantius_cox3-i2_intron_with_orf327 AGATGACATAGTCTAACAAGATGAAAATTTATGATAATCTCTTTTATTTG
102 Fusarium_solani_cox3-i2 AGATGACATAGTCTAACAAGATGAAAATTTATGATAATCTCTTTTATTTG
103 *****

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Figure S3. Alignment between cox3-i2 of *Fusarium solani* and downstream intron in cox3-i2 of *Hypomyces aurantius*. Among 1538 base pairs compared, 1234 were identify (80.2%). Consensus sequences were marked by asterisks. Gaps were indicated by dash lines. Comparison was performed by CLUSTAL O 1.2.1.

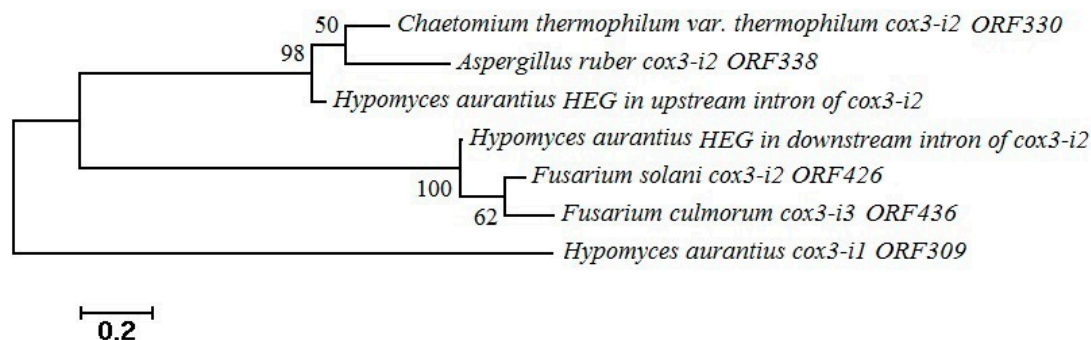


Figure S4. Phylogenetic analysis for translated protein sequences of six ORFs/HEGs. HEG in upstream intron of cox3-i2 showed highest similarity with ORF330 in cox3-i2 of *C. thermophilum* var. *thermophilum* and ORF338 in cox3-i2 of *A. ruber*, and HEG in downstream intron matched well with ORF426 in cox3-i2 of *F. solani* and ORF436 in cox3-i3 of *F. culmorum*. ORF309 in cox3-i1 (group IB) of *H. aurantius* was used as an outgroup. Comparing with the ORF309, the relationship between two HEGs in cox3-i2 of *H. aurantius* was much closer.

Table S1. Conserved protein-coding genes and ribosomal RNA subunits in *H. aurantius* mitochondrial genome.

Gene	Start	End	Strand	Gene Size, bp	CDSSize, bp	Intron Number	Average Intron Length, bp	Percent of Intronic Sequences, %	Stop Codon
<i>rns</i>	1	1505	+	1505				0	
<i>cox3</i>	3516	8330	+	4815	810	2	2003	83.2	TAA
<i>nad6</i>	8565	9242	+	678	678			0	TAA
<i>rnl</i>	11,589	22,088	+	10,500		4	1993	80	
<i>rps3</i>	17,762	19,186	+	1425	1425			0	TAG
<i>nad2</i>	30,234	31,901	+	1668	1668			0	TAA
<i>nad3</i>	33,265	33,678	+	414	414			0	TAA
<i>atp9</i>	33,856	34,050	+	195	195			0	TAG
<i>cox2</i>	35,317	39,361	+	4045	750	1	1795	81.5	TAA
<i>nad4L</i>	41,027	41,296	+	270	270			0	TAA
<i>nad5</i> ^a	41,296	44,055	+	2760	2760			0	TAA
<i>cob</i>	44,271	49,297	+	5027	1170	3	1286	76.7	TAA
<i>cox1</i>	49,947	60,590	+	10,644	1587	5	1811	85.1	TAA
<i>nad1</i>	61,752	62,852	+	1101	1101			0	TAA
<i>nad4</i>	63,999	65,456	+	1458	1458			0	TAA
<i>atp8</i>	66,581	66,727	+	147	147			0	TAA
<i>atp6</i>	66,860	70,630	+	3771	783	2	711	79.2	TAA

^a *Nad5* gene uses the last A of *nad4L* termination codon as first nt, blank space means no present or observed, or unable to calculate.

Table S2. The frequency of utilization of the 64 standard codons.

Amino acid	Codon	Frequency	Amino acid	codon	Frequency	Amino acid	codon	Frequency	Amino acid	codon	Frequency
Phe	UUU	571	Ser	UCU	426	Tyr	UAU	568	Cys	UGU	87
	UUC	256		UCC	48		UAC	165		UGC	19
Leu	UUA	1158		UCA	269	TER	UAA	34	Trp	UGA	130
	UUG	104		UCG	27		UAG	10		UGG	11
	CUU	201	Pro	CCU	254	His	CAU	200	Arg	CGU	93
	CUC	8		CCC	22		CAC	69		CGC	4
	CUA	139		CCA	91	Gln	CAA	264		CGA	3
	CUG	29		CCG	16		CAG	35		CGG	1
Ile	AUU	501	Thr	ACU	350	Asn	AAU	836	Ser	AGU	387
	AUC	117		ACC	24		AAC	216		AGC	73
	AUA	791		ACA	339	Lys	AAA	955		Arg	AGA
Met	AUG	277	ACG	20	AAG		153	AGG	18		
	Val	GUU	307	Ala	GCU	328	Asp	GAU	448	Gly	GGU
GUC		32	GCC		72	GAC		73	GGC		24
GUA		367	GCA		186	Glu	GAA	537	GGA		279
GUG		68	GCG		31		GAG	136	GGG		65

14,074 codons in Average of genes (used Filamentous fungi Mito).