AMOEBAE command line interface documentation

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Version of November 13, 2020

Contents

1 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.

1.1 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.

Miscellaneous commands:
   csv_to_fasta      Generate a fasta file from sequences detailed in a
                     spreadsheet of similarity search results.
   check_depend      Check that all the dependencies are properly installed and
                     useable.
   check_imports     Check that all the import statements used in the AMOEBAE
                     repository run without error.
   regen_genome_info Write a new genome info spreadsheet file using filenames
                     from the Genomes directory.

```

```
positional arguments:
  command     Specify one of the functionalities of amoebae.

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

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express or implied. See the License for the specific language governing
permissions and limitations under the License.
```

1.2 amoebae mkdatadir

```
usage: amoebae [-h] new_dir_path

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

positional arguments:
  new_dir_path  Specify the full file path that you want the new directory to
                have.

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

1.3 amoebae setup_hmmdb

```
usage: amoebae [-h] indirpath

Construct an HMM database (with hmmpress). This is for later sorting of given
sequences into categories based on which HMM the score highest against.

positional arguments:
  indirpath   Path to directory containing amino acid sequence alignment
              file(s) to be constructed into an HMM database using hmmpress
              from the HMMer3 software package.

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

1.4 amoebae add_to_dbs

```
usage: amoebae [-h] [--split_char SPLIT_CHAR] [--split_pos SPLIT_POS]
```

```
               [--skip_header_reformat] [--auto_extract_accs]
               new_file main_data_dir

Format and add a file to a formatted directory.

positional arguments:
  new_file              Can be a fasta file (prot or nucl) or HMM databases,
                        generated using the hmmpress program in the HMMer
                        software package. Or a GFF3 annotation file.
  main_data_dir         Path to main data directory (with Genomes, Queries,
                        and Models subdirectories).

optional arguments:
  -h, --help            show this help message and exit
  --split_char SPLIT_CHAR
                        Character to split the header string on for extracting
                        the accession. (default: )
  --split_pos SPLIT_POS
                        Position that the accession will be in after
                        splitting. (default: 0)
  --skip_header_reformat
                        Skip reformatting of header lines in input fasta file.
                        (default: False)
  --auto_extract_accs   Automatically identify accessions/IDs in sequence
                        headers (overrides split_char and split_pos options
                        above). (default: False)
```

1.5 amoebae list_dbs

```
usage: amoebae [-h] main_data_dir

Print a list of all usable query files in the query directory as defined in a
given AMOEBAE data directory.

positional arguments:
  main_data_dir  Path to main data directory (with Genomes, Queries, and
                 Models subdirectories).

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

1.6 amoebae add_to_queries

```
usage: amoebae [-h] query_file main_data_dir

Add a query file to a formatted directory. This command adds a given sequence
file to the directory with the path that you have specified in the settings.py
file, and appends a corresponding line to the CSV file that you specified
```

```
(e.g., '0_query_info.csv') to indicate the query title, etc.

positional arguments:
  query_file     Path to a sequence file in FASTA format that can be used as a
                 similarity search query file. Or path to a directory
                 containing only files for addition to the queries. Note: By
                 default, the portion of the input filename preceding the
                 first underscore character will be recorded as the "query
                 title", the remaining substring preceding the second
                 underscore character will be recorded as the taxon (e.g.,
                 "Hsapiens"), and the rest of the filename preceding the
                 filename extension will be recorded as the sequence ID. So
                 the filename might look like this:
                 "QUERYTITLE_HSAPIENS_SEQUENCEID.fa". However, the relevant
                 information can be revised in the "Queries/0_query_info.csv"
                 file afterward if necessary.
  main_data_dir  Path to main data directory (with Genomes, Queries, and
                 Models subdirectories).

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

1.7 amoebae list_queries

```
usage: amoebae [-h] main_data_dir

Print a list of all usable query files in the query directory as defined in a
given AMOEBAE data directory.

positional arguments:
  main_data_dir  Path to main data directory (with Genomes, Queries, and
                 Models subdirectories).

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

1.8 amoebae get_redun_hits

```
usage: amoebae [-h] [--query_name QUERY_NAME]
               [--query_list_file QUERY_LIST_FILE] [--db_name DB_NAME]
               [--db_list_file DB_LIST_FILE] [--query_title QUERY_TITLE]
               [--blast_report_evalue_cutoff BLAST_REPORT_EVALUE_CUTOFF]
               [--blast_max_target_seqs BLAST_MAX_TARGET_SEQS]
               [--hmmer_report_evalue_cutoff HMMER_REPORT_EVALUE_CUTOFF]
               [--hmmer_report_score_cutoff HMMER_REPORT_SCORE_CUTOFF]
               [--max_number_of_hits_to_summarize MAX_NUMBER_OF_HITS_TO_SUMMARIZE]
               [--num_threads_similarity_searching NUM_THREADS_SIMILARITY_SEARCHING
    ]
```

```
               [--predict_redun_hit_selection] [--csv_file CSV_FILE]
               out_dir_path main_data_dir

Run searches with queries to find redundant hits in databases (for
interpreting results).

positional arguments:
  out_dir_path          Path to directory to write search results to.
  main_data_dir         Path to main data directory (with Genomes, Queries,
                        and Models subdirectories).

optional arguments:
  -h, --help            show this help message and exit
  --query_name QUERY_NAME
                        Query filename to use (not full path). (default: None)
  --query_list_file QUERY_LIST_FILE
                        Path to file containing a list of query files to use,
                        if no query_name is specified (or all queries by
                        default). (default: None)
  --db_name DB_NAME     Name of database file in the database directory in
                        which to do searches (not full path). (default: None)
  --db_list_file DB_LIST_FILE
                        Path to file containing a list of database files to
                        use (if no db_name specified). (default: None)
  --query_title QUERY_TITLE
                        Name to be assigned to hits in databases that may be
                        considered redundant with a search query to which the
                        same title is assigned, otherwise it is taken from the
                        query info spreadsheet specified in the settings.py
                        file ('query_info_csv'). (default: None)
  --blast_report_evalue_cutoff BLAST_REPORT_EVALUE_CUTOFF
                        Maximum E-value for reporting BLAST hits. (default:
                        0.05)
  --blast_max_target_seqs BLAST_MAX_TARGET_SEQS
                        Maximum BLAST target sequences to consider. (default:
                        500)
  --hmmer_report_evalue_cutoff HMMER_REPORT_EVALUE_CUTOFF
                        Maximum E-value for reporting HMMer hits. (default:
                        0.05)
  --hmmer_report_score_cutoff HMMER_REPORT_SCORE_CUTOFF
                        Minimum sequence score for reporting HMMer hits.
                        (default: 5)
  --max_number_of_hits_to_summarize MAX_NUMBER_OF_HITS_TO_SUMMARIZE
                        Absolute maximum number of hits (BLAST, HMMer, etc) to
                        summarize in the output spreadsheet. This is important
                        when working with sequences with WD40 domains, for
                        example. (default: 50)
  --num_threads_similarity_searching NUM_THREADS_SIMILARITY_SEARCHING
                        Number of threads to use for running searches.
                        (default: 4)
  --predict_redun_hit_selection
                        Write a copy of the output spreadsheet with '+' in
                        rows for hits that may be specific to each query
                        title, due to not being retrieved as top hits by
                        queries associated with different query titles.
                        (default: False)
  --csv_file CSV_FILE   Path to spreadsheet to append summary of result to for
                        manual annotation. (default: None)

Recommendation: For most analyses, use the --query_name option and the
--db_name option, and run the get_redun_hits command for each query
separately. Otherwise, there will be redundant information in the output
spreadsheet(s).
```

1.9 amoebae setup_fwd_srch

```
usage: amoebae [-h] [--outdir OUTDIR] srch_dir query_list_file db_list_file

Make a directory in which to write output files from similarity searches.

positional arguments:
  srch_dir         Path to directory that will contain output directory as a
                   subdirectory.
  query_list_file  Path to file with list of queries to search with.
  db_list_file     Path to file with list of databases to search with.

optional arguments:
  -h, --help       show this help message and exit
  --outdir OUTDIR  Path to directory to put search results into (so that this
                   step can be piped together with other commands). (default:
                   None)

Note: Use the bash script to run forward searches on a remote server.
```

1.10 amoebae run_fwd_srch

```
usage: amoebae [-h] [--blast_report_evalue_cutoff BLAST_REPORT_EVALUE_CUTOFF]
               [--blast_max_target_seqs BLAST_MAX_TARGET_SEQS]
               [--hmmer_report_evalue_cutoff HMMER_REPORT_EVALUE_CUTOFF]
               [--hmmer_report_score_cutoff HMMER_REPORT_SCORE_CUTOFF]
               [--num_threads_similarity_searching NUM_THREADS_SIMILARITY_SEARCHING
    ]
               fwd_srch_dir main_data_dir

Perform searches with original queries into subject databases.

positional arguments:
  fwd_srch_dir          Path to directory that will contain forward search
                        output files.
  main_data_dir         Path to main data directory (with Genomes, Queries,
                        and Models subdirectories).

optional arguments:
  -h, --help            show this help message and exit
  --blast_report_evalue_cutoff BLAST_REPORT_EVALUE_CUTOFF
                        Maximum E-value for reporting BLAST hits. (default:
                        0.05)
  --blast_max_target_seqs BLAST_MAX_TARGET_SEQS
                        Maximum BLAST target sequences to consider. (default:
                        500)
  --hmmer_report_evalue_cutoff HMMER_REPORT_EVALUE_CUTOFF
                        Maximum E-value for reporting HMMer hits. (default:
                        0.05)
  --hmmer_report_score_cutoff HMMER_REPORT_SCORE_CUTOFF
                        Minimum sequence score for reporting HMMer hits.
                        (default: 5)
  --num_threads_similarity_searching NUM_THREADS_SIMILARITY_SEARCHING
                        Number of threads to use for running searches.
                        (default: 4)
```

1.11 amoebae sum_fwd_srch

```
usage: amoebae [-h] [--csv_file CSV_FILE] [--max_evalue MAX_EVALUE]
               [--max_gap_between_tblastn_hsps MAX_GAP_BETWEEN_TBLASTN_HSPS]
               [--do_not_use_exonerate]
               [--exonerate_score_threshold EXONERATE_SCORE_THRESHOLD]
               [--max_hits_to_sum MAX_HITS_TO_SUM]
               [--max_length_diff MAX_LENGTH_DIFF]
               fwd_srch_out csv_out_path main_data_dir

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

positional arguments:
  fwd_srch_out          Path to directory where forward search results were
                        written.
  csv_out_path          Path to output summary spreadsheet (CSV) file.
  main_data_dir         Path to main data directory (with Genomes, Queries,
                        and Models subdirectories).

optional arguments:
  -h, --help            show this help message and exit
  --csv_file CSV_FILE   Path to summary spreadsheet (CSV) file, which already
                        contains search summaries. If such a file is
                        specified, then the output CSV file will contain the
                        columns from this CSV file with additional columns
                        summarizing additional forward search results.
                        (default: None)
  --max_evalue MAX_EVALUE
                        Maximum E-value threshold for reporting forward search
                        hits. (default: 0.0005)
  --max_gap_between_tblastn_hsps MAX_GAP_BETWEEN_TBLASTN_HSPS
                        Maximum number of nucleotide bases between TBLASTN
                        HSPs to be considered part of the same gene locus.
                        This is important, because it will be assumed that HSP
                        separated by more than this number of nucleotide bases
                        are not part of the same gene or TBLASTN "hit".
                        (default: 10000)
  --do_not_use_exonerate
                        Override the default use of exonerate to identify
                        coding sequences and translations, and just use
                        TBLASTN instead. This option is provided because
                        concatenated TBLASTN HSPs may be more inclusive of
                        sequences within the target sequence, and the results
                        of TBLASTN and exonerate may need to be compared.
                        Also, note that HSPs identified by TBLASTN but for
                        which exonerate yields no alignments will be ignored
                        if exonerate is used. (default: False)
  --exonerate_score_threshold EXONERATE_SCORE_THRESHOLD
                        Set score threshold to be applied when running
                        exonerate on nucleotide sequences identified by
                        TBLASTN. The default for setting of exonerate is 100,
                        but a lower score is set as default here, because
                        otherwise exonerate cannot identify some of the
                        seqeunces identified by TBLASTN. This option is only
                        relevant if using exonerate. (default: 10)
  --max_hits_to_sum MAX_HITS_TO_SUM
                        Maximum number of forward search hits to list in the
                        summary spreadsheet. If zero, then reverse searches
                        will be performed for all hits. (default: 0)
  --max_length_diff MAX_LENGTH_DIFF
                        Maximum number of amino acid residues length
                        difference allowed between the original query and the
                        forward hit sequence. If -1, then a maximum length
                        cutoff will not be applied. (default: -1)
```

1.12 amoebae setup_rev_srch

```
usage: amoebae [-h] [--outdir OUTDIR] [--aasubseq] [--nafullseq]
               srch_dir csv_file databases main_data_dir

Make directory in which to write results of reverse searches.

positional arguments:
  srch_dir         Path to directory that will contain output directory as a
                   subdirectory.
  csv_file         Path to summary spreadsheet (CSV) file, which contains a
                   summary of forward search(es).
  databases        Database filename (in database directory) or path to file
                   with list of database filenames. Note that filenames are
                   needed, not file paths.
  main_data_dir    Path to main data directory (with Genomes, Queries, and
                   Models subdirectories).

optional arguments:
  -h, --help       show this help message and exit
  --outdir OUTDIR  Path to directory to put search results into (so that this
                   step can be piped together with other commands). (default:
                   None)
  --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.
                   (default: False)
  --nafullseq      Use the full (nucleic acid) forward hit sequence. This is
                   default for amino acid hits. (default: False)
```

1.13 amoebae run_rev_srch

```
usage: amoebae [-h] [--blast_report_evalue_cutoff BLAST_REPORT_EVALUE_CUTOFF]
               [--blast_max_target_seqs BLAST_MAX_TARGET_SEQS]
               [--hmmer_report_evalue_cutoff HMMER_REPORT_EVALUE_CUTOFF]
               [--hmmer_report_score_cutoff HMMER_REPORT_SCORE_CUTOFF]
               [--num_threads_similarity_searching NUM_THREADS_SIMILARITY_SEARCHING
    ]
               rev_srch_dir main_data_dir

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

positional arguments:
  rev_srch_dir          Path to directory that will contain output of
                        searches.
  main_data_dir         Path to main data directory (with Genomes, Queries,
                        and Models subdirectories).

```

```
optional arguments:
  -h, --help            show this help message and exit
  --blast_report_evalue_cutoff BLAST_REPORT_EVALUE_CUTOFF
                        Maximum E-value for reporting BLAST hits. (default:
                        0.05)
  --blast_max_target_seqs BLAST_MAX_TARGET_SEQS
                        Maximum BLAST target sequences to consider. (default:
                        500)
  --hmmer_report_evalue_cutoff HMMER_REPORT_EVALUE_CUTOFF
                        Maximum E-value for reporting HMMer hits. (default:
                        0.05)
  --hmmer_report_score_cutoff HMMER_REPORT_SCORE_CUTOFF
                        Minimum sequence score for reporting HMMer hits.
                        (default: 5)
  --num_threads_similarity_searching NUM_THREADS_SIMILARITY_SEARCHING
                        Number of threads to use for running searches.
                        (default: 4)
```

1.14 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]
               fwd_srch_csv rev_srch_out csv_out_path main_data_dir

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

positional arguments:
  fwd_srch_csv          Path to summary spreadsheet (CSV) file, which contains
                        forward search summaries and also may already contain
                        reverse search summaries.
  rev_srch_out          Path to directory where reverse search results were
                        written.
  csv_out_path          Path output spreadsheet (CSV) file with reverse search
                        results appended to previous results.
  main_data_dir         Path to main data directory (with Genomes, Queries,
                        and Models subdirectories).

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 any and all reverse
                        search hits are equivalent to/redundant with 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)
```

1.15 amoebae interp_srchs

```
usage: amoebae [-h] [--fwd_only] [--fwd_evalue_cutoff FWD_EVALUE_CUTOFF]
               [--rev_evalue_cutoff REV_EVALUE_CUTOFF]
               [--hmmer_cutoff HMMER_CUTOFF] [--no_overlapping_hits]
               [--out_csv_path OUT_CSV_PATH]
               csv_file

Interpret search results based on final summary, which provides a basis for
further analyses of positive hits.

positional arguments:
  csv_file              Path to spreadsheet with forward and reverse search
                        results.

optional arguments:
  -h, --help            show this help message and exit
  --fwd_only            Interpret forward searches based on score (HMMer)
                        cutoff. (default: False)
  --fwd_evalue_cutoff FWD_EVALUE_CUTOFF
                        Specify an (more stringent) E-value cutoff for forward
                        search results. (default: None)
  --rev_evalue_cutoff REV_EVALUE_CUTOFF
                        Specify an (more stringent) E-value cutoff for reverse
                        search results. (default: None)
  --hmmer_cutoff HMMER_CUTOFF
                        Specify a score that hits must exceed to be included.
                        (default: 20)
  --no_overlapping_hits
                        If more than one query (query title) retrieves a given
                        sequence as a positive hit based on the search
                        criteria, make the sequence a negative hit for all
                        queries (query titles), except for the one that
                        retrieved the sequence with the lowest (strongest)
                        E-value. Warning: Do not use this option if you are
                        searching sequences that include genomic sequences
                        that may include more than one genomic locus per
                        sequence. False-negative results could occur in this
                        case, because different queries for non-orthologous
                        genes could retrieve subsequences in the same subject
                        sequence. (default: False)
  --out_csv_path OUT_CSV_PATH
                        Optionally specify an output file path, so that this
                        command can be piped together with others. (default:
                        None)
```

1.16 amoebae find_redun_seqs

```
usage: amoebae [-h] [--out_csv_path OUT_CSV_PATH]
               [--remove_tblastn_hits_at_annotated_loci]
               [--just_look_for_genes_in_gff3] [--ignore_gff3]
               [--allow_internal_stops ALLOW_INTERNAL_STOPS]
               [--min_length MIN_LENGTH]
               [--min_percent_length MIN_PERCENT_LENGTH]
               [--min_percent_query_cover MIN_PERCENT_QUERY_COVER]
               [--overlap_required] [--max_percent_ident MAX_PERCENT_IDENT]
               [--min_alig_res_in_overlap MIN_ALIG_RES_IN_OVERLAP]
               [--min_ident_res_in_overlap MIN_IDENT_RES_IN_OVERLAP]
               [--min_sim_res_in_overlap MIN_SIM_RES_IN_OVERLAP]
               [--min_ident_span_len MIN_IDENT_SPAN_LEN]
               [--min_sim_span_len MIN_SIM_SPAN_LEN]
               [--min_percent_ident_in_overlap MIN_PERCENT_IDENT_IN_OVERLAP]
               [--min_percent_sim_in_overlap MIN_PERCENT_SIM_IN_OVERLAP]
               [--min_percent_overlap MIN_PERCENT_OVERLAP]
               [--plot_hit_exclusion] [--add_ali_col]
               csv_file main_data_dir

Identify hit sequences likely encoded by the same gene loci in the genome of a
given species, or otherwise not representing paralogous genes. Criteria are
applied in this order: 1. Peptide hits with the same ID as higher-ranking hits
for the same query (query title) are excluded. 2. Nucleotide hits for the same
loci as peptide sequence hits are excluded. 3. Sequences with internal stop
codons are excluded, as these are potentially pseudogenes. 4. Sequences are
excluded if they do not meet several minimum length criteria: Absolute minimum
length (in amino acids) and percent query cover. 5. Sequences are excluded if
they do not overlap to a specified degree with all included higher-ranking
hits for the same query (query title) in sequence data for the same
species/genome. This is determined by algorithmically comparing pairs of
sequences aligned to a reference alignment of homologues, and several minimum
```

```
measures of alignment overlap may be specified. 6. Secondary hit sequences are
excluded if they do not meet a specified maximum percent identity threshold.
Highly identical sequences may result from false segmental duplications in the
genome assembly, may represent alleles, etc. Note: Applying these criteria
requires a column to be manually added to the input csv file prior to running
with the header "Alignment for sequence comparison" and filled with the
appropriate alignment name to use (one for each query title, as listed in the
"Query title" column). Alternatively, you can run this command with the
--add_ali_col option to automatically identify appropriate alignments among
your aligned FASTA queries used for running HMMer searches. If no alignment
(.afaa) file can be found, then the first single sequence query file (.faa)
that appears in the summary CSV file will be used instead.

positional arguments:
  csv_file              Path to spreadsheet with interpreted search results
                        outputted by the interp_srchs command.
  main_data_dir         Path to main data directory (with Genomes, Queries,
                        and Models subdirectories).

optional arguments:
  -h, --help            show this help message and exit
  --out_csv_path OUT_CSV_PATH
                        Optionally specify an output file path, so that this
                        command can be piped together with others. (default:
                        None)
  --remove_tblastn_hits_at_annotated_loci
                        Ignore tblastn hits that overlap with any previously
                        annotated loci. The rationale for this would be that
                        the corresponding protein sequences should have been
                        retrieved if the tblastn hit were a true positive
                        anyway. If this option is not specified, then
                        sequences will still be excluded if they specifically
                        correspond to the same loci as do higher-ranking hits.
                        (default: False)
  --just_look_for_genes_in_gff3
                        When looking for records in GFF3 annotation files that
                        overlap with subsequences identified by similarity
                        searching (TBLASTN), ignore records that are not
                        explicitly "gene" (for example, "CDS", "mRNA", and
                        "exon"). This option should probably not be selected,
                        because in some GFF3 annotation files do not include
                        "gene" records, but do include predicted coding
                        sequences for genes. (default: False)
  --ignore_gff3         Disregard any information regarding redundancy of
                        identified nucleotide sequences with identified
                        protein sequences that may be found in GFF3 annotation
                        files. (default: False)
  --allow_internal_stops ALLOW_INTERNAL_STOPS
                        Include sequences that have internal stop codons
                        (anywhere other than the N-terminal position).
                        (default: True)
  --min_length MIN_LENGTH
                        Absolute minimum length (in AA) of a hit sequence to
                        be considered a potential distinct paralogue.
                        (default: 55)
  --min_percent_length MIN_PERCENT_LENGTH
                        Minimum length (in AA) of a hit sequence as a
                        percentage of query length for the hit to be
                        considered a potential distinct paralogue. (default:
                        15)
  --min_percent_query_cover MIN_PERCENT_QUERY_COVER
                        Minimum number of residues aligning with the original
                        query as a percentage of the original query sequence
                        length. (default: 0)
  --overlap_required    True if hits must overlap with a higher-ranking hit to
                        be considered potential unique paralogues. (default:
                        False)
  --max_percent_ident MAX_PERCENT_IDENT
                        Maximum percent identity (among aligning residues) for
                        evaluating whether two sequences are redundant or not
                        (secondary hits showing a percent identity with a
                        higher-ranking hit exceeding this value will be
                        excluded). (default: 98.0)
  --min_alig_res_in_overlap MIN_ALIG_RES_IN_OVERLAP
                        Minimum number of residues which must align for two
                        sequences to be considered as potentially distinct
                        hits. This is only relevant if the overlap_required
                        option is specified. (default: 20)
  --min_ident_res_in_overlap MIN_IDENT_RES_IN_OVERLAP
                        Minimum number of aligning residues which must be
                        identical for two sequences to be considered as
                        potentially distinct hits. This is only relevant if
                        the overlap_required option is specified. (default:
                        10)
  --min_sim_res_in_overlap MIN_SIM_RES_IN_OVERLAP
                        Minimum number of aligning residues which must be
                        similar for two sequences to be considered as
                        potentially distinct hits. This is only relevant if
                        the overlap_required option is specified. (default:
                        15)
  --min_ident_span_len MIN_IDENT_SPAN_LEN
                        Minimum number of aligning residues which are
                        identical that must exist in at least one continuous
                        span for two sequences to be considered as potentially
                        distinct hits (not counting positions where both
                        sequences have gaps). This is only relevant if the
                        overlap_required option is specified. (default: 0)
  --min_sim_span_len MIN_SIM_SPAN_LEN
```

```
                        Minimum number of aligning residues which are similar
                        (or identical) that must exist in at least one
                        continuous span for two sequences to be considered as
                        potentially distinct hits (not counting positions
                        where both sequences have gaps). This is only relevant
                        if the overlap_required option is specified. (default:
                        0)
  --min_percent_ident_in_overlap MIN_PERCENT_IDENT_IN_OVERLAP
                        Minimum percent identity between the two sequences of
                        interest in the alignment.This is only relevant if the
                        overlap_required option is specified. (default: 0)
  --min_percent_sim_in_overlap MIN_PERCENT_SIM_IN_OVERLAP
                        Minimum percent similarity (including identity)
                        between the two sequences of interest in the
                        alignment.This is only relevant if the
                        overlap_required option is specified. (default: 0)
  --min_percent_overlap MIN_PERCENT_OVERLAP
                        Minimum number of aligning residues between the two
                        sequences of interest as a percentage of the length of
                        the second sequence (the last sequence in the
                        alignment), not including gaps, for the two sequences
                        to be considered as potentially distinct hits. This is
                        only relevant if the overlap_required option is
                        specified. (default: 0)
  --plot_hit_exclusion  Plot number of hits excluded by the various criteria
                        applied. (default: False)
  --add_ali_col         Add a column to the csv file listing which alignment
                        file in the queries directory to use for comparing
                        sequences. Aligned FASTA queries are selected that
                        match the query titles of the original queries used to
                        retrieve each of the relevant hits listed in the csv
                        file. No other options need to be specified in this
                        case. (default: False)
```

1.17 amoebae plot

```
usage: amoebae [-h] [--csv_file2 CSV_FILE2] [--complex_info COMPLEX_INFO]
               [--row_order ROW_ORDER] [--out_pdf OUT_PDF]
               csv_file

Plot results of similarity search and sequence classification analyses. The
outputs are PDF files.

positional arguments:
  csv_file              Path to a spreadsheet with the relevant results to be
                        plotted. This can be either a CSV file output of the
                        sum_rev_srch command or from the find_redun_seqs
                        command. If the output of the sum_rev_srch command is
                        used, however, redundant hits will be counted (e.g.,
                        BLASTP and TBLASTN hits corresponding to the same or
                        highly identical genomic loci).

optional arguments:
  -h, --help            show this help message and exit
  --csv_file2 CSV_FILE2
                        Path to a second spreadsheet with relevant results to
                        be compared to the first and plotted. (default: None)
  --complex_info COMPLEX_INFO
                        Path to file that specifies which query titles
                        represent components of which protein complexes (or
                        otherwise grouped proteins). (default: None)
  --row_order ROW_ORDER
                        Path to file that specifies the order in which data
                        for each species will be displayed. (default: None)
  --out_pdf OUT_PDF     Path to output pdf file. (default: None)
```

1.18 amoebae csv_to_fasta

```
usage: amoebae [-h] [--output_dir OUTPUT_DIR] [--abbrev] [--paralogue_names]
               [--only_descr] [--subseq] [--all_hits] [--split_by_query_title]
               [--split_by_top_rev_srch_hit SPLIT_BY_TOP_REV_SRCH_HIT]
               [--split_to_query_fastas]
               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)
  --paralogue_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)
  --split_to_query_fastas
                        Write sequences to separate files with filenames that
                        can be easily parsed for loading the the files as
                        queries using the add_to_queries command. (default:
                        False)
```

1.19 amoebae check_depend

```
usage: amoebae [-h]

Check that all the dependencies (other than python modules) are properly
installed and useable.

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

1.20 amoebae check_imports

```
usage: amoebae [-h]

Check that all the import statements used in the AMOEBAE repository run
without error.

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

1.21 amoebae regen_genome_info

```
usage: amoebae [-h] data_dir_path

Write a new genome info spreadsheet (0_genome_info.csv) file using filenames
from the Genomes directory.

positional arguments:
  data_dir_path  Specify the full path to an existing AMOEBAE data directory,
                 which contains a 'Genomes' subdirectory. The new genome info
                 file will be added to this subdirectory.

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