Appendix B

Software Manual

codonPhyML

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GNU General Public License V3

created by

Marcelo Serrano Zanetti

contact: www.marceloserranozanetti.org

&

Stefan Zoller, Manuel Gil, Louis Du Plessis and Maria Anisimova

based on PhyML

Stéphane Guindon

guindon@stat.auckland.ac.nz

B.1 Download

codonPhyML is an open source project hosted by sourceforge. To download its source code, access the project's website at https://sourceforge.net/projects/codonphyml/.

B.2 Installation

Support is guaranteed to LINUX/UNIX environments. Only source code is provided which makes possible compilation in any other environment. The software makes use of optimized linear algebra libraries that improve its performance, and therefore the installation of those libraries in your local environment is recommended though the source code can be compiled without them. If your computer is a multi-core machine (most of current computers are), you can enable the OPENMP support and the expected running time will be greatly decreased when running CODONPHYML with site classes codon model.

B.2.1 Preliminary steps

The first step is to unzip the source code. A directory codonPhyML_dev will be created in the process.

Additional Libraries

The CODONPHYML performance can be increased by using the well known mathematical libraries BLAS and LAPACK. You can check which implementations are available for your environment at `http://www.netlib.org/`. For Linux users, Tte best option is to install the ATLAS library which provides support to BLAS and LAPACK and moreover, will deliver a version optimized to your hardware, automatically. An installation shortcut is available for Ubuntu/Kubuntu/Debian Linux users. It is enough to install the ATLAS package which can be easily done by typing the following command in a terminal window: *sudo apt-get install libatlas-base-dev libatlas-headers*

After the installation is complete check your directory /usr/lib and search for the files libatlas.a, libblas.a, liblapack-atlas.a and liblapack.a. In some systems you might find liblapack-3.a instead of just liblapack.a. If that is your case you must edit few lines of the file src/Makefile.am. Open it and replace all occurrences of "-llapack" by "-llapack-3". If you cannot find any of those files and if you cannot install the libraries yourself you can still compile CODONPHYML without the BLAS/LAPACK support. You should now be done and ready to continue.

If you are using a recent version of MacOSX, LAPACK and BLAS are already installed. Just use the "–enable-blas" switch when configuring the source code.

One (optional) configuration file format for CODONPHYML is YAML (see `http://www.yaml.org/`), and we use libyaml (`http://pyyaml.org/wiki/LibYAML`) to parse this for-

mat. Again, for UBUNTU/KUBUNTU/DEBIAN LINUX users, installation is as simple as typing *sudo apt-get install libyaml-dev* in a terminal window. In OSX, you could, e.g., use homebrew (`http://mxcl.github.com/homebrew/`) to install this library. If you want to use the YAML format in CODONPHYML, use '–with-yaml' with './configure'. If you have your YAML library installed in some other location than /usr/local/lib or /usr/local/include, change this default value using

$$'--with-yaml-path=/YOUR/LIBYAML/ROOTDIR'$$

B.2.2 Compilation Troubleshoots

The source code and make files were configured to be used with the GCC compiler. If you need to use another compiler you must edit the files configure.ac and src/Makefile.am, which can be found in the installation directory.

There are four options for the compilation of the source code which differ in the extra libraries that are going to be linked together with the main program. For that you will execute one of the four make scripts in the directory codonPhyML_dev/. Table B.1 depicts all options and respective features.

Table B.1: The "make" scripts and auxiliary library requirements

Script	OpenMP	BLAS+LAPACK
make_phyml	NO	NO
make_phyml_omp	YES	NO
make_phyml_blas	NO	YES
make_phyml_blas_omp	YES	YES

Select one of the scripts above and execute it in a Linux/Unix terminal window. You might need to add file execution permission, which can be easily done with the command: *chmod a+wrx script*.

During the compilation check for error messages. If none appears, the installation is complete and you can execute the file CODONPHYML to start using CODONPHYML. You can find it in the directory codonPhyML_dev/src/. One common error that stops the compilation at the very beginning is the absence of the package AUTOMAKE. To install AUTOMAKE in Ubuntu/Kubunt/Debian Linux environments simply type *sudo apt-get install automake* in a terminal window. Users of other environments are recommended to access `http://www.gnu.org/software/automake/` for further details.

B.3 Program Usage

The codonPhyML supports command line input and it also allows the user to set up its execution through a self-explanatory menu. For more detailed program options related to nucleotide and amino acid models read the original PhyML user guide, which you can find within the codonPhyML installation folder. In this section, only the options required for the execution of codonPhyML codon models will be described.

B.3.1 Command Line

The basic commands and respective options are described below.

-i filename

Read an input sequence alignment in the standard formats.

-q

Sequence alignment is sequential. Default is interleaved.

--expm option

The choice of the method used to perform the matrix exponentiation. The options are "EIGEN" for the eigenvalues/eigenvectors decomposition and "SSPADE" for the Padé approximation (see [57] and [32]). The "SSPADE" is preferable for non-reversible models. The default option is "EIGEN". Under research is an heuristic based on the Taylor expression of the matrix exponential, which can executed with option "TAYLOR".

--optBrent option

Use Brent search to optimize each free parameter individually instead of using the BFGS algorithm which does multi-variate simultaneous optimization (default method). At each parameter optimization step, the Brent algorithm will cycle through all parameters the number of times specified by "option".

-g option

The genetic code. All genetic codes listed in `http://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi` are available. The default option is the standard genetic code, other codes and their respective command line option are listed in Table B.2. When using a genetic code different from the standard one must remember to provide a sequence

alignment without stop codons. Also, empirical and semi-parametric models can only use the genetic code with which its empirical matrices were generated.

Table B.2: The available genetic codes.

Option	Description
STANDARD	Standard
TVMC	Vertebrate Mitochondrial
TYMC	Yeast Mitochondrial
THMPCMCMSC	Mold, Protozoan, and Coelenterate Mit. Code and Myco/Spiroplasma
THIMC	Invertebrate Mitochondrial
THCDHNC	Ciliate, Dasycladacean and Hexamita Nuclear
THEFMC	Echinoderm and Flatworm Mitochondrial
THENC	Euplotid Nuclear
THBAPPC	Bacterial, Archaeal and Plant Plastid
THAYNC	Alternative Yeast Nuclear
THAMC	Ascidian Mitochondrial
THAFMC	Alternative Flatworm Mitochondrial
BLNC	Blepharisma Nuclear
CHMC	Chlorophycean Mitochondrial
TRMC	Trematode Mitochondrial
SCOMC	Scenedesmus obliquus mitochondrial
THMC	Thraustochytrium Mitochondrial

-m option

Selects a substitution model. Nucleotide or amino-acid based models are the same as in PhyML; the additional possible codon models of codonPhyML are "GY" [according to 26], "MG" [according to 47], "YAP" [according to 73] or "PCM" [according to 75].

--pcs option

If you choose PCM as your codon model, specify the number of principal components (up to 100) to use.

--qrates option

If you choose GY, MG or YAP as your basic model of substitution, you can additionally use one of the following initial Q matrices to create a (semi-) empirical codon model: KOSI07 as given in [43], "SCHN05" as used by [56] or "ECMUSR" for a user defined initial rate matrix. The use of a rate matrix defined by the user ("ECMUSR") requires a file

containing the exchange rates and equilibrium frequencies in (also base frequencies for MG based models). The exchange rates must be provided as a lower triangular matrix, including the rows corresponding to stop codons, from/to which the exchange rates must be specified to zero. Separated by one line after the exchange rates the user must provide the equilibrium frequencies of the codons (including stop codons) and if a MG model is being applied, after another line the user must provide the base frequencies within the F3X4 framework. The file must be called "usermatrix.ecm".

--fmodel option

Define the frequency model. Can be either one of "F1XCODONS", "F1X4", "F3X4" or "CF3X4".

-f option

Equilibrium frequency estimation: If option is "empirical" the frequencies are estimated by counting directly from the input data. If option is "optimize" the frequencies will be estimated using maximum likelihood approach and finally if option is "model" the equilibrium frequencies are assumed to be taken from the given model (applies for amino acid and codon data). At last, the user can provided the frequencies values separated by comma, without spaces. For example, if the frequencies are modeled within the F3x4 framework the user must provide: fT1, fC1, fA1, fG1, fT2, fC2, fA2, fG2, fT3, fC3, fA3, fG3. Where "f" refers to frequency, {T,C,A,G} are the nucleotide bases, and the index {1,2,3} corresponds to the respective codon position. The model F1x4 and F1xSenseCodons (depending on the genetic code) are also available. This option can also be used to provide the equilibrium frequencis of nucleotide and amino acid models. For nucleotides one uses the cannonical ordering "fT, fC, fA, fG" and for amino acids one must use the ordering "fA, fR, fN, fD, fC, fQ, fE, fG, fH, fI, fL, fK, fM, fF, fP, fS, fT, fW, fY, fV".

-t option

The κ parameter or transition/transversion rate ratio. For parametric codon models, option can be "e" for estimation by maximum likelihood or can be a fixed value given by the user. For semi–parametric models option refers to the $\kappa(i, j)$ in [43]. The possibilities are displayed in Table B.3. By default, κ is kept fixed and equal to 1.0 unless stated otherwise.

--dist_tree_model option

Selects a codon model to be used in the estimation of the pairwise distances used to assemble the initial tree topology. Option can be "ECMK07" (see [43]) or "ECMS05" (see

Table B.3: $\kappa(i,j)$ according to [43].

Option	Description
KAP1	$\kappa(i,j) = 1$
KAP2	$\kappa(i,j) = \kappa^{nts}$, where nts is the number of transitions between codon i and j
KAP3	$\kappa(i,j) = \kappa^{ntv}$, where nts is the number of transversions between codon i and j
KAP4	$\kappa(i,j) = \kappa_1^{nts}\kappa_2^{ntv}$, that is two different parameters
KAP5	$\kappa(i,j) = \kappa_k$, where k is one of the nine possible (t_S, t_V) combinations. See [43]

[56]) or "JC69", which is a codon model using a single exchange rate first used in nucleotide models (see [37]). The default option is "ECMK07".

-w option

The ω parameter or non-synonymous/synonymous rate ratio. Option can be "s" or "DM0" for single ω model, "d" or "DMODEL" for discrete unconstrained distribution model or "g" or "DGAMMA" for discrete gamma model.

--wclasses option

If the "w" option is set to chose a discrete unconstrained distribution model or to a discrete gamma model, this option decides how many classes there are. The default value is to use four classes.

--wvals option

Per default, all values for ω are estimated by ML. If you want to fix the values to your own choices use this option. Depending on your model of ω, you can provide on single value (for the single ω model) or a comma separated list; do not set spaces between commas and values. If your ω model is the discrete unconstrained distribution model, provide first the values for the ω categories, followed by the probabilities for each category. If you want to use three categories, your list would look like this: $\omega_1, \omega_2, \omega_3, p_1, p_2, p_3$. If you are using the discrete gamma model, you can provide the application with values for α and β that configure the gamma distribution: α, β

--optHeuristic option

Under research is an optimization heuristic that runs "n" rounds of parameter/branch length optimization at the start of an execution and "m" at its end. In between only tree topology swapping is performed (NNI/SPR). The user must provide "n,m" which will replace the option token above.

--NT2AA

Translates nucleotide data into amino acid data, using an specific genetic code defined by the user.

--oformat option

Defines the output format. Possible values are "txt" (text format, default), "darwin" (darwin format) or "yaml" (YAML output). YAML output can be chosen independent of compiling CODONPHYML with libyaml.

-- darwinconfig filepath

Path to an optional configuration file in darwin format. If this is set, no other command line option is parsed.

-- yamlconfig filepath

Path to an optional configuration file in yaml format. If this is set, no other command line option is parsed. Only available if CODONPHYML has been compiled with libyaml support.

-- logtree option

Should the intermediate trees be logged or not? Possible option values are 0 (no logging, default value), 1 (one single log file; only the most recent tree is written to that file) or 2 (for each "best" tree, a new file is created). Useful for running long computations on systems that have a runtime limit; you can write your most recent tree to the file system and later restart CODONPHYML with that topology. Option 2 is mainly useful for people who want to analyse the tree optimization in more detail (now we are looking in your direction, Toni...).

To summarize all options and to show how they fit together suppose you wish to run GY model with three ω categories drawn from the discrete gamma distribution, optimizing its α and β parameters using ML, with equilibrium frequencies estimated directly from the data using the F3X4 model and having κ estimated by ML as well. Given a sequence alignment file, named Inputfile, you must type the following line in a Linux/Unix terminal to start your execution.

```
codonphyml -i Inputfile -m GY --fmodel F3X4 -t e -f empirical -w g -a e --wclasses 3
```

B.3.2 User Interface Menu

Alternative to the command line input, which is very convenient for multiple executions, there is also an option to start CODONPHYML using a self-explanatory interactive menu. The user does not need to worry about which options combination are forbidden for it is controlled by the software. The options correspond to the same functionalities described for the command line user input, as described above.

Some options are contracdictory. For example, it is not possible to use a non-standard genetic code and KOSI07 as initial rate matrix at the same time. If you choose a combination that is not feasible, the interface will respect your choice in the current parameter but might automatically set another option to its appropriate value. If you have set your init rate matrix for an empirical model to SCHN05, e.g., and are about to change the genetic code to "invertebrate mitochondrial", the choice for the genetic code will be respected, but the init rate matrix will be reset to ECMUSR.

B.3.3 Output and Results

At start CODONPHYML will display a summary with settings selected for the execution of the program, and then the calculation begins. Figure B.1 illustrates how the settings screen is displayed in the terminal.

```
oooooooooooooooooooooooo        CURRENT   SETTINGS        oooooooooooooooooooooooo
                                ..................

. Sequence filename:                               codon
. Number of taxa:                                  25
. Sequence length:                                 100
. Data type:                                       Codon
. Alphabet size:                                   61
. Genetic code:                                    Standard
. Sequence format:                                 Interleaved
. Number of data sets:                             1
. Number of bootstrapped data sets:                0
. Compute aLRT:                                    Yes (SH-like branch supports)
. Model type:                                      GY
. Equilibrium frequencies model:                   Empirical
. Prop. of invariable sites:                       0.00
. Number of subst. rate categs:                    1
. Number of dn/ds rate categs:                     1 - (M0)
. Optimise tree topology:                          Yes
. Heuristic during tree search:                    Original
. Parameter optimization strategy:                 Multi-variate (BFGS)
. Tree topology search:                            NNIs
. Starting tree:                                   BioNJ + GYECMK07
. Add random input tree:                           No
. Optimise branch lengths:                         Yes
. Optimise subst. model params:                    Yes
. Matrix exponential:                              Eigenvalue problem
. Run ID:                                          None
. Random seed:                                     1130535063
. Code optimization:                               None
. Version:                                         1.00 201407.24

oooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooooo
```

Figure B.1: Settings of a CODONPHYML execution, displayed at its start.

The output will be displayed on the terminal window used to execute the program, and two files will be created after the execution. One file will contain the reconstructed tree and the other will contain summary statistics.

B.4 Feedback

The authors of CODONPHYML greatly encourage feedback, suggestions and feature request. Please, access the project's website at `https://sourceforge.net/projects/codonphyml/`, where you can leave a message in the forum and also find up-to-date contact information of the project administrators.

Appendix C

codonPhyML Software License

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Version 3, 29 June 2007

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