# ReproPhylo in an Ipython notebook Docker container

## Getting there

This is a simple use case for ReproPhylo, analysing three genes. In order to get to the live version of this notebook you will need to start a Docker container:

1. Go to '/home/generic/Docker':
   `$cd /home/generic/Docker`
2. Start a Docker container while specifiying your working directory
   `$./startDocker.sh /home/generic/tutorial`
3. Google chrome will start up and show you the Ipython notebook login page.
4. Key in the password, which is `password`
5. You will next get the Ipython notebook navigation page. It will treat `/home/generic/tutorial` as the home directory.
6. Start a new empty notebook
7. To download the tutorial copy and paste the following line to the first gray cell and click `shift + enter`:
   `!wget some_gross_lie_there_is_nothing_to_download; tar -xvzf tutorial.tar.gz`
8. Close this noebook
9. Refresh your web page, you will then see a link to the directory `Tutorial_files` appear, click it.
10. Click the link to the `Tutorial` notebook, and here you are.

You are now working inside a virtual machine (VM). Only canges to your working directory will be saved. If you change directory and save things in other locations on the virtual machine, they will be lost.

When done, use your terminal (on the local machine, not the virtual one) to stop the VM as follows:

1. `$cd /home/generic/Docker`
2. `$./stopDocker.sh`

## Working with ReproPhylo in Ipython notebook

This is an Ipython notebook. It consists of text (markdown) cells like this one, which contain comments and explanations, but do not affect the program. Actual script is written in code cells, which have a shaded background. The code in the code cells can be executed by placing the curser anywhere inside a code cell and clicking `shift+enter`.

**Task: Run the first code cell bellow.** It will upload ReproPhylo and its dependencies. There is no output to expect, except for a number that will appear or change in the square brackets on the left hand side of the code cell.

In [55]:
```
from reprophylo import *
```

## Version control in Ipython notebook

A version control program called git is incorperated in the ReproPhylo code. This ensures that you will always be able to role back to older versions of files which might have been overwritten.
**Task: To get git to start working at the background, run the next cell.**

```
[master (root-commit) 2a79b8c] 2 script file(s) from Sun Nov  9 21:06:10 2014
 2 files changed, 3386 insertions(+)
 create mode 100644 .ipynb_checkpoints/Tutorial-checkpoint.ipynb
 create mode 100644 Tutorial.ipynb
```

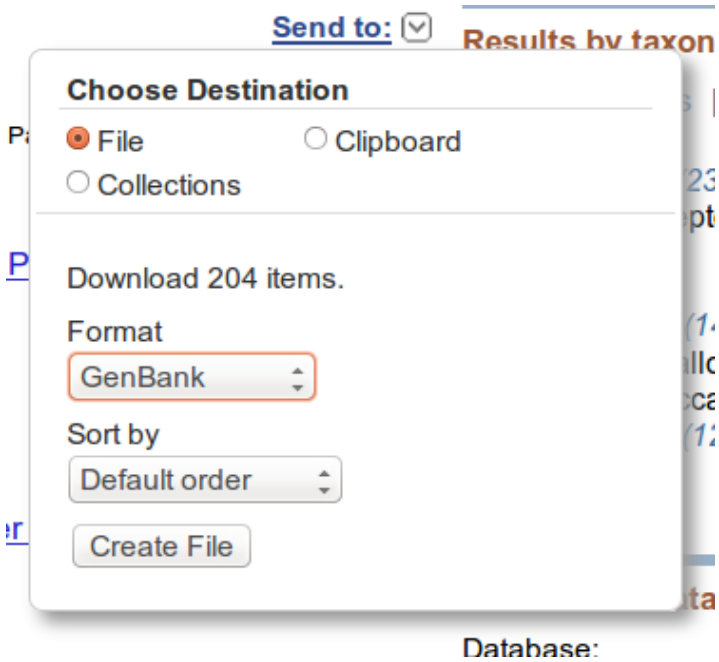
The massage recived was generated by git and it is letting us know that a repositpry has been created and that this Ipython notebook has been recorded in it and will be tracked for changes. Input data files and python scripts, should you write any, will be controlled as well.

## Working with a GenBank file

ReproPhylo is designed to easily handle genbank files. The first step would be to make an online NCBI search in a the Nucleotide database and saving the search results as a genbank file, by using menu on the right hand side of the Nucleotide webpage.

In [14]:
```
#execute if figure is small
from IPython.display import Image
Image(filename='Selection_003.png')
```

Out[14]:




In most cases, entries in the NCBI Protein database are included in their counterpart Nucleotide database entry under the `translation` qualifier. ReproPhylo allows you to switch easily between the datatypes, as long as you provide this sort of a **Nucleotide** genbank file. For the ocassions in which a protein family is not well represented in the Nucleotide database, it is also possible to work with protein (or nucleotide) fasta files. For the purpose of this tutorial, we have included a genbank file, `Tetillidae.gb`. ## Exploring the locus content of a genbank file

The first step in ReproPhylo would be to list the genes in a genbank file and to quantify their occurance. This is done with the `list_loci_in_genbnk()` function. As arguments, it takes the genbank file name and an output filename. The output file will be a CSV file, written in a format which can be read by ReproPhylo (details below). CSV files (Comma Seperated Values) are text representation of tables. We use the name loosly to also include tab delimited text.

The `list_loci_in_genbnk()` function will print the counts and names of the loci in alphabetical order and then by descending counts. It will allow you to choose which genes to carry forward in the analysis and also to check if there are several synonyms for any given

gene. This function is used as follows:

```
list_loci_in_genbank("input.gb","output_loci.csv")
```

**Task: Write all the loci from `Tetillidae.gb` to this notebook, as well as a CSV output, using `list_loci_in_genbank()`**

```
list_loci_in_genbank('Tetillidae.gb', 'Tetillidae_loci.csv')
```

```
There are 57 gene names (or gene product names) detected
---------------------------------
Gene and count sorted by gene name
---------------------------------
1 instances of CDS,ALD
2 instances of CDS,alg11
1 instances of CDS,ATP synthase beta subunit
3 instances of CDS,atp6
1 instances of CDS,ATP6
3 instances of CDS,atp8
1 instances of CDS,ATP8
1 instances of CDS,ATP9
2 instances of CDS,atp9
1 instances of CDS,catalase
1 instances of CDS,CchGa
1 instances of CDS,CchGb
1 instances of CDS,CchGc
1 instances of CDS,CchGd
1 instances of CDS,CchGe
1 instances of CDS,CchGf
2 instances of CDS,cob
1 instances of CDS,coi
11 instances of CDS,COI
78 instances of CDS,cox1
1 instances of CDS,COX1
3 instances of CDS,cox2
1 instances of CDS,COX2
3 instances of CDS,cox3
1 instances of CDS,COX3
3 instances of CDS,coxI
1 instances of CDS,CYTB
1 instances of CDS,elongation factor 1 alpha
1 instances of CDS,Hsp70
1 instances of CDS,MAT
2 instances of CDS,nad1
2 instances of CDS,nad2
2 instances of CDS,nad3
2 instances of CDS,nad4
2 instances of CDS,nad4L
2 instances of CDS,nad5
2 instances of CDS,nad6
1 instances of CDS,ND1
1 instances of CDS,ND2
1 instances of CDS,ND3
2 instances of CDS,ND4
1 instances of CDS,ND4L
1 instances of CDS,ND5
1 instances of CDS,ND6
7 instances of CDS,putative LAGLIDADG protein
1 instances of CDS,TPI
41 instances of rRNA,12S ribosomal RNA
```

## The loci CSV file

The additional output file can be veiwed by executing the next cell, using the linux `cat` command. Note that terminal commands can be executed from within this notebook by prefixing them with an exclamation mark `!`.

**Task: Use the cat command to write Tetillidae_loci.csv in this notebook**

This output is the loci CSV file which can be used to instruct ReproPhylo which loci to include in the analysis and which data type (DNA or protein) to analyse.

In [16]:
```
!cat Tetillidae_loci.csv
```

```
dna,rRNA,18s,18S ribosomal RNA,18S rRNA,small subunit 18S ribosomal RNA
dna,rRNA,28s,28S large subunit ribosomal RNA,28S ribosomal RNA
dna,rRNA,5.8S_rRNA,5.8S rRNA
dna,rRNA,5.8S_ribosomal_RNA,5.8S ribosomal RNA
dna,rRNA,5S_rRNA,5S rRNA
dna,CDS,ALD,ALD
dna,CDS,ATP9,ATP9,atp9
dna,CDS,ATP_synthase_beta_subunit,ATP synthase beta subunit
dna,CDS,CchGa,CchGa
dna,CDS,CchGb,CchGb
dna,CDS,CchGc,CchGc
dna,CDS,CchGd,CchGd
dna,CDS,CchGe,CchGe
dna,CDS,CchGf,CchGf
dna,CDS,Hsp70,Hsp70
dna,CDS,MAT,MAT
dna,CDS,MT-ATP6,atp6,ATP6
dna,CDS,MT-ATP8,atp8,ATP8
dna,CDS,MT-CO1,coi,COI,cox1,COX1,coxI
dna,CDS,MT-CO2,cox2,COX2
dna,CDS,MT-CO3,cox3,COX3
dna,CDS,MT-CYB,CYTB
dna,CDS,MT-ND1,nad1,ND1
dna,CDS,MT-ND2,nad2,ND2
dna,CDS,MT-ND3,nad3,ND3
dna,CDS,MT-ND4,nad4,ND4
dna,CDS,MT-ND4L,ND4L
dna,CDS,MT-ND5,nad5,ND5
dna,CDS,MT-ND6,nad6,ND6
dna,CDS,TPI,TPI
dna,CDS,alg11,alg11
dna,CDS,catalase,catalase
dna,CDS,cob,cob
dna,CDS,ef1a,elongation factor 1 alpha
dna,CDS,nad4L,nad4L
dna,CDS,putative_LAGLIDADG_protein,putative LAGLIDADG protein
dna,rRNA,rnl,rnl
dna,rRNA,rns,rns
dna,rRNA,rrnL,rrnL
```



This file has one line for each locus, where each line contains at least four, comma separated values. The first value is the character type

(either `dna` or `prot`). The second is the type of locus (feature type in genbank terminology). It can be CDS, rRNA, tRNA or anything else. It has to match to at least one feature type in your genebank file in order for it to have any effect (more below). The third value is the locus name, and any value thereafter is a synonym which might be used in genbank as a gene name or product name. The name cannot have any white spaces in it, while the aliases need to appear as they do in the genbank file. Note that in some cases, such as in the first line (the 18S gene) synonyms have been pooled together into one line. In other cases, such as in the last line, (the rrnL gene, which also apears as rnl two lines above) synonyms were not recognised by the `list_loci_in_genbank()` function and have remained in seperate lines.
In order to tell ReproPhylo that these two lines are the same gene, a shared integer can be added to both lines in this manner:

```
dna,rRNA,rnl,rnl,5
dna,rRNA,rns,rns
dna,rRNA,rrnL,rrnL,5
```

I have used the number five to show that as long as the lines to join share a number, it could be any number at all. Also, it's important to remember the comma before the integer. Otherwise the number will be taken as a part of the last alias. Another possible way to influence the analysis by editing the loci CSV is to change the first value from `dna` to `prot`, or to delete lines containing loci which are not interesting. Note that loci matching less than four unique sequences in the genbank file will be dropped automatically in subsequent stages of the analysis.
**Task: It is possible to edit the loci CSV within this notebook, by starting a code cell with the line `%%file filename`. When this type of cell is executed with `shift+enter`, its content, excluding the first line, will be written to the file `filename`. In the code cell below, copy and pase the loci CSV, edit it so that it will contain only the 18s, 28s and cox1 genes, and save it to a file called Tetillidae_loci_edited.csv. We will carry these three loci through the analysis.**

In [17]:
```
%%file Tetillidae_loci_edited.csv
dna,rRNA,18s,18S ribosomal RNA,18S rRNA,small subunit 18S ribosomal RNA
dna,rRNA,28s,28S large subunit ribosomal RNA,28S ribosomal RNA
dna,CDS,MT-CO1,coi,COI,cox1,COX1,coxI
```

```
Overwriting Tetillidae_loci_edited.csv
```

## Starting a ReproPhylo Project

Now that we have a grasp of the loci content of our data and have prepared a loci file, we can use it to start a ReproPhylo `Project`. This is achived by saving a `Project()` instance into a variable and providing it with a loci file name:
`pj = Project("loci_file_name")`

**Task: Use the loci CSV file you have edited above ("Tetillidae_loci_edited.csv") to start a `Project` instance in the cell below**

In [57]:
```
pj = Project("Tetillidae_loci_edited.csv")
```

## Reading data from a genbank file into the `Project` instance

The `Project` object has a bunch of methods that allow to read data, manage it, configure analyses and runing them. In python, methods are called using a dot notation: `object.method(the, method, variables)`. Variables can be positional, ie their meaning is determined by their position. These are required. In additional, sometimes there are optional variables, in which case they will be assigned using the format `keyword=value`. Reading a genbank file, or several genbank files is done with the method `read_embl_genbank`. It recieves one positional argumnet, which is a list of one or more genbank filenames, as follows:

```
pj.read_embl_genbank(["file1.gb"])
```


or

As the files are read, only loci that correspond to loci that were indicated in the loci CSV file will be read, and others will be dropped.
**Task: Read the file `Tetillidae.gb` into the `Project` using the method `read_embl_genbank()`.**
As its name conveys, embl formatted files are just as welcome.

In [58]:
```
pj.read_embl_genbank(["Tetillidae.gb"])
```

```
[master a084006] 1 genbank/embl data file(s) from Sun Nov  9 21:06:43 2014
 1 file changed, 15099 insertions(+)
 create mode 100644 Tetillidae.gb
```

Since we are running git at the background, reading the file invoked a massage from git saying the file was registered in the repository. ## Exploring the OTU content of the data Now that we specified loci to analyse and also provided sequence data and its associated metadata, we can write a table summarising the species repesentation for each locus. This is done with the `species_vs_loci` method, which is used as follows:

```
pj.species_vs_loci("species_vs_loci_table_output.csv")
```

**Task: Write a summary table to the file "species.csv" using the `species_vs_loci` method.**

In [20]:
```
pj.species_vs_loci("species.csv")
```

A utility function called `view_csv_as_table` can be used to print the table into this notebook for observation. It is not a `Project` method and is thus simply used as follows:

```
view_csv_as_table("input.csv","separator")
```

where separator is the character used to separate the values to cloumns. In this case tha separator is `"\t"` (tab).
**Task: Use the `view_csv_as_table` function to print "species.csv" in this notebook**

[] In [21]:
```
view_csv_as_table("species.csv","\t")
```

| species | 18s | 28s | MT-CO1 |
|---|---|---|---|
| Acanthotetilla celebensis | 0 | 1 | 1 |
| Acanthotetilla cf. seychellensis 0CDN8107-V | 1 | 0 | 0 |
| Acanthotetilla walteri | 0 | 1 | 3 |
| Amphitethya cf. microsigma AS-2012 | 0 | 1 | 1 |
| Calthropella geodioides | 0 | 1 | 1 |
| Cinachyra antarctica | 0 | 1 | 3 |
| Cinachyra barbata | 1 | 1 | 1 |
| Cinachyrella alloclada | 3 | 1 | 2 |
| Cinachyrella anomala | 0 | 2 | 5 |
| Cinachyrella apion | 1 | 1 | 4 |
| Cinachyrella australiensis | 3 | 4 | 16 |
| Cinachyrella cf. paterifera 0M9H2022-P | 1 | 0 | 0 |
| Cinachyrella cf. schulzei | 0 | 1 | 0 |
| Cinachyrella kuekenthali | 3 | 0 | 5 |
| Cinachyrella levantinensis | 4 | 4 | 6 |
| Cinachyrella paterifera | 0 | 1 | 2 |
| Cinachyrella schulzei | 2 | 1 | 2 |
| Cinachyrella sp. 1A KRC-2012 | 0 | 0 | 1 |
| Cinachyrella sp. 1B KRC-2012 | 0 | 0 | 1 |
| Cinachyrella sp. 22-XI-02-1-13 N25 | 1 | 0 | 0 |
| Cinachyrella sp. 24-XI-02-3-2 N28 | 1 | 0 | 0 |
| Cinachyrella sp. 3-1 | 1 | 0 | 0 |
| Cinachyrella sp. 3-10 | 1 | 0 | 0 |
| Cinachyrella sp. 3-9 | 1 | 0 | 0 |
| Cinachyrella sp. 3473 | 1 | 1 | 1 |
| Cinachyrella sp. AS-2010 | 0 | 0 | 2 |
| Cinachyrella sp. MI-2012 | 2 | 0 | 0 |
| Cinachyrella sp. USNM 1204826 | 1 | 0 | 0 |
| Cinachyrella sp. USNM 1204829 | 1 | 0 | 0 |
| Cinachyrella sp. sp11 | 1 | 0 | 0 |
| Craniella cf. leptoderma AS-2012 | 4 | 7 | 6 |
| Craniella cranium | 1 | 1 | 1 |
| Craniella neocaledoniae | 1 | 0 | 0 |
| Craniella sagitta | 1 | 1 | 3 |
| Craniella sp. 0CDN5142-X | 1 | 0 | 0 |
| Craniella sp. 2-7 | 1 | 0 | 0 |
| Craniella sp. 3318 | 1 | 0 | 1 |
| Craniella sp. 3878 | 2 | 1 | 2 |
| Craniella sp. AS-2010 | 0 | 0 | 1 |
| Craniella sp. PC-2011 | 0 | 1 | 1 |
| Craniella zetlandica | 1 | 1 | 2 |
| Fangophilina sp. MK-2012 | 3 | 3 | 2 |
| Geodia barretti | 0 | 0 | 1 |
| Geodia cydonium | 1 | 0 | 0 |
| Geodia neptuni | 1 | 0 | 1 |
| Pachymatisma johnstonia | 0 | 1 | 1 |
| Paratetilla bacca | 2 | 3 | 6 |
| Paratetilla sp. 2209 | 0 | 1 | 0 |
| Paratetilla sp. 2656 | 0 | 1 | 1 |
| Paratetilla sp. KRC-2012 | 0 | 0 | 1 |
| Tetilla sp. | 2 | 1 | 1 |

## Reading denovo sequence data

Usually, when reconstructing a phylogenetic tree, we would like to include our on novel sequecnes. The `Porject` method `read_denovo` is desined to do that. It has two positional paraameters. The first is a list of file names to read. The files have to be in the same format and to have the same sequence type. The second parameter is a string representing the sequence type, and could be either `dna` or `prot`. An optional parameter is the `format` parameter. By default it is set to `format="fasta"`, however it can be any format recognised by Biopython, including sequence alignments. If aligned sequences are read, the gaps will be removed. Here is a usage example:

```
pj.read_denovo(["filename1.fasta","filename.2.fasta"],'dna')
pj.read_denovo(["filename3.fasta"],'prot')
```

**Task: Read a novel DNA sequence from the file `Tetillidae_denovo_sequence.fasta` using the `Project` method `read_denovo()`**

In [59]:
```
pj.read_denovo(["Tetillidae_denovo_sequence.fasta"], "dna")
```

```
[master 999c808] 1 denovo data file(s) from Sun Nov  9 21:06:54 2014
 1 file changed, 2 insertions(+)
 create mode 100644 Tetillidae_denovo_sequence.fasta
```

Out[59]: 1

Once again we are informed that git has recorded the new data file. Now the sequence is in the `Project`. However, it discloses no information that will allow us to utilize it. In order for ReproPhylo to make sense of it we need to provide information about the gene it contains. If it contains a coding sequence, we might want to specify an open reading frame so that it will be translated and provide us with a protein sequence. Even if we want to analyse DNA sequences, if a protein sequence is available we then have the option of codon-aligning our sequences. To get a protein sequence automatically, we must provide the reading frame, the location of the exons, the strand and the translation table, as feature qualifiers.

The next code cell will add a feature to our denovo sequence and will include all the relevant information to allow a translation to be crated for us.

In [60]:
```
qualifiers={'gene': 'cox1',
            'transl_table': 4,
            'codon_start': 1,
            'organism': 'Craniella microsigma'}
for record in pj.records:
    if 'denovo' in record.id: # New sequences are assigned with IDs starting
                              # with 'denovo'. See below.
        pj.add_feature_to_record(record.id, 'CDS',
                                 #The location is specified as a list
                                 # of lists. Every sub-list is an exon
                                 # and has the start, the end and the strand.
                                 # The numbers are "real" positions and not
                                 # machine. ie, counting starts from 1.
                                 location=[[1,786,1],[1742,2092,1]],
                                 qualifiers=qualifiers)
```



## Editing the metadata programatically

The GenBank file comes with a lot of metadata. However, this data needs to be organized a bit and also extended for it to be useful. Only feature qualifiers can be manipulated (ie, not source qualifiers). The following cell shows how to copy a qualifier from the source to the features, where it can be manipulated. For this we'll use the `add_qualifier_from_source` method.

In [61]:
```
pj.add_qualifier_from_source('organism')
```

We need to use the info in the `organism` qualifier to create a new qualifier containing only the genus. The code below loops over the tetillid genera and puts the genus name in the new `genus` qualifier, if this name is found in the `organism` qualifier. This is done by the `if_this_than_that` method. We will use the `part` mode to indicate that while searching for the genus name in the species name we do not require the whole `organism` value to fit.

In [62]:
```
for genus in ['Cinachyrella','Cinachyra','Craniella','Tetilla',
              'Acanthotetilla','Amphitethya','Fangophilina',
              'Paratetilla']:
    pj.if_this_then_that(genus, 'organism', genus, 'genus',
                         mode='part')
```

Several additional methods for editing the metadata programatically can be used. In the next cell, place the curser right after the dot and click the tab key. A list of all the `Project` methods will appear. You may review it to see all the methods related to metadata manipulations as well as others.

In []:
```
pj.
```

## Editing the metadata manually

Next we are going to print out a spreadheet of all the metadata in the `Project`:

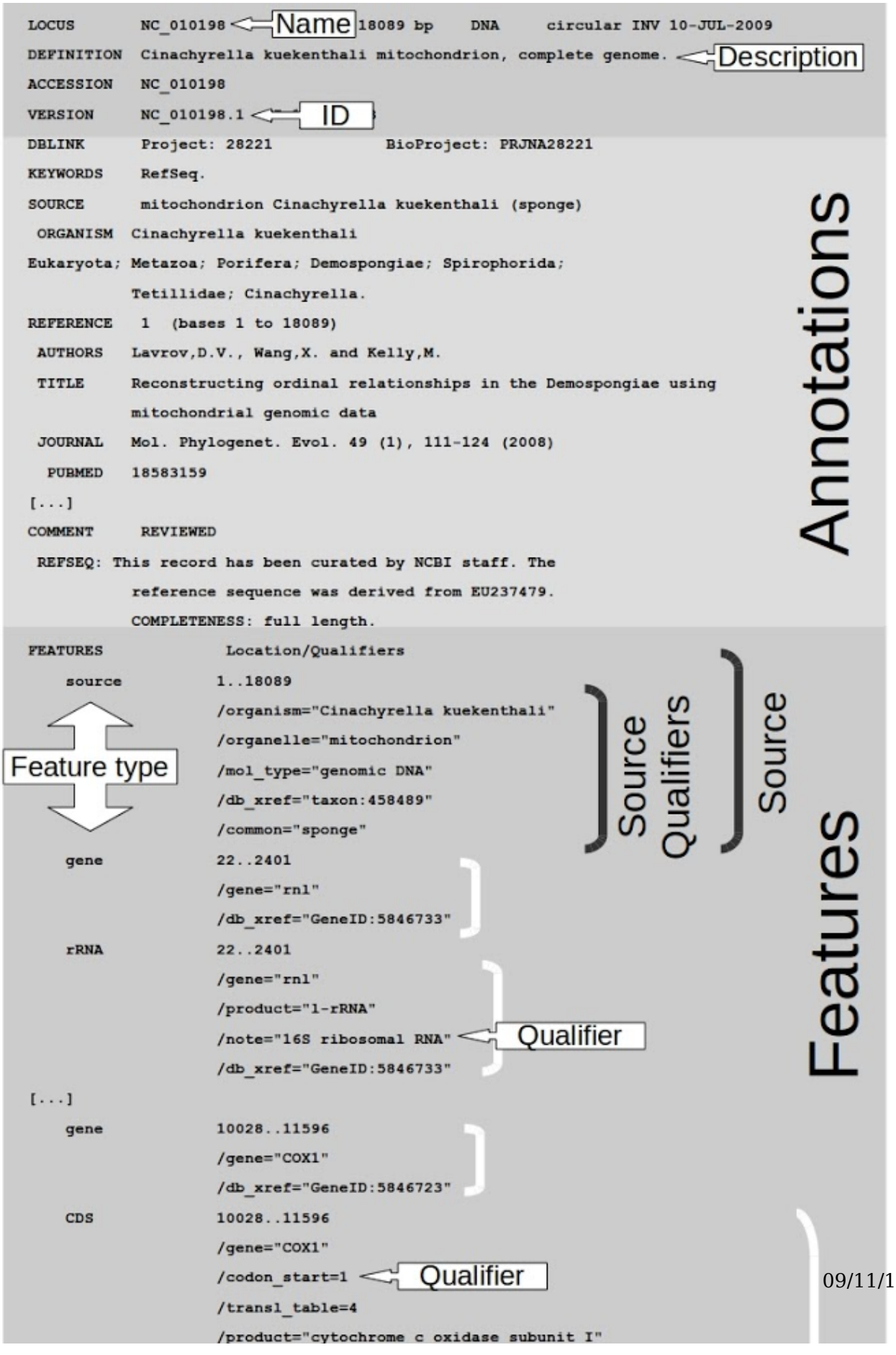
In [54]:
```
pj.write('Tetillidae_metadata.csv',format='csv')
```

The metadata can be also controlled by editing the metadata spreadsheet and reading it back to into the `Project`. This can be done in Excel or Libreoffice. However, while using any of those programs, be very aware to the errors (http://nsaunders.wordpress.com/2012/10/22/gene-name-errors-and-excel-lessons-not-learned/) that might be introduced by the autocorrect, autocomplete and autoformat functions running in there.

So, now that we know we need to be carefull, open the file 'Tetillidae_metadata.csv' in Excel. Try to spot the new **genus** qualifier you have created above. In order to describe the content of these file we'll be using Biopython and GenBank terminology, as illustrated in this figure:

[] In [27]:
```
# execute if figure is small
from IPython.display import Image
Image(filename='genbank_terminology.jpg')
```

Out[27]:



**Record ID**. Column **B** is a short representation of the **Seq**, showing it's 5' and 3' portions. This can help validate the record's identity if a doubt arises. Columns **C** through **W** are the source feature qualifiers. Column **X** is the taxonomy line from the **ORGANISM** field in the **Annotations** section of the GenBank record.

A GenBank file may have several sequence features (ie, loci) described. However, it will always have only a single source feature. The information in the source feature is relevant to all the features in the record. For this reason, each and every line in the metadata spreadsheet, starts with the srouce feature qualifiers. Say a GenBank record includes a source feature and five additional features. This will be represented in the spreadsheet as five lines, one for each non-source feature. Across the five lines, the first few columns will be identical as they are derived from the shared source feature. The five lines (representing the five features) will be assigned feature IDs, which consist of the record ID and the suffixes _f0 to _f4 (eg, **NC_010198_f3**).

Now scroll down to the very end of the file, to see your denovo sequence. You'll notice that it has been assigned the record ID **denovo0**, a source ID **denovo0_source** and a feature ID **denovo_f0**. The first word in the fasta header is recorded as **original_id** and the remaining of the header as **original_desc**, short for **Description**. Find the **translation** column to make sure that a protein sequence has been created. You may move or copy these values to other columns, programmatically as we did above, or manually, by editing the spreadsheet as we will do next. At the moment, source qualifiers cannot be edited programatically. They can be copied to the feature qualifiers and edited there. It is, however, possible to edit them in the spreadsheet.

In order to proceed with the analysis there are a few additional things we need to tidy up in our metadata. First, we should include some morphological information so that we can annotate our trees with it (it will also be available to anyone wanting to carry the trees out of reprophylo to do some character evolution tests). To do this, first add the three columns **porocalyx**, **cortex** and **calthrops** at the right hand side of the table. These are tetillid morphological features. Now sort the spreadsheet according to the **genus** clounm. Add the values bellow to each feature, based of it's genus. You can copy the values to the first row in each genus and then drag them down all the way to the last line of this genus. Can you think of a way to this in a script using the `if_this_than_that` method?

| genus | porocalyx | cortex | calthrops |
|---|---|---|---|
| Cinachyrella | 2 | 1 | 1 |
| Paratetilla | 2 | 1 | 2 |
| Amphitethya | 2 | 1 | 1 |
| Acanthotetilla | 2 | 1 | 1 |
| Fangophilina | 2 | 1 | 1 |
| Craniella | 1 | 2 | 1 |
| Cinachyra | 2 | 2 | 1 |
| Tetilla | 1 | 1 | 1 |

Next, we want to indicate our outgroup species. In fact, any line that has not been assigned with the genus (ie, it says 'null') is in the outgroup order 'Astrophorida'. In the column genus, replace 'null' with 'Astrophorida'. The word 'null' represents a qualifier that does not exist in the feature. Therefore, we cannot use the word 'null' as the outgroup value in the genus column. The genus qualifier will not exist for features with the value 'null', unless we change it to something else.

In addition, we want to make sure we will be able to label our new sequence with it's species name. So copy the sepcies name from the **original_desc** column to the **source:_organism** column, ommiting the gene name, cox1.



Last, there is a coding sequence, **AM076987.1_f1**, encoded from within a cox1 mitochondrial intron, which does not belong to the cox1

CDS. Yet, it has the value 'cox1' in its gene qualifier. To make sure this sequence does not end up in our cox1 dataset, change its gene qualifier value from cox1 to laglidadg.

Now, save the file and make sure you retain the tab delimited CSV format.
The method `correct_metadata_from_file` will modify the data feature qualifiers according to the canges made to the CSV file. It used as follows:

In [63]:
```
pj.correct_metadata_from_file("Tetillidae_metadata_edited.csv")
```

## Reporting sequence statistics

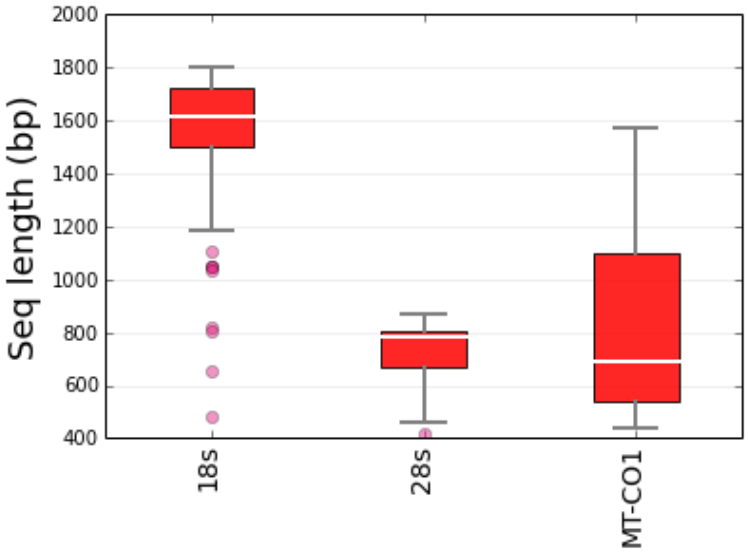
After making sure the sequences are assigned with the correct gene or product name, we can split them to their repsective datasets by using this method:

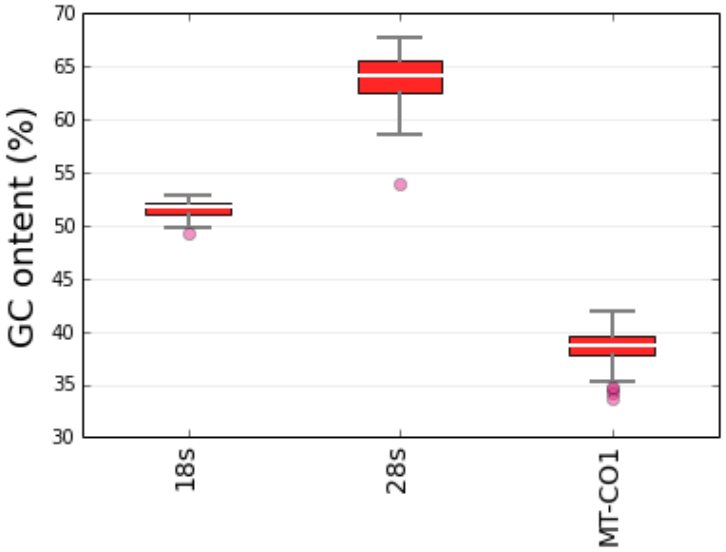
In [64]:
```
pj.extract_by_locus()
```

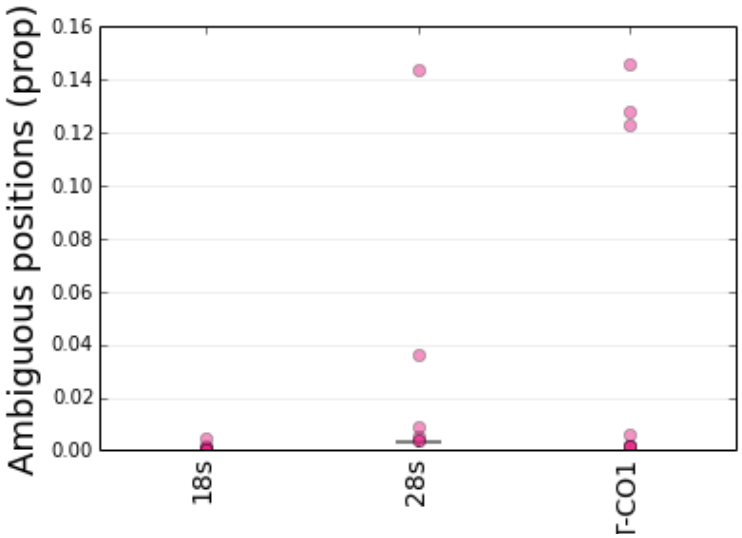
Once this is done, the first thing ReproPhylo can do with the data is to report some sequence statistics:

[] In [65]:
```
%matplotlib inline
pj.report_seq_stats()
```

```
Distribution Of Sequence Lengths
Distribution Of Sequence Statistic "Gc_Content"
Distribution Of Sequence Statistic "Nuc_Degen_Prop"
Distribution Of Sequence Statistic "Prot_Degen_Prop"
```



## Configuring and running the sequence alignment

Analyses in ReproPhylo are configured using `Conf` objects and are then run by passing the `Conf` object to the respective `Project` method. For example, a sequence alignment run will be configured by making an `AlnConf` object and then passing it to the `align` method. The next two code cells make two `AlnConf` objects. The first one configures the program `MAFFT` to run the `L-ins-i` algorithm to align the MT-CO1 sequences. `MAFFT` is the default program so it is not explisitly specified.

In [66]:
```
# Mafft linsi algorithm
mafftLinsi = AlnConf(pj,
                     method_name='mafftLinsi',
                     loci=['MT-CO1'],
                     cline_args=dict(localpair=True, maxiterate=1000))
```

```
mafft --localpair --maxiterate 1000 906441415567281.93_CDS_proteins_MT-CO1.fasta
```

The `AlnConf` object reports the command lines that will be run. The next cell configures the program `MUSCLE` to align the rRNA loci 18s and 28s.

In [67]:
```
rRNA = ["18s","28s"]

muscle = AlnConf(pj, loci=rRNA,
                 method_name='MuscleDefaults',
                 program_name='muscle')
```

```
muscle -in 902871415567283.41_18s.fasta
muscle -in 902871415567283.41_28s.fasta
```

The complete set of parameters in the `AlnConf` object are described here:

```
AlnConf(pj,                          # This is the Project object
        method_name='mafftDefault',  # Any string
        CDSAlign=True,               # By default, CDSs will be codon aligned,
        program_name='mafft',        # Currently takes "mafft" or "muscle"
        cmd='mafft',                 # Or if not the env var $path, change to the full path
        loci='all',                  # Or a list of loci names, as appear in the CSV
        cline_args={})               # Any mafft or muscle keyward and argument
```

We can run both the `AlnConf` objects we have configured in one go, using the `align` method, which takes a single parameter, an `AlnConf` object(s) list.

In [68]:
```
pj.align([mafftLinsi,
          muscle])
```

## Checkpoingting the analysis

One the sequence alignment is done, the results only exist in the `Project` object in memory. It's a good idea to checkpoint the analysis by saving it to a file. `Project` objects are saved to pickle files using the `pickle_pj` function as follows:

```
pickle_pj(pj, "pickle_filename")
```

The `Project` can be revived from a pickle file with the `unpickle_db` function:

```
pj = unpickle_pj("pickle_filename")
```

In [69]:
```
pickle_pj(pj, "tetillidae_tutorial")
```

```
[master ff58852] A pickled Project from Sun Nov  9 21:08:47 2014
 1 file changed, 0 insertions(+), 0 deletions(-)
 create mode 100644 tetillidae_tutorial
```

Out[69]:
```
'tetillidae_tutorial'
```

because we are using version control, we can overwrite the pickle file each time we checkpoint. If we need to role back to any overwritten version, git will allow us to do that.

## Configuring and running the alignment trimming

As of now, trimAl is the only program used for alignment trimming in ReproPhylo. Trimming is carried out in a similar way to the sequence alignment stage. These are the default settings of the `TrimalConf` object.

```
TrimalConf(pj,                                  # The Project file
           method_name='gappyout',              # Any string
           program_name='trimal',               # The program name, no alternative to trimal a
s of no
           cmd='default',                       # Can be overidden if trimal is not in $PATH
           alns='all',                          # Or a list of loci names, as appear in the CS
V
           trimal_commands={'gappyout': True})  # Any trimal keyword and arg which are
                                                # related to trimming. Others will be ignored.
```

We'll trim the alignments using the default settings which is the gappyout option in trimAl:

In [70]:
```
gappyout = TrimalConf(pj)
```

```
trimal -in 906581415567328.09_28s@MuscleDefaults.fasta -gappyout
trimal -in 906581415567328.09_18s@MuscleDefaults.fasta -gappyout
trimal -in 906581415567328.09_MT-CO1@mafftLinsi.fasta -gappyout
```

In [71]:
```
pj.trim([gappyout])
```

## Configuring and running the tree reconstruction

RAxML is currently the only phylogenetic reconstruction program. The algorithm if set using the `preset=` keyword and takes either the value `fa` for a single ML search with rapid bootstrap, `fD_fb` for ML search(es) with relBootstrap or `fd_b_fb` for ML search(es) with thorough bootstrap. The model is passed with `model=` keyword and a protein substitution matrix with `matrix=`. The number of threads to run is set with `threads=` any additioanl argument can be passed in a dictionary to `cline_args=`. The number of ML searches would be passed as `-N: 100` in this dictionary, and the number of bootstrap replicates as `-#: 100`. The full default settings are:

```
[]  RaxmlConf(pj,
            method_name='fa',
            program_name='raxmlHPC-PTHREADS-SSE3',
            cmd='default',
            preset='fa',
            alns='all',
            model='GAMMA',
            matrix='JTT',
            threads=4,
            cline_args={})
```

Since this is a first pass of the data we can choose `fD_fb` as the preset as relBootstrap is extremely fast.

In [72]:
```
raxml = RaxmlConf(pj, method_name="fD_fb", preset="fD_fb")
```

```
raxmlHPC-PTHREADS-SSE3 -f D -m GTRGAMMA -n 856861415567330.81_28s@MuscleDefaults@gappyout
0 -p 139 -s 856861415567330.81_28s@MuscleDefaults@gappyout.fasta -T 4 -N 1
raxmlHPC-PTHREADS-SSE3 -f b -m GTRGAMMA -n 856861415567330.81_28s@MuscleDefaults@gappyout
1 -p 878 -s 856861415567330.81_28s@MuscleDefaults@gappyout.fasta -t RAxML_bestTree.856861
415567330.81_28s@MuscleDefaults@gappyout0 -T 4 -z RAxML_rellBootstrap.856861415567330.81_
28s@MuscleDefaults@gappyout0
raxmlHPC-PTHREADS-SSE3 -f D -m GTRGAMMA -n 856861415567330.81_18s@MuscleDefaults@gappyout
0 -p 217 -s 856861415567330.81_18s@MuscleDefaults@gappyout.fasta -T 4 -N 1
raxmlHPC-PTHREADS-SSE3 -f b -m GTRGAMMA -n 856861415567330.81_18s@MuscleDefaults@gappyout
1 -p 187 -s 856861415567330.81_18s@MuscleDefaults@gappyout.fasta -t RAxML_bestTree.856861
415567330.81_18s@MuscleDefaults@gappyout0 -T 4 -z RAxML_rellBootstrap.856861415567330.81_
18s@MuscleDefaults@gappyout0
raxmlHPC-PTHREADS-SSE3 -f D -m GTRGAMMA -n 856861415567330.81_MT-CO1@mafftLinsi@gappyout0
 -p 437 -s 856861415567330.81_MT-CO1@mafftLinsi@gappyout.fasta -T 4 -N 1
raxmlHPC-PTHREADS-SSE3 -f b -m GTRGAMMA -n 856861415567330.81_MT-CO1@mafftLinsi@gappyout1
 -p 839 -s 856861415567330.81_MT-CO1@mafftLinsi@gappyout.fasta -t RAxML_bestTree.85686141
5567330.81_MT-CO1@mafftLinsi@gappyout0 -T 4 -z RAxML_rellBootstrap.856861415567330.81_MT-
CO1@mafftLinsi@gappyout0
```

In [73]:
```
pj.tree([raxml])
```

## Printing tree figures

The `Project` method `annotate` annotates all the trees uniformly. It has the following options:

[]
```
annotate(fig_folder,                    # A local path in which to write figures - requiered
         root_meta,                     # A qualifier name to get root value from - requiered
         root_value,                    # The value indicating outgourp taxa - required
                                        # use 'mid' in root_meta and root_value for midpoint ro
ot

         leaf_labels_txt_meta,          # A list of qualifiers to use in leaf labels - required
         leaf_node_color_meta=None,     # To color labels, provide a qualifier which will deter
mine the color
         leaf_label_colors=None,        # and provide a dictionary with those values as keys an
d color
                                        # as values

         node_bg_meta=None,             # To color clade backgrounds, provide a qualifier which
 will determine the color
         node_bg_color=None,            # and provide a dictionary with those values as keys an
d color
                                        # as values

         node_support_dict=None,        # A dictionary of the form ("color": [high_value,low_va
lue]

         heat_map_meta = None,          # list of qualifies with numeric values
         heat_map_colour_scheme=2,

         multifurc=None,                # A support value under which a node will bo collapsed

         scale = 1000                   # The tree width

         html = None                    # A file name
         )
```

we'll make a simple annotation at this stage.

In [74]:
```
supports = {'black':[100,99],
            'dimgray':[99,75],
            'silver':[75,50]}

pj.annotate('.', 'genus','Astrophorida',['organism'],
            node_support_dict=supports,
            html="tetillidae_figures")
```

In [75]:
```
pickle_pj(pj, "tetillidae_tutorial")
```

```
[master 847a87e] A pickled Project from Sun Nov  9 21:09:20 2014
 1 file changed, 0 insertions(+), 0 deletions(-)
```

Out[75]:
```
'tetillidae_tutorial'
```

## Making a concatenated matrix

The final product in a phylogenetic analysis would usually be a phylogenetic tree based on a supermatrix. ReproPhylo can handle the concatenation of sequence alignments for this purpose.

To do that, the user needs to provide information that indicates which sequences shoudl be concatenated into a single line in the supermatrix. In this tutorial we will use the `specimen_vaucher` to tie together sequences of different genes that belong to the same sample or OTU. However, some of the genbank records miss this qualifiers. In othe cases, the voucher numbers are spelled slightly differently in different records. All of this could be fixed manually in the metadata CSV, or programatically.

First, we add the `specimen_voucer` to records that miss it:

In [82]:
```
pj.add_qualifier_from_source('specimen_voucher')

# Add missing/ correct wrong specimen vouchers according to feature id

add_specimen_voucher = [[['JX177968.1_f0'],'specimen_voucher','QMG_321405'],
                        [['JX177913.1_f0',
                          'JX177935.1_f0',
                          'JX177965.1_f0'],'specimen_voucher','TAU_25617'],
                        [['JX177903.1_f0',
                          'JX177938.1_f0'],'specimen_voucher','TAU_25618'],
                        [['HM032740.1_f0',
                          'JX177964.1_f0'],'specimen_voucher','TAU_25621'],
                        [['HM032739.1_f0',
                          'JX177962.1_f0'],'specimen_voucher','TAU_25622'],
                        [['JX177968.1_f0'],'specimen_voucher','QMG_321405'],
                        [['JX177891.1_f0'],'specimen_voucher','RMNH_POR_3100'],
                        [['JX177900.1_f0',
                          'JX177926.1_f0'],'specimen_voucher','TAU_25620'],
                        [['JX177901.1_f0',
                          'JX177961.1_f0',
                          'JX177956.1_f0'],'specimen_voucher','TAU_25619'],
                        [['HM032742.1_f0',
                          'JX177957.1_f0'],'specimen_voucher','MNRJ_576']]
for add in add_specimen_voucher:
    pj.add_qualifier(add[0],add[1],add[2])

# Reformat specimen voucher according to the specimen voucher
```

Then, we correct spelling differences:

```
correct_specimen_voucher = [['QMG321405','specimen_voucher','QMG_321405','specimen_voucher'],
                            ['MHNM 16194','specimen_voucher','MHNM_16194','specimen_voucher'],
                            ['TAU 25456','specimen_voucher','TAU_25456','specimen_voucher'],
                            ['QMG320636','specimen_voucher','QMG_320636','specimen_voucher'],
                            ['QMG320270','specimen_voucher','QMG_320270','specimen_voucher'],
                            ['ZMBN:85239','specimen_voucher','ZMBN_85239','specimen_voucher'],
                            ['QMG318785','specimen_voucher','QMG_318785','specimen_voucher'],
                            ['QMG316342','specimen_voucher','QMG_316342','specimen_voucher'],
                            ['QMG314224','specimen_voucher','QMG_314224','specimen_voucher'],
                            ['VM14754','specimen_voucher','VM_14754','specimen_voucher'],
                            ['ZMBN:85240','specimen_voucher','ZMBN_85240','specimen_voucher'],
                            ['ZMBN:81789','specimen_voucher','ZMBN_81789','specimen_voucher'],
                            ['ZMBN:81787','specimen_voucher','ZMBN_81787','specimen_voucher'],
                            ['ZMBN:81785','specimen_voucher','ZMBN_81785','specimen_voucher']]
for correction in correct_specimen_voucher:
    pj.if_this_then_that(correction[0],correction[1],correction[2],correction[3])

# Make a qualifier that will be used to concatenate OTU sequences
```

Now, for the outgroup, we have no prior knowlage of their voucher numbers nor does it exists in the genbank file. We still want to tie together sequences of the same species. We therefore add a new qualifier, `OTU_dict` that will include the `specimen_voucher` info where it exists, and species names for outgroup speceis:

In [84]:
```
pj.copy_paste_within_feature('specimen_voucher','OTU_dict')

# Add missing values to the OTU dictionary

add_to_concatenation_dict=[[['AY737635.1_f0',
                             'AY320032.1_f0'],'OTU_dict','Geodia_neptuni'],
                           [['EF564339.1_f0',
                             'HM592832.1_f0'],'OTU_dict','Pachymatisma_johnstonia'],
                           [['HM592717.1_f0',
                             'HM592765.1_f0'],'OTU_dict','Thenea_levis'],
                           [['HM592745.1_f0',
                             'HM592820.1_f0'],'OTU_dict','Theonella_swinhoei'],
                           [['KC762708.1_f0',
                             'NC_010198.1_f0'],'OTU_dict','Cinachyrella_kuekenthali'],
                           [['HM592705.1_f0',
                             'HM592826.1_f0'],'OTU_dict','Calthropella_geodioides']]

for add in add_to_concatenation_dict:
    pj.add_qualifier(add[0],add[1],add[2])
```

The metadata is sorted and we can design the super matrix. The `Concatenation` class takes care of this:

In [85]:
```
combined = Concatenation(name='combined',                          # any string
                         loci=pj.loci,                             # In this case we want
 to include all the loci.
                         otu_meta='OTU_dict',                      # As explained above
                         concat_must_have_all_of=['MT-CO1'],       # Only species that ha
ve the genes in this list
                         concat_must_have_one_of =[['18s','28s']]) # Only species that ha
ve at least on gene in each sub-list
```

In [88]:
```
pj.add_concatenation(combined)
```

In [89]:
```
pj.make_concatenation_alignments()
```

```
52 individuals will be included in the concatenations combined
```

And now a tree can be built:

In [90]:
```
raxml_method_concat = RaxmlConf(pj, method_name='fD_fb_combined',
                                program_name='raxmlHPC-PTHREADS-SSE3',
                                preset = 'fD_fb', alns=['combined'],
                                model='GAMMA', matrix='JTT', threads=4,
                                cline_args={'-N': 1})
```

```
raxmlHPC-PTHREADS-SSE3 -f D -m PROTGAMMAJTT -n 154301415570224.36_combined0 -q 1543014155
70224.36_combined_partfile -p 98 -s 154301415570224.36_combined.fasta -T 4 -N 1
raxmlHPC-PTHREADS-SSE3 -f b -m PROTGAMMAJTT -n 154301415570224.36_combined1 -q 1543014155
70224.36_combined_partfile -p 762 -s 154301415570224.36_combined.fasta -t RAxML_bestTree.
154301415570224.36_combined0 -T 4 -z RAxML_rellBootstrap.154301415570224.36_combined0
```

In [91]:
```
pj.tree([raxml_method_concat])
```

In [92]:
```
pj.clear_tree_annotations()
```

## Figures with heatmaps

In [93]:
```
supports = {'black':[100,99],
            'dimgray':[99,75],
            'silver':[75,50]}

pj.annotate('.', 'genus','Astrophorida',['organism'], html="figures_with_heatmap",
            heat_map_meta = ['porocalyx','cortex','calthrops'],
            heat_map_colour_scheme = 1,
            node_support_dict=supports)
```

In [52]:
```
pj.clear_tree_annotations()
```

## Figures with clade background colours

In [51]:
```
supports = {'black':[100,99],
            'dimgray':[99,75],
            'silver':[75,50]}

genera_colors = {'Tetilla':'purple',
                 'Cinachyra':'steelblue',
                 'Cinachyrella':'crimson',
                 'Craniella':'royalblue',
                 'Paratetilla':'darkred',
                 'Fangophilina':'mediumslateblue',
                 'Amphitethya':'firebrick',
                 'Acanthotetilla':'rosybrown'
                 }



pj.annotate('.', 'genus','Astrophorida',['organism'], html="figures_with_background",
            node_bg_meta="genus",
            node_bg_color=genera_colors,
            node_support_dict=supports)
```

## Archiving the results

As a final step, ReproPhylo conviniently archives essential outputs: A report describing the methods, a nexml file containing the sequence alignments and trees, a pickled `Project` file, a genbank file containing the records that were used in the analysis with the metadata

In [56]:
```
publish(pj, 'Tutorial_results', '.')
```

```
checking if file exists
reporter was called by publish
now printing species table
now making sequence statistics plots
now reporting concatenations
now reporting methods
now reporting alignment statistics
making RF matrix
reporting trees
pickling

[master de0c316] A pickled Project from Fri Nov  7 14:35:02 2014
 1 file changed, 0 insertions(+), 0 deletions(-)
 create mode 100644 Tutorial_results/Fri_07_Nov_2014_14:35:01.pkl

archiving
report ready

/usr/local/lib/python2.7/dist-packages/pandas/io/excel.py:626: UserWarning: Installed ope
npyxl is not supported at this time. Use >=1.6.1 and <2.0.0.
  .format(openpyxl_compat.start_ver, openpyxl_compat.stop_ver))
```

In []:
```

```