

A DATABASE FOR TAXONOMIC AND PHYLOGENETIC IDENTIFICATION OF THE GENUS BRADYRHIZOBIUM USING MULTILOCUS SEQUENCE ANALYSIS

1 TUTORIAL

In this tutorial we'll show you how to navigate through some of the available options.

2 ABOUT

On the main page of our database, you will find an overview of the work that led to this site.

Biological nitrogen fixation, with an emphasis on the legume-rhizobia symbiosis, is a key process for agriculture and the environment, allowing the replacement of nitrogen fertilizers, reducing water pollution by nitrate as well as emission of greenhouse gases. Soils contain numerous strains belonging to the bacterial genus *Bradyrhizobium*, which establish symbioses with a variety of legumes. However, due to the high conservation of *Bradyrhizobium* 16S rRNA genes-considered as the backbone of the taxonomy of prokaryotes-few species have been delineated. The multilocus sequence analysis (MLSA) methodology, which includes analysis of housekeeping genes, has been shown to be promising and powerful for defining bacterial species, and, in this study, it was applied to *Bradyrhizobium* species, increasing our understanding of the diversity of nitrogen-fixing bacteria.

Classification of bacteria of agronomic importance is relevant to biodiversity, as well as to biotechnological manipulation to improve agricultural productivity. We propose construction of an on-line database that will provide information and tools using MLSA to improve phylogenetic and taxonomic characterization of *Bradyrhizobium*, allowing the comparison of genomic sequences with those of type and representative strains of each species.

A database for the taxonomic and phylogenetic identification of the *Bradyrhizobium* genus, using MLSA, will facilitate the use of biological data available through an intuitive web interface. Sequences stored in the on-line database can be compared with multiple sequences of other strains with simplicity and agility through multiple alignment algorithms and computational routines integrated into the database. The proposed database and software tools can be used, free of charge, by researchers worldwide to classify *Bradyrhizobium* strains; the database and software can be applied to replicate the experiments presented in this study as well as to generate new experiments. The next step will be expansion of the database to include other rhizobial species.

2.1 URL, AND CONTACT

Clicking on the About tab will show you the Contact sub-menu, which contains contact data of the main researcher.

2.1.1 URL: <http://mlsa.cnpso.embrapa.br>

2.1.2 **Important:** This website and its applications are best viewed in **Firefox browser** version 30 or later.

2.1.3 **Contact:** Contact for information about the website and database.

Name: Fabrício Martins Lopes

E-mail: fabricao@utfpr.edu.br

URL: <http://www.utfpr.edu.br/cornelioprocopio>

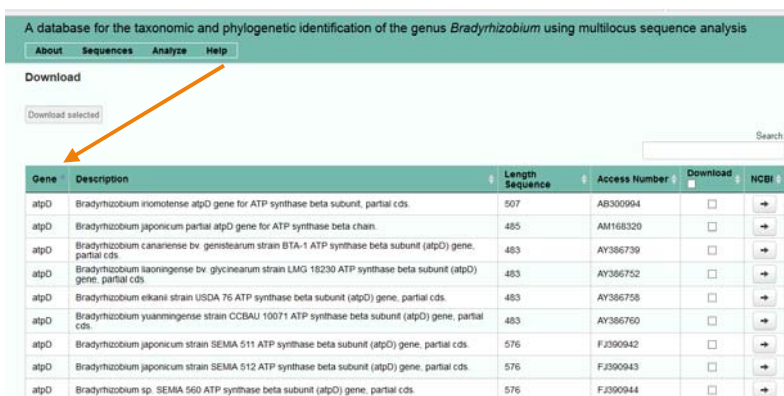
3 SEQUENCES DOWNLOAD

Clicking on the Sequences tab will show you the Download sub-menu, which will link you to the Data Table component, with all sequences stored in our database.

The database currently has 286 entries, distributed as follows:

- For all (57) strains stored, we provide four genes (atpD, dnaK, glnII and recA);
- For 30 strains stored, we provide five different genes (atpD, dnaK, glnII, recA and gyrB);
- For 28 strains stored, we provide four different genes (atpD, dnaK, glnII, recA, gyrB and rpoB).

3.1.1 Ordering can be changed by clicking on the arrows that appear to the right of each field in the table's first row.



A database for the taxonomic and phylogenetic identification of the genus *Bradyrhizobium* using multilocus sequence analysis

Download

Download selected

Search

Gene	Description	Length Sequence	Access Number	Download	NCBI
atpD	Bradyrhizobium innotense atpD gene for ATP synthase beta subunit, partial cds.	507	AB300994	<input type="checkbox"/>	↔
atpD	Bradyrhizobium japonicum partial atpD gene for ATP synthase beta chain.	485	AM168320	<input type="checkbox"/>	↔
atpD	Bradyrhizobium canariense bv. genistearum strain BTA-1 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY386739	<input type="checkbox"/>	↔
atpD	Bradyrhizobium liaoningense bv. glycinearum strain LMG 18230 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY386752	<input type="checkbox"/>	↔
atpD	Bradyrhizobium elkanii strain USDA 76 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY386758	<input type="checkbox"/>	↔
atpD	Bradyrhizobium yuanmingense strain CCBAU 10071 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY386760	<input type="checkbox"/>	↔
atpD	Bradyrhizobium japonicum strain SEMA 511 ATP synthase beta subunit (atpD) gene, partial cds.	576	FJ390942	<input type="checkbox"/>	↔
atpD	Bradyrhizobium japonicum strain SEMA 512 ATP synthase beta subunit (atpD) gene, partial cds.	576	FJ390943	<input type="checkbox"/>	↔
atpD	Bradyrhizobium sp. SEMA 560 ATP synthase beta subunit (atpD) gene, partial cds.	576	FJ390944	<input type="checkbox"/>	↔

3.1.2 You can also search for specific organisms using keywords in the Search field on the right.

A database for the taxonomic and phylogenetic identification of the genus *Bradyrhizobium* using multilocus sequence analysis

Download

Download selected

Search: betae

Gene	Description	Length Sequence	Access Number	Download	NCBI
atpD	Bradyrhizobium betae partial mRNA for ATP synthase F1 (atpD gene), type strain LMG 21987T.	504	FM253129	<input type="checkbox"/>	→
dnaK	Bradyrhizobium betae strain PL7HG1 Hsp70 class chaperone (dnaK) gene, partial cds.	598	AY923046	<input type="checkbox"/>	→
glnI	Bradyrhizobium betae glnI gene for glutamine synthetase II, partial cds, strain: LMG 21987	637	AB353733	<input type="checkbox"/>	→
gyrB	Bradyrhizobium betae partial gyrB gene for DNA gyrase, B subunit, type strain LMG 21987T.	669	FM253217	<input type="checkbox"/>	→
recA	Bradyrhizobium betae recA gene for recombinase A, partial cds, strain: LMG 21987	508	AB353734	<input type="checkbox"/>	→
rpoB	Bradyrhizobium betae partial rpoB gene for RNA polymerase, beta subunit, type strain LMG 21987T.	1440	FM253260	<input type="checkbox"/>	→

Showing 1 to 6 of 6 entries (filtered from 296 total entries)

Download selected

3.1.3 By clicking on the download check box (on the right of each strain) will give file in Fasta format, for one or more sequences.

A database for the taxonomic and phylogenetic identification of the genus *Bradyrhizobium* using multilocus sequence analysis

Download

Download selected

Search: betae

Gene	Description	Length Sequence	Access Number	Download	NCBI
atpD	Bradyrhizobium betae partial mRNA for ATP synthase F1 (atpD gene), type strain LMG 21987T.	504	FM253129	<input type="checkbox"/>	→
dnaK	Bradyrhizobium betae strain PL7HG1 Hsp70 class chaperone (dnaK) gene, partial cds.	598	AY923046	<input checked="" type="checkbox"/>	→
glnI	Bradyrhizobium betae glnI gene for glutamine synthetase II, partial cds, strain: LMG 21987	637	AB353733	<input checked="" type="checkbox"/>	→
gyrB	Bradyrhizobium betae partial gyrB gene for DNA gyrase, B subunit, type strain LMG 21987T.	669	FM253217	<input checked="" type="checkbox"/>	→
recA	Bradyrhizobium betae recA gene for recombinase A, partial cds, strain: LMG 21987	508	AB353734	<input checked="" type="checkbox"/>	→
rpoB	Bradyrhizobium betae partial rpoB gene for RNA polymerase, beta subunit, type strain LMG 21987T.	1440	FM253260	<input type="checkbox"/>	→

Showing 1 to 6 of 6 entries (filtered from 296 total entries)

Download selected

3.1.4 By clicking the download check box button will give file in Fasta Format, for all sequences displayed.

A database for the taxonomic and phylogenetic identification of the genus *Bradyrhizobium* using multilocus sequence analysis

Download

Download selected

Search: betae

Gene	Description	Length Sequence	Access Number	Download	NCBI
atpD	Bradyrhizobium canariense bv. genisteorum strain BTA-1 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY386730	<input checked="" type="checkbox"/>	→
atpD	Bradyrhizobium liaoningense bv. glycinearum strain LMG 18230 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY386752	<input checked="" type="checkbox"/>	→
atpD	Bradyrhizobium eikani strain USDA 76 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY386758	<input checked="" type="checkbox"/>	→
atpD	Bradyrhizobium yuanmingense strain CCBBAU 10071 ATP synthase beta subunit (atpD) gene, partial cds.	483	AY386760	<input checked="" type="checkbox"/>	→
atpD	Bradyrhizobium japonicum partial atpD gene for ATP synthase beta chain.	485	AM168320	<input checked="" type="checkbox"/>	→
atpD	Bradyrhizobium inmolense atpD gene for ATP synthase beta subunit, partial cds.	507	AB300994	<input checked="" type="checkbox"/>	→
atpD	Bradyrhizobium pachythai strain PAC48 AtpD (atpD) gene, partial cds.	512	FJ428208	<input checked="" type="checkbox"/>	→
atpD	Bradyrhizobium jcamae strain PAC68 AtpD (atpD) gene, partial cds.	512	FJ428211	<input checked="" type="checkbox"/>	→
atpD	Bradyrhizobium betae partial mRNA for ATP synthase F1 (atpD gene), type strain LMG 21987T.	504	FM253129	<input checked="" type="checkbox"/>	→
atpD	Bradyrhizobium japonicum SEMA 5079 ATP synthase beta subunit (atpD) gene, partial cds.	576	FJ990956	<input checked="" type="checkbox"/>	→
atpD	Bradyrhizobium diazoefficiens SEMA 5080 ATP synthase beta subunit (atpD) gene, partial cds.	576	FJ990957	<input checked="" type="checkbox"/>	→

Showing 1 to 12 of 12 entries (filtered from 296 total entries)

Download selected

3.1.5 The Next Step is selected click download button to download the desired sequences into a single file.

Opening sequences.fasta

You have chosen to open:

sequences.fasta
which is: TXT file (2.1 KB)
from: blob:

What should Firefox do with this file?

Open with Notepad (default)

Save File

Do this automatically for files like this from now on.

OK Cancel

3.1.6 Clicking on the NCBI button (on the right of each strain) will give you access to the desired sequence in the NCBI.

A database for the taxonomic and phylogenetic identification of the genus *Bradyrhizobium* using multilocus sequence analysis

Download selected

Search: betae

Gene	Description	Length Sequence	Access Number	Download	NCBI
atpD	Bradyrhizobium betae partial mRNA for ATP synthase F1 (atpD gene), type strain LMG 21987T	504	FM253129	✓	→
dnaK	Bradyrhizobium betae strain FL7HG1 hsp70 class chaperone (dnaK) gene, partial cds.	598	AY923046	✓	→
glnII	Bradyrhizobium betae glnII gene for glutamine synthetase II, partial cds. strain: LMG 21987	637	AB353733	✓	→
gyrB	Bradyrhizobium betae partial gyrB gene for DNA gyrase, B subunit, type strain LMG 21987T.	669	FM253217	✓	→
recA	Bradyrhizobium betae recA gene for recombinase A, partial cds. strain: LMG 21987	508	AB353734	✓	→
rpoB	Bradyrhizobium betae partial rpoB gene for RNA polymerase, beta subunit, type strain LMG 21987T	1440	FM253260	✓	→

Showing 1 to 6 of 6 entries (filtered from 286 total entries)

Download selected

3.1.7 On the upper corner of this page you find the possible format options for the GenBank.

NCBI Resources How To Sign in to NCBI

Nucleotide Nucleotide Search

Display Settings: GenBank Send

Change region shown

Customize view

Basic Features

Default features

Gene, RNA, and CDS features only

Features added by NCBI

1 conserved domain

Display options

Show reverse complement Update View

Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

LinkOut to external resources

LMG 21987 (strain passport) [StrainInfo]

Related information

Related Sequences

Full text in PMC

Pubmed

PubMed

Taxonomy

Recent activity

Bradyrhizobium betae partial gyrB gene for DNA gyrase, B subunit, type strain LMG 21987T

GenBank: FM253217.1

FASTA Graphics

Go to

LOCUS FM253217 669 bp DNA linear BCT 25-FEB-2009

DEFINITION Bradyrhizobium betae partial gyrB gene for DNA gyrase, B subunit, type strain LMG 21987T.

ACCESSION FM253217

VERSION FM253217.1 GI:224174489

KEYWORDS .

SOURCE Bradyrhizobium betae

ORGANISM Bradyrhizobium betae

Bacteria; Proteobacteria; Alphaproteobacteria; Rhizobiales; Bradyrhizobiales; Bradyrhizobium.

REFERENCE 1

AUTHORS Silva, R., Martens, M., de Lajudie, F. and Willems, A.

TITLE Multilocus sequence analysis of the genus Bradyrhizobium

JOURNAL Syst. Appl. Microbiol. 32 (2), 101-110 (2009)

REFERENCE 2 (bases 1 to 669)

AUTHORS Willems, A.M.C.G.

TITLE Direct Submission

JOURNAL Submitted (21-OCT-2008) Willems A.M.C.G., Laboratory of Microbiology, MBI, University Gent, Ledeganckstraat 35, B-9000, BELGIUM

FEATURES

Location/Qualifiers

source

1..669

/organism="Bradyrhizobium betae"

/mol_type="genomic DNA"

/strain="type strain: LMG 21987"

/culture_collection="IMB:21987"

/db_xref="taxon:214734"

gene

1..669

/gene="gyrB"

cds

1..669

/gene="gyrB"

/codon_start=3

/transl_table=11

/product="DNA gyrase, B subunit"

/protein_id="224174489.1"

/db_xref="GI:224174489"

/db_xref="GI:224174489"

3.1.8 There are two ways to transfer the data to your device:

3.1.8.1 By selecting and copying the sequence data right clicking the mouse;

Display Settings: FASTA Send

Bradyrhizobium betae partial gyrB gene for DNA gyrase, B subunit, type strain LMG 21987T

GenBank: FM253217.1

GenBank Graphics

>gi|224174489|emb|FM253217.1| Bradyrhizobium betae partial gyrB gene for DNA gyrase, B subunit, type strain LMG 21987T

TTCTCCGCGCCGTCACCGCGCCGCGCTCCGCTCCACACCGCTGTCGAGCAAGCTCCGCGTCCGAT

CTGCGCGCAACAAGGACATACATCGAATTCGCCATGCGGATGCGGTCGACCGCTCGTGTGTGTC

GCGGATGACCCGCGCGCGCGCCACCGAGGTGACGTTCCAGGCTCGACGGAACCTTCAAGAACATCG

AATATGATTTCGACCGCTGAGCACCGGCTGCGGAGCTCGCCTTCTCAATTCCGCGGTCACAACTCG

GCTCTCCGACATCGTCCGCGGTGAGAAAGCGGAGGATGCACTATTGGGGCGCGTCCGAGAAATC

GTCAATATCTCGACCGCAACAAGGCGGATCGTCCGCGCCGATCATGCGCGCGGAAAGCAACG

GCAATCGGCTCGAGGCGCGTGTGTGTGGAACGAGCTACCGAGAACGTAAGTGTCTTACCAACAA

CATTCCGCGCGTGTGCGCGCACCATCTGCGCGGTTCCGCGCGCGCTGACGCGCAGTCAACGTT

TATGCGAGGCCACGCGCAAGAAAGAAAGATCGCGCTGACCGCGGACACTGCGCGGAGGCTCACCG

CCGTGCTTCGTGAGGTGCCCATCCGAAATTCCTGT

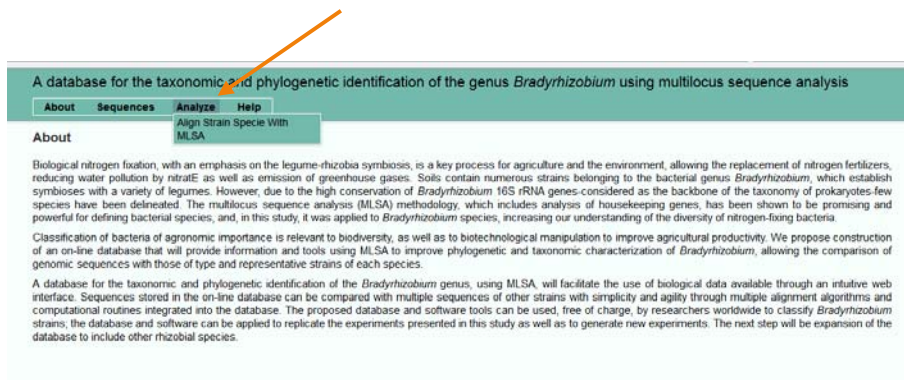
3.1.8.2 Or by the Send dialog box in the right upper corner of the screen and selecting “complete record” and “file”.



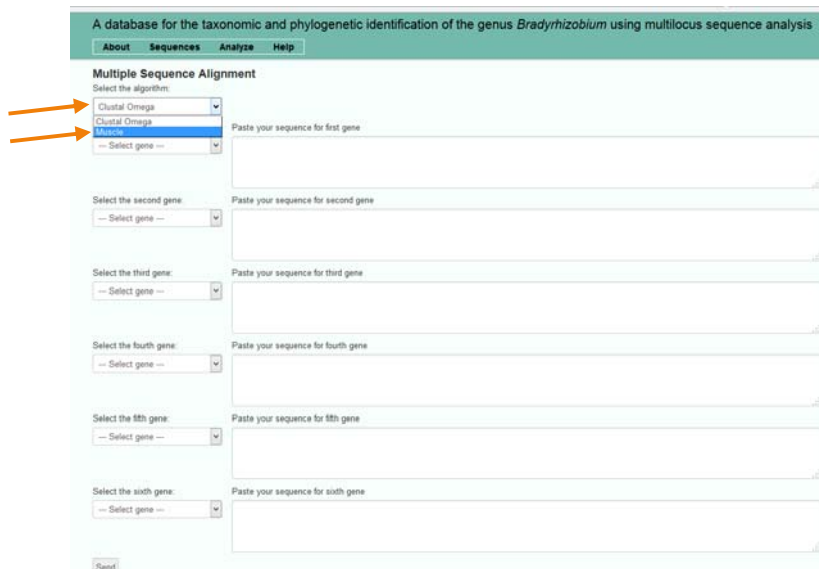
4 ANALYZE

In this part of tutorial, we will show you the process of multilocus sequence analysis.

4.1.1 Clicking the context menu “analysis” will show you the option “align with strain MLSA”.



4.1.2 In the first dialogue box on your left, clicking on the arrow will give you the choice between Clusta Omega or Muscle.



4.1.3 The next six dialogue boxes on left allow you to select each of the one to six genes to be used for analysis.

4.1.4 In this example we must select 3 different genes and paste the sequences for each of them in the "field" paste your sequence for your first (second / third) gene.

4.1.5 When selecting a particular gene, information on the smallest size of the sequence stored: min xxx bp (example: min 369 bp) which means that the smallest sequence for gene **dnaK** in the databank has 369 base pairs.

4.1.6 After filling in your sequences, you can click the **send** button and wait for results.

A database for the taxonomic and phylogenetic identification of the genus *Bradyrhizobium* using multilocus sequence analysis

About Sequences Analyze Help

Multiple Sequence Alignment

Select the algorithm:
Clustal Omega

Select the first gene:
atpD
Paste your sequence for first gene (min 455 bp):
CGTCAAGTGAAGTGGCGGCTGGTGTTCGGCCAGATGAACGAGCCGCCGGGGCCDGGCCDGGTGGG
CTACAGGGCTGAGACATCGGGGAAGACTCGGGGCAAGGGCCAGGACGTGCTGTCTGTGCGACAAEA
TCTCCGGTTCAC

Select the second gene:
dnaK
Paste your sequence for second gene (min 369 bp):
ACGCCGTGCATCGAGGATGCCCTACGGACCTCAAGGAAGCCCTGAAGGGCCAGCATGCCGAGCCGATC
AAGGCCAAGACCCAGAGCGCTGCCACGGCTCGATGAAGCTCGGCBAGGCCATGACACCCAGCAGGCCG
AGGCCGACGCCAAGAGGATCGGCCAAGGACGATC

Select the third gene:
gdh
Paste your sequence for third gene (min 538 bp):
CGTGGAAAGCATCTCGACCTCTGCCTCGCGCCGGGATCAACCTGAAGGCATCAACCGGGAAGTGGCC
AAGGGCCAGTGGGAATCCAGATCTCGGCAAGGGCTCCAAGAAGCCCGCCGACGAAATGTGGATGGCC
GCTACTGATGCTGGGCTGACGGAGAGTACGGGCTGAGATGAGTTTCACTGCAAGCCGCTGGCGCA
CAGCGAC

Select the fourth gene:
-- Select gene --
Paste your sequence for fourth gene

Select the fifth gene:
-- Select gene --
Paste your sequence for fifth gene

Select the sixth gene:
-- Select gene --
Paste your sequence for sixth gene

Send

4.1.7 A screen informs the procedure being performed.

A database for the taxonomic and phylogenetic identification of the genus *Bradyrhizobium* using multilocus sequence analysis

About Sequences Analyze Help

Please wait...

Data are being processed, please wait a moment

Selecting sequences: done!
Performing alignment: done!
Performing cutoffs: done!
Performing alignment: done!
Performing cutoffs: done!
Performing concatenation: done!
Performing alignment:

4.1.8 The results will be shown in the following order (Alignment, Identity Matrix and Parameters for the Generation of the Phylogenetic Tree), and include download option to alignments, the identity matrix and the newick tree.

4.1.8.1 ALIGNMENT

A database for the taxonomic and phylogenetic identification of the genus *Bradyrhizobium* using multilocus sequence analysis

About Sequences Analyze Help

Results

Alignment

```
>B_cenX18186_LMG_22249
CGGCCATCCACAGAGCCGCGGCTACCCGACGATCGACCGAAGCTGAATTTCT
GTCAAGACATCAAGTGTGTGATTTCTCTCCGCTACGCAAGGGCCGAAATGCG
CTGTTGCGCGCCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG
CGGAAAGGCGCAAGTGTGACT---CGTGTTCGGCGGCGTGGTGAAGTACCGCG
-AGGCGACGACTTATACGAAATTCATCGTTCAGGTCAGGTCAGCCGATCCGCAAT
CGAATCGGAGTCAAGTGTGAAATGCGGCTGTGTTTGGCGCATCAAGAGCGCG
GGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG
GATCAGAGC-----AAGGATGATGATGATGATGATGATGATGATGATGATG
AAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
GAGAGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATG
CTGACTGCGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
AGCAGTTCGAGAG--T-CGCGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
AGCAGC--TCAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
```

Download

Opening Alignment.fasta

You have chosen to open:

Alignment.fasta
which is: TXT file (0.1 kb)
from: local

What should Firefox do with this file?

Open with: Notepad (default)

Save File

Do this automatically for files like this from now on.

OK Cancel

4.1.8.2 IDENTITY MATRIX

The screenshot shows a table titled "Identity Matrix" with columns for various species and their corresponding LMG sequences. The rows represent different SEMIA sequences. Below the table is a "Download" button. An orange arrow points from the "Download" button to a file opening dialog box titled "Opening Identity-Matrix.csv". The dialog box shows the file "Identity-Matrix.csv" selected, with options to "Open with Microsoft Excel (default)", "Save File", or "Do this automatically for files like this from now on".

	Usher SEQUENCE	B.cannariense LMG 22265	B.lissoningense LMG 18230	B.elkanii USDA 76	B.yuanmingense LMG 21827	B.japonicum USDA 6	B.vivianense ERM5	B.pachytrisi PAC 48	B.sicariae PAC 68	B.betulae LMG 21987	SEMIA 5079	SEMIA 5080	SEMIA 6089	B.cytisii CTAN71
SEMIA-6192	95.28	94.06	95.58	89.05	94.55	95.13	94.09	88.05	95.16	95.28	95.29	96.98	97.06	95.30
SEMIA-6319	93.31	92.53	93.86	89.24	97.05	94.27	93.54	88.06	91.33	93.31	94.42	94.94	95.62	92.21
SEMIA-6374	94.89	94.70	96.13	89.08	94.95	95.13	93.46	88.69	95.40	94.89	95.29	96.44	96.62	93.38
SEMIA-6434	94.71	93.85	93.23	89.90	93.22	94.35	95.58	88.95	91.58	94.71	94.51	94.71	94.79	94.86
SEMIA-6442	87.76	87.62	88.82	97.51	89.42	88.82	88.88	97.86	89.89	87.76	88.82	88.63	88.79	89.03
SEMIA-6144	94.27	94.15	95.36	89.08	95.18	94.03	93.07	88.45	89.05	94.27	94.19	95.59	95.67	92.21

4.1.8.3 PARAMETERS FOR GENERATION OF THE PHYLOGENETIC TREE.

The screenshot shows a phylogenetic tree visualization with a "Download" button. An orange arrow points from the "Download" button to a file opening dialog box titled "Opening Phylogenetic-Tree.txt". The dialog box shows the file "Phylogenetic-Tree.txt" selected, with options to "Open with Notepad (default)", "Save File", or "Do this automatically for files like this from now on".

```

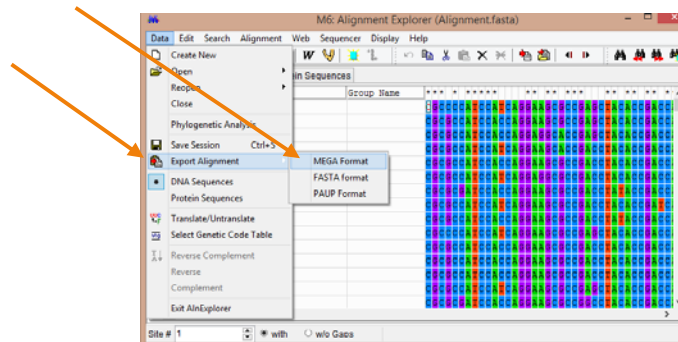
Phylogenetic tree
((((((USDA_SEQUENCE:0.00000,B.cannariense_LMG_22265:0.00000),B.lissoningense_LMG_18230:0.02541),{((((((B.elkanii_USDA_76:0.01142,SEMIA_495:0.01500):0.00460),(B.pachytrisi:
  
```

4.1.9 After downloading, you can use programs for data processing.

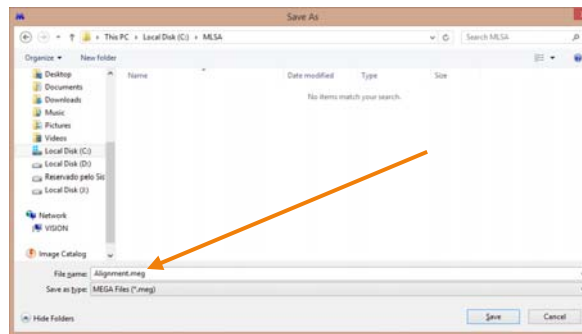
4.1.10 Next step is an example alignment and phylogenetic tree analysis using MEGA software version 6.

The screenshot shows the MEGA Alignment Explorer software interface. The window title is "MEGA: Alignment Explorer (Alignment.fasta)". The interface includes a menu bar (Data, Edit, Search, Alignment, View, Sequencer, Display, Help) and a toolbar. The main area displays a list of DNA sequences with their species names and group names, alongside a color-coded sequence alignment. The sequences listed are: 1. USDA_SEQUENCE, 2. B.cannariense_LMG_22265, 3. B.lissoningense_LMG_18230, 4. B.elkanii_USDA_76, 5. B.yuanmingense_LMG_21827, 6. B.japonicum_USDA_6, 7. B.lissoningense_ERM5, 8. B.pachytrisi_PAC_48, 9. B.sicariae_PAC_68, 10. B.betulae_LMG_21987, 11. SEMIA_5079, 12. SEMIA_5080, 13. SEMIA_6089, 14. B.cytisii_CTAN71, 15. B.rifense_CTAN71.

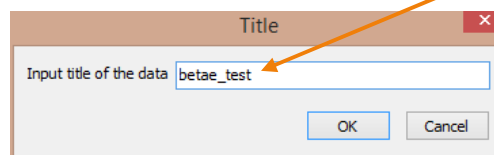
4.1.11 Use option DATA > Export Alignment > MEGA Format



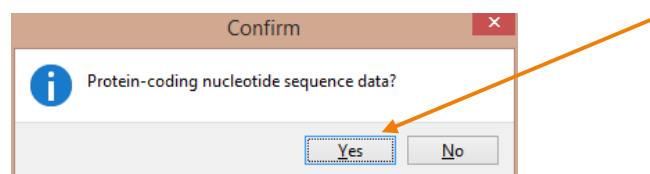
4.1.12 Save as alignment.meg



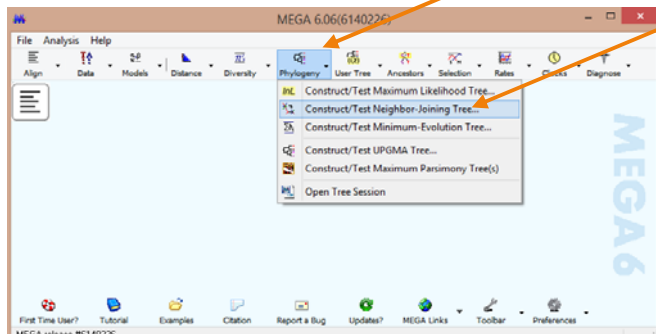
4.1.13 Input title of the data: "betae_test"



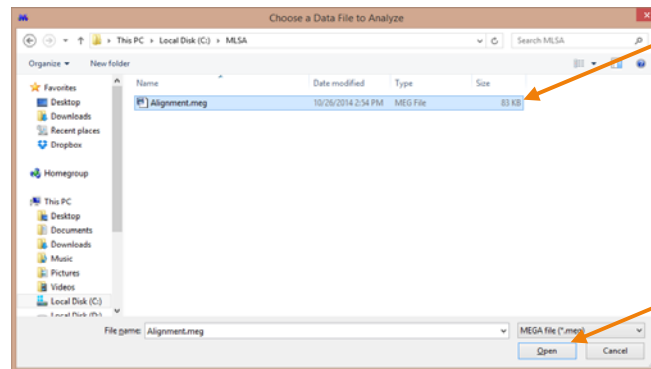
4.1.14 Protein-coding nucleotide sequence data? "Yes"



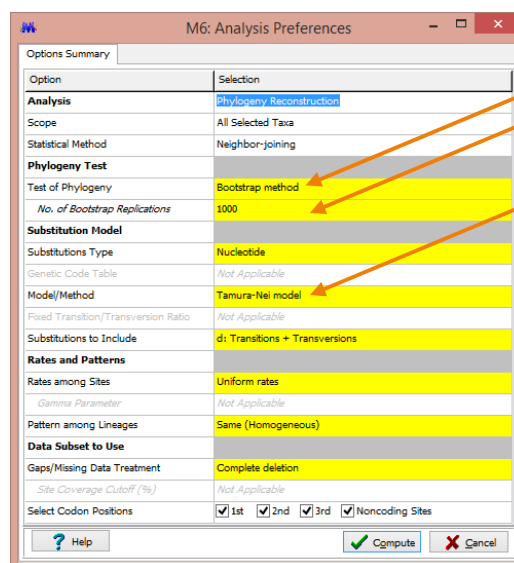
4.1.15 Now Open Phylogeny Tab in Software Mega.



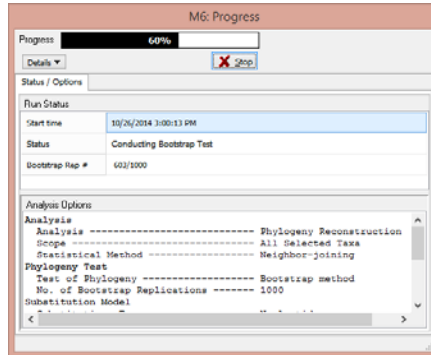
4.1.16 Choose a Data File to Analyze: "alignment.meg"



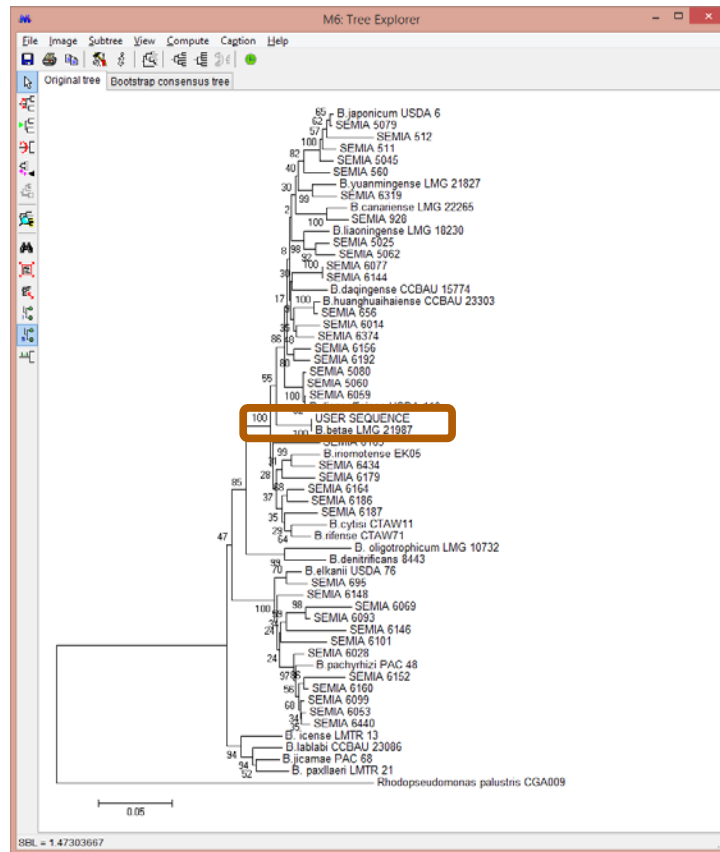
4.1.17 In Options Summary, input 3 parameters different of default analysis preferences: "Test Phylogeny: Bootstrap method", "No. of Bootstrap Replications: 1000" and "Model/Method: Tamura-Nei model". Click in compute.



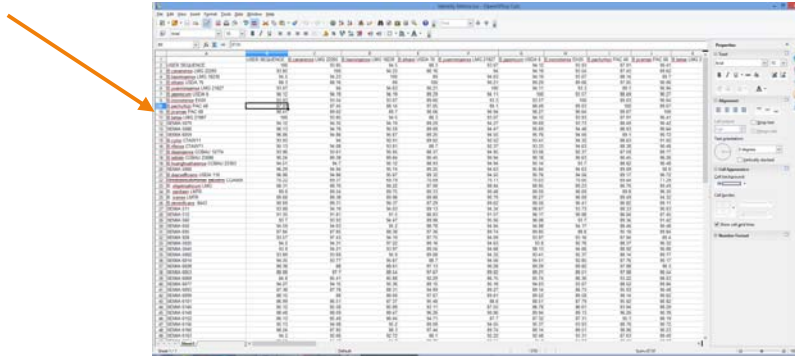
4.1.18 A screen informs the procedure being performed



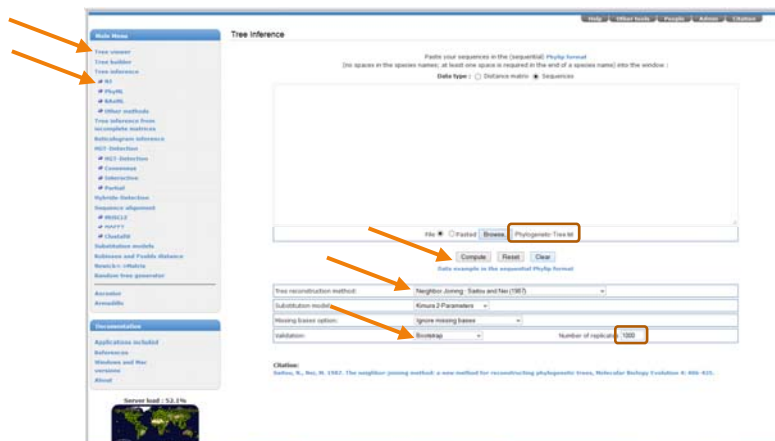
4.1.19 Result of analysis:



4.1.20 To make an analysis of Identity-Matrix.csv file, can be used a software Open Calc of Apache OpenOffice software available in: <http://www.openoffice.org/>



4.1.21 To the phylogenetic analysis of the file “Phylogenetic-Tree.txt” an option can be found in the services available on the website: <http://www.trex.uqam.ca/>



5 COMMENTS AND SUGGESTIONS

5.1 WE WOULD BE VERY HAPPY WITH YOUR COMMENTS OR SUGGESTIONS.

PLEASE CONTACT US.