

AMOEBAE documentation

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1 Introduction

1.1 What is AMOEBAE?

Analysis of MOlecular Evolution with BAtch Entry (AMOEBAE) is a bioinformatics software toolkit composed primarily of scripts written in the Python3 language. AMOEBAE scripts use existing Python packages including Biopython (Cock *et al.*, 2009), the Environment for Tree Exploration (ETE3) (Huerta-Cepas *et al.*, 2016), pandas, and Matplotlib (Hunter, 2007) for setting up, running, and summarizing analyses of molecular evolution using bioinformatics software packages including MUSCLE (Edgar, 2004), BLAST+ (Camacho *et al.*, 2009), HMMer3 (Eddy, 1998), and IQ-Tree (Nguyen *et al.*, 2015). Applications include identifying and classifying predicted peptide sequences according to their evolutionary relationships with homologues. All dependencies are freely available, and AMOEBAE code is open-source (see subsection 1.8) and available on GitHub (<https://github.com/laelbarlow/amoebae>).

1.2 Why AMOEBAE?

Webservices such as those provided by NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Camacho *et al.*, 2009) provide a means to investigate the evolution of one or a few genes via similarity searching, and automated pipelines such as orthoMCL (Li, 2003) attempt to rapidly perform orthology prediction for all genes in several genomes. AMOEBAE addresses the problem mid-scale analyses which are too cumbersome to be done via webservices and yet requiring a level of detail and flexibility not offered by automated pipelines. AMOEBAE may be useful for analyzing the distribution of orthologues of up to perhaps 30 genes/proteins among a sampling of no more than approximately 100 eukaryotic genomes. However, you may need to carefully define the scope of your analysis depending on what additional steps you may find necessary beyond those that may be performed using AMOEBAE (30 queries and 100 genomes may in fact be unmanageable). AMOEBAE provides many options which can be tailored to the specific genes/proteins being analyzed, and allow analyses using complex sets of customized criteria to be reproduced more practically.

1.3 Key features

The core functionality is to run sequence similarity searches with multiple algorithms, multiple queries, and multiple databases simultaneously and facilitate efficient and highly customizable implementation of reciprocal-best-hit search strategies. The output includes detailed summaries of results in the form of a spreadsheet and plots.

1.4 User support

For specific issues with the code, please use the issue tracker on the GitHub webpage here:
<https://github.com/laelbarlow/amoebae/issues>.

If you have general questions regarding AMOEBAE, please email the author at lael (at) ualberta.ca.

1.5 Documentation

This document provides an overview of AMOEBAE and describes the functionality of the various commands/scripts. For a tutorial which includes a working example of a similarity search analysis run using AMOEBAE, see the Jupyter Notebook: `amoebae/notebooks/similarity_search_tutorial.ipynb`. For code documentation, please see the html file(s), which can be opened with your web browser: `amoebae/doc/code_documentation/html/index.html`.

1.6 How to cite AMOEBAE

Please cite the GitHub webpage <https://github.com/laelbarlow/amoebae> (or alternative permanent repositories if relevant). Also, the first publication to make use of a version of AMOEBAE was an analysis of Adaptor Protein subunits in embryophytes by Larson *et al.* (2019).

Also, you may wish to cite the software packages which are key dependencies of AMOEBAE, since AMOEBAE would not work without these (see subsection 2.2).

1.7 Acknowledgments

1.8 License

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2 How to start using AMOEBAE

2.1 System requirements

Please note that the commands shown likely only work on macOS or Linux operating systems (you may have trouble running AMOEBAE directly on Windows).

2.2 Dependencies

All dependencies are free and open-source, and can be automatically installed in a virtual environment (see subsection 2.3).

These are the main dependencies of AMOEBAE:

- Python3 (the Anaconda distribution works well).
- Biopython, a Python package for bioinformatics (Cock *et al.*, 2009).
- The Environment for Tree Exploration 3 (ETE3), a Python package for working with phylogenetic trees (Huerta-Cepas *et al.*, 2016).
- Matplotlib, a Python package for generating plots (Hunter, 2007).
- (gffutils).
- NCBI BLAST+, a software package for sequence similarity searching (Camacho *et al.*, 2009).
- HMMer3, a software package for profile sequence similarity searching (Eddy, 1998).
- MUSCLE, for multiple sequence alignment (Edgar, 2004).
- IQ-TREE, for phylogenetic analysis (Nguyen *et al.*, 2015).

2.3 Setting up an environment for AMOEBAE using Docker

Follow the steps below to set up AMOEBAE on your personal computer. This setup process will take approximately 1 hour to complete, however, most of the process is automated, so only about 20 minutes or less is required for the steps that require manual input. Instructions for setting up AMOEBAE on a remote server will soon be added as well.

1. Ensure that Git is installed on your computer This program may already be installed by default on your operating system. If you have a newer version of macOS it may prompt you to install Git. Documentation for Git is available here: <https://git-scm.com/doc>. You can check which version you have by running the command below.

```

1      >>> git --version
2
3  2. Clone the AMOEBAE repository using Git. If you simply download the code from
4  GitHub, instead of cloning the repository, then AMOEBAE cannot record specifically
5  what version of the code you use, and will not run properly. Make sure to use the
6  appropriate directory path (the path shown is just an example). Please note: Here
7  ">>>" is used to indicate that the following text in the line is to be entered in you
8  terminal command prompt.
9
10     >>> cd /path/to/directory/where/you/keep/scripts
11     >>> git clone https://github.com/laelbarlow/amoebae.git
12
13  3. Make a copy of the settings.py.example file as settings.py. This will be customized later.
14
15     >>> cd amoebae
16     >>> cp settings.py.example settings.py
17
18  4. Download and install the appropriate version of Docker from this website: https://www.docker.com/products/docker-desktop.
19
20  5. Add the amoebae directory to the list of directories that can be shared with Docker con-
21  tainers using the Docker graphical user interface by selecting Preferences > Resources
22  > File sharing.
23
24  6. Customize the CPUs, memory, etc. that you wish to make available to docker contain-
25  ers using the Docker graphical user interface by selecting Preferences > Resources >
26  Advanced.
27
28  7. Build a Docker image (virtual environment) using the build_env.sh script. This
29  uses the continuumio/anaconda3 image from DockerHub (https://hub.docker.com/r/continuumio/anaconda3), and extends it by downloading and installing several soft-
30  ware packages that AMOEBAE depends on. The details of this process are defined in
31  the Dockerfile file in the amoebae repository. This step will take approximately 40
32  minutes to complete.
33
34     >>> bash build_env.sh
35
36  8. Run the Docker using the run_env.sh script. This generates a Docker container from
37  the Docker image built in the preceding step.
38
39     >>> bash run_env.sh
40
41  9. Copy and past the resulting URL into the address bar of your web browser (either
42  Firefox, Chrome, or Safari will work). This should launch a Jupyter session with
43  an interface where you can navigate within the amoebae directory. Documentation on
44  Jupyter is available here: https://jupyter-notebook.readthedocs.io/en/stable/.
45
46  10. Click on the "notebooks" directory to open it. Then open one of the tutorial files.

```

3 Command reference

Documentation for each AMOEBAE command and the various options may be accessed from the command line via the "-h" options. The following command reference information is the output of running amoebae (and each command) with the "-h" option.

3.1 amoebae

usage: amoebae <command> [<args>]

Commands for setting up data structure:

mkdatadir	Make a directory with subdirectories and CSV files for storing sequence data, etc.
-----------	--

Commands for similarity searching:

setup_hmmdb	Construct an HMM database (with hmmpress).
add_to_dbs	Format and add a file to a formatted directory.
list_dbs	Print a list of all usable database files in the database directory as defined in the settings file.
add_to_queries	Add a query file to a formatted directory.
list_queries	Print a list of all usable query files in the query directory as defined in the settings file.
get_redun_hits	Run searches with queries to find redundant hits in databases (for interpreting results).
setup_fwd_srch	Make directory in which to perform forward searches.
run_fwd_srch	Perform searches with given queries into given dbs.
sum_fwd_srch	Append information about forward searches to csv summary file (this is used to organize reverse searches).
setup_rev_srch	Make a directory in which to perform reverse searches.
run_rev_srch	Perform searches with given forward search hits into given db.
sum_rev_srch	Append information about reverse searches to csv summary file.
interp_srchs	Interpret search results based on summary.
find_redun_seqs	Identify sequences likely encoded on redundant loci predicted for the same species.
plot	Plot search results.

Commands for phylogenetic analysis using a reference tree:

add_to_models	Add an alignment, tree, substitution model, names of clade-defining sequences to a directory with other models.
list_models	Print a list of all usable model/reference tree names in the models directory as defined in the settings file.
get_alt_topos	Take a tree and make copies with every alternative topology for the branches connecting the clades of interest.

Commands for phylogenetic analysis without a reference tree:


```

1      prune          Identify sequences in a tree, and remove them from a
2                      given alignment for further phylogenetic analysis.
3      auto_prune      Automatically identify sequences in a tree, and remove
4                      them from a given alignment for further phylogenetic
5                      analysis.
6      reduce_tree     Remove terminal nodes from a given tree if there are
7                      not any sequences with the same name in a given multiple
8                      sequence alignment file.
9      constrain_mb     Add constraint commands to MrBayes input file based on a
10                     given tree topology.
11     visualize_tree   Parse phylogenetic analysis output files for a single
12                     alignment in a given directory, and write human-readable
13                     tree figures to PDF files.
14     replace_seqs     Replace sequences in an alignment with their top hits in a
15                     given fasta file (useful if genomes or taxon selection has
16                     been updated).
17
18 Miscellaneous commands:
19     csv_to_fasta      Generate a fasta file from sequences detailed in a
20                     spreadsheet of similarity search results.
21     check_depend      Check that all the dependencies are properly installed and
22                     useable.
23     check_imports     Check that all the import statements used in the AMOEBAE
24                     repository run without error.
25
26 positional arguments:
27     command           Specify one of the functionalities of amoebae.
28
29 optional arguments:
30     -h, --help        show this help message and exit
31
32 Copyright 2018 Lael D. Barlow Licensed under the Apache License, Version 2.0
33 (the "License"); you may not use this file except in compliance with the
34 License. You may obtain a copy of the License at
35 http://www.apache.org/licenses/LICENSE-2.0 Unless required by applicable law
36 or agreed to in writing, software distributed under the License is distributed
37 on an "AS IS" BASIS, WITHOUT WARRANTIES OR CONDITIONS OF ANY KIND, either
38 express or implied. See the License for the specific language governing
39 permissions and limitations under the License.

```

40 3.2 amoebae mkdatadir

```

41 usage: amoebae [-h] new_dir_path
42
43 Make a directory with subdirectories and CSV files for storing sequence data,
44 etc.
45
46 positional arguments:
47     new_dir_path       Specify the full file path that you want the new directory to

```

```

1             have.
2
3 optional arguments:
4   -h, --help    show this help message and exit

```

5 3.3 amoebae setup_hmmdb

```

6 usage: amoebae [-h] indirpath
7
8 Construct an HMM database (with hmmpress). This is for later sorting of given
9 sequences into categories based on which HMM the score highest against.
10
11 positional arguments:
12   indirpath    Path to directory containing amino acid sequence alignment
13                file(s) to be constructed into an HMM database using hmmpress
14                from the HMMer3 software package.
15
16 optional arguments:
17   -h, --help  show this help message and exit

```

18 3.4 amoebae add_to_dbs

```

19 usage: amoebae [-h] [--split_char SPLIT_CHAR] [--split_pos SPLIT_POS]
20                [--skip_header_reformat] [--auto_extract_accs]
21                new_file
22
23 Format and add a file to a formatted directory.
24
25 positional arguments:
26   new_file      Can be a fasta file (prot or nucl) or HMM databases,
27                 generated using the hmmpress program in the HMMer
28                 software package. Or a GFF3 annotation file.
29
30 optional arguments:
31   -h, --help    show this help message and exit
32   --split_char SPLIT_CHAR
33                 Character to split the header string on for extracting
34                 the accession. (default: )
35   --split_pos SPLIT_POS
36                 Position that the accession will be in after
37                 splitting. (default: 0)
38   --skip_header_reformat
39                 Skip reformatting of header lines in input fasta file.
40                 (default: False)
41   --auto_extract_accs
42                 Automatically identify accessions/IDs in sequence
43                 headers (overrides split_char and split_pos options
44                 above). (default: False)

```

1 3.5 amoebae list_db

```
2 usage: amoebae [-h]
3
4 Print a list of all usable query files in the query directory as defined in
5 the settings file.
6
7 optional arguments:
8   -h, --help  show this help message and exit
```

9 3.6 amoebae add_to_queries

```
10 usage: amoebae [-h] query_file
11
12 Add a query file to a formatted directory. This command adds a given sequence
13 file to the directory with the path that you have specified in the settings.py
14 file, and appends a corresponding line to the CSV file that you specified
15 (e.g., '0_query_info.csv') to indicate the query title, etc.
16
17 positional arguments:
18   query_file  Path to a sequence file in FASTA format that can be used as a
19               similarity search query file. Or path to a directory containing
20               only files for addition to the queries. Note: By default, the
21               portion of the input filename preceding the first underscore
22               character will be recorded as the "query title", the remaining
23               substring preceding the second underscore character will be
24               recorded as the taxon (e.g., "Hsapiens"), and the rest of the
25               filename preceding the filename extension will be recorded as
26               the sequence ID. So the filename might look like this:
27               "QUERYTITLE_HSAPIENS_SEQUENCEID.fa". However, the relevant
28               information can be revised in the "Queries/0_query_info.csv"
29               file afterward if necessary.
30
31 optional arguments:
32   -h, --help  show this help message and exit
```

33 3.7 amoebae list_queries

```
34 usage: amoebae [-h]
35
36 Print a list of all usable query files in the query directory as defined in
37 the settings file.
38
39 optional arguments:
40   -h, --help  show this help message and exit
```

41 3.8 amoebae get_redun_hits

```

1  usage: amoebae [-h] [--csv_file CSV_FILE] [--query_name QUERY_NAME]
2                  [--query_list_file QUERY_LIST_FILE] [--db_name DB_NAME]
3                  [--db_list_file DB_LIST_FILE] [--query_title QUERY_TITLE]
4                  [--outdir OUTDIR]
5                  [--blast_report_evalue_cutoff BLAST_REPORT_EVALUE_CUTOFF]
6                  [--blast_max_target_seqs BLAST_MAX_TARGET_SEQS]
7                  [--hmmmer_report_evalue_cutoff HMMER_REPORT_EVALUE_CUTOFF]
8                  [--hmmmer_report_score_cutoff HMMER_REPORT_SCORE_CUTOFF]
9                  [--num_threads_similarity_searching NUM_THREADS_SIMILARITY_SEARCHING]
10 ]
11     srch_dir
12
13 Run searches with queries to find redundant hits in databases (for
14 interpreting results).
15
16 positional arguments:
17     srch_dir            Path to directory that will contain output directory
18                        as a subdirectory.
19
20 optional arguments:
21     -h, --help          show this help message and exit
22     --csv_file CSV_FILE Path to spreadsheet to append summary of result to for
23                        manual annotation. (default: None)
24     --query_name QUERY_NAME
25                        Query filename to use (not full path). (default: None)
26     --query_list_file QUERY_LIST_FILE
27                        Path to file containing a list of query files to use,
28                        if no query_name is specified (or all queries by
29                        default). (default: None)
30     --db_name DB_NAME   Name of database file in the database directory in
31                        which to do searches (not full path). (default: None)
32     --db_list_file DB_LIST_FILE
33                        Path to file containing a list of database files to
34                        use (if no db_name specified). (default: None)
35     --query_title QUERY_TITLE
36                        Name to be assigned to hits in databases that may be
37                        considered redundant with a search query to which the
38                        same title is assigned, otherwise it is taken from the
39                        query info spreadsheet specified in the settings.py
40                        file ('query_info_csv'). (default: None)
41     --outdir OUTDIR     Path to directory to write search results to.
42                        (default: None)
43     --blast_report_evalue_cutoff BLAST_REPORT_EVALUE_CUTOFF
44                        Maximum E-value for reporting BLAST hits. (default:
45                        0.05)
46     --blast_max_target_seqs BLAST_MAX_TARGET_SEQS
47                        Maximum BLAST target sequences to consider. (default:
48                        500)
49     --hmmmer_report_evalue_cutoff HMMER_REPORT_EVALUE_CUTOFF

```

```

1             Maximum E-value for reporting HMMer hits. (default:
2             0.05)
3  --hmmreport_score_cutoff HMMER_REPORT_SCORE_CUTOFF
4             Minimum sequence score for reporting HMMer hits.
5             (default: 5)
6  --num_threads_similarity_searching NUM_THREADS_SIMILARITY_SEARCHING
7             Number of threads to use for running searches.
8             (default: 4)
9
10 Recommendation: For most analyses, use the --query_name option and the
11 --db_name option, and run the get_redun_hits command for each query
12 separately. Otherwise, there will be redundant information in the output
13 spreadsheet(s).

```

3.9 amoebae setup_fwd_srch

```

14 usage: amoebae [-h] [--outdir OUTDIR] srch_dir query_list_file db_list_file
15
16 Make a directory in which to write output files from similarity searches.
17
18 positional arguments:
19  srch_dir              Path to directory that will contain output directory as a
20                       subdirectory.
21  query_list_file       Path to file with list of queries to search with.
22  db_list_file          Path to file with list of databases to search with.
23
24 optional arguments:
25  -h, --help            show this help message and exit
26  --outdir OUTDIR       Path to directory to put search results into (so that this
27                       step can be piped together with other commands). (default:
28                       None)
29
30 Note: Use the bash script to run forward searches on a remote server.

```

3.10 amoebae run_fwd_srch

```

33 usage: amoebae [-h] [--blast_report_evalue_cutoff BLAST_REPORT_EVALUE_CUTOFF]
34                [--blast_max_target_seqs BLAST_MAX_TARGET_SEQS]
35                [--hmmreport_evalue_cutoff HMMER_REPORT_EVALUE_CUTOFF]
36                [--hmmreport_score_cutoff HMMER_REPORT_SCORE_CUTOFF]
37                [--num_threads_similarity_searching NUM_THREADS_SIMILARITY_SEARCHING]
38                ]
39                fwd_srch_dir
40
41 Perform searches with original queries into subject databases.
42
43 positional arguments:
44  fwd_srch_dir          Path to directory that will contain forward search

```

```

1             output files.
2
3 optional arguments:
4   -h, --help             show this help message and exit
5   --blast_report_evalue_cutoff BLAST_REPORT_EVALUE_CUTOFF
6                           Maximum E-value for reporting BLAST hits. (default:
7                           0.05)
8   --blast_max_target_seqs BLAST_MAX_TARGET_SEQS
9                           Maximum BLAST target sequences to consider. (default:
10                          500)
11  --hmmmer_report_evalue_cutoff HMMER_REPORT_EVALUE_CUTOFF
12                          Maximum E-value for reporting HMMer hits. (default:
13                          0.05)
14  --hmmmer_report_score_cutoff HMMER_REPORT_SCORE_CUTOFF
15                          Minimum sequence score for reporting HMMer hits.
16                          (default: 5)
17  --num_threads_similarity_searching NUM_THREADS_SIMILARITY_SEARCHING
18                          Number of threads to use for running searches.
19                          (default: 4)

```

20 3.11 amoebae sum_fwd_srch

```

21 usage: amoebae [-h] [--max_evalue MAX_EVALUE]
22                [--max_gap_between_tblastn_hsps MAX_GAP_BETWEEN_TBLASTN_HSPS]
23                [--do_not_use_exonerate]
24                [--exonerate_score_threshold EXONERATE_SCORE_THRESHOLD]
25                [--max_hits_to_sum MAX_HITS_TO_SUM]
26                fwd_srch_out csv_file
27

```

28 Append information about forward searches to csv summary file (this is used to
29 organize reverse searches). For TBLASTN searches (protein queries, nucleotide
30 target sequences), HSPs are clustered into groups that are close enough within
31 the target sequence to potentially represent exons from the same coding
32 sequence. The nucleotide subsequences in which these clusters of HSPs are
33 found are then analyzed using exonerate to identify and translate potential
34 exons, in "protein2genome" mode, because exonerate, unlike TBLASTN, attempts
35 to identify exon boundaries, yielding translations that are less likely to
36 include translations of non-coding regions outside exons (which might include
37 apparent stop codons).

```

38
39 positional arguments:
40   fwd_srch_out          Path to directory where forward search results were
41                          written.
42   csv_file              Path to summary spreadsheet (CSV) file, which may
43                          already contain search summaries, or may not exist
44                          yet.
45

```

```

46 optional arguments:
47   -h, --help            show this help message and exit

```

```

1  --max_evalue MAX_EVALUE
2      Maximum E-value threshold for reporting forward search
3      hits. (default: 0.0005)
4  --max_gap_between_tblastn_hsps MAX_GAP_BETWEEN_TBLASTN_HSPS
5      Maximum number of nucleotide bases between TBLASTN
6      HSPs to be considered part of the same gene locus.
7      This is important, because it will be assumed that HSP
8      separated by more than this number of nucleotide bases
9      are not part of the same gene or TBLASTN "hit".
10     (default: 10000)
11  --do_not_use_exonerate
12      Override the default use of exonerate to identify
13      coding sequences and translations, and just use
14      TBLASTN instead. This option is provided because
15      concatenated TBLASTN HSPs may be more inclusive of
16      sequences within the target sequence, and the results
17      of TBLASTN and exonerate may need to be compared.
18      Also, note that HSPs identified by TBLASTN but for
19      which exonerate yields no alignments will be ignored
20      if exonerate is used. (default: False)
21  --exonerate_score_threshold EXONERATE_SCORE_THRESHOLD
22      Set score threshold to be applied when running
23      exonerate on nucleotide sequences identified by
24      TBLASTN. The default for setting of exonerate is 100,
25      but a lower score is set as default here, because
26      otherwise exonerate cannot identify some of the
27      sequences identified by TBLASTN. This option is only
28      relevant if using exonerate. (default: 10)
29  --max_hits_to_sum MAX_HITS_TO_SUM
30      Maximum number of forward search hits to list in the
31      summary spreadsheet. If zero, then reverse searches
32      will be performed for all hits. (default: 0)

```

3.12 amoebae setup_rev_srch

```

34 usage: amoebae [-h] [--outdir OUTDIR] [--aasubseq] [--nafullseq]
35      srch_dir csv_file databases
36
37 Make directory in which to write results of reverse searches.
38
39 positional arguments:
40   srch_dir      Path to directory that will contain output directory as a
41                 subdirectory.
42   csv_file      Path to summary spreadsheet (CSV) file, which contains a
43                 summary of forward search(es).
44   databases     Database filename (in database directory) or path to file
45                 with list of database filenames. Note that filenames are
46                 needed, not file paths.
47

```

```

1 optional arguments:
2   -h, --help          show this help message and exit
3   --outdir OUTDIR    Path to directory to put search results into (so that this
4                       step can be piped together with other commands). (default:
5                       None)
6   --aasubseq          Use only the portion of each (amino acid) forward hit
7                       sequence that aligns to the original query used (top HSP
8                       subject sequence). This is default for nucleotide hits.
9                       (default: False)
10  --nafullseq          Use the full (nucleic acid) forward hit sequence. This is
11                       default for amino acid hits. (default: False)

```

12 3.13 amoebae run_rev_srch

```

13 usage: amoebae [-h] [--blast_report_evalue_cutoff BLAST_REPORT_EVALUATE_CUTOFF]
14                [--blast_max_target_seqs BLAST_MAX_TARGET_SEQS]
15                [--hmmmer_report_evalue_cutoff HMMER_REPORT_EVALUATE_CUTOFF]
16                [--hmmmer_report_score_cutoff HMMER_REPORT_SCORE_CUTOFF]
17                [--num_threads_similarity_searching NUM_THREADS_SIMILARITY_SEARCHING]
18                ]
19                rev_srch_dir

```

21 Perform searches with forward search hit sequences as queries into the
22 original query databases.

24 positional arguments:

```

25   rev_srch_dir        Path to directory that will contain output of
26                       searches.

```

28 optional arguments:

```

29   -h, --help          show this help message and exit
30   --blast_report_evalue_cutoff BLAST_REPORT_EVALUATE_CUTOFF
31                       Maximum E-value for reporting BLAST hits. (default:
32                       0.05)
33   --blast_max_target_seqs BLAST_MAX_TARGET_SEQS
34                       Maximum BLAST target sequences to consider. (default:
35                       500)
36   --hmmmer_report_evalue_cutoff HMMER_REPORT_EVALUATE_CUTOFF
37                       Maximum E-value for reporting HMMer hits. (default:
38                       0.05)
39   --hmmmer_report_score_cutoff HMMER_REPORT_SCORE_CUTOFF
40                       Minimum sequence score for reporting HMMer hits.
41                       (default: 5)
42   --num_threads_similarity_searching NUM_THREADS_SIMILARITY_SEARCHING
43                       Number of threads to use for running searches.
44                       (default: 4)

```


3.14 amoebae sum_rev_srch

```
usage: amoebae [-h] [--redun_hit_csv REDUN_HIT_CSV]
               [--min_evaldiff MIN_EVALDIFF] [--aasubseq] [--nafullseq]
               [--max_rev_srchs MAX_REV_SRCHS]
               csv_file rev_srch_out

Append information about reverse searches to csv summary file. Use information
from redundant hit csv file to interpret results.

positional arguments:
  csv_file              Path to summary spreadsheet (CSV) file, which may
                        already contain reverse search summaries.
  rev_srch_out          Path to directory where reverse search results were
                        written.

optional arguments:
  -h, --help            show this help message and exit
  --redun_hit_csv REDUN_HIT_CSV
                        Path to spreadsheet (CSV) file, which specifies which
                        hits are redundant positive hits for a given query
                        (query title) in a given database. If this is not
                        provided, then it is assumed that the top reverse
                        search hit is equivalent to the original query.
                        (default: None)
  --min_evaldiff MIN_EVALDIFF
                        Minimum difference in E-value order of magnitude
                        between top reverse search hit and first reverse
                        search hit that is not redundant with the original
                        query. (default: 5)
  --aasubseq            Use only the portion of each (amino acid) forward hit
                        sequence that aligns to the original query used (top
                        HSP subject sequence). This is default for nucleotide
                        hits. Must be selected if selected when the
                        setup_rev_srch command was run. (default: False)
  --nafullseq           Use the full (nucleic acid) forward hit sequence. This
                        is default for amino acid hits. Must be selected if
                        selected when the setup_rev_srch command was run.
                        (default: False)
  --max_rev_srchs MAX_REV_SRCHS
                        Maximum number of forward search hits to perform
                        reverse searches for per query database. If zero, then
                        reverse searches will be performed for all hits.
                        (default: 0)
```

3.15 amoebae interp_srchs

```
usage: amoebae [-h] [--fwd_only] [--fwd_evalue_cutoff FWD_EVALUE_CUTOFF]
               [--rev_evalue_cutoff REV_EVALUE_CUTOFF]
```

```

1      [--hmmmer_cutoff HMMER_CUTOFF] [--redun_hits]
2      [--out_csv_path OUT_CSV_PATH]
3      csv_file
4
5  Interpret search results based on final summary, which provides a basis for
6  further analyses of positive hits.
7
8  positional arguments:
9      csv_file          Path to spreadsheet with forward and reverse search
10                      results.
11
12  optional arguments:
13      -h, --help        show this help message and exit
14      --fwd_only        Interpret forward searches based on score (HMMer)
15                      cutoff. (default: False)
16      --fwd_evalue_cutoff FWD_EVALUATE_CUTOFF
17                      Specify an (more stringent) E-value cutoff for forward
18                      search results. (default: None)
19      --rev_evalue_cutoff REV_EVALUATE_CUTOFF
20                      Specify an (more stringent) E-value cutoff for reverse
21                      search results. (default: None)
22      --hmmmer_cutoff HMMER_CUTOFF
23                      Specify a score that hits must exceed to be included.
24                      (default: 20)
25      --redun_hits      Interpret which hits are redundant in output of
26                      get_redun_hits command. (default: False)
27      --out_csv_path OUT_CSV_PATH
28                      Optionally specify an output file path, so that this
29                      command can be piped together with others. (default:
30                      None)

```

3.16 amoebae find_redun_seqs

```

32  usage: amoebae [-h] [--out_csv_path OUT_CSV_PATH]
33                [--remove_tblastn_hits_at_annotated_loci]
34                [--just_look_for_genes_in_gff3] [--ignore_gff3]
35                [--allow_internal_stops ALLOW_INTERNAL_STOPS]
36                [--min_length MIN_LENGTH]
37                [--min_percent_length MIN_PERCENT_LENGTH]
38                [--min_percent_query_cover MIN_PERCENT_QUERY_COVER]
39                [--overlap_required] [--max_percent_ident MAX_PERCENT_IDENT]
40                [--min_alig_res_in_overlap MIN_ALIG_RES_IN_OVERLAP]
41                [--min_ident_res_in_overlap MIN_IDENT_RES_IN_OVERLAP]
42                [--min_sim_res_in_overlap MIN_SIM_RES_IN_OVERLAP]
43                [--min_ident_span_len MIN_IDENT_SPAN_LEN]
44                [--min_sim_span_len MIN_SIM_SPAN_LEN]
45                [--min_percent_ident_in_overlap MIN_PERCENT_IDENT_IN_OVERLAP]
46                [--min_percent_sim_in_overlap MIN_PERCENT_SIM_IN_OVERLAP]
47                [--min_percent_overlap MIN_PERCENT_OVERLAP] [--add_ali_col]

```

```

1         csv_file
2
3 Identify hit sequences likely encoded by the same gene loci in the genome of a
4 given species, or otherwise not representing paralogous genes. Criteria are
5 applied in this order: 1. Peptide hits with the same ID as higher-ranking hits
6 for the same query (query title) are excluded. 2. Nucleotide hits for the same
7 loci as peptide sequence hits are excluded. 3. Sequences with internal stop
8 codons are excluded, as these are potentially pseudogenes. 4. Sequences are
9 excluded if they do not meet several minimum length criteria: Absolute minimum
10 length (in amino acids) and percent query cover. 5. Sequences are excluded if
11 they do not overlap to a specified degree with all included higher-ranking
12 hits for the same query (query title) in sequence data for the same
13 species/genome. This is determined by algorithmically comparing pairs of
14 sequences aligned to a reference alignment of homologues, and several minimum
15 measures of alignment overlap may be specified. 6. Secondary hit sequences are
16 excluded if they do not meet a specified maximum percent identity threshold.
17 Highly identical sequences may result from false segmental duplications in the
18 genome assembly, may represent alleles, etc. Note: Applying these criteria
19 requires a column to be manually added to the input csv file prior to running
20 with the header "Alignment for sequence comparison" and filled with the
21 appropriate alignment name to use (one for each query title, as listed in the
22 "Query title" column). Alternatively, you can run this command with the
23 --add_ali_col option to automatically identify appropriate alignments among
24 your aligned FASTA queries used for running HMMer searches.
25
26 positional arguments:
27     csv_file            Path to spreadsheet with interpreted search results
28                         outputted by the interp_srchs command.
29
30 optional arguments:
31     -h, --help          show this help message and exit
32     --out_csv_path OUT_CSV_PATH
33                         Optionally specify an output file path, so that this
34                         command can be piped together with others. (default:
35                         None)
36     --remove_tblastn_hits_at_annotated_loci
37                         Ignore tblastn hits that overlap with any previously
38                         annotated loci. The rationale for this would be that
39                         the corresponding protein sequences should have been
40                         retrieved if the tblastn hit were a true positive
41                         anyway. If this option is not specified, then
42                         sequences will still be excluded if they specifically
43                         correspond to the same loci as do higher-ranking hits.
44                         (default: False)
45     --just_look_for_genes_in_gff3
46                         When looking for records in GFF3 annotation files that
47                         overlap with subsequences identified by similarity
48                         searching (TBLASTN), ignore records that are not
49                         explicitly "gene" (for example, "CDS", "mRNA", and

```

```

1         "exon"). This option should probably not be selected,
2         because in some GFF3 annotation files do not include
3         "gene" records, but do include predicted coding
4         sequences for genes. (default: False)
5  --ignore_gff3      Disregard any information regarding redundancy of
6                     identified nucleotide sequences with identified
7                     protein sequences that may be found in GFF3 annotation
8                     files. (default: False)
9  --allow_internal_stops ALLOW_INTERNAL_STOPS
10                     Include sequences that have internal stop codons
11                     (anywhere other than the N-terminal position).
12                     (default: True)
13  --min_length MIN_LENGTH
14                     Absolute minimum length (in AA) of a hit sequence to
15                     be considered a potential distinct paralogue.
16                     (default: 55)
17  --min_percent_length MIN_PERCENT_LENGTH
18                     Minimum length (in AA) of a hit sequence as a
19                     percentage of query length for the hit to be
20                     considered a potential distinct paralogue. (default:
21                     15)
22  --min_percent_query_cover MIN_PERCENT_QUERY_COVER
23                     Minimum number of residues aligning with the original
24                     query as a percentage of the original query sequence
25                     length. (default: 0)
26  --overlap_required True if hits must overlap with a higher-ranking hit to
27                     be considered potential unique paralogues. (default:
28                     False)
29  --max_percent_ident MAX_PERCENT_IDENT
30                     Maximum percent identity (among aligning residues) for
31                     evaluating whether two sequences are redundant or not
32                     (secondary hits showing a percent identity with a
33                     higher-ranking hit exceeding this value will be
34                     excluded). (default: 98.0)
35  --min_alig_res_in_overlap MIN_ALIG_RES_IN_OVERLAP
36                     Minimum number of residues which must align for two
37                     sequences to be considered as potentially distinct
38                     hits. This is only relevant if the overlap_required
39                     option is specified. (default: 20)
40  --min_ident_res_in_overlap MIN_IDENT_RES_IN_OVERLAP
41                     Minimum number of aligning residues which must be
42                     identical for two sequences to be considered as
43                     potentially distinct hits. This is only relevant if
44                     the overlap_required option is specified. (default:
45                     10)
46  --min_sim_res_in_overlap MIN_SIM_RES_IN_OVERLAP
47                     Minimum number of aligning residues which must be
48                     similar for two sequences to be considered as
49                     potentially distinct hits. This is only relevant if

```

```

1         the overlap_required option is specified. (default:
2         15)
3     --min_ident_span_len MIN_IDENT_SPAN_LEN
4         Minimum number of aligning residues which are
5         identical that must exist in at least one continuous
6         span for two sequences to be considered as potentially
7         distinct hits (not counting positions where both
8         sequences have gaps). This is only relevant if the
9         overlap_required option is specified. (default: 0)
10    --min_sim_span_len MIN_SIM_SPAN_LEN
11        Minimum number of aligning residues which are similar
12        (or identical) that must exist in at least one
13        continuous span for two sequences to be considered as
14        potentially distinct hits (not counting positions
15        where both sequences have gaps). This is only relevant
16        if the overlap_required option is specified. (default:
17        0)
18    --min_percent_ident_in_overlap MIN_PERCENT_IDENT_IN_OVERLAP
19        Minimum percent identity between the two sequences of
20        interest in the alignment. This is only relevant if the
21        overlap_required option is specified. (default: 0)
22    --min_percent_sim_in_overlap MIN_PERCENT_SIM_IN_OVERLAP
23        Minimum percent similarity (including identity)
24        between the two sequences of interest in the
25        alignment. This is only relevant if the
26        overlap_required option is specified. (default: 0)
27    --min_percent_overlap MIN_PERCENT_OVERLAP
28        Minimum number of aligning residues between the two
29        sequences of interest as a percentage of the length of
30        the second sequence (the last sequence in the
31        alignment), not including gaps, for the two sequences
32        to be considered as potentially distinct hits. This is
33        only relevant if the overlap_required option is
34        specified. (default: 0)
35    --add_ali_col          Add a column to the csv file listing which alignment
36                           file in the queries directory to use for comparing
37                           sequences. Aligned FASTA queries are selected that
38                           match the query titles of the original queries used to
39                           retrieve each of the relevant hits listed in the csv
40                           file. No other options need to be specified in this
41                           case. (default: False)

```

3.17 amoebae plot

```

43 usage: amoebae [-h] [--csv_file2 CSV_FILE2] [--complex_info COMPLEX_INFO]
44               [--row_order ROW_ORDER] [--out_pdf OUT_PDF]
45               csv_file
46
47 Plot results of similarity search and sequence classification analyses. The

```

```

1 outputs are PDF files.
2
3 positional arguments:
4     csv_file          Path to a spreadsheet with the relevant results to be
5                       plotted. This can be either a CSV file output of the
6                       sum_rev_srch command or from the find_redun_seqs
7                       command. If the output of the sum_rev_srch command is
8                       used, however, redundant hits will be counted (e.g.,
9                       BLASTP and TBLASTN hits corresponding to the same or
10                      highly identical genomic loci).
11
12 optional arguments:
13     -h, --help        show this help message and exit
14     --csv_file2 CSV_FILE2
15                       Path to a second spreadsheet with relevant results to
16                       be compared to the first and plotted. (default: None)
17     --complex_info COMPLEX_INFO
18                       Path to file that specifies which query titles
19                       represent components of which protein complexes (or
20                       otherwise grouped proteins). (default: None)
21     --row_order ROW_ORDER
22                       Path to file that specifies the order in which data
23                       for each species will be displayed. (default: None)
24     --out_pdf OUT_PDF  Path to output pdf file. (default: None)

```

25 3.18 amoebae add_to_models

```

26 usage: amoebae [-h]
27                model_name alignment tree_topology subs_model type_seqs taxon
28
29 Add a phylogenetic model for relationships between members of a gene family
30 (sequence_data matrix, data type, tree topology, type sequence defining each
31 clade of interest, and substitution model) to a directory for use in
32 classifying sequence (via the 'phylo_class' command).
33
34 positional arguments:
35     model_name        An arbitrary name for the model (which will refer to the
36                       alignment, tree, substitution model, etc. collectively).
37     alignment         A multiple amino acid sequence alignment in nexus format.
38     tree_topology     Text file containing a tree (identified previously using
39                       MrBayes, etc) containing the names of all the sequences in
40                       the alignment, in newick format.
41     subs_model        The name of the substitution model used to recover the
42                       provided topology (chosen with ModelFinder or similar
43                       software).
44     type_seqs         Names of sequences (sequence headers) that are to be used to
45                       define clades of interest. A csv file with seq names in one
46                       column and clade names in the next column.
47     taxon             Taxonomic group represented in the model (e.g., "Eukaryotes",

```

```

1         or "Amorphea").
2
3 optional arguments:
4   -h, --help      show this help message and exit

```

5 3.19 amoebae list_models

```

6 usage: amoebae [-h]
7
8 Print a list of all usable model/reference tree names in the models directory
9 as defined in the settings file.
10
11 optional arguments:
12   -h, --help      show this help message and exit

```

13 3.20 amoebae get_alt_topos

```

14 usage: amoebae [-h] [--polytomy] [--not_polytomy_clades]
15                [--keep_original_backbone] [--iqtree_au_test]
16                model_name out_dir_path
17
18 Take a tree and make copies with every alternative topology for the branches
19 connecting the clades of interest. Output as additional models in the Models
20 directory.
21
22 positional arguments:
23   model_name          Name of model/backbone tree to modify (other info
24                       provided in the model info csv file).
25   out_dir_path        Path to directory in which output directory will be
26                       written.
27
28 optional arguments:
29   -h, --help          show this help message and exit
30   --polytomy          Just make one big polytomy connecting the clades of
31                       interest instead of making alternative bifurcating
32                       trees. (default: False)
33   --not_polytomy_clades
34                       Do not make subtrees/clades of interest polytomies in
35                       output topologies. (default: False)
36   --keep_original_backbone
37                       Keep the original backbone topology instead of
38                       generating a polytomy or alternative resolved
39                       topologies. (default: False)
40   --iqtree_au_test    Test all the relevant alternative topologies against
41                       each other using Approximately Unbiased (AU) test with
42                       IQ-tree. (default: False)

```

3.21 amoebae prune

```
usage: amoebae [-h] [--include_seqs] [--output_file OUTPUT_FILE]
              tree_file alignment name_replace_table

Identify sequences in a tree, and remove them from a given alignment for
further phylogenetic analysis.

positional arguments:
  tree_file              Tree in newick format (coded names, because ETE3
                        cannot parse taxon names with space characters without
                        quotation marks around them).
  alignment              Dataset used to make the tree (nexus alignment)
                        (original alignment with original taxon names either
                        trimmed or untrimmed).
  name_replace_table     File for decoding names in input tree file.

optional arguments:
  -h, --help            show this help message and exit
  --include_seqs         Include only listed sequences/nodes instead of
                        removing them. (default: False)
  --output_file OUTPUT_FILE
                        Path to output file. (default: None)
```

3.22 amoebae auto_prune

```
usage: amoebae [-h]
              [--max_bl_iqr_above_third_quartile MAX_BL_IQR_ABOVE_THIRD_QUARTILE]
              [--remove_redun_seqs REMOVE_REDUN_SEQS]
              [--remove_redun_seqs_threshold REMOVE_REDUN_SEQS_THRESHOLD]
              [--output_file OUTPUT_FILE]
              in_dir

Automatically identify sequences in a tree, and remove them from a given
alignment for further phylogenetic analysis.

positional arguments:
  in_dir                Path to directory that contains the phylogenetic
                        analysis output files (sequence name conversion table
                        file and original nexus alignment file can be in the
                        parent directory to this directory as long as their
                        names are mostly identical.

optional arguments:
  -h, --help            show this help message and exit
  --max_bl_iqr_above_third_quartile MAX_BL_IQR_ABOVE_THIRD_QUARTILE
                        Inclusion threshold for number of interquartile ranges
                        above the third quartile of terminal branch lengths
                        the length of a terminal branch can be before it is
```



```

1             considered an outlier (length is total distance from
2             root node after rooting on midpoint, or the longest
3             terminal branch on either side of the midpoint).
4             (default: 1.5)
5  --remove_redun_seqs REMOVE_REDUN_SEQS
6             Remove taxonomically redundant sequences (longest
7             branch of two sister branches when both are sequences
8             from the same species. (default: True)
9  --remove_redun_seqs_threshold REMOVE_REDUN_SEQS_THRESHOLD
10            Minimum support required to consider one of two sister
11            branches/sequences taxonomically redundant. Note: only
12            used if the remove_redun_seqs option is specified.
13            (default: 0.95)
14  --output_file OUTPUT_FILE
15            Path to output file. (default: None)

```

16 3.23 amoebae reduce_tree

```

17 usage: amoebae [-h] [--output_file OUTPUT_FILE] alignment tree_file
18
19 Remove terminal nodes from a given tree if there are not any sequences with
20 the same name in a given alignment.
21
22 positional arguments:
23   alignment            Alignment in nexus format with sequences representing
24                       a subset of those represented in the input tree.
25   tree_file            Tree in newick format.
26
27 optional arguments:
28   -h, --help            show this help message and exit
29   --output_file OUTPUT_FILE
30                       Path to output file. (default: None)

```

31 3.24 amoebae constrain_mb

```

32 usage: amoebae [-h] [--out_alignment OUT_ALIGNMENT] alignment tree
33
34 Add constraint commands to MrBayes input file.
35
36 positional arguments:
37   alignment            Nexus alignment for input to MrBayes (without any
38                       constraint commands).
39   tree                 Tree in newick format with same taxon names as in
40                       alignment. To be used as a topology constraint (all
41                       nodes).
42
43 optional arguments:
44   -h, --help            show this help message and exit

```

```

1  --out_alignment OUT_ALIGNMENT
2                                Path to nexus alignment for input to Mrbayes with
3                                constraints added. (default: None)

4  3.25 amoebae visualize_tree

5  usage: amoebae [-h] [--root_taxon ROOT_TAXON] [--highlight_paralogues]
6                [--add_clade_names_from_file]
7                input_directory method
8
9  Parse phylogenetic analysis output files in a given directory, and write
10 human-readable tree figures to PDF files.
11
12 positional arguments:
13   input_directory      Path to directory containing input files (must contain
14                        a .table file for decoding taxon names.
15   method               Name of tree searching program used. Either iqtree,
16                        raxml, or mrbayes accepted.
17
18 optional arguments:
19   -h, --help           show this help message and exit
20   --root_taxon ROOT_TAXON
21                        Name of species to root on (e.g.,
22                        "Klebsormidium_nitens").
23   --highlight_paralogues
24                        Highlight clades that contain paralogues found in at
25                        least one other clade in the tree.
26   --add_clade_names_from_file
27                        Use a file in the parent directory with clade names
28                        corresponding to representative sequences to add clade
29                        names to all the taxon names in the output trees.

```

30 **3.26 amoebae replace_seqs**

```

31 usage: amoebae [-h] [--fasta_file FASTA_FILE] alignment
32
33 Replace sequences in an alignment the full-length sequences from the relevant
34 file(s) in the Genomes directory, or with their top hits in a given fasta
35 file. And, align, mask, and trim the identified sequences to the input
36 alignment
37
38 positional arguments:
39   alignment            Path to multiple sequence alignment file in nexus
40                        format (trimmed alignment).
41
42 optional arguments:
43   -h, --help           show this help message and exit
44   --fasta_file FASTA_FILE

```

Path to file containing sequences with which to replace sequences in the alignment. If this option is not specified, then full-length sequences will be retrieved from files in the Genomes directory.

3.27 amoebae csv_to_fasta

```
usage: amoebae [-h] [--output_dir OUTPUT_DIR] [--abbrev] [--parologue_names]
               [--only_descr] [--subseq] [--all_hits] [--split_by_query_title]
               [--split_by_top_rev_srch_hit SPLIT_BY_TOP_REV_SRCH_HIT]
               csv_file
```

Extract sequences described in a spreadsheet output by AMOEBAE, and write to a file in FASTA format.

positional arguments:

csv_file Path to csv file listing sequences.

optional arguments:

-h, --help show this help message and exit

--output_dir OUTPUT_DIR

Path for output directory to contain FASTA files.
(default: None)

--abbrev Add species name instead of sequence description from fasta header. Applicable when the output file is to be used for alignment and phylogenetic analysis.
(default: False)

--parologue_names Use species name, query title, and paralogue number instead of sequence description from fasta header. Applicable when the output file is to be used for alignment and phylogenetic analysis. Does not work if the abbrev option is specified. (default: False)

--only_descr Use the description but not the ID as the new fasta sequence header. Does not work if the abbrev option is specified. (default: False)

--subseq Write subsequences that aligned to forward search query, instead of the full sequences. (default: False)

--all_hits Write all forward hits listed in the input csv file.
(default: False)

--split_by_query_title Write sequences to files according to the query title of the query which retrieved them in a similarity search. (default: False)

--split_by_top_rev_srch_hit SPLIT_BY_TOP_REV_SRCH_HIT

Write sequences to files according to the top hit that they retrieve in a reverse search, for each sequence that meets the reverse search criteria. (Provide the reverse search identifier, eg, "rev_srch_20180924122402-1") (default: None)

1 3.28 amoebae check_depend

```
2 usage: amoebae [-h]
3
4 Check that all the dependencies (other than python modules) are properly
5 installed and useable.
6
7 optional arguments:
8   -h, --help  show this help message and exit
```

9 3.29 amoebae check_imports

```
10 usage: amoebae [-h]
11
12 Check that all the import statements used in the AMOEBAE repository run
13 without error.
14
15 optional arguments:
16   -h, --help  show this help message and exit
```

17 4 Miscellaneous scripts

18 see amoebae/misc_scripts...

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