This document contains Figs. S1 and S2 of Nilsson et al., "*A comprehensive, automatically updated fungal ITS sequence dataset for reference-based chimera control in environmental sequencing efforts*".

Fig. S1 (page below). Graphical illustration of problematic sequences with further explanations on how to manually identify compromised sequences.

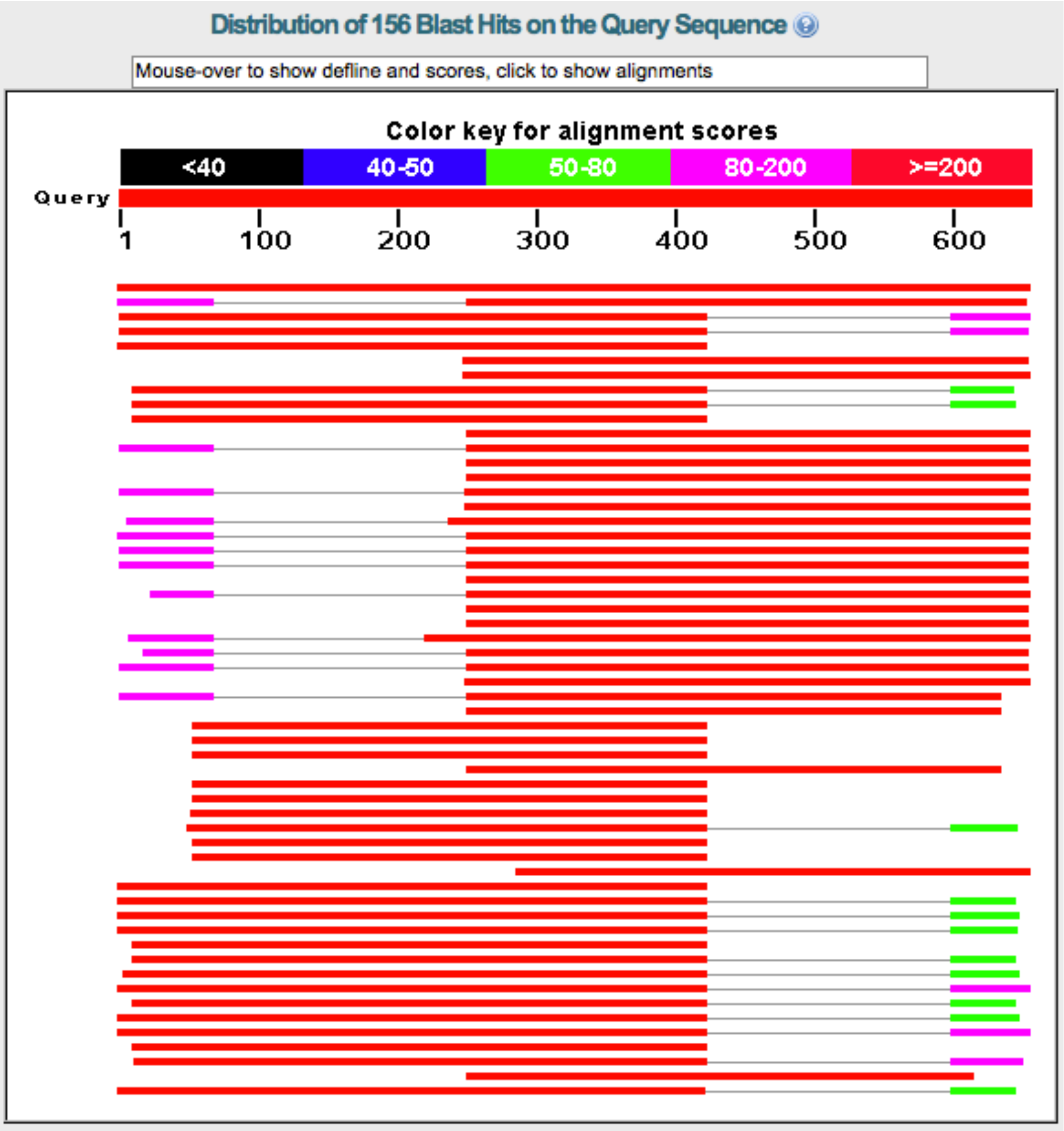
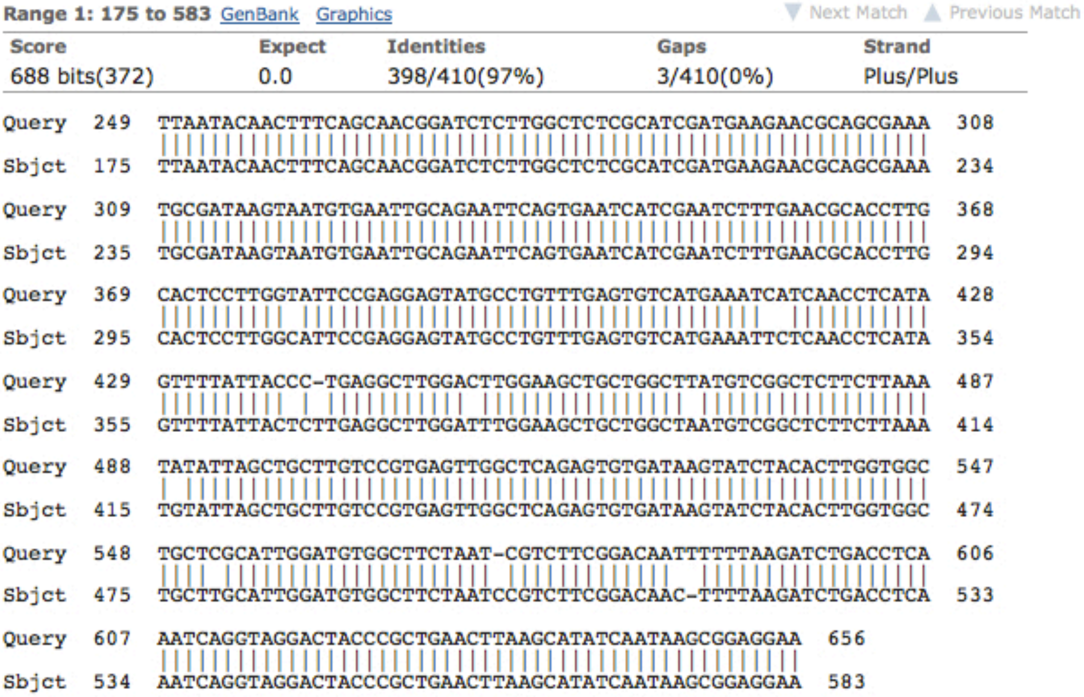


Figure S1-1a. Graphical BLAST overview in GenBank of a chimera (JQ272336). The chimera itself was used as query sequence, such that it is both query sequence and its own best BLAST match. The remaining sequences show a very alarming pattern: alternation of matching regions. Either the ITS1-5.8S part matches the query sequence well, or the 5.8S-ITS2 part matches the query sequence well. No non-self GenBank sequence matches the query sequence over its full length. This is never a good sign and should always be taken to mean that manual examination is necessary.

```
Range 1: 1 to 414 GenBank  Graphics                           ▼ Next Match  ▲ Previous
Score            Expect    Identities        Gaps          Strand
682 bits(369)    0.0       399/414(96%)      0/414(0%)     Plus/Plus

Query  11    TTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAT  70
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1     TTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAT  60

Query  71    CGTACAACAGGAGGTGCCGAGGTTATCGCTGACCCTAGGTCGTGCACGCCTCGAGCATTC  130
             |||||||||||||||||||||||| |||||||||||||||||||||||||||||||||||
Sbjct  61    CGTACAACAGGAGGTGCCGAGGTTGTCGCTGACCCTAGGTCGTGCACGCCTCGAGCATTC  120

Query  131   TTACTCCTCATCCACTTCACCCCTTGTGCATCCCCGCGTGGGCTCTCTCGAAAGAGAGAG  190
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121   TTACTCCTCATCCACTTCACCCCTTGTGCATCCCCGCGTGGGCTCTCTCGAAAGAGAGAG  180

Query  191   CCGGCGTCATTACACAAACACAAAGTAGCAGTAGAATGTTCTTTGCATTTGTATGCAGTT  250
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181   CCGGCGTCATTACACAAACACAAAGTAGCAGTAGAATGTTCTTTGCATTTGTATGCAGTT  240

Query  251   AATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATG  310
             ||||||||||||| |||||||||||||||||||||||||||||||| |||||||||||||
Sbjct  241   AATACAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGGACGCAGCGAAATG  300

Query  311   CGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCA  370
             ||||| |||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301   CGATACGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCG  360

Query  371   CTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTGTCATGAAATCATCAACCT  424
             | |||||||||||||| | | |  || |||||||||||||||| |  |||||||
Sbjct  361   CCCCTTGGTATTCCGAAGGGCACACCCGTTTGAGTGTCATGAAGTTCTCAACCT  414
```

Figure S1-1b. Alternation of matching regions as seen from the BLAST alignments (same accession number). Here, it is the ITS2 region that does not match the query sequence well, whereas the first part of the sequence is aligned well over more than 400 bp.



```
Range 1: 175 to 583 GenBank  Graphics                 ▼ Next Match  ▲ Previous Match
Score            Expect    Identities        Gaps          Strand
688 bits(372)    0.0       398/410(97%)      3/410(0%)     Plus/Plus

Query  249   TTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAA  308
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  175   TTAATACAACTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAA  234

Query  309   TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTG  368
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  235   TGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTG  294

Query  369   CACTCCTTGGTATTCCGAGGAGTATGCCTGTTTGAGTGTCATGAAATCATCAACCTCATA  428
             |||||||||| ||||||||||||||||||||||||||||||||||||  |||||||||||
Sbjct  295   CACTCCTTGGCATTCCGAGGAGTATGCCTGTTTGAGTGTCATGAAATTCTCAACCTCATA  354

Query  429   GTTTATTACCC-TGAGGCTTGGACTTGGAAGCTGCTGGCTTATGTCGGCTCTTCTTAAA  487
             ||||||||| | ||||||||||| |||||||||||||||| |||||||||||||||||||
Sbjct  355   GTTTATTACTCTTGAGGCTTGGATTTGGAAGCTGCTGGCTAATGTCGGCTCTTCTTAAA  414

Query  488   TATATTAGCTGCTTGTCCGTGAGTTGGCTCAGAGTGTGATAAGTATCTACACTTGGTGGC  547
             | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  415   TGTATTAGCTGCTTGTCCGTGAGTTGGCTCAGAGTGTGATAAGTATCTACACTTGGTGGC  474

Query  548   TGCTCGCATTGGATGTGGCTTCTAAT-CGTCTTCGGACAATTTTTTAAGATCTGACCTCA  606
             |||| ||||||||||||||||||||| |||||||||||||  ||||||||||||||||||
Sbjct  475   TGCTTGCATTGGATGTGGCTTCTAATCCGTCTTCGGACAAC-TTTTAAGATCTGACCTCA  533

Query  607   AATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA  656
             ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  534   AATCAGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAA  583
```

Figure S1-1c. A few entries down the list (same accession number), it is the ITS1 of the query sequence that does not match the reference sequence well (no alignment produced between the query and the subject for the first few hundred base-pairs). This bespeaks a 5.8S-based chimeric union. We recommend examining the BLAST alignment to learn of the specifics of each putative chimera. In cases where the graphical summary is not as clear-cut as above (and indeed when the graphical summary does not indicate any problem at all), the BLAST alignment is the best source for further examination.

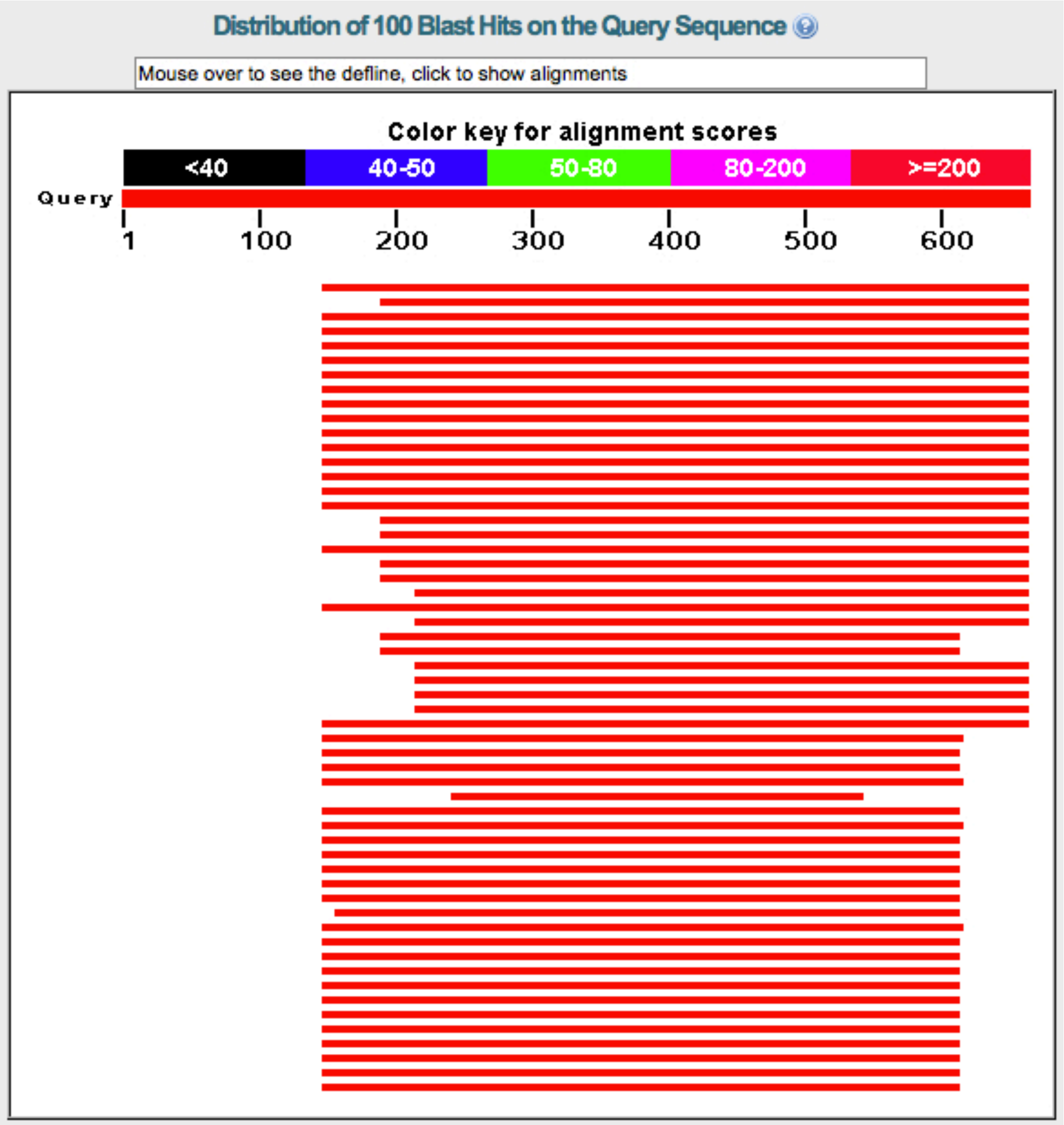


Figure S1-2. Entry JN035638 shows a very alarming pattern: a complete lack of matches for the first part of the query sequence. This is not to be expected for a high-quality fungal ITS sequence, and manual examination of the BLAST results are always needed in these cases. BLAST:ing the first part of the sequence separately is one way forward, as is examining the first part of the query sequence for indications of low read quality.

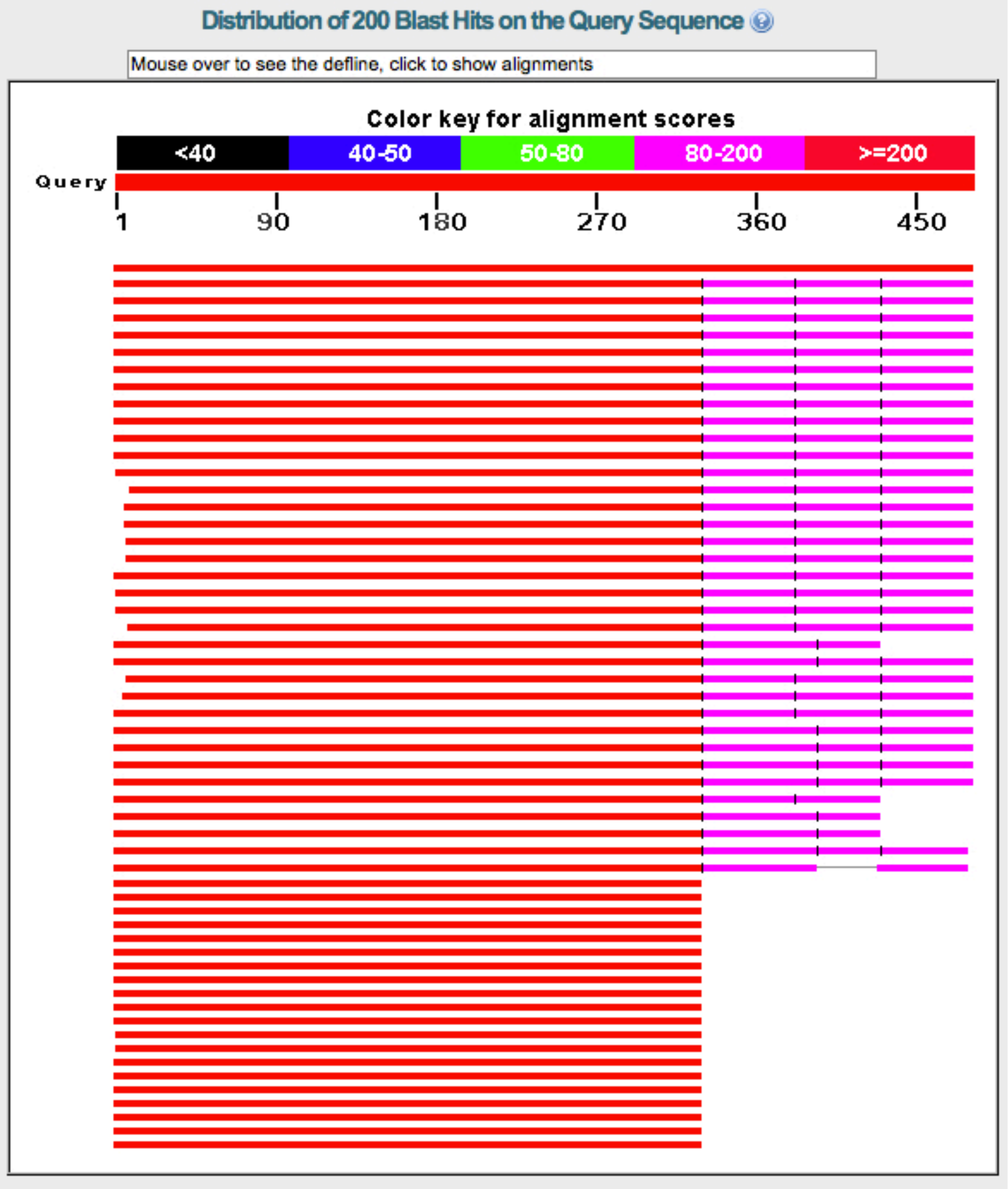


Figure S1-3. Entry FM180040 shows another alarming pattern: breaks in the BLAST alignment. This typically means that the BLAST results cannot be shown in a straightforward way, such as when a sequence was assembled incorrectly. Assembly chimeras produce BLAST results like this.

Nilsson et al. Fig. S1

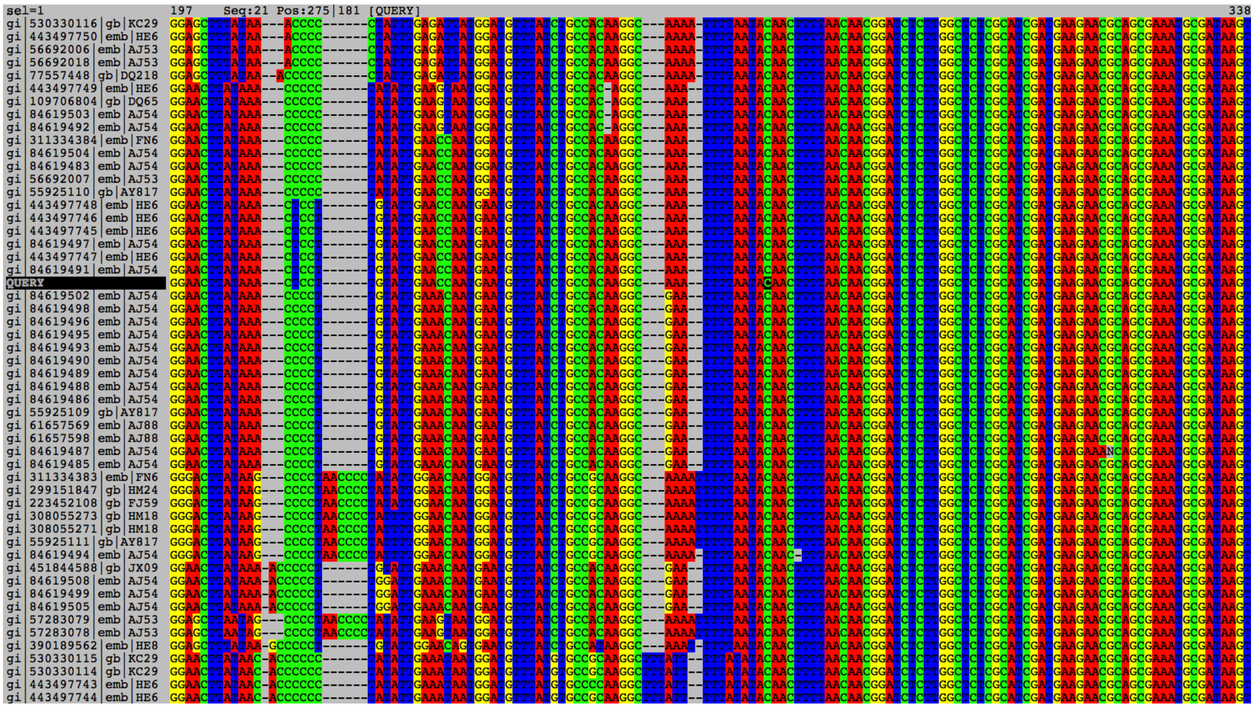


Figure S1-4a. The sequence QUERY (highlighted) is chimeric. The sequence aligns perfectly in the ITS1 and the first part of the 5.8S – the screenshot shows the last part of the ITS1 and the first part of the 5.8S. The cursor approximately in the middle of the screen indicates the first base-pair of the 5.8S. The sequences come from the genus *Hydnum*.

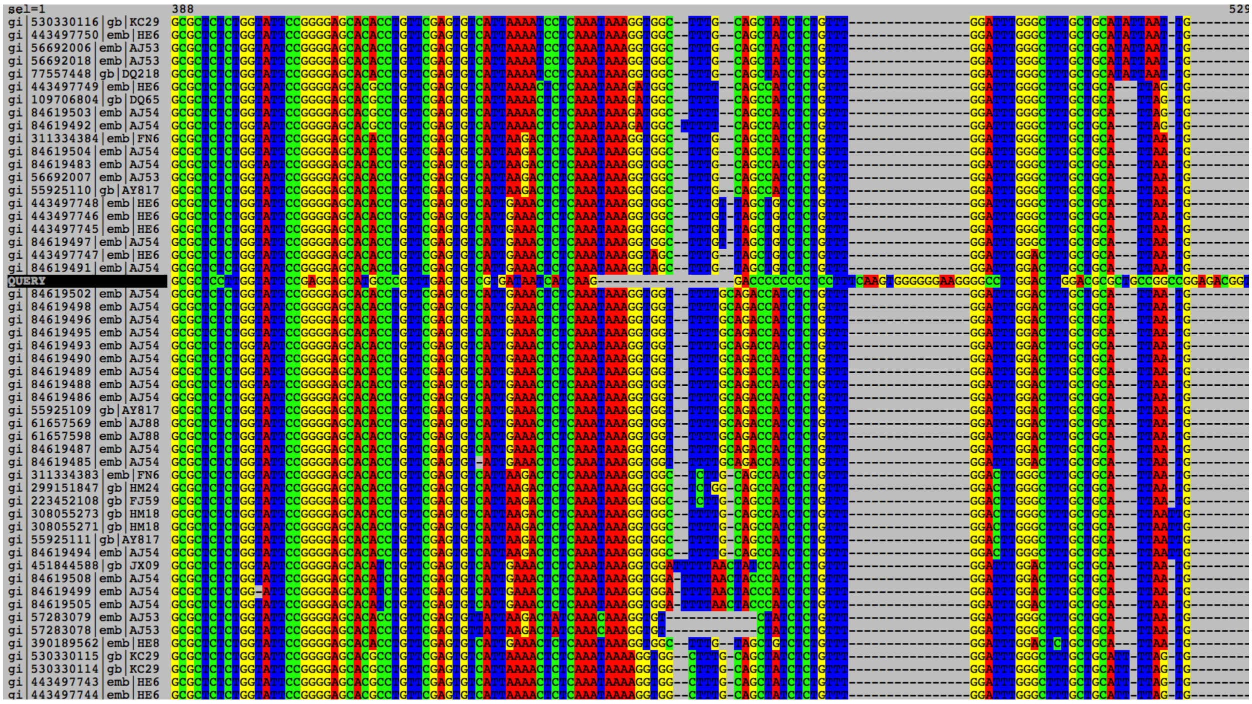
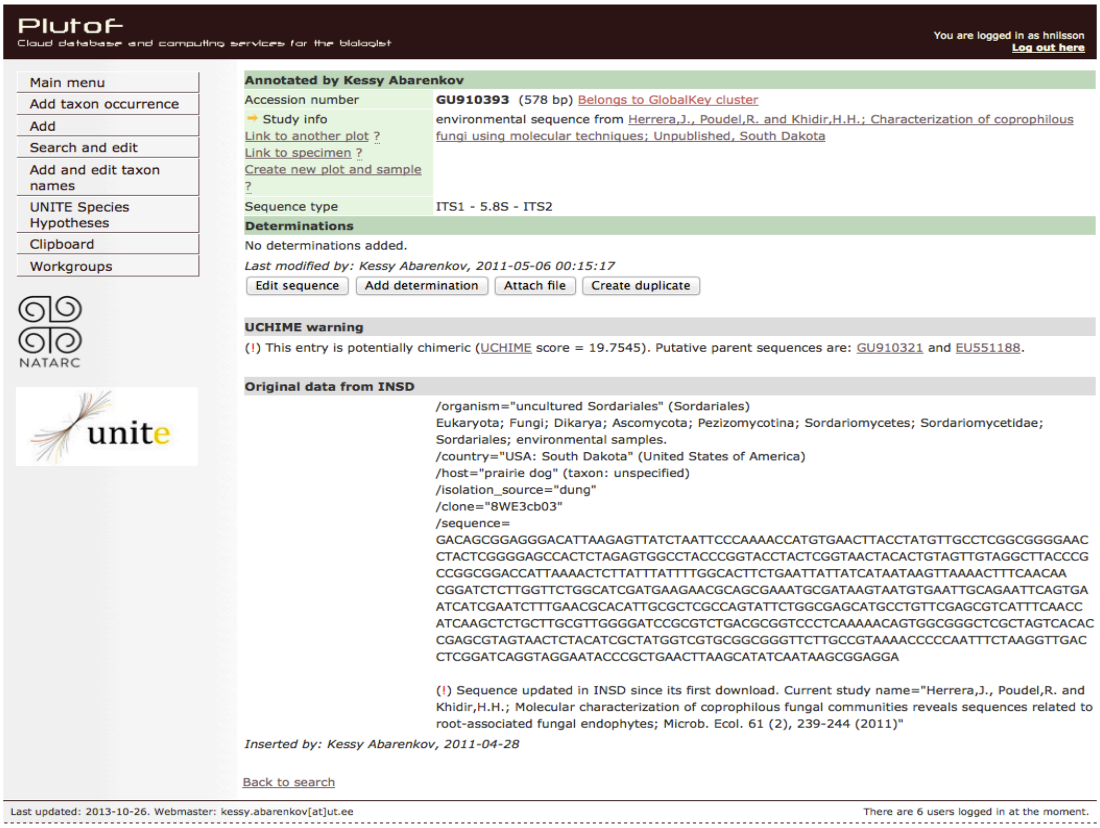


Figure S1-4b. However, as one scrolls through the 5.8S gene, the chimeric nature of the entry QUERY becomes evident. This screenshot shows the last base-pairs of the 5.8S and the start of ITS2. A separate BLAST search of the ITS2 shows it to belong to a *Botryobasidium* species (same order as *Hydnum,* but a different family). The alignment was computed in MAFFT and displayed in SeaView.

ITS Primer Maps – ITS primer maps are available at

`https://unite.ut.ee/primers.php`,
`http://sites.biology.duke.edu/fungi/mycolab/primers.htm`, and
`http://lutzonilab.org/nuclear-ribosomal-dna/`

Fig. S2. Screenshot showing how potentially chimeric sequences are indicated in UNITE through the use of UCHIME.



Annotated by Kessy Abarenkov

Accession number: **GU910393** (578 bp) Belongs to GlobalKey cluster

Study info: environmental sequence from Herrera,J., Poudel,R. and Khidir,H.H.; Characterization of coprophilous fungi using molecular techniques; Unpublished, South Dakota

Link to another plot ?
Link to specimen ?
Create new plot and sample ?

Sequence type: ITS1 - 5.8S - ITS2

Determinations

No determinations added.

Last modified by: Kessy Abarenkov, 2011-05-06 00:15:17

UCHIME warning

(!) This entry is potentially chimeric (UCHIME score = 19.7545). Putative parent sequences are: GU910321 and EU551188.

Original data from INSD

```
/organism="uncultured Sordariales" (Sordariales)
Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina; Sordariomycetes; Sordariomycetidae;
Sordariales; environmental samples.
/country="USA: South Dakota" (United States of America)
/host="prairie dog" (taxon: unspecified)
/isolation_source="dung"
/clone="8WE3cb03"
/sequence=
GACAGCGGAGGGACATTAAGAGTTATCTAATTCCCAAAACCATGTGAACTTACCTATGTTGCCTCGGCGGGGAAC
CTACTCGGGGAGCCACTCTAGAGTGGCCTACCCGGTACCTACTCGGTAACTACACTGTAGTTGTAGGCTTACCCG
CCGGCGGACCATTAAAACTCTTATTTATTTTGGCACTTCTGAATTATTATCATAATAAGTTAAAACTTTCAACAA
CGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGA
ATCATCGAATCTTTGAACGCACATTGCGCTCGCCAGTATTCTGGCGAGCATGCCTGTTCGAGCGTCATTTCAACC
ATCAAGCTCTGCTTGCGTTGGGGATCCGCGTCTGACGCGGTCCCTCAAAAACAGTGGCGGGCTCGCTAGTCACAC
CGAGCGTAGTAACTCTACATCGCTATGGTCGTGCGGCGGGTTCTTGCCGTAAAACCCCCAATTTCTAAGGTTGAC
CTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATATCAATAAGCGGAGGA
```

(!) Sequence updated in INSD since its first download. Current study name="Herrera,J., Poudel,R. and Khidir,H.H.; Molecular characterization of coprophilous fungal communities reveals sequences related to root-associated fungal endophytes; Microb. Ecol. 61 (2), 239-244 (2011)"

Inserted by: Kessy Abarenkov, 2011-04-28

Last updated: 2013-10-26. Webmaster: kessy.abarenkov[at]ut.ee

There are 6 users logged in at the moment.