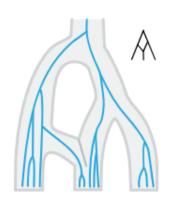
#### Parametric methods

BP&P Version 4.7



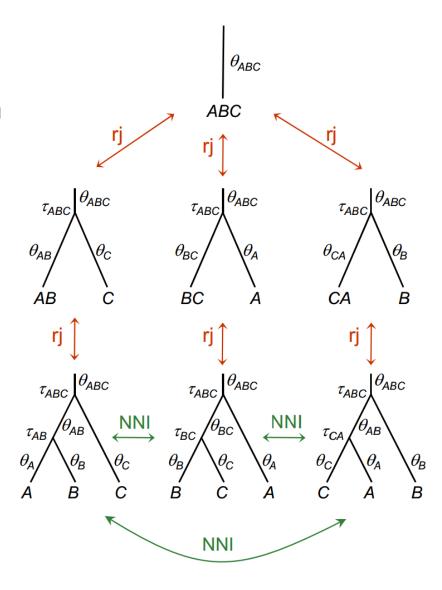
- Bayesian (MCMC) program for analyzing DNA sequence alignments from multiple loci under the multispecies coalescent (MSC)
- https://github.com/bpp/bpp

# Unguided Species Delimitation Using DNA Sequence Data from Multiple Loci

Ziheng Yang<sup>1,2</sup> and Bruce Rannala\*,1,3

#### **Bayesian Phylogenetics and Phylogeography (BPP)**

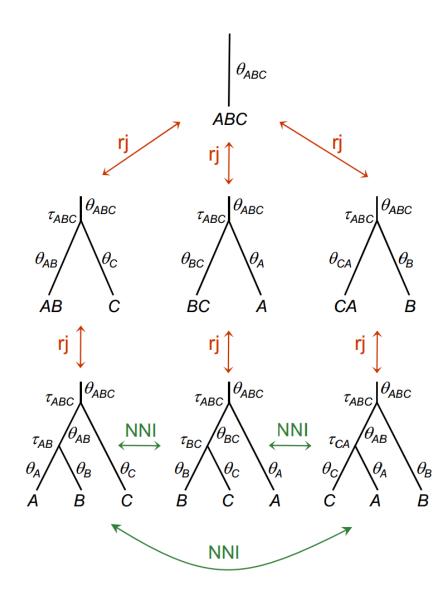
- Bayesian modeling approach generating speciation posterior probabilities
- Sequence data as input
- Multiple loci
- Uncertainty in gene phylogeny
- With or without guide species tree as input
- Prior for  $\theta$ : effective population size parameter
- Prior for τ: species divergence time parameter
- Available at: <a href="https://github.com/bpp/bpp">https://github.com/bpp/bpp</a>



# **Bayesian Phylogenetics and Phylogeography (BPP)**

 Nearest-neighbor interchange (NNI) used to move between species phylogenies with the species delimitation fixed

 Reversible-jump Markov Chain Monte Carlo (rjMCMC) to move between species delimitations when the underlying guide tree is fixed



#### Assumptions of the Coalescent Model

- 1) No recombination within a locus
- 2) Free recombination between loci
- 3) No migration (gene flow) between species
- 4) Neutral evolution
- 5) clock-like evolution.

#### Recent implementations of BPP can accommodate for:

1) Gene-flow



RESEARCH ARTICLE

**EVOLUTION** 





# Efficient Bayesian inference under the multispecies coalescent with migration

Tomáš Flouri<sup>a</sup>, Xiyun Jiao<sup>b</sup>, Jun Huang<sup>c</sup>, Bruce Rannala<sup>d,1</sup>, and Ziheng Yang<sup>a,1</sup>

Edited by Rasmus Nielsen, University of California, Berkeley, CA; received June 25, 2023; accepted August 15, 2023

#### Recent implementations of BPP can accommodate for:

- 1) Gene-flow
- 2) Non-clockwise evolution

# Bayesian Phylogenetic Inference using Relaxed-clocks and the Multispecies Coalescent

Tomáš Flouri (10,1 Jun Huang (10,1,2 Xiyun Jiao,1,3 Paschalia Kapli (10,1 Bruce Rannala (10,4 and Ziheng Yang (10\*\*)

#### Recent implementations of BPP can accommodate for:

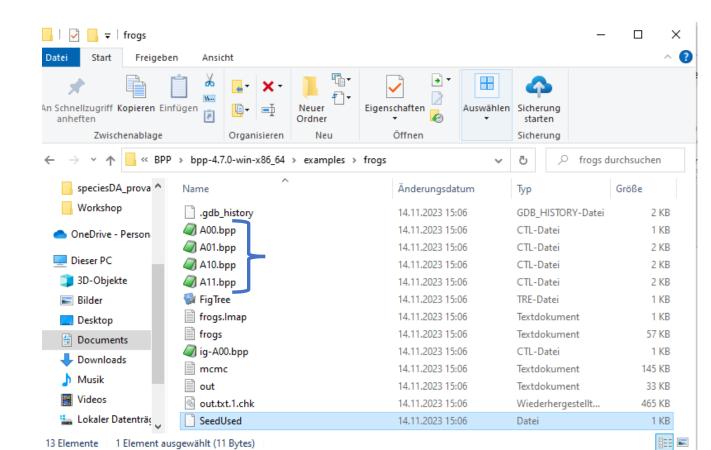
- 1) Gene-flow
- 2) Non-clockwise evolution
- 3) Scalable to phylogenomic data

- <u>A00</u> (speciesdelimitation = 0, speciestree = 0): estimation of the parameters  $\tau$  (species divergence times) and  $\theta$  (effective population sizes) under the MSC when the species tree model is given (Rannala and Yang, 2003; Flouri et al., 2020a);
- <u>A01</u> (speciesdelimitation = 0, speciestree = 1): inference of the species tree when the assignments are given by the user (Rannala and Yang, 2017);
- A10 (speciesdelimitation = 1, speciestree = 0): species delimitation using a user-specified guide tree (Yang and Rannala, 2010; Rannala and Yang, 2013);
- A11 (speciesdelimitation = 1, speciestree = 1): joint species delimitation and species tree inference of unguided species delimitation (Yang and Rannala, 2014).

- Command line software.
- No graphical interface (unlike BEAST)
   take a look to the software folder and examples...

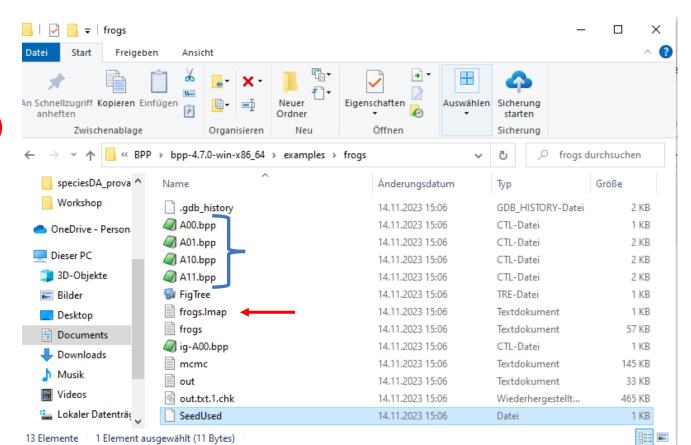
- Command line software.
- No graphical interface (unlike BEAST)

Settings file



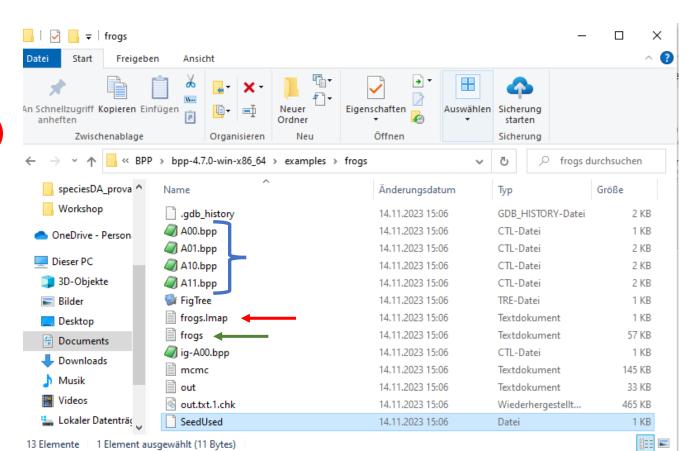
- Command line software.
- No graphical interface (unlike BEAST)

- Settings file
- Imap (sequence-taxon association)



- Command line software.
- No graphical interface (unlike BEAST)

- Settings file
- Imap (sequence-taxon association)
- Alignments file



#### Sequence alignments must be in phylip format

First line contains information on sequence number and length of the alignment



# Sequence alignments must be in phylip format

21 489		
6 c66	GGAGCCAACAGAGTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCTAAGTCAAACCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	ACCATCACTGTTTAGC
rd1	GGAGCCAACAG Sequence names must be preceded by ^	ACCATCACTGTTTAGC
hn24	GGAGCCAACAG	ACCATCACTGTTTAGC
gs250	GGAGCCAACAG Names are separated from sequences by more than one space or tab	ACCATCACTGTTTAGC
kiz1375	GGAGCCAACAG	ACCATCACTGTTTAGCA
kiz2305	GGAGCCAACAGAGTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCTAAGTCAAACCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
kiz2321	GGAGCCAACAGAGTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCTAAGTCAAACCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
kiz2405	GGAGCCAACAGAGTTTAACGTTCTGTTTTRGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCTAAGTCAAACYTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
bj64	GGAGCCAACAGAGTTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCAAGCCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
d14	GGAGCCCACAGAGTTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCAAGCCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
wlht2	GGAGCCAACAGAGTTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCAAGTCAAACCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
ypx2767	GGAGCCAaCAGAGTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCAAGTCAAACCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
ypx2732	GGAGCCAACAGAGTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCAAGCCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
ypx3876	GGAGCCAACAGAGTTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCAAGCCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
ypx4225	GGAGCCAACAGAGTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCAAGCCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
gs49	GGAGCCAACAGAGTTTAACGTTCTGTTTTAGATGGTAACAtAtAGGTCTCCCCCAATTCTGTGTCCAAGCCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
gs132	GGAGCCAACAGAGTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCAAGCCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
nx4	GGAGCCAACAGAGTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCAAGCCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
ypx3462	GGAGCCAACAGAGTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCAAGCCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	
ypx3893	GGAGCCACCAGAGTTTAACGTTCTGTTTTAGATGGTAACATATAGGTCTCCCCCAATTCTGTGTCCTAAGTCAAACCTTTCTGTTATTATGATCCTATGTCAGTGCTTTACATGTGTCCATTGAACC	TTACCATCACTGTTTAGCA
8 455		
6	TCCCTTTCTCGGGCATTGATGATCGAGAAAACTGGCCCATTGTCTTCTACAACAGGACRTGCCAATGCCAGGGGAACTTCATGGGCTACAATTGCGGTGAGTGCAGGTTTGGCTACACGGGACCGAA	CTGCACCGTTAGACGCAA
-66	T	CTGCACCGTTAGACGCAAA

#### Sequence alignments must be in phylip format



# The Imap files contain information of sequence-taxa assignment:

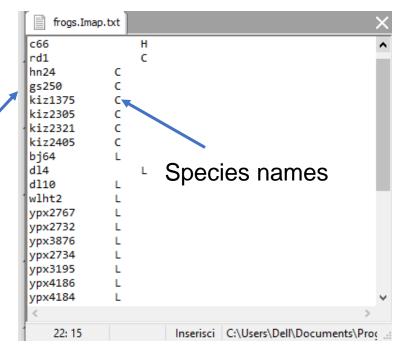
sequences from a sample can have different names in different analyses

 One could assign sequence from two or more samples to the same taxon (e.g., two individuals of the same population). They will not be divided

during the analyses

 Two columns separated by spaces or tabs

Sequence names



```
seed = -1
     segfile = ../frogs.txt
                                    File Names
    Imapfile = ../frogs.Imap.txt
     outfile = out.txt
    mcmcfile = mcmc.txt
 speciesdelimitation = 0  # fixed species delimitation
        speciestree = 1 0 0 0
                                   # estimate species tree
  speciesmodelprior = 1
                             # 0:uniform LH; 1:uniform rooted trees; 2:uniformSLH
3:uniformSRooted
                              # number of species and list of species labels
  species&tree = 4 K C L H
                   9 7 14 2 # max number of sequences from each species at a loci
                  ((K, C), (L, H)); # initial species phylogeny
       diploid = 1 1 1 1
                                    # 0: phased sequences; 1: diploid unphased sequences.
    checkpoint = 0
                             * 0: nothing; 1: save; 2: read
       usedata = 1 # 0: no data (prior); 1: seq like
         nloci = 5
                             # number of data sets to read in segfile
     cleandata = 0
                             # remove sites with ambiguity data (1: yes, 0: no)
    thetaprior = 3 0.002 e
                             # invgamma(a,b) for theta parameters
      tauprior = 3 0.004
                             # invgamma(a,b) for root tau
      heredity = 1 4 4 # (0: no variation, 1: estimate, 2: from file)
     locusrate = 0 2.0 # (0: no variation, 1: estimate, 2: from file)
      finetune = 1: .01 .01 .01 .01 .01 .01 .01 # auto (0 or 1): MCMC step lengths
                             # print MCMC samples, locusrate, heredity scalars, gene trees
         print = 1 0 0 0
        burnin = 8000
                             # burn-In
                             # frequency of sampling (sample every second MCMC iteration)
      sampfreq = 2
       nsample = 100000
                             # total number of samples to log
```

```
seed = -1
     segfile = ../frogs.txt
    Imapfile = ../frogs.Imap.txt
     outfile = out.txt
                                      If or not you want to perform species delimitation (1=yes)
    mcmcfile = mcmc.txt
 speciesdelimitation = 0
                             # fixed species delimitation
        speciestree = 1 0 0 0
                                    # estimate species tree
  speciesmodelprior = 1
                             # 0:uniform LH; 1:uniform rooted trees; 2:uniformSLH
3:uniformSRooted
  species&tree = 4 K C L H
                                     # number of species and list of species labels
                    9 7 14 2
                               # max number of sequences from each species at a loci
                  ((K, C), (L, H)); # initial species phylogeny
       diploid = 1 1 1 1
                                     # 0: phased sequences; 1: diploid unphased sequences.
                             * 0: nothing; 1 : save; 2: read
    checkpoint = 0
       usedata = 1
                             # 0: no data (prior); 1: seg like
         nloci = 5
                             # number of data sets to read in segfile
     cleandata = 0
                             # remove sites with ambiguity data (1: yes, 0: no)
    thetaprior = 3 0.002 e
                             # invgamma(a,b) for theta parameters
      tauprior = 3 0.004
                             # invgamma(a,b) for root tau
      heredity = 1 4 4 # (0: no variation, 1: estimate, 2: from file)
     locusrate = 0 2.0 # (0: no variation, 1: estimate, 2: from file)
      finetune = 1: .01 .01 .01 .01 .01 .01 .01 # auto (0 or 1): MCMC step lengths
                              # print MCMC samples, locusrate, heredity scalars, gene trees
         print = 1 0 0 0
        burnin = 8000
                              # burn-In
                             # frequency of sampling (sample every second MCMC iteration)
      sampfreq = 2
       nsample = 100000
                             # total number of samples to log
```

```
seed = -1
      segfile = ../frogs.txt
    Imapfile = ../frogs.Imap.txt
     outfile = out.txt
    mcmcfile = mcmc.txt
                                      If or not you want to infer the species tree (1=yes)
 speciesdelimitation = 0
                                fixed species delimitation
        speciestree = 1 0 0 0
                                     # estimate species tree
   speciesmodelprior = 1
                              # 0:uniform LH; 1:uniform rooted trees; 2:uniformSLH
3:uniformSRooted
   species&tree = 4 K C L H
                                      # number of species and list of species labels
                    9 7 14 2
                                     # max number of sequences from each species at a loci
                  ((K, C), (L, H)); # initial species phylogeny
       diploid = 1 1 1 1
                                      # 0: phased sequences; 1: diploid unphased sequences.
    checkpoint = 0
                              * 0: nothing; 1: save; 2: read
       usedata = 1
                              # 0: no data (prior); 1: seg like
         nloci = 5
                              # number of data sets to read in segfile
                              # remove sites with ambiguity data (1: yes, 0: no)
     cleandata = 0
    thetaprior = 3 0.002 e
                              # invgamma(a,b) for theta parameters
      tauprior = 3 0.004
                              # invgamma(a,b) for root tau
      heredity = 1 4 4 # (0: no variation, 1: estimate, 2: from file)
     locusrate = 0 2.0 # (0: no variation, 1: estimate, 2: from file)
      finetune = 1: .01 .01 .01 .01 .01 .01 .01 # auto (0 or 1): MCMC step lengths
                              # print MCMC samples, locusrate, heredity scalars, gene trees
         print = 1 0 0 0
         burnin = 8000
                              # burn-In
                              # frequency of sampling (sample every second MCMC iteration)
       sampfreq = 2
       nsample = 100000
                              # total number of samples to log
```

```
seed = -1
     segfile = ../frogs.txt
    Imapfile = ../frogs.Imap.txt
     outfile = out.txt
    mcmcfile = mcmc.txt
 speciesdelimitation = 0 # fixed species delimitation
        speciestree = 1 0 0 0
                                    # estimate species tree
  speciesmodelprior = 1
                             # 0:uniform LH; 1:uniform rooted trees; 2:uniformSLH
3:uniformSRooted
                                  Number and names of the samples/taxa/pre-defined species
  species&tree = 4 K C L H
                                     # max number or sequences from each species at a foci
                  ((K, C), (L, H)); # initial species phylogeny
       diploid = 1 1 1 1
                                     # 0: phased sequences; 1: diploid unphased sequences.
    checkpoint = 0
                             * 0: nothing; 1: save; 2: read
       usedata = 1
                             # 0: no data (prior); 1: seg like
         nloci = 5
                             # number of data sets to read in segfile
     cleandata = 0
                             # remove sites with ambiguity data (1: yes, 0: no)
                             # invgamma(a,b) for theta parameters
    thetaprior = 3 0.002 e
      tauprior = 3 0.004
                             # invgamma(a,b) for root tau
      heredity = 1 4 4 # (0: no variation, 1: estimate, 2: from file)
     locusrate = 0 2.0 # (0: no variation, 1: estimate, 2: from file)
      finetune = 1: .01 .01 .01 .01 .01 .01 .01 .01 # auto (0 or 1): MCMC step lengths
         print = 1 0 0 0
                             # print MCMC samples, locusrate, heredity scalars, gene trees
        burnin = 8000
                             # burn-In
                             # frequency of sampling (sample every second MCMC iteration)
      sampfreq = 2
       nsample = 100000
                             # total number of samples to log
```

```
seed = -1
     segfile = ../frogs.txt
    Imapfile = ../frogs.Imap.txt
     outfile = out.txt
    mcmcfile = mcmc.txt
speciesdelimitation = 0 # fixed species delimitation
        speciestree = 1 0 0 0
                                    # estimate species tree
  speciesmodelprior = 1
                             # 0:uniform LH; 1:uniform rooted trees; 2:uniformSLH
3:uniformSRooted
  species&tree = 4 K C L
                                  Max. number of sequences per samples/taxa/pre-defined species
                                     # INITITAL SPECIES PHYLOGENY
       diploid = 1 1 1 1
                                     # 0: phased sequences; 1: diploid unphased sequences.
    checkpoint = 0
                             * 0: nothing; 1: save; 2: read
       usedata = 1
                             # 0: no data (prior); 1: seg like
         nloci = 5
                             # number of data sets to read in segfile
                             # remove sites with ambiguity data (1: yes, 0: no)
     cleandata = 0
                             # invgamma(a,b) for theta parameters
    thetaprior = 3 0.002 e
      tauprior = 3 0.004
                             # invgamma(a,b) for root tau
      heredity = 1 4 4 # (0: no variation, 1: estimate, 2: from file)
     locusrate = 0 2.0 # (0: no variation, 1: estimate, 2: from file)
      finetune = 1: .01 .01 .01 .01 .01 .01 .01 # auto (0 or 1): MCMC step lengths
         print = 1 0 0 0
                             # print MCMC samples, locusrate, heredity scalars, gene trees
        burnin = 8000
                             # burn-In
                             # frequency of sampling (sample every second MCMC iteration)
      sampfreq = 2
       nsample = 100000
                             # total number of samples to log
```

```
seed = -1
     segfile = ../frogs.txt
    Imapfile = ../frogs.Imap.txt
     outfile = out.txt
    mcmcfile = mcmc.txt
 speciesdelimitation = 0 # fixed species delimitation
        speciestree = 1 0 0 0
                                    # estimate species tree
  speciesmodelprior = 1
                             # 0:uniform LH; 1:uniform rooted trees; 2:uniformSLH
3:uniformSRooted
  species&tree = 4 K C L H
                                     # number of species and list of species labels
                                     Guide species tree (if not inferred) or starting tree
       diploid =
                                     # v: phased sequences; i: diploid unphased sequences.
    checkpoint = 0
                             * 0: nothing; 1: save; 2: read
       usedata = 1 # 0: no data (prior); 1: seq like
         nloci = 5
                             # number of data sets to read in segfile
                             # remove sites with ambiguity data (1: yes, 0: no)
     cleandata = 0
                             # invgamma(a,b) for theta parameters
    thetaprior = 3 0.002 e
      tauprior = 3 0.004
                             # invgamma(a,b) for root tau
      heredity = 1 4 4 # (0: no variation, 1: estimate, 2: from file)
     locusrate = 0 2.0 # (0: no variation, 1: estimate, 2: from file)
      finetune = 1: .01 .01 .01 .01 .01 .01 .01 # auto (0 or 1): MCMC step lengths
         print = 1 0 0 0
                             # print MCMC samples, locusrate, heredity scalars, gene trees
        burnin = 8000
                             # burn-In
                             # frequency of sampling (sample every second MCMC iteration)
      sampfreq = 2
       nsample = 100000
                             # total number of samples to log
```

```
seed = -1
      segfile = ../frogs.txt
    Imapfile = ../frogs.Imap.txt
     outfile = out.txt
    mcmcfile = mcmc.txt
 speciesdelimitation = 0 # fixed species delimitation
        speciestree = 1 0 0 0
                                     # estimate species tree
   speciesmodelprior = 1
                              # 0:uniform LH; 1:uniform rooted trees; 2:uniformSLH
3:uniformSRooted
   species&tree = 4 K C L H # number of species and list of species labels
                  9 7 14 2 # max number of sequences from each species at a loci ((K, C), (L, H)); # initial species phylogeny
       diploid = 1 1 1 1
                                      # 0: phased sequences; 1: diploid unphased sequences.
    checkpoint = 0
                              * 0: nothing; 1: save; 2: read
       usedata = 1 # 0: no data (prior); 1: seq like
         nloci = 5
                              # number of data sets to read in segfile
                              # remove sites with ambiguity data (1: yes, 0: no)
      cleandata = 0
     thetaprior = 3 \cdot 0.002 e
                              Priors for \tau and \theta theta parameters
      tauprior = 3 0.004
      heredity = 1 4 4 # (0: no variation, 1: estimate, 2: from file)
      locusrate = 0 2.0 # (0: no variation, 1: estimate, 2: from file)
      finetune = 1: .01 .01 .01 .01 .01 .01 .01 # auto (0 or 1): MCMC step lengths
                              # print MCMC samples, locusrate, heredity scalars, gene trees
         print = 1 0 0 0
         burnin = 8000
                              # burn-In
                              # frequency of sampling (sample every second MCMC iteration)
       sampfreq = 2
        nsample = 100000
                              # total number of samples to log
```

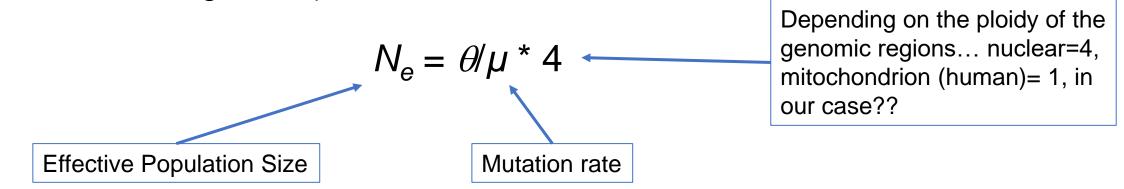
# Prior on $\theta$

- Since BPP3.4, both the  $\theta$  and  $\tau$  parameters have inverse gamma priors
- The mean of an inverse gamma distribution  $G(\alpha, \beta)$  is:

$$\beta/(\alpha-1)$$

#### Prior on $\theta$

- The effective population size  $(N_e)$  is an estimate of the population size base sequence data
- It differs among species and even among different genomic regions (e.g., nuclear vs. organellar)



# Genome Wide Analyses Reveal Little Evidence for Adaptive Evolution in Many Plant Species

Toni I. Gossmann,<sup>1</sup> Bao-Hua Song,<sup>2</sup> Aaron J. Windsor,<sup>2</sup> Thomas Mitchell-Olds,<sup>2</sup> Christopher J. Dixon,<sup>3</sup> Maxim V. Kapralov,<sup>3</sup> Dmitry A. Filatov,<sup>3</sup> and Adam Eyre-Walker\*,<sup>1</sup>

# Prior on $\theta$

- Since BPP3.4, both the  $\theta$  and  $\tau$  parameters have inverse gamma priors
- The mean of an inverse gamma distribution  $IG(\alpha, \beta)$  is:

$$\beta/(\alpha-1)$$

- Say you have an effective population size of 100.000, and a mutation rate of 1\*10-8
- What would you expect for  $\theta$ ?
- giving  $\alpha = 3$  (for a diffuse prior) what would you give to  $\alpha$  in order to center the distribution to the expected value?

#### Prior on $\tau$

- Since BPP3.4, both the  $\theta$  and  $\tau$  parameters have inverse gamma priors
- The mean of an inverse gamma distribution  $IG(\alpha, \beta)$  is:

$$\beta / (\alpha - 1)$$
 Generations (from root to leaves =  $\tau / \mu$ 

- And for  $\tau$ ? Let's say the samples used diverged 3 millions year ago, the mutation rate of 1\*10<sup>-8</sup> (mutations per site per year)
- Xanthium is annual
- What would you expect for  $\tau$ ?
- giving  $\alpha = 3$  (for a diffuse prior) what would you give to  $\alpha$  in order to center the distribution to the expected value?

```
seed = -1
     segfile = ../frogs.txt
    Imapfile = ../frogs.Imap.txt
     outfile = out.txt
    mcmcfile = mcmc.txt
 speciesdelimitation = 0 # fixed species delimitation
        speciestree = 1 0 0 0
                                     # estimate species tree
  speciesmodelprior = 1
                              # 0:uniform LH; 1:uniform rooted trees; 2:uniformSLH
3:uniformSRooted
                               # number of species and list of species labels
  species&tree = 4 K C L H
                  9 7 14 2 # max number of sequences from each species at a loci ((K, C), (L, H)); # initial species phylogeny
       diploid = 1 1 1 1
                                      # 0: phased sequences; 1: diploid unphased sequences.
    checkpoint = 0
                              * 0: nothing; 1: save; 2: read
       usedata = 1 # 0: no data (prior); 1: seq like
         nloci = 5
                              # number of data sets to read in segfile
                              # remove sites with ambiguity data (1: yes, 0: no)
     cleandata = 0
    thetaprior = 3 0.002 e
                              # invgamma(a,b) for theta parameters
      tauprior = 3 0.004
                              # invgamma(a,b) for root tau
                                if you want to scale \theta according to the genomic region
      heredity = 1 4 4
                                  (e.g., nuclear organellar, X or Y chromosomes...)
      finetune = 1: .01 .01 .01 .01 .01 .01 .01 .01 # auto (0 or 1): MCMC step lengths
         print = 1 0 0 0
                              # print MCMC samples, locusrate, heredity scalars, gene trees
        burnin = 8000
                              # burn-In
                              # frequency of sampling (sample every second MCMC iteration)
       sampfreq = 2
       nsample = 100000
                              # total number of samples to log
```

# heredity

• scale the  $\theta$  parameter in different genomic regions

```
heredity = 0 (no variation)
= 1 (estimated from gamma distribution (provide \alpha, \beta)
= 2 (from file)
```

Genome	Heredity scalar
Nuclear autosome	1
X chromosome	0.75
Y chromosome	0.25
Mitochondrial	0.25

What in our case?

```
seed = -1
     segfile = ../frogs.txt
    Imapfile = ../frogs.Imap.txt
     outfile = out.txt
    mcmcfile = mcmc.txt
speciesdelimitation = 0 # fixed species delimitation
        speciestree = 1 0 0 0
                                    # estimate species tree
  speciesmodelprior = 1
                             # 0:uniform LH; 1:uniform rooted trees; 2:uniformSLH
3:uniformSRooted
                              # number of species and list of species labels
  species&tree = 4 K C L H
                  9 7 14 2 # max number of sequences from each species at a loci ((K, C), (L, H)); # initial species phylogeny
       diploid = 1 1 1 1
                                     # 0: phased sequences; 1: diploid unphased sequences.
    checkpoint = 0
                             * 0: nothing; 1: save; 2: read
       usedata = 1 # 0: no data (prior); 1: seq like
         nloci = 5 # number of data sets to read in segfile
     cleandata = 0
                            # remove sites with ambiguity data (1: yes, 0: no)
    thetaprior = 3 0.002 e
                             # invgamma(a,b) for theta parameters
      tauprior = 3 0.004
                             # invgamma(a,b) for root tau
      heredity = 1 4 4 # (0: no variation, 1: estimate, 2: from file)
     locusrate = 0 2.0 # (0: no variation, 1: estimate, 2: from file)
      finetune = 1: .01 .01 .01 .01 .01 .01 .01 # auto (0 or 1): MCMC step lengths
                             # print MCMC samples, locusrate, heredity scalars, gene trees
         print = 1 0 0 0
        burnin = 8000
                              mcmc settings (chain length, sampling every, etc...)
      sampfreq = 2
       nsample = 100000
                             # total number of samples to log
```

Run the program from a command box (rather than double-clicking the executable) so that you will see the error messages. In the bpp/ folder, run the program by typing the following command:

On WINDOWS	On LINUX/UNIX/MAC OSX
bin\bppcfile bpp.5s.ctl	bin/bppcfile bpp.5s.ctl

- Copy the sequence, Imap and setting files in the BPP folder
- Navigate to the bpp folder and run:

# >bpp.exe --cfile SettingFile.ctl

#### Screen output

```
Initial parameters, np = 3.
Genetrees generated from the MSC density.
lnpG0 = 711.7015 lnL0 = -3350.8540
-3% 0.70 0.13 0.00 0.22 0.35
                              4 3 0.0039 0.1455 P(4)=0.9965 -1.0000 0.0007
                                                                            775.17 -3126.4790
 -2% 0.70 0.31 0.00 0.21 0.28
                              4 3 0.0100 0.1260 P(4)=0.9925
                                                             -1.0000 0.0008
                                                                            745.60 -3125.8524
 -1% 0.70 0.30 0.00 0.22 0.35
                              4 3 0.0039 0.1000 P(4)=0.9980
                                                             -1.0000 0.0007 750.41 -3126.2339
 0% 0.70 0.30 0.00 0.23 0.25
                              4 3 0.0077 0.0920 P(4)=0.9845
                                                             -1.0000 0.0009
                                                                            775.04 -3126.2217
                                                                                               0:13
                              4 3 0.0231 0.0967 P(4)=0.9368
 5% 0.70 0.30 0.00 0.24 0.34
                                                             -1.0000 0.0007
                                                                            750.67 -3125.9098
                                                                                               0:30
10% 0.70 0.30 0.00 0.24 0.34
                              4 3 0.0189 0.0954 P(4)=0.9474
                                                             -1.0000 0.0007
                                                                            732.19 -3125.9363
                                                                                               0:46
    ^^ Pjump for MCMC moves ^^
                                  Sp
                                         Pri PSPR P(S=4)
                                                                 theta
                                                                               1npG
                                                                                       E(lnL)
                                                                         tau0
```

Acceptance proportions of the step lengths for MCMC moves:

Should be values around 0.3, between 0.1 and 0.8. If some of the values is too low, the respective step lengths should be increased in the settings file and vice versa

```
tauprior = 3 0.004  # invgamma(a,b) for root tau

heredity = 1 4 4  # (0: no variation, 1: estimate, 2: from file)
locusrate = 0 2.0  # (0: no variation, 1: estimate, 2: from file)

finetune = 1: .01 .01 .01 .01 .01 .01 .01 .01 # auto (0 or 1): MO

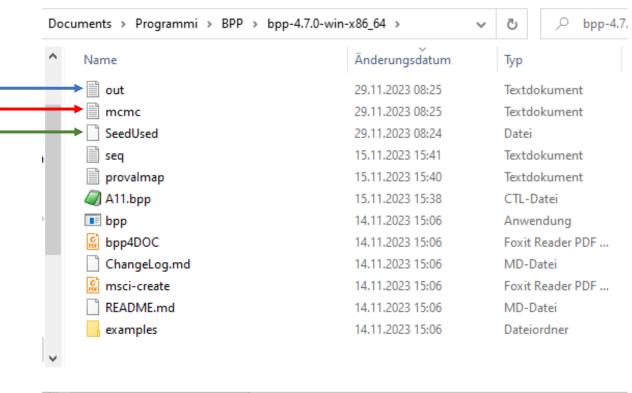
print = 1 0 0 0  # print MCMC samples, locusrate, heredity
burnin = 8000  # burn-In

sampfreq = 2  # frequency of sampling (sample every seconds applied = 1000000  # total number of samples to log
```

output with species delimitation and species tree results

mcmc file

 seed used (useful if one wants to perform repeat the analyses with another seed (to see if results are consistent)



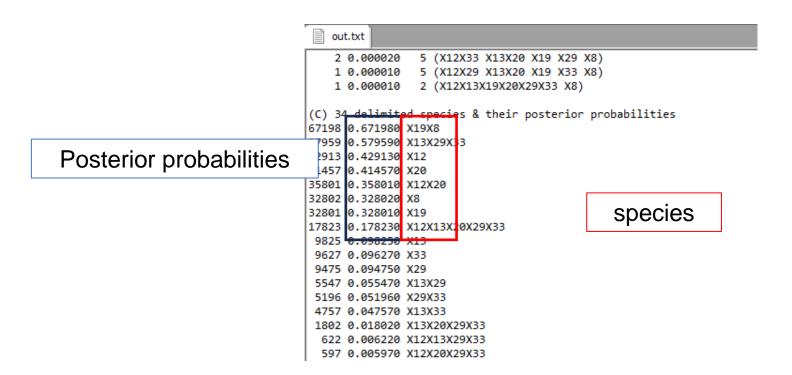
 List of models (species delimitation and species tree with the posterior probabilities)

```
out.txt
(A) List of best models (count postP #species SpeciesTree)
16898 0.168980 0.168980 3
                          (X12X20 X13X29X33 X19X8) ((X12X20, X13X29X33), X19X8);
14648 0.146480 0.315460 4
                           (X12 X13X29X33 X19X8 X20) (((X12, X20), X13X29X33), X19X8);
12391 0.123910 0.439370 2
                           (X12X13X20X29X33 X19X8) (X12X13X20X29X33, X19X8);
7884 0.078840 0.518210 4
                           (X12X20 X13X29X33 X19 X8) ((X12X20, X13X29X33), (X19, X8));
 6949 0.069490 0.587700 5
                           (X12 X13X29X33 X19 X20 X8) (((X12, X20), X13X29X33), (X19, X8));
 6358 0.063580 0.651280 3
                           (X12X13X20X29X33 X19 X8) (X12X13X20X29X33, (X19, X8));
 2351 0.023510 0.674790 4
                           (X12 X13X29X33 X19X8 X20) (((X12, X13X29X33), X20), X19X8);
 2224 0.022240 0.697030 4
                           (X12 X13X29X33 X19X8 X20)
                                                     ((X12, (X13X29X33, X20)), X19X8);
                                                     ((X12X20, (X13X33, X29)), X19X8);
 1464 0.014640 0.711670 4
                           (X12X20 X13X33 X19X8 X29)
 1382 0.013820 0.725490 4
                           (X12X20 X13 X19X8 X29X33)
                                                      ((X12X20, (X13, X29X33)), X19X8);
                                                      ((X12X20, (X13X29, X33)), X19X8);
 1337 0.013370 0.738860 4
                           (X12X20 X13X29 X19X8 X33)
                                                       (((X12, X20), (X13X29, X33)), X19X8);
 1116 0.011160 0.750020 5
                           (X12 X13X29 X19X8 X20 X33)
 1065 0.010650 0.760670 5
                           (X12 X13X29X33 X19 X20 X8)
                                                       (((X12, X13X29X33), X20), (X19, X8));
                                                       ((X12, (X13X29X33, X20)), (X19, X8));
 1052 0.010520 0.771190 5
                           (X12 X13X29X33 X19 X20 X8)
 1032 0.010320 0.781510 5
                           (X12 X13 X19X8 X20 X29X33)
                                                       (((X12, X20), (X13, X29X33)), X19X8);
 1019 0.010190 0.791700 5
                           (X12 X13X33 X19X8 X20 X29) (((X12, X20), (X13X33, X29)), X19X8);
 891 0.008910 0.800610 3
                           (X12 X13X20X29X33 X19X8) ((X12, X13X20X29X33), X19X8);
  809 0.008090 0.808700 3
                           (X12X13X29X33 X19X8 X20) ((X12X13X29X33, X20), X19X8);
 701 0.007010 0.815710 5
                           (X12X20 X13 X19 X29X33 X8) ((X12X20, (X13, X29X33)), (X19, X8));
  638 0.006380 0.822090 5
                           (X12X20 X13X29 X19 X33 X8)
                                                       ((X12X20, (X13X29, X33)), (X19, X8));
  620 0.006200 0.828290 5
                           (X12X20 X13X33 X19 X29 X8) ((X12X20, (X13X33, X29)), (X19, X8));
  524 0.005240 0.833530 4
                           (X12 X13X20X29X33 X19 X8) ((X12, X13X20X29X33), (X19, X8));
 466 0.004660 0.838190 3
                           (X12X20X29X33 X13 X19X8) ((X12X20X29X33, X13), X19X8);
  461 0.004610 0.842800 6
                           (X12 X13 X19 X20 X29X33 X8) (((X12, X20), (X13, X29X33)), (X19, X8));
  447 0.004470 0.847270 6
                           (X12 X13X33 X19 X20 X29 X8) (((X12, X20), (X13X33, X29)), (X19, X8));
                           (X12 X13X29 X19 X20 X33 X8) (((X12, X20), (X13X29, X33)), (X19, X8));
  428 0.004280 0.851550 6
                           (X12 X13X29X33 X19X8 X20) (((X12, X20), X19X8), X13X29X33);
 426 0.004260 0.855810 4
                           (X12X13X29X33 X19 X20 X8) ((X12X13X29X33, X20), (X19, X8));
  386 0.003860 0.859670 4
                           (X12X20 X13 X19X8 X29 X33) ((X12X20, ((X13, X33), X29)), X19X8);
— Trova & sostituisci
```

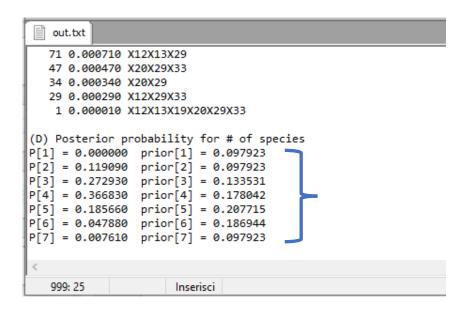
List the best species delimitation models (just species delimitation!)

out.txt (X12X13X19X20X29X33 X8) (X12X13X19X20X29X33, X8); Posterior probability of (B) 93 species delimitations & their posterior probabilities the model <del>2094</del> 0.209440 4 (X12 X13X29X33 X19X8 X20) 3 (X12X20 X13X29X33 X19X8) 18400 0.184000 11908 0.119080 2 (X12X13X20X29X33 X19X8) 5 (X12 X13X29X33 X19 X20 X8) 9804 0.098040 8811 0.088110 4 (X12X20 X13X29X33 X19 X8) 3 (X12X13X20X29X33 X19 X8) 5915 0.059150 model 1725 0.017250 5 (X12 X13X29 X19X8 X20 X33) 5 (X12 X13 X19X8 X20 X29X33) 1653 0.016530 4 (X12X20 X13 X19X8 X29X33) 1608 0.016080 1595 0.015950 4 (X12X20 X13X29 X19X8 X33) 1494 0.014940 5 (X12 X13X33 X19X8 X20 X29) 1466 0.014660 6 (X12 X13 X19X8 X20 X29 X33) 1433 0.014330 4 (X12X20 X13X33 X19X8 X29) 3 (X12 X13X20X29X33 X19X8) 1190 0.011900 1110 0.011100 5 (X12X20 X13 X19X8 X29 X33) 6 (X12 X13X29 X19 X20 X33 X8) 949 0.009490 6 (X12 X13X33 X19 X20 X29 X8) 838 0.008380 837 0.008370 6 (X12 X13 X19 X20 X29X33 X8) 5 (X12X20 X13X29 X19 X33 X8) 795 0.007950 7 (X12 X13 X19 X20 X29 X33 X8) 761 0.007610 741 0.007410 5 (X12X20 X13X33 X19 X29 X8) 5 (X12X20 X13 X19 X29X33 X8) 701 0.007010 612 0.006120 4 (X12 X13X20X29X33 X19 X8) 6 (X12X20 X13 X19 X29 X33 X8) 607 0.006070 416 0.004160 3 (X12X13X29X33 X19X8 X20) 390 0.003900 3 (X12X20X29X33 X13 X19X8) 3 (X12X13X20X33 X19X8 X29) 306 0.003060 4 (X12X20X29X33 X13 X19 X8) 206 0.002060 4 (X12X13X29X33 X19 X20 X8) 3 (X12X20X33 X13X29 X19X8) 183 0.001830

delimited species and their posterior probabilities

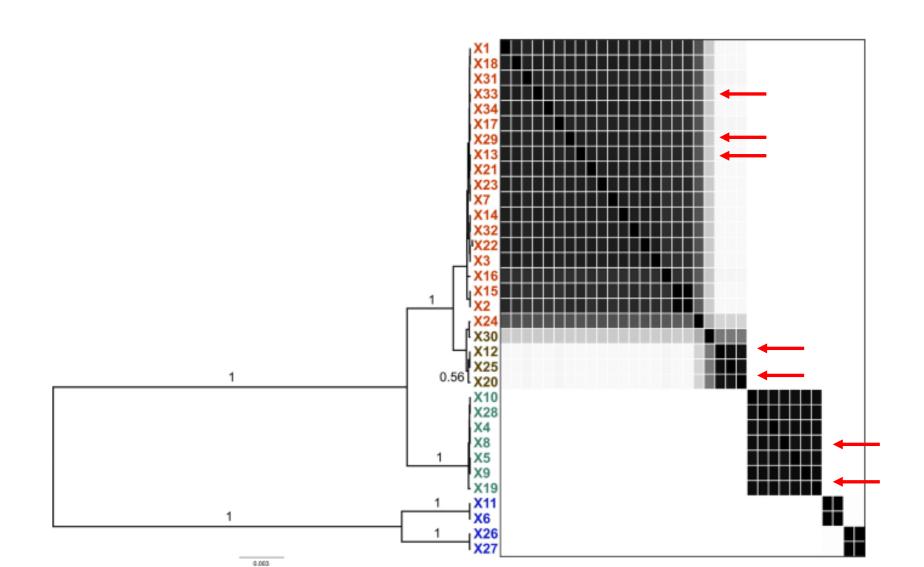


 posterior probabilities (and their respective priors) of species delimitation models of n species



- What come next?
  - Are different analyses, with different starting tree producing the same results?
  - Do different values of influence heavily the results?

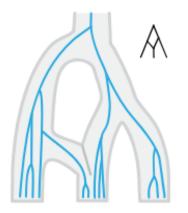
• Are results consistent with those in Tomasello (2018)?



### Coffe?



#### Integrative approach



- Extension on BPP capable to co-analyse molecular and morphometric data
- https://github.com/cecileane/iBPP/

doi:10.1111/evo.12582

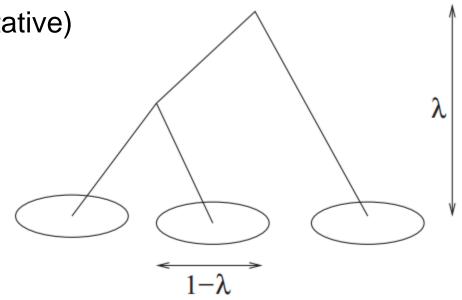


# Bayesian species delimitation combining multiple genes and traits in a unified framework

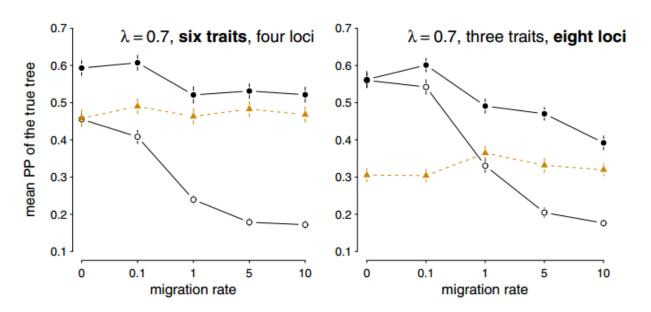
Claudia Solís-Lemus, 1 L. Lacey Knowles, 2 and Cécile Ané 1,3,4

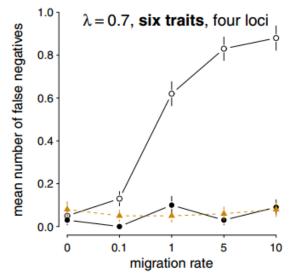
## <u>Integrative Bayesian Phylogenetics and Phylogen</u>

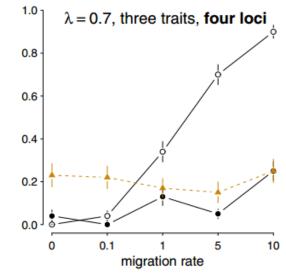
- Extension of BPP
- Sequence and morphological (continuous quantitative) data as input
- Multiple loci
- Each trait is assumed to have a normal distribution
- A parameter λ models the between-to-within species variance ratio
- Each trait is allowed to have its own λ parameter



## <u>Integrative Bayesian Phylogenetics and Phylogen</u>







Integrating morphological traits increase accuracy!!

It uses BPP, basically it works in the same way

#### <u>but</u>

- It is based on an older version of BPP, layout of inputs and outputs might be a bit different
- Not all model implemented in BPP v4 are supported in iBPP (e.g., MSC with gene flow, etc...)
- The setting files need a few adjustments for the analyses of morphometric data

#### The following settings need to be added to the setting file:

```
traitfile = 5s.morph.txt replace with name of file with the trait data useseqdata = 1 0 if sequence data should not be used in the analysis, and 1 if it should usetraitdata = 1 0 if trait data should not be used in the analysis, and 1 if it should number of traits in the trait file prior parameter for \sigma^2 kappa0 = 0 prior parameter for \mu given \sigma^2
```

#### Change also:

- "speciesmodelprior" in the control file of BPP 4.7 with "uniformrootedtrees" in the control of the iBPP
- "useseqdata" instead of "usedata"



Ind	Population	length	width	beaks_length	spines_length	spines	Tomento
X13_	X13	1.9342	0.7334	0.4805	0.32504	6.6	2
X29_	X29	2.166	0.8384	0.5847	0.49068	12.4	3
X33_	X33	1.86875	0.81375	0.493875	0.36105	12.5	5
X8_	X8	0.9154	0.4918	0.2038	0.16696	14.2	1
X12_	X12	1.5122	0.6432	0.3623	0.32412	9.8	0
X19_	X19	1.024	0.6048	0.2703	0.24212	10.6	1
X20_	X20	1.0568	0.5278	0.3459	0.25172	15.2	0



• Bur length



- Bur length
- Bur width



- Bur length
- Bur width
- Beaks' length



- Bur length
- Bur width
- Beaks' length
- Spines' length



- Bur length
- Bur width
- Beaks' length
- Spines' length
- Spines / 0.5 cm<sup>2</sup>
- Tomentosity

#### Prior on $\theta$

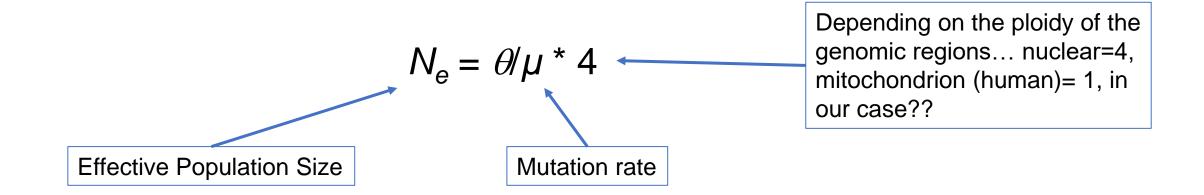
- In older version of BPP a gamma (not inverse gamma!!!) distribution was given to both the  $\theta$  and  $\tau$  parameters
- The mean of an gamma distribution  $G(\alpha, \beta)$  is:

$$\alpha/\beta$$

- Say you have an effective population size of 100.000, and a mutation rate of 1\*10-8
- What would you expect for  $\theta$ ?
- giving  $\beta = 2$  (for a diffuse prior) what would you give to  $\alpha$  in order to center the distribution to the expected value?

#### Prior on $\theta$

 Perform different analyses with different values of θ, for both big and small effective population size



#### possible prior values:

- G(2, 50) Large
- G(2, 1000) Small

#### The setting file

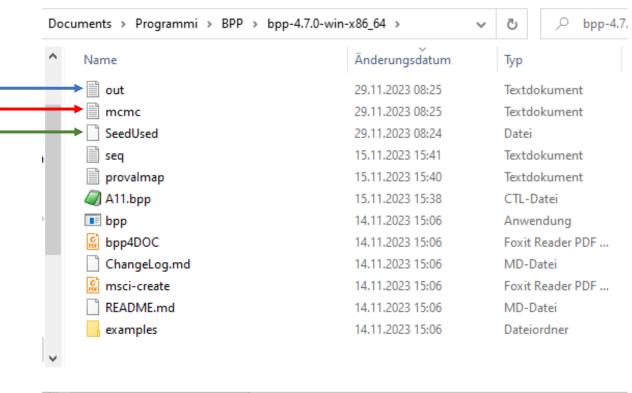
- Copy the sequence, Imap and setting files in the iBPP folder
- Navigate to the iBPP folder and run:

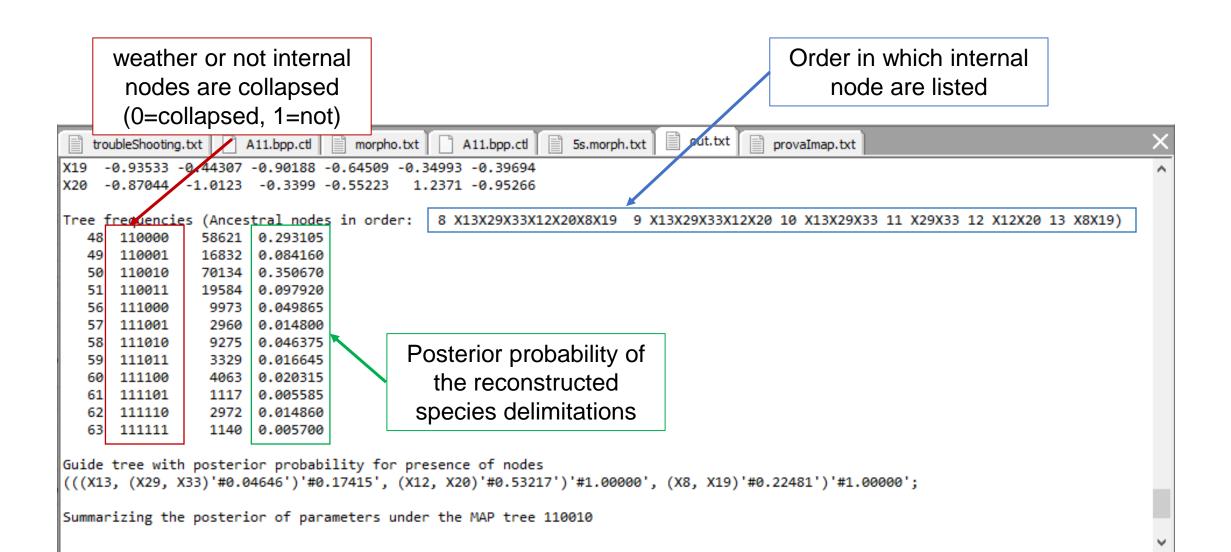
>ibpp.exe SettingFile.ctl

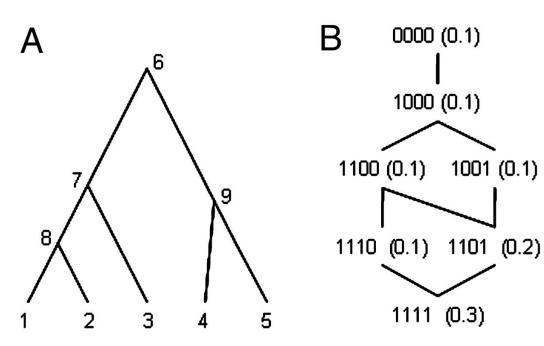
output with species delimitation and species tree results

mcmc file

 seed used (useful if one wants to perform repeat the analyses with another seed (to see if results are consistent)





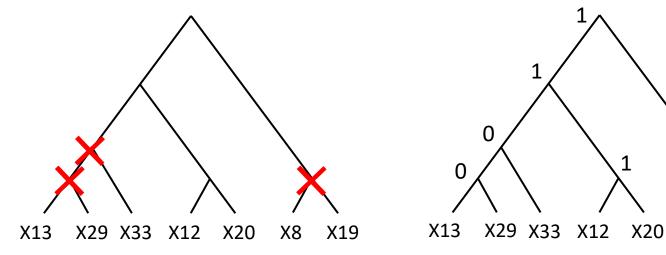


A guide tree with five species

Seven species delimitations

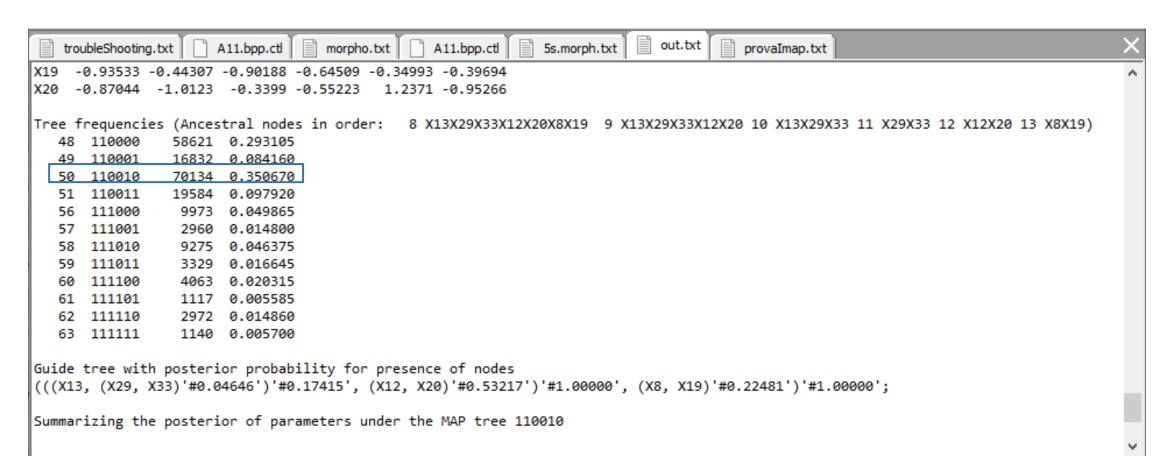
1001 (0.1)

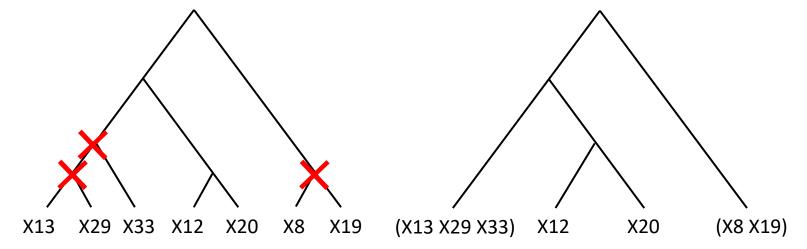
- Given the guide species tree (fixed to the one provided in the setting files
- Each species delimitation is represented by a set of flags indicating whether each of the four ancestral nodes (6, 7, 8, 9) is collapsed (0) or resolved (1)

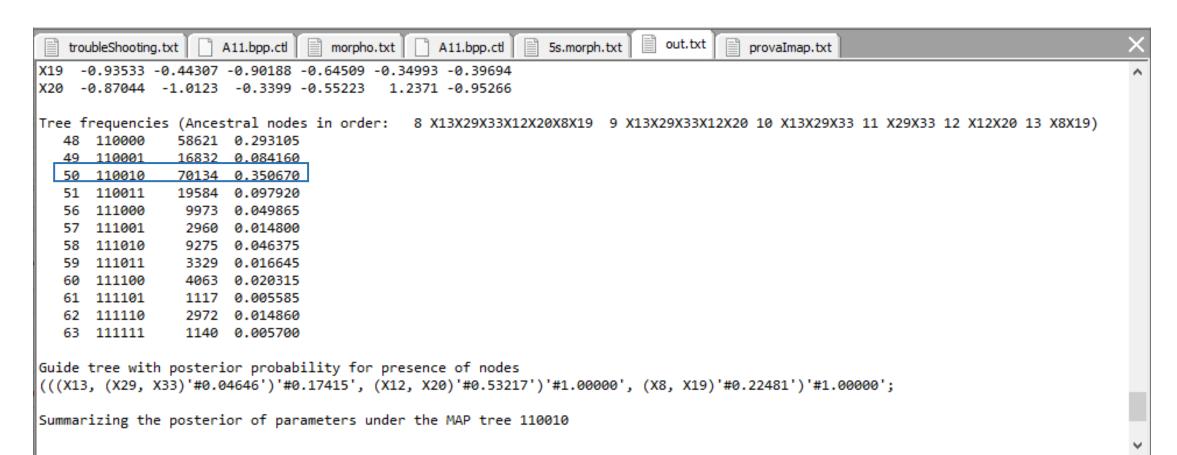


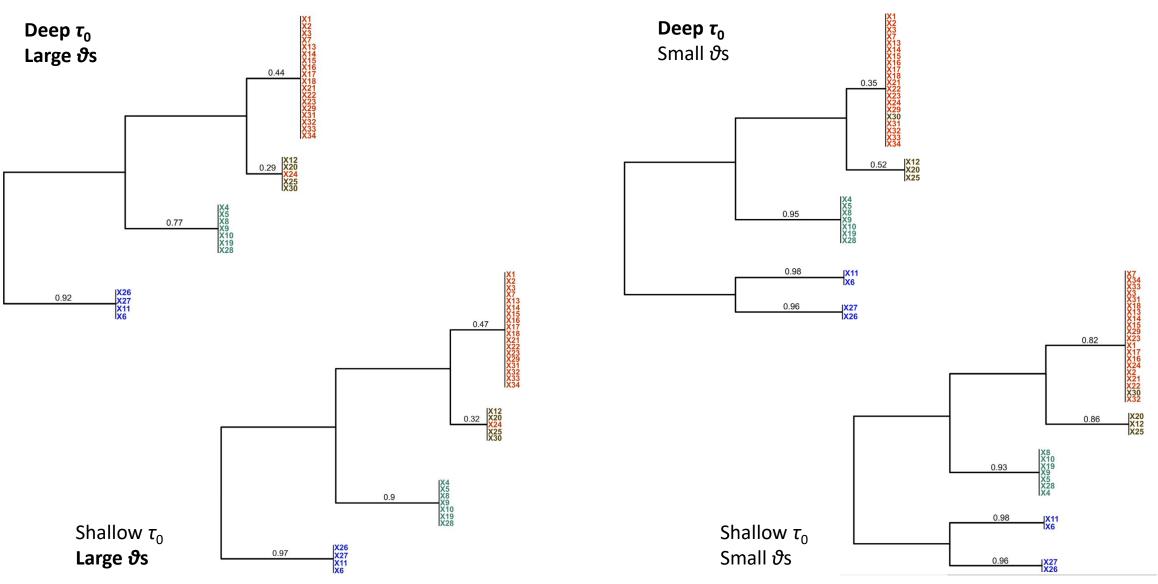
X8

X19









Tomasello 2018. Molecular Phylogenetics and Evolution 127: 135-145. https://doi.org/10.1016/j.ympev.2018.05.024

#### contacts:

https://www.uni-goettingen.de/de/staff/185774.html

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