GLOSSary USER GUIDE

GLOSSary (GLobal Ocean 16S Subunit web accessible resource) is a metagenomics platform that allows user friendly explorations of 16S rRNA related sequences from environmental metagenomics efforts.

GLOSSary was conceived and is maintained by the BIOIN*for*MA service at the Stazione Zoologica Anton Dohrn.

Description

GLOSSary can be accessed by two main entry points: a "Keyword search" and a "BLAST search". Both pages can be reached from the GLOSSary main page (Figure 1) and they are cross-linked.

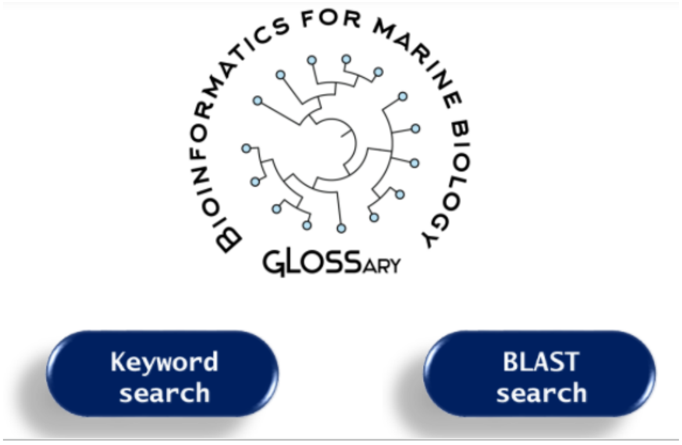


Figure 1. GLOSSary main page. The dark blue buttons redirect to the "Keyword search" and to the "BLAST search" web pages, respectively.

GLOSSary currently contains the 16S miTAGs collections from the Tara project [1] and processed sequences from the BIOIN*for*MA service. The pipeline of the work is available here.

“Keyword search” Web Page

The GLOSSary data can be accessed in the “Keyword search” page by an interactive map and searched by taxonomy affiliation, by sequence ID, by Tara station ID (Figure 2).

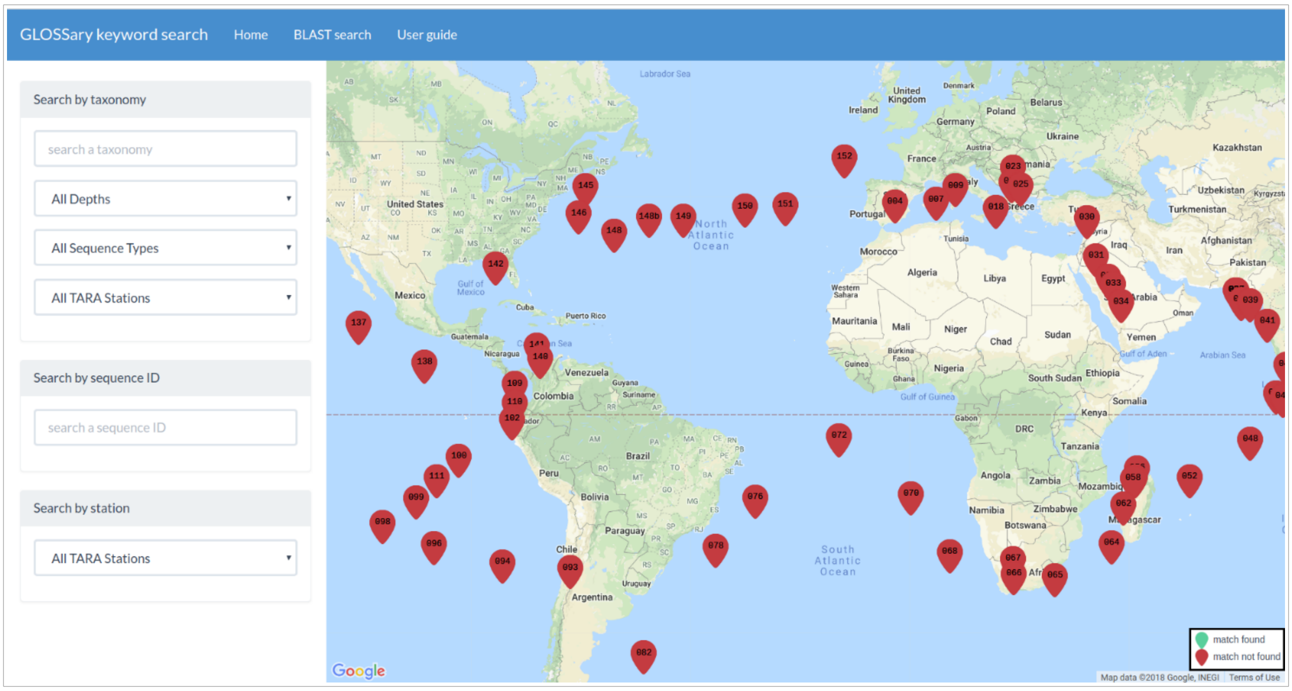
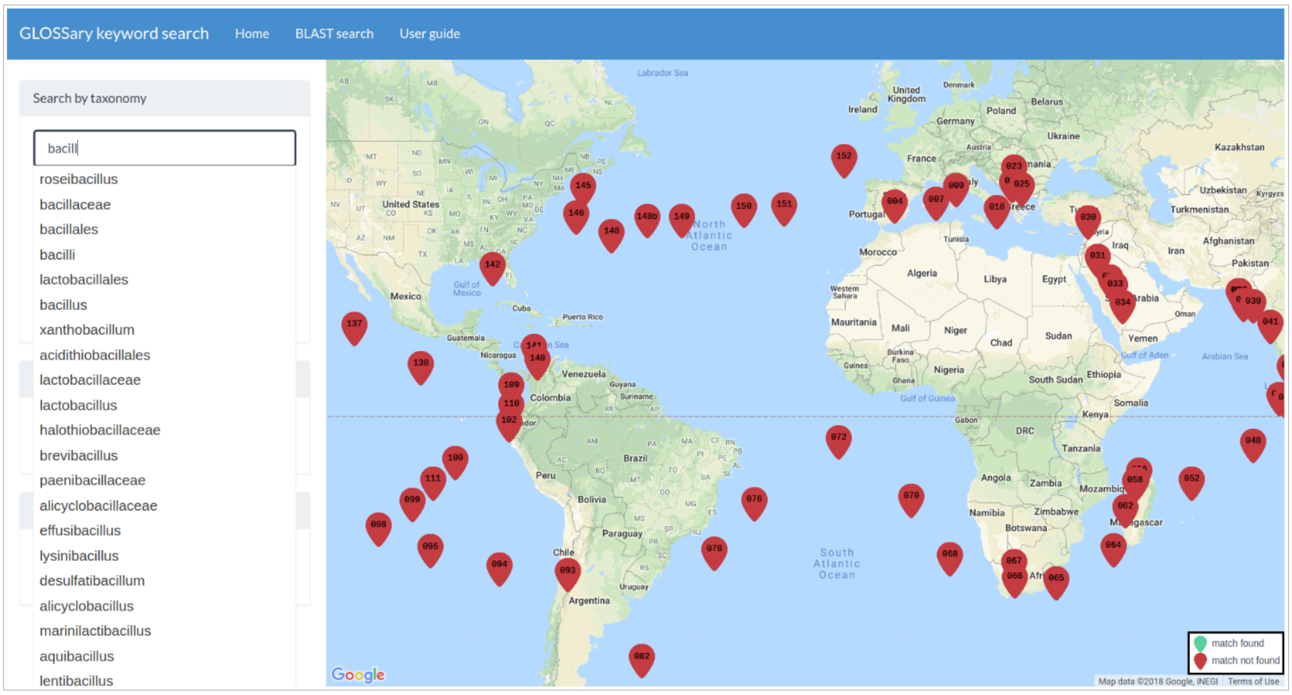


Figure 2. GLOSSary “Keyword search” web page. On the left, there are the “Search by taxonomy”, “Search by sequence ID” and the “Search by station” options; on the right, a map with red pointers, indicating the geographical position of the Tara stations in the world, is also displayed.

1) *“Search by taxonomy”*

The “*Search by taxonomy*” option allows to search by the available taxonomic affiliations. While typing in the *taxonomy* search field, a dropdown list will appear, allowing the user to narrow the search to the related terms (Figure 3). The list includes all the taxa that are associated to at least one of the sequences included in the database. Please be aware: if the selected taxonomic affiliation is highly inclusive (eg. a domain) the query could take a while to be performed. Please be patient!



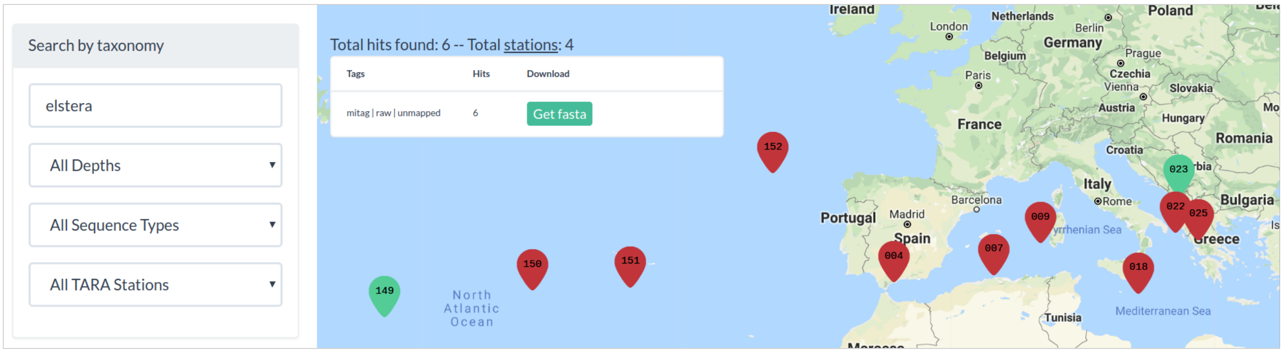
Search by taxonomy

bacill

roseibacillus
bacillaceae
bacillales
bacilli
lactobacillales
bacillus
xanthobacillum
acidithiobacillales
lactobacillaceae
lactobacillus
halothiobacillaceae
brevibacillus
paenibacillaceae
alicyclobacillaceae
effusibacillus
lysinibacillus
desulfatibacillum
alicyclobacillus
marinilactibacillus
aquibacillus
lentibacillus

Figure 3. Typing a name into the taxonomy field of the "Search by taxonomy" area. All the matching taxonomy affiliation (i.e. containing the inserted chars, for example "*bacill*" in the figure) will be displayed.

The page will show the corresponding results: the map will be updated with green pointers indicating the Tara stations including hits matching the query; the central section of the page will list all the sequences having the selected taxonomic affiliation. The total number of hits found is shown on the top together with the numner of involved stations. The results, grouped by Tag, are listed below (Figure 4). The map can be dragged an moved to better explore the resulting stations by holding the left button of the mouse.



Search by taxonomy

elstera

All Depths

All Sequence Types

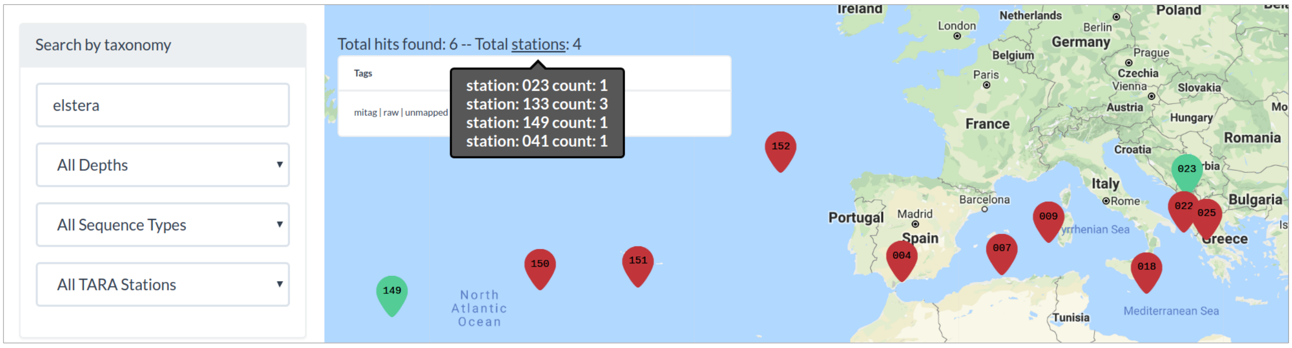
All TARA Stations

Total hits found: 6 -- Total stations: 4

Tags	Hits	Download
mitag | raw | unmapped	6	Get fasta

Figure 4. Results of a search by taxonomy. The hits found are grouped by tag. Pointers on the map are updated accordingly (green pointers).

By clicking with the mouse on the “stations” link of the summary line, it is possible to visualize the list counts for each station (Figure 5). This information helps the user in further refining the search by using the dropdown station filter as explained in the following.



Search by taxonomy

elstera

All Depths ▾

All Sequence Types ▾

All TARA Stations ▾

Total hits found: 6 -- Total stations: 4

Tags

mitag | raw | unmapped

station: 023 count: 1
station: 133 count: 3
station: 149 count: 1
station: 041 count: 1

Figure 5. Details on the station counts. Clicking with the mouse on the “stations” link shows the list of the resulting stations with the corresponding hit count.

The Tags are labels used to identify features of the extracted sequences according to the processing pipeline. They include, in the case of the contigs, the classification, the length and the contamination status. The miTAGs, instead, are classified in “mapped” or “unmapped”, indicating if they mapped or not on the contigs, using the VSEARCH aligner [2] (minimum coverage accepted 97%).

Detail of the possible values are described in Table 1.

sequence type	classification	length	contamination status
Contig	long	> 900bp	chimeraFree chimera borderline
	medium	> 800bp and < 900bp	
	short	< 800bp	
miTAG	mapped		
	unmapped		

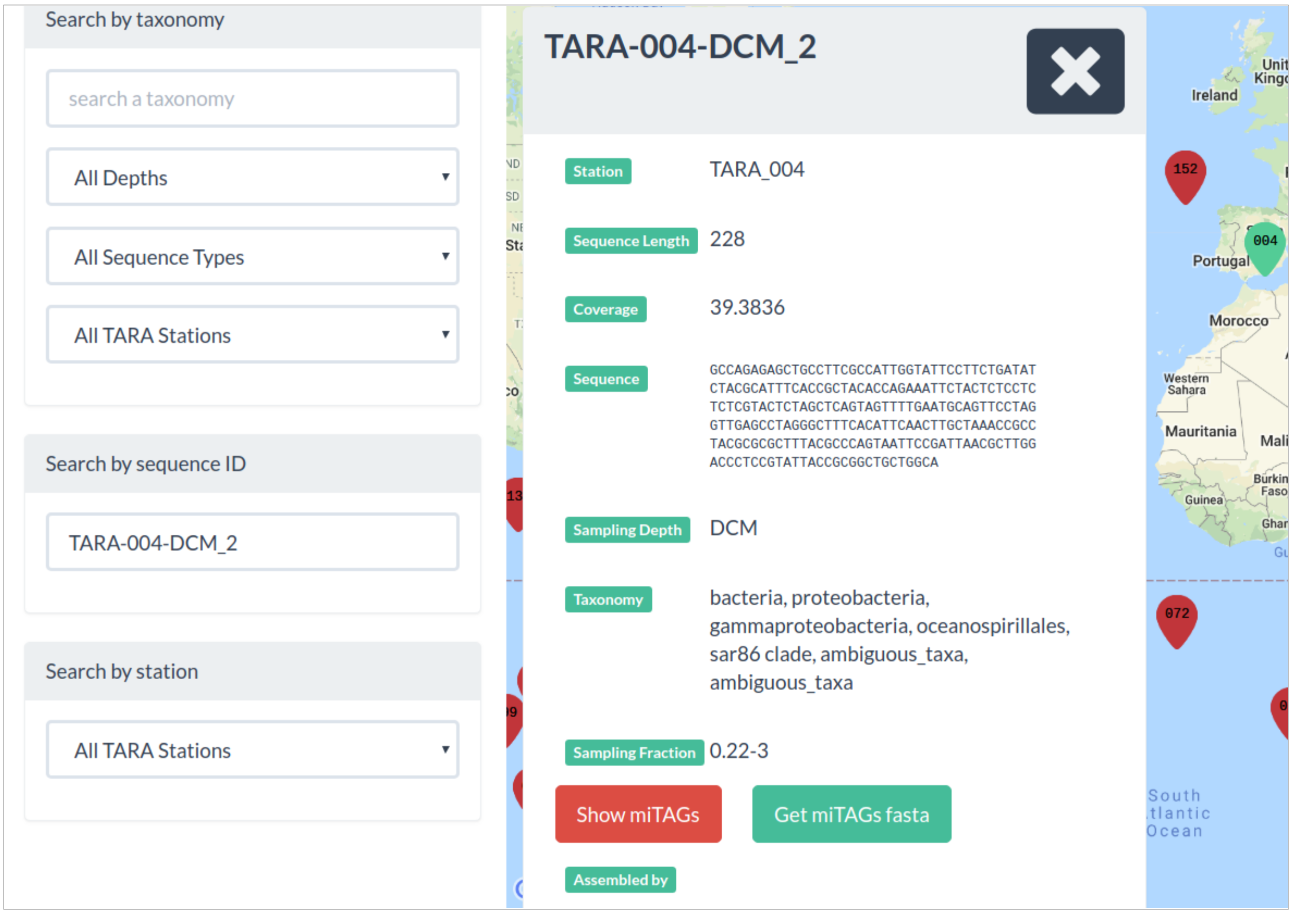
Table 1. Possible values of the Tags fields.

It is possible to further refine the query by selecting specific values for the following fields (Figure 4):

- “**All Depths**”. Select from the dropdown list the available sampling depths: “*Surface Oceanic*”, “*Deep-Chlorophyll Maximum*”, “*Epipelagic*”, “*Mesopelagic*” (metadata from the Tara project).
- “**All Sequence Types**”. Either the Tara “*miTAGs*” or the “*16S Contigs*” from the GLOSSARY collection.
    - If “*16S Contigs*” is chosen, the new “*All Contigs Length*” dropdown list will appear. From here it is possible to select the contigs by classification (i.e. length): “*All Contigs Length*”, “*Long (> 900bp)*”, “*Medium (> 800bp and < 900bp)*”, “*Short (< 800 bp)*”;
    - If “*miTAGs*” is chosen, the new “*All miTAGs*” dropdown list will appear. From here it is possible to select among the “*Mapped*” or “*Unmapped*” miTAGs;
- “**All TARA Stations**”. A specific Tara station ID, chosen from the dropdown list. The links to primary resources per libraries are provided (both miTAGs and raw reads).

2) *“Search by Sequence ID”*

In the “*Search by sequence ID*” box it is possible to search for a specific sequence among miTAG or contigs by its identifier (e.g. TARA-004-DCM_2) (Figure 6).



TARA-004-DCM_2

Station TARA_004

Sequence Length 228

Coverage 39.3836

Sequence

```
GCCAGAGAGCTGCCTTCGCCATTGGTATTCCTTCTGATAT
CTACGCATTTCACCGCTACACCAGAAATTCTACTCTCCTC
TCTCGTACTCTAGCTCAGTAGTTTTGAATGCAGTTCCTAG
GTTGAGCCTAGGGCTTTCACATTCAACTTGCTAAACCGCC
TACGCGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGG
ACCCTCCGTATTACCGCGGCTGCTGGCA
```

Sampling Depth DCM

Taxonomy bacteria, proteobacteria, gammaproteobacteria, oceanospirillales, sar86 clade, ambiguous_taxa, ambiguous_taxa

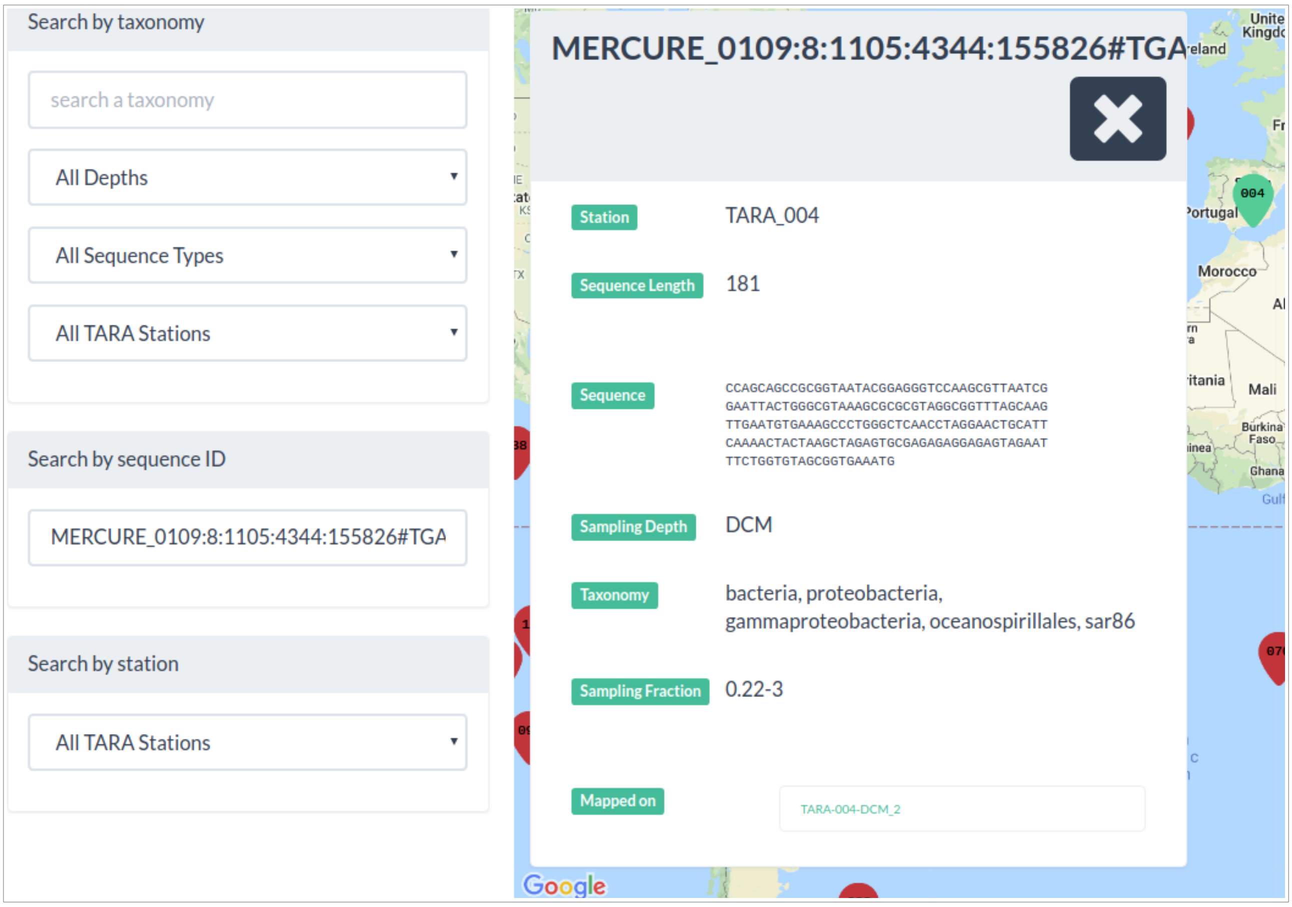
Sampling Fraction 0.22-3

Assembled by

Figure 6. Searching by contig ID. Details on the contig, the coverage, and its features are provided, including the possibility of viewing and downloading the associated miTAGs.

Details on the sequence including taxonomic affiliation, the sequence and its length are shown (see Figure 6 and Figure 7). If the query is a contig, it is possible to have information on the coverage, and to view the list of miTAGs sequences that map onto the contig. The sequences can also be downloaded as FASTA formatted files (Figure 6).

If an assembled miTAG sequence is searched, the crosslink to the corresponding contig ID is provided (Figure 7).



MERCURE_0109:8:1105:4344:155826#TGA

Station: TARA_004

Sequence Length: 181

Sequence:

```
CCAGCAGCCGCGGTAATACGGAGGGTCCAAGCGTTAATCG
GAATTACTGGGCGTAAAGCGCGCGTAGGCGGTTTAGCAAG
TTGAATGTGAAAGCCCTGGGCTCAACCTAGGAACTGCATT
CAAAACTACTAAGCTAGAGTGCGAGAGAGGAGAGTAGAAT
TTCTGGTGTAGCGGTGAAATG
```

Sampling Depth: DCM

Taxonomy: bacteria, proteobacteria, gammaproteobacteria, oceanospirillales, sar86

Sampling Fraction: 0.22-3

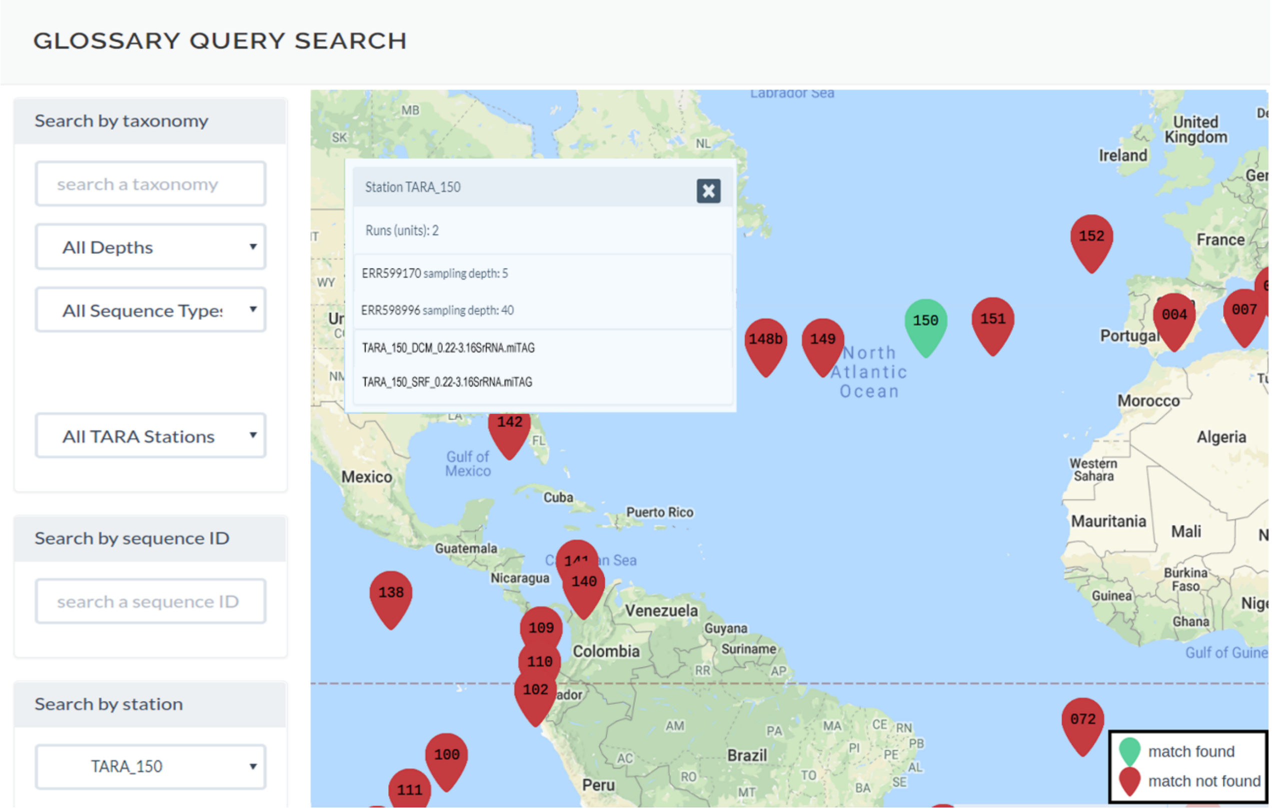
Mapped on: TARA-004-DCM_2

Figure 7. Searching by miTAG ID. Details on the miTAG and its features are given. If the miTAG is mapped on a contig, then the ID of the contig is also shown.

3) *"Search by station"*

In the "*Search by station*" box it is possible to search for a specific Tara station ID selecting from the dropdown list (Figure 8). The results shown include:

- the list of the runs, linked to the libraries at the SRA archives of the NCBI for that station;
- the list of miTAG libraries for the station. Clicking on the link starts the download of the corresponding miTAGs libraries in FASTA format.



GLOSSARY QUERY SEARCH

Search by taxonomy

search a taxonomy

All Depths ▾

All Sequence Type: ▾

All TARA Stations ▾

Search by sequence ID

search a sequence ID

Search by station

TARA_150 ▾

Station TARA_150 ☒

Runs (units): 2

ERR599170 sampling depth: 5

ERR598996 sampling depth: 40

TARA_150_DCM_0.22-3.16SrRNA.miTAG

TARA_150_SRF_0.22-3.16SrRNA.miTAG

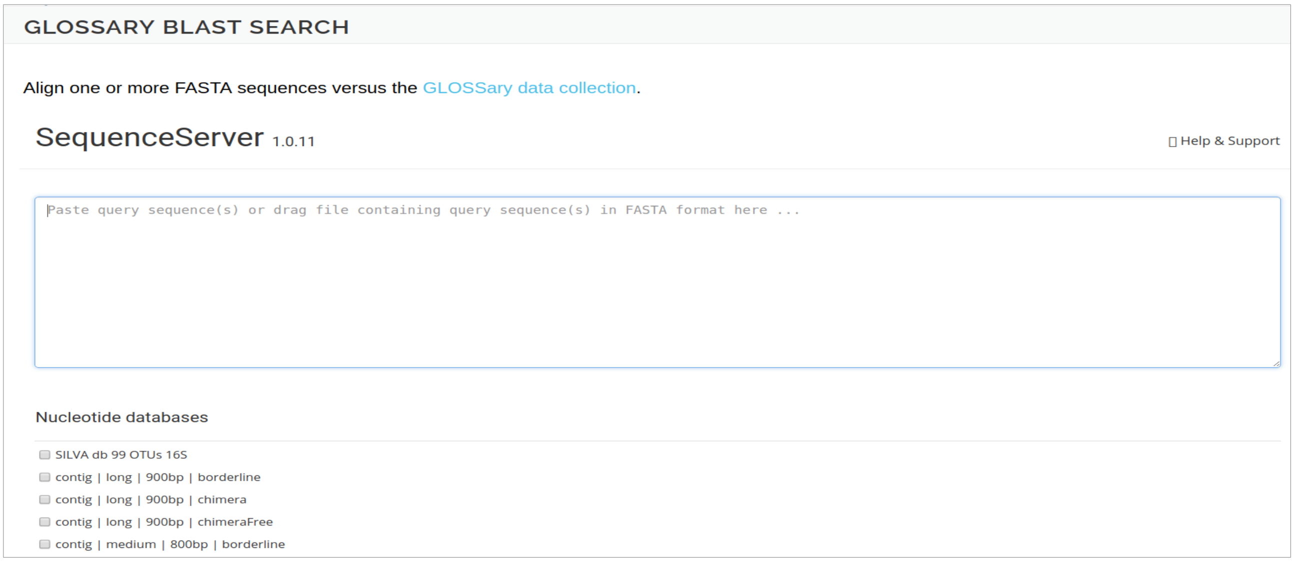
🟩 match found

🟥 match not found

Figure 8. Searching by station ID. The list of the runs and the list of the miTAG libraries for the selected station are shown. Items in the first list link to the SRA archives of the NCBI, the second ones directly download the libraries in FASTA format.

“BLAST search” Web Page

The GLOSSary data can be searched by alignment similarity using the “BLAST search” service (Figure 9).



GLOSSARY BLAST SEARCH

Align one or more FASTA sequences versus the GLOSSary data collection.

SequenceServer 1.0.11

Paste query sequence(s) or drag file containing query sequence(s) in FASTA format here ...

Nucleotide databases

- □ SILVA db 99 OTUs 16S
- □ contig | long | 900bp | borderline
- □ contig | long | 900bp | chimera
- □ contig | long | 900bp | chimeraFree
- □ contig | medium | 800bp | borderline

Figure 9. GLOSSary “BLAST search” web page.

The user can query one or more sequences (in FASTA format), choosing one or more databases among the available ones:

- 16S Contigs:

  — “long” (>900 bp) chimera free;

  — “long” (>900 bp) chimera;

  — “long” (>900 bp) borderline;

  — “medium” (800 bp<>900 bp) chimera free;

  — “medium” (800 bp<>900 bp) chimera;

  — “medium” (800 bp<>900 bp) borderline;

  — “short” (<800 bp).

- miTAGs:

  — mapped on contigs (i.e. assembled);

  — unmapped on contigs (i.e. not assembled).

- SILVA DB:

  — SSU Ref NR 99 (version 128).

References

1. Sunagawa, S., et al., *Structure and function of the global ocean microbiome.* Science, 2015. **348**(6237): p.1261359.
2. Rognes, T., et al., *VSEARCH: a versatile open source tool for metagenomics.* PeerJ, 2016. **4**: p. e2584.

Please cite

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For further information please visit our web site http://bioinfo.szn.it/ or contact us at bioinfo@szn.it