

**File S1 – Supporting information for****PACo: a novel Procrustes application to cophylogenetic analysis****PACo in R – User Guide**Juan Antonio Balbuena<sup>1\*</sup>, Raúl Míguez-Lozano<sup>1</sup>, Isabel Blasco-Costa<sup>2</sup><sup>1</sup> Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Valencia, Spain.<sup>2</sup> Institute of Parasitology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic.\*E-mail: [j.a.balbuena@uv.es](mailto:j.a.balbuena@uv.es)**Introduction**

We present an R [1] script to carry out PACo (**P**rocrustes **A**pproach to **C**ophylogeny), an application of Procrustes analysis for comparison of phylogenetic trees of associated organisms, such as hosts and parasites. PACo provides a residual sum of squares of the Procrustean fit that measures the congruence between two given phylogenies and uses a permutation approach to test its significance. The analysis allows for multiple host-parasite associations and different number of hosts and parasites. Because in the Procrustean superimposition the host matrix is kept fixed, whereas the parasite matrix is rotated and scaled to fit the former, PACo tests the classical view of whether the parasite phylogeny is constrained by the host phylogeny [2]. This implies that the null hypothesis tested is slightly different from that of previous tests of phylogenetic congruence [3-5]. In addition to hypothesis testing, PACo provides a superimposition plot enabling a graphical comparison of the fit of the host-parasite associations, and a residual bar chart for evaluation of the contribution of the individual host-parasite associations to the global fit.

**The R script**

All the computations described in the accompanying paper can be carried out with the R script below. R runs on a wide variety of Linux/Unix platforms, Windows and MacOS and can be downloaded at <http://www.r-project.org/>. In addition to the basic R install, two dedicated packages need to be installed to implement PACo: `ape` [6], required for handling of phylogenetic data and Principal Coordinates Ordination and `vegan` [7], required for Procrustes fitting. (See <http://cran.r-project.org/doc/manuals/R-admin.html#Installing-packages> for details on how to install R packages). In order to assist users with little or no experience with R, we provide annotations to the script. The analyses can be implemented by cutting and pasting the code below in an opened R console. The text in red identifies parameters that can be customized to adapt the analysis to the user's needs.

The script is demonstrated with the phylogenies of pocket gophers and their chewing lice based on the mitochondrial cytochrome oxidase I sequences of Hafner et al. [8], which represents a classical example of host-parasite cospeciation [9]. The input files required are shown in the Appendix below and can be downloaded, together with a fully annotated R script, at <http://www.uv.es/cophylpaco/index.html>.

First, load the two packages required. At the R prompt, write

```
library(ape)
library(vegan)
```

### *Data input*

Input files should be in plain text format either space- or tab-delimited. (See Appendix for examples). Three files are required. Two of them encapsulate the host and parasite phylogenies, respectively, and will eventually be transformed into distance matrices between host or parasite taxa. The third one consists of a binary matrix coding the host-parasite associations, where host species are arranged in rows and parasites in columns; 1's indicate occurrence of a given parasite in a given host and 0's denote parasite absences in the host. The input files should **include taxa labels that have to match exactly in the three files**. Any mismatch will cause execution errors and/or incorrect results. (For clarity of the graphical output, short labels are recommended.)

The following syntax reads the matrix of host-parasite associations and computes the number of associations (NLinks) required for further computations:

```
HP <- as.matrix(read.table("PACo/example/gophers/g-1_links.txt", header=TRUE))
NLinks = sum(HP)
```

As illustrated by the foregoing code, the path pointing to the file system location should be indicated in quotation marks. [Windows users should also note that folder names are separated by forward (/), instead of backward (\) slashes.] Alternatively, one can open the input files interactively with `file.choose()`, e.g.

```
HP <- as.matrix(read.table(file.choose(), header=TRUE))
```

The syntax to load the phylogenetic input depends on the type of data used. There are three possibilities: (a) phylogenetic trees, (b) aligned sequences or (c) distance matrices.

#### *a) Phylogenetic trees*

Use the `read.tree` or `read.nexus` functions to open tree files in Newick or Nexus formats, respectively.

For instance,

```
TreeH <- read.tree("PACo/example/gophers/phylo_gopher.tre")
TreeP <- read.tree("PACo/example/gophers/phylo_lice.tre")
```

The host and parasite trees are then transformed for further analysis into respective matrices of patristic distances (host.D and para.D):

```
host.D <- cophenetic(TreeH)
para.D <- cophenetic(TreeP)
```

### b) *Aligned sequences*

The `read.dna` function can read sequences in different formats. (Consult the `ape` package tutorial for details). In this example the files are in fasta format:

```
seqH <- read.dna("PACo/example/gophers/align_gopher.fasta", format="fasta")
seqP <- read.dna("PACo/example/gophers/align_lice.fasta", format="fasta")
```

Next, the sequence data is used to compute genetic distance matrices of hosts and parasites:

```
host.D <- dist.dna(seqH, model = "F84", as.matrix=TRUE)
para.D <- dist.dna(seqP, model = "F84", as.matrix=TRUE)
```

The `dist.dna` function allows choosing among a range of evolutionary models [6]. The example above uses the Felsenstein's substitution model (F84) [10].

### c) *Distance matrices*

Text files with square distance or dissimilarity matrices can also be used as input:

```
host.D <- as.matrix(read.table("PACo/example/gophers/dist_gopher.txt",
header=TRUE))
para.D <- as.matrix(read.table("PACo/example/gophers/dist_lice.txt",
header=TRUE))
```

The files `dist_gopher.txt` and `dist_lice.txt` (see Appendix), contain genetic distances computed with PAUP\* [11] using the HKY85 [12] substitution model.

**All the demonstrations below use this third input option (HKY85 genetic distances).**

### *Procustean Superimposition*

The host and parasite distance matrices are first sorted according, respectively, to the order of rows (hosts) and columns (parasites) of the host-parasite association matrix:

```
host.D <- host.D[rownames(HP), rownames(HP)]
para.D <- para.D[colnames(HP), colnames(HP)]
```

The `PACo` function, defined below, transforms the host and parasite distance matrices into the respective matrices of Principal Coordinates (`pcoa` of `ape`) and duplicates taxa (if necessary) to accommodate multiple host-parasite associations:

```
PACo <- function (H.dist, P.dist, HP.bin)
{HP.bin <- which(HP.bin > 0, arr.in=TRUE)
H.PCo <- pcoa(H.dist, correction="cailliez")$vectors
```

```
P.PCo <- pcoa(P.dist, correction="cailliez")$vectors
H.PCo <- H.PCo[HP.bin[,1],]
P.PCo <- P.PCo[HP.bin[,2],]
list (H.PCo = H.PCo, P.PCo = P.PCo)}
```

PACo is then applied to the input and a Procrustes fit is carried out:

```
PACo.fit <- PACo(host.D, para.D, HP)
HP.proc <- procrustes(PACo.fit$H.PCo, PACo.fit$P.PCo)
```

The program produces the following output:

```
Warning message:
In procrustes(PACo.fit$H.PCo, PACo.fit$P.PCo) :
  X has fewer axes than Y: X adjusted to conform Y.
```

This indicates that the host input matrix has fewer columns than the parasite counterpart. No action by the user is required since the narrower matrix is completed with zero columns [13].

To visualize the host-parasite superimposition plot, as show in Figure S1.1:

```
HostX <- HP.proc$X
ParY <- HP.proc$Yrot
plot(HostX, asp=1, pch=46)
points(ParY, pch=1)
arrows(ParY[,1], ParY[,2], HostX[,1], HostX[,2], length=0.12, angle=15,
xpd=FALSE)
HostX <- unique(HP.proc$X)
ParY <- unique(HP.proc$Yrot)
identify(ParY[,1], ParY[,2], rownames(ParY), offset=0.3, xpd=FALSE, cex=0.8)
identify(HostX[,1], HostX[,2], rownames(HostX),offset=0.3, xpd=TRUE, cex= 0.8)
```

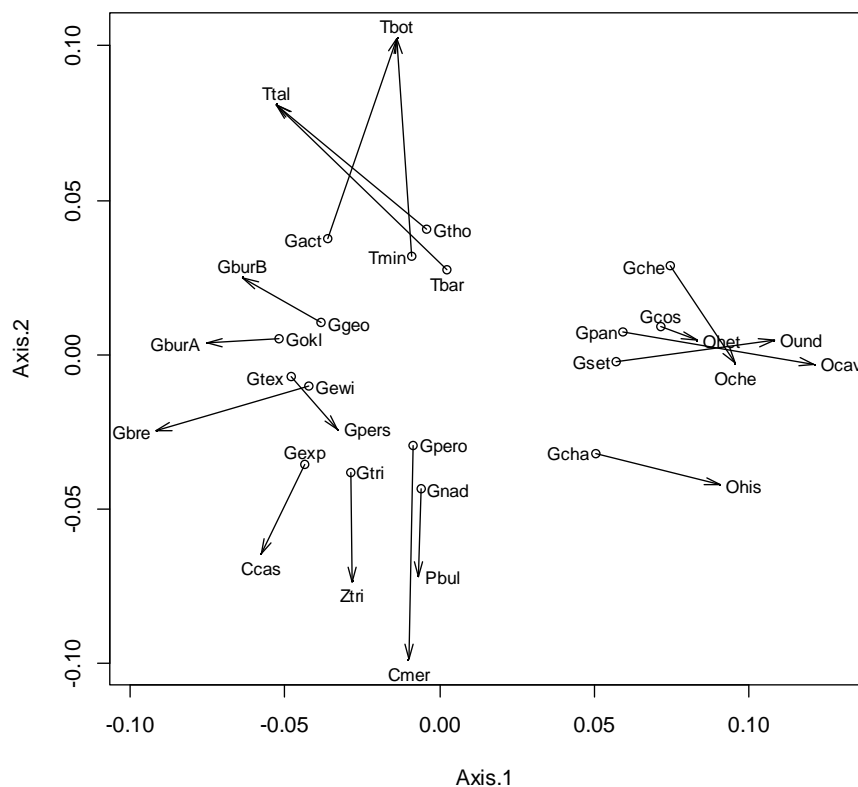
With `identify` the user can label the data points interactively by clicking near each data point on the plot. First label the parasite locations and then those of the hosts.

### ***Goodness-of-fit test***

The following code computes the residual sum of squares  $m_{XY}^2$  and performs a randomization of the host-parasite association matrix to establish the probability  $P$  under  $H_0$ :

```
m2.obs <- HP.proc$ss
N.perm = 100000
```

where `N.perm` sets the number of random permutations of the host-parasite association matrix. For high precision of the  $P$  estimate, 100,000 permutations were used in the accompanying paper. Although computing time was not prohibitive (some 24 min. on a PC equipped with an Intel Core 2 CPU 6600 @ 2.40GHz processor), in most situations  $\leq 10,000$  permutations would be sufficient for hypothesis testing.



**Figure S1. Procrustean superimposition plot of pocket gophers and chewing lice.** The ordinations of gopher and lice are Principal Correspondence Coordinates of HKY85 genetic distances. The lice configuration (dots) has been rotated and scaled to fit the gopher ordination (arrow tips). See Appendix for abbreviations of taxa.

```
P.value = 0
```

```
set.seed(.Random.seed[trunc(runif(1,1,626))])
```

The last line sets the randomization seed. To obtain reproducible sets of permutations, use instead an integer of choice, for instance `set.seed(5)`.

```
for (n in c(1:N.perm))
{if (NLinks <= nrow(HP) | NLinks <= ncol(HP))
  {flag2 <- TRUE
  while (flag2 == TRUE)
  {HP.perm <- t(apply(HP,1,sample))
  if(any(colSums(HP.perm) == NLinks)) flag2 <- TRUE
  else flag2 <- FALSE}
```

```

} else { HP.perm <- t(apply(HP,1,sample))}
PACo.perm <- PACo(host.D, para.D, HP.perm)
m2.perm <- procrustes(PACo.perm$H.PCo, PACo.perm$P.PCo)$ss
write(m2.perm, file="PACo/example/m2_perm.txt", sep="\t", append=TRUE)
if (m2.perm <= m2.obs){P.value = P.value + 1}
}

```

Although the `write` function above is not essential for the analysis (and can be omitted), it is useful if one wishes to save the set of residual sum of squares generated at each permutation for further reference. Note the path pointing to the location where the file will be saved. Given that `append=TRUE`, the file created (`m2_perm.txt`) should be deleted or renamed prior to a new analysis. Otherwise the values generated in the new run will be appended to those produced in the previous one.

To conclude the goodness-of-fit test:

```

P.value <- P.value/N.perm
cat(" The observed m2 is ", m2.obs, "\n", P.value, " based on ", N.perm, "
permutations.")

```

The following output is produced:

```

There were 50 or more warnings (use warnings() to see the first 50)
The observed m2 is  0.1158733
P-value =  1e-05 based on  1e+05  permutations.

```

(The warnings are originated by each of the Procrustes analyses with the permuted host-parasite association matrix and result from the different number of columns in the host and parasite matrices. As noted above this has no effect on the analysis).

So  $m_{XY}^2 = 0.1159$ . In only one of the 100,000 random permutations the residual sum of squares was smaller than this value (i.e.,  $P = 10^{-5}$ ) and congruence between the host and parasite phylogenies is statistically significant at the conventional significance level of 0.05.

### ***Evaluation of host-parasite links***

As justified in the accompanying article, the contribution of each host-parasite to the global fit can be assessed with a jackknife procedure that estimates the squared residual and its 95% confidence interval of each individual link:

```

HP.ones <- which(HP > 0, arr.in=TRUE)
SQres.jackn <- matrix(rep(NA, NLinks**2), NLinks)

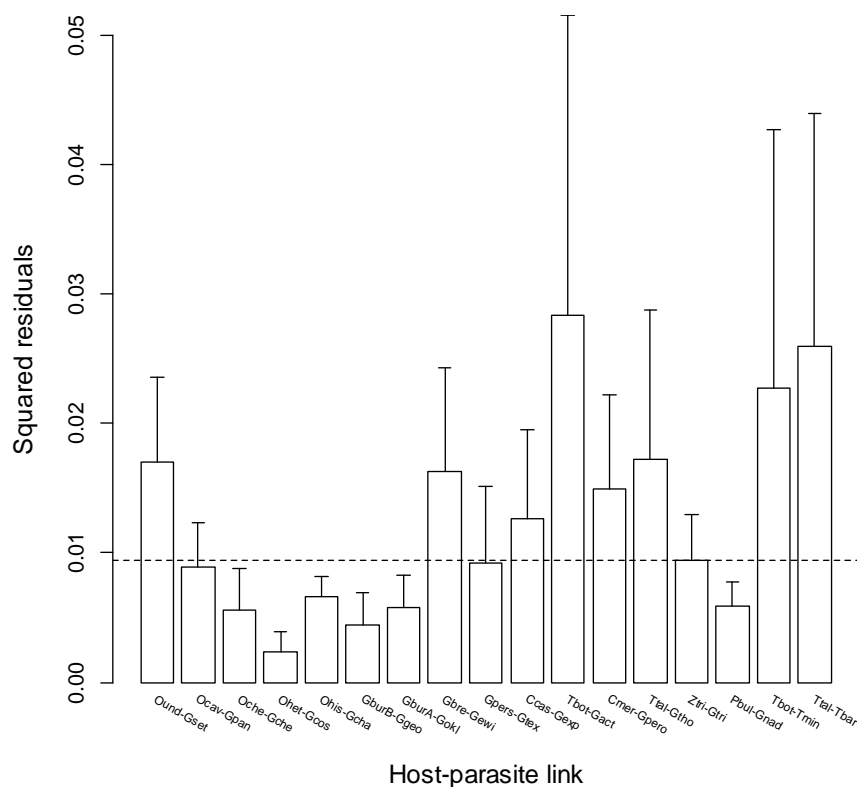
```

```

colnames (SQres.jackn) <- paste(rownames(HostX),rownames(ParY), sep="-")
t.critical = qt(0.975,NLinks-1)
for(i in c(1:NLinks))
{HP.ind <- HP
HP.ind[HP.ones[i,1],HP.ones[i,2]]=0
PACo.ind <- PACo(host.D, para.D, HP.ind)
Proc.ind <- procrustes(PACo.ind$H.PCo, PACo.ind$P.PCo)
res.Proc.ind <- c(residuals(Proc.ind))
res.Proc.ind <- append (res.Proc.ind, NA, after= i-1)
SQres.jackn [i, ] <- res.Proc.ind}
SQres.jackn <- SQres.jackn**2
SQres <- (residuals (HP.proc)**2
SQres.jackn <- SQres.jackn*(-(NLinks-1))
SQres <- SQres*NLinks
SQres.jackn <- t(apply(SQres.jackn, 1, "+", SQres))
phi.mean <- apply(SQres.jackn, 2, mean, na.rm = TRUE)
phi.UCI <- apply(SQres.jackn, 2, sd, na.rm = TRUE)
phi.UCI <- phi.mean + t.critical * phi.UCI/sqrt(NLinks)
pat.bar <- barplot(phi.mean, names.arg = " ", space = 0.25, col="white", xlab=
"Host-parasite link", ylab= "Squared residuals", ylim=c(0, max(phi.UCI)),
cex.lab=1.2)
text(pat.bar, par("usr")[3] - 0.001, srt = 330, adj = 0, labels =
colnames(SQres.jackn), xpd = TRUE, font = 1, cex=0.6)
arrows(pat.bar, phi.mean, pat.bar, phi.UCI, length= 0.05, angle=90)
abline(a=median(phi.mean), b=0, lty=2)

```

The foregoing code produces a bar chart of squared residuals (Fig. S1.2). Most links related to gopher species of *Orthogeomys*, *Geomys* and *Pappogeomys* contributed relatively little to  $m_{XY}^2$  and thus likely represent coevolutionary links. The links related to *Thomomys* spp. showed the highest residuals but their confidence intervals were quite broad (Fig. S1.2). Thus, it is difficult to evaluate their contribution to the cophylogenetic pattern observed.



**Figure S2. Jackknifed squared residuals (bars) and upper 95% confidence intervals (error bars) associated to each gopher-lice link.** PACo was applied to HKY85 genetic distances. The dashed line indicates the median squared residual value. See Appendix for abbreviations of taxa.

## References

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## Appendix

This appendix illustrates the format of the input files used in the present document. **Files should have matching taxa labels.** The following labels were used:

**Pocket gophers:** Ccas: *Cratogeomys castanops*; Cmer: *C. merriami*; GburA: *Geomys bursarius halli*; GburB: *G. bursarius majusculus*; Gbre: *G. breviceps*; Gpers: *G. personatus*; Ocav: *Orthogeomys cavator*; Oche: *O. cherriei*; Ohet: *O. heterodus*; Ohis: *O. hispidus*; Ound: *O. underwoodii*; Pbul: *Pappogeomys bulleri*; Ztri: *Zygoeomys trichopus*; Tbot: *Thomomys bottae*; Ttal: *T. talpoides*. **Chewing lice:** Gact: *Geomydoecus actuosus*; Gcha: *G. chapini*; Gche: *G. cherriei*; Gcos: *G. costaricensis*; Gewi: *G. ewingi*; Gexp: *G. expansus*; Ggeo: *G. geomydis*; Gnad: *G. nadleri*; Gokl: *G. oklahomensis*; Gpan: *G. panamensis*; Gpero: *G. perotensis*; Gset: *G. setzeri*; Gtex: *G. texanus*; Gtho: *G. thomomyus*; Gtri: *G. trichopi*; Tbar: *Thomomydoecus barbarae*; Tmin: *T. minor*.

### A1. Host-parasite association matrix (g-l\_links.txt)

Binary matrix with host and parasite species in rows and columns, respectively; 1's represent presence of a given parasite in a given host in nature, whereas 0's denote otherwise.

	Gset	Gpan	Gche	Gcos	Gcha	Ggeo	Gokl	Gewi	Gtex	Gexp	Gact	Gpero	Gtho	Gtri	Gnad	Tmin	Tbar
GburA	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
GburB	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Gbre	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Gpers	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Ound	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ocav	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oche	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ohet	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Ohis	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Pbul	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Ccas	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Cmer	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Ztri	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Tbot	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
Ttal	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1

## A2. Tree files

Newick format files with branch lengths and taxon labels.

### A2.1 Pylogeny of pocket gophers (*phylo\_gopher.tre*):

```
(Ttal:0.07713,Tbot:0.08101,((((((Oche:0.02354,Ohet:0.01733):0.01634,(Ocav:0.04239,Ound:0.04272):0.01389):0.01327,Ohis:0.05788):0.03688,Pbul:0.08235):0.00364,(Cmer:0.04659,Ccas:0.05507):0.03224):0.0047,Ztri:0.10476):0.00323,(Gpers:0.05442,(Gbre:0.07104,(GburA:0.03003,GburB:0.04034):0.02281):0.01448):0.01172):0.04061);
```

### A2.2 Pylogeny of chewing lice (*phylo\_lice.tre*):

```
(Tbar:0.07682,Tmin:0.10403,((((((Gtho:0.14298,Gact:0.10454):0.01104,Gpero:0.10711):0.00819,(((Gche:0.07859,Gcos:0.05567):0.03793,(Gpan:0.07292,Gset:0.07992):0.01251):0.01527,Gcha:0.10553):0.01919):0.00187,((Gnad:0.11749,Gtri:0.09812):0.0122,(Gexp:0.11179,((Gtex:0.03366,Gewi:0.03409):0.06301,(Gokl:0.02618,Ggeo:0.02384):0.07929):0.01325):0.01303):0.0524):0.0262);
```

## A3. Aligned sequences

### A3.1 Sequences of pocket gophers (*alig\_gopher.fasta*):

>GburA

```
TGAAGTTTATATCTTAATTCTACCTGGATTTCGGAATAATTTACACATTGTTACTTATTATTCAGGAAAAAAGAACCCTTTTGGCTACATAGGCATAGTTTGAGCTATGATATCAA
TTGGATTTCCTAGGCTTTTATTGTATGAGCCCATCATATATTTACAGTAGGTATGGATGTAGACACCCGAGCCTATTTTACATCTGCAACTATAATCATTGCTATCCCAACAGGAGTA
AAAGTGTTTAGCTGACTAGCTACTTTACACGGAGGTAATATTTAAATGATCTCCTGCTATACTGTGAGCATTAGGCTTTATTTTCTTTTCACTATTGGCGGATTAACCTGGAATCGT
CCTATTCAACTCATCATTAGATATTGTATTA
```

>GburB

```
TGAAGTTTACATCTTAATCCTACCCGGATTTCGGAATAATTTCCCATATTGTTACCTATTACTCAGGAAAAAAGAGCCTTTTGGCTACATAGGCATAGTTTGAGCCATGATATCAA
TTGGATTTCCTAGGCTTTTATTGTTTGGAGCCCATCATATATTTACAGTAGGTATGGACGTAGATACCCGAGCCTATTTTACATCTGCAACTATAATCATTGCTATCCCAACAGGAGTA
AAAGTATTCAGCTGATTAGCTACTTTACACGGGGTAATATTTAAATGATCACCTGCTATACTGTGAGCATTAGGCTTTATTTTCTTTTCACTATTGGCGGGTTAACTGGAATTGT
CCTGTCCAACCTCATCATTAGACATTGTTCTA
```

>Gbre

TGAAGTTTTATATCTTGATCCTGCCAGGATTTGGAATAATTTACATATTTGTTACTTACTATTTCCGGAAAAAAGAGCCTTTTGGCTATATAGGTATAGTTTGGAGCTATAATATCAA  
TCGGATTTTTAGGCTTTATCGTGTGAGCCCACCATATATTTACAGTAGGTATGGATGTAGACACACGAGCTTACTTTACATCTGCTACTATAAATTATCGCCATCCCAACTGGAGTG  
AAAGTATTTAGCTGATTAGCCACTTTACACGGAGGTAATATTTAAATGGTCACCTGCTATATTTGTGAGCGCTAGGTTTTATTTTCTTTTCACTATCGGCGGATTAACCTGGAATCGT  
CCTGTCCAACCTCATCACTAGACATTGTTACTG

>Gpers

TGAGGTTTTACATCCTAATTCTACCAGGATTCGGTATAATTTACATATTTGTCACCTTATTATTTCAGGAAAAAAGGAACCCCTTTGGCTATATAGGCATAGTTTGGAGCTATAATATCAA  
TTGGGTTCTTAGGTTTTATTGTATGAGCCCACCATATATTTACAGTAGGCATGGACGTAGATACCCGAGCCTACTTTACATCTGCAACTATAAATTATCGCTATTTCTACAGGAGTA  
AAAGTATTTAGCTGACTAGCCACTCTACACGGAGGTAATATTTAAATGATCCCCCTGCTATACTATGAGCACTAGGCCTTTATTTTCTTTTCACTATTTGGTGGGCTAAGCTGGTATCGT  
CTTATCCAACCTCATCATTAGACATCGTACTG

>Ound

TGAAGTTTTATATCTTGATCCTCCCAGGATTCGGAATGATTTCTCATATTTGTCACCTACTATTTACAGGTAAAAAAGAACCCTTTGATTATATAGGCATGGTATGAGCTATAATATCTA  
TTGGTTTTCTTAGGTTTTATAGTATGAGCCCACCATATATTTACAGTAGGGATAGACGTAGATACACGAGCCTACTTTACATCCGCTACTATGATTATCGCTATCCCTACCGGAGTA  
AAAGTGTTCAGTTGACTAGCTACCATGAACGGAGGTAATATTTAAATGATCTCCTGCCATATTTATGAGCCTTAGGTTTTATTTTCTTTTCACTATTTGGTGGACTAACAGGCATTGT  
ATTATCTAATTCATCCTTAGACATTATTCTA

>Ocav

TGAAGTTTTATATCTTAATTCTCCCAGGCTTCGGAATGATTTCTCATATTTGTCACCTTACTACTCAGGTAAAAAAGAACCCTTTGATTATATAGGCGTGGTATGAGCTATAATATCCA  
TCGGATTCCTAGGTTTTGATAGTATGAGCCCACCATATATTTACAGTAGGAATAGACGTAGACACACGAGCCTATTTTACATCCGCTACTATAAATTATTGCTATTTCCACCGGAGTA  
AAAGTATTCAGTTGACTGGCTACCATGAACGGAGGTAATATTTAAATGATCTCCTGCCATATTTATGAGCCTTTAGGCTTTATCTTTCTATTTACAATTTGGTGGCCTAACCGGCATTGT  
ATTATCAAATTCATCCTTAGATATTATTCTA

>Oche

TGAAGTTTTACATCTTAATCCTCCCAGGCTTCGAAATAATCTCTCATATTTGTCACCTTATTATTTCAGGTAAAAAAGAACCCTTTGGCTATATAGGTATGGTATGAGCTATAATATCAA  
TTGGTTTTCTTAGGTTTTAATGGTATGAGCCCACCATATATTTACAGTAGGAATAGACGTAGATACACGAGCCTACTTTACATCCGCTACTATAAATTATTGCTATTTCTACCGGAGTA  
AAAGTATTCAGTTGACTAGCTACCATGAACGGAGGCAATATTTAAATGATCCCCCTGCCATATTTATGAGCCTTAGGTTTTATTTTCTATTTACAATTTGGTGGCCTAACCGGCATTGT  
ATTATCAAACCTCATCCTTAGATATTGTCCTA

>Ohet

TGAAGTTTTACATCTTAATCCTCCCAGGCTTCGGAATGATTTCTCATATTTGTCACCTTATTATTTCAGGTAAAAAAGAACCCTTTGGCTATATAGGCATGGTATGAGCTATAATATCAA  
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ACTATCCAACCTCATCATTGGATATTGTTCTA

>Ohis

TGAGGTTTTATATCTTAATCCTCCCAGGCTTCGGTATAATTTCTCATATCGTCACTTATTACTCAGGCAAAAAAGAACCCTTTGGATATATAGGCATGGTATGAGCCATAATATCTA  
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AAAGTATTCAGTTGACTGGCTACACTGCATGGAGGTAATATTTAAATGATCTCCTGCCATATTTATGAGCCTTAGGTTTTATTTTCTATTTACTATCGGTGGTCTAACGGGCATTGT  
CCTATCAAATTCATCTTTGGATATCGTTCTA

>Pbul

TGAAGTTTTATATTTAATTCTCCCAGGTTTTGGAATAATTTACATATCGTAACCTATTATTCTGGTAAAAAAGAGCCATTCGGTTACATAGGTATAGTATGGGCTATAATATCAA  
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>Ccas

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>Cmer

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>Ztri

AGAAGTTTACATTCTAATCCTCCCTGGCTTCGGTATAATCTCACACATTGTCACTTACTTACTCAGGCAAGAAAGAACCATTTCCGATATATAGGTATAGTATGAGCTATAATGTCCA  
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>Tbot

TGAAGTTTATATCCTAATTTCTCCAGGGTTTGGAAATAATTTCCCATATTTGTTACCTACTTACTCAGGCAAGAAAGAACCTTTTCGATTATATGGGAATAGTATGAGCTATAATATCTA  
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>Ttal

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 TCTATCTAACTCATCACTAGATATTGTATTA

### ***A3.2 Sequences of chewing lice (alig\_lice.fasta):***

>Gset

CGAAGTTTATATTTTGGATTTTACCAGGTTTTGGGTTAATTTCTCAAATTTATTTTATTCGAGAGAGGAAAGCAAGTTTTTGGAACTGTAGGAATAATCTATGCTATAATAGCAA  
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>Gpan

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 AAGGTGTTTAGGTGATTGGCCACTTCTTTTGGAAAGCCGAATTTTCATATTCGGTTTTCGATGCTCTGAACATTTAGGATTCGTATTTCTGTTTACCGTTGGAGGGATAACGGGGTTAGT  
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>Gche

AGAAGTCTATATTTTGGATTTTACCCGGATTTGGTTTAATTTCCCAAATTTATTTTATTCGAAAGAGGGAAAAAGCAGGTTTTTGGTACCCTGGGTATAATTTATGCGATAATAGCAA  
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>Gcos

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>Gcha

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>Tmin

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>Tbar

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>Gokl

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>Gewi

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>Gpero

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>Gtho

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>Gact

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GTTGGCAAATTCATGTGTAGACGTCGTCTTC

## A4. Genetic distances

### A4.1 HKY85 distance matrix of pocket gophers (*dist\_gopher.txt*):

	GburA	GburB	Gbre	Gpers	Ound	Ocav	Oche	Ohet	Ohis	Pbul	Ccas	Cmer	Ztri	Tbot	Ttal
GburA	0	0.07036	0.12782	0.115	0.20408	0.20273	0.19576	0.17476	0.21425	0.15956	0.16259	0.17248	0.20074	0.19232	0.17722
GburB	0.07036	0	0.13025	0.12348	0.19983	0.21755	0.19538	0.1709	0.20616	0.18745	0.19825	0.20861	0.20414	0.18526	0.20606
Gbre	0.12782	0.13025	0	0.14761	0.20842	0.24667	0.21918	0.20787	0.2265	0.1779	0.18249	0.1972	0.19659	0.22756	0.20515
Gpers	0.115	0.12348	0.14761	0	0.17846	0.19938	0.15333	0.14377	0.17094	0.17019	0.15947	0.15104	0.16814	0.18461	0.20125
Ound	0.20408	0.19983	0.20842	0.17846	0	0.08511	0.0948	0.08823	0.13279	0.20057	0.23937	0.19607	0.21642	0.20669	0.23728
Ocav	0.20273	0.21755	0.24667	0.19938	0.08511	0	0.09557	0.09427	0.11633	0.1757	0.21863	0.18528	0.22771	0.19941	0.23171
Oche	0.19576	0.19538	0.21918	0.15333	0.0948	0.09557	0	0.04087	0.11613	0.17989	0.20765	0.17847	0.19638	0.19917	0.20537
Ohet	0.17476	0.1709	0.20787	0.14377	0.08823	0.09427	0.04087	0	0.10577	0.18019	0.19657	0.18165	0.20042	0.19946	0.19587
Ohis	0.21425	0.20616	0.2265	0.17094	0.13279	0.11633	0.11613	0.10577	0	0.17213	0.18182	0.16737	0.2097	0.21779	0.22399
Pbul	0.15956	0.18745	0.1779	0.17019	0.20057	0.1757	0.17989	0.18019	0.17213	0	0.1868	0.14119	0.19351	0.21842	0.22155
Ccas	0.16259	0.19825	0.18249	0.15947	0.23937	0.21863	0.20765	0.19657	0.18182	0.1868	0	0.10166	0.20622	0.21578	0.18191
Cmer	0.17248	0.20861	0.1972	0.15104	0.19607	0.18528	0.17847	0.18165	0.16737	0.14119	0.10166	0	0.17967	0.2126	0.22256
Ztri	0.20074	0.20414	0.19659	0.16814	0.21642	0.22771	0.19638	0.20042	0.2097	0.19351	0.20622	0.17967	0	0.23958	0.22094
Tbot	0.19232	0.18526	0.22756	0.18461	0.20669	0.19941	0.19917	0.19946	0.21779	0.21842	0.21578	0.2126	0.23958	0	0.15814
Ttal	0.17722	0.20606	0.20515	0.20125	0.23728	0.23171	0.20537	0.19587	0.22399	0.22155	0.18191	0.22256	0.22094	0.15814	0

**A4.2 HKY85 distance matrix of chewing lice (*dist\_lice.txt*):**

	Gset	Gpan	Gche	Gcos	Gcha	Tmin	Tbar	Gtri	Gnad	Ggeo	Gokl	Gewi	Gtex	Gexp	Gpero	Gtho	Gact
Gset	0	0.15285	0.20774	0.18508	0.20672	0.28637	0.20558	0.25492	0.25773	0.23895	0.25154	0.24683	0.24684	0.26487	0.25636	0.2713	0.27781
Gpan	0.15285	0	0.20307	0.18012	0.21644	0.24089	0.23687	0.26877	0.23214	0.23289	0.22842	0.23888	0.24289	0.26214	0.24513	0.26842	0.25225
Gche	0.20774	0.20307	0	0.13426	0.24437	0.28942	0.27768	0.31042	0.28377	0.24705	0.27571	0.2955	0.30766	0.30526	0.26057	0.24025	0.26306
Gcos	0.18508	0.18012	0.13426	0	0.20368	0.26451	0.22371	0.27052	0.25355	0.25926	0.28869	0.25906	0.26638	0.2914	0.23581	0.23521	0.27029
Gcha	0.20672	0.21644	0.24437	0.20368	0	0.25833	0.21789	0.22702	0.26045	0.27646	0.28893	0.24521	0.24124	0.25057	0.23825	0.29333	0.26947
Tmin	0.28637	0.24089	0.28942	0.26451	0.25833	0	0.18085	0.23405	0.27406	0.26303	0.25934	0.24557	0.22602	0.26036	0.24257	0.31228	0.2839
Tbar	0.20558	0.23687	0.27768	0.22371	0.21789	0.18085	0	0.21219	0.23126	0.25821	0.25332	0.28135	0.25983	0.2348	0.20537	0.26172	0.23509
Gtri	0.25492	0.26877	0.31042	0.27052	0.22702	0.23405	0.21219	0	0.21561	0.26088	0.2447	0.24405	0.24713	0.22154	0.2393	0.28044	0.24128
Gnad	0.25773	0.23214	0.28377	0.25355	0.26045	0.27406	0.23126	0.21561	0	0.26134	0.27782	0.2428	0.25414	0.25364	0.26446	0.28466	0.26895
Ggeo	0.23895	0.23289	0.24705	0.25926	0.27646	0.26303	0.25821	0.26088	0.26134	0	0.05002	0.21445	0.20119	0.20727	0.26013	0.31126	0.22115
Gokl	0.25154	0.22842	0.27571	0.28869	0.28893	0.25934	0.25332	0.2447	0.27782	0.05002	0	0.19903	0.19007	0.20666	0.24403	0.3197	0.22055
Gewi	0.24683	0.23888	0.2955	0.25906	0.24521	0.24557	0.28135	0.24405	0.2428	0.21445	0.19903	0	0.06775	0.25981	0.2385	0.26712	0.21988
Gtex	0.24684	0.24289	0.30766	0.26638	0.24124	0.22602	0.25983	0.24713	0.25414	0.20119	0.19007	0.06775	0	0.22878	0.24655	0.29137	0.23083
Gexp	0.26487	0.26214	0.30526	0.2914	0.25057	0.26036	0.2348	0.22154	0.25364	0.20727	0.20666	0.25981	0.22878	0	0.24703	0.31316	0.22625
Gpero	0.25636	0.24513	0.26057	0.23581	0.23825	0.24257	0.20537	0.2393	0.26446	0.26013	0.24403	0.2385	0.24655	0.24703	0	0.24623	0.2376
Gtho	0.2713	0.26842	0.24025	0.23521	0.29333	0.31228	0.26172	0.28044	0.28466	0.31126	0.3197	0.26712	0.29137	0.31316	0.24623	0	0.24752
Gact	0.27781	0.25225	0.26306	0.27029	0.26947	0.2839	0.23509	0.24128	0.26895	0.22115	0.22055	0.21988	0.23083	0.22625	0.2376	0.24752	0