**R script used for the reconstruction of ancestral states and test of evolutionary rates**

library(mvMORPH)

library(ape)

library(phytools)

Xipho\_tree <- read.nexus(file="xiphbayesFrontiers\_trees.nex")

Pruned\_tree=drop.tip(Xipho\_tree, c("Alanops\_magnifica","Tasmaniolimulus\_patersoni","Paleolimulus\_jakovlevi","Palaeocharinus\_sp\_","Madagascan\_limulid","Mesolimulus\_sibiricus","Mesolimulus\_tafraoutensis","Moravurus\_rehori","Liomesaspis\_laevis","Stilpnocephalus\_pontebbanus","Pickettia\_carteri","Fuxianhuia\_protensa","Emeraldella\_brocki","Olenoides\_serratus","Sydneyia\_inexpectans","Leanchoilia\_illecebrosa","Alalcomenaeus\_cambricus","Yohoia\_tenuis","Pycnogonum\_litorale","Haliestes\_dasos","Palaeoisopus\_problematicus","Offacolus\_kingi","Dibasterium\_durgae","Anderella\_parva","Camanchia\_grovensis","Venustulus\_waukeshaensis","Legrandella\_lombardi","Bembicosoma\_pomphicus","Bunodes\_lunula","Limuloides\_limuloides","Pasternakevia\_podolica","Cyamocephalus\_loganensis","Pseudoniscus\_roosevelti","Winneshiekia\_youngae","Houia\_yueya","Stoermeropterus\_conicus","Moselopterus\_ancylotelson","Onychopterella\_kokomoensis","Erieopterus\_macrophthalmus","Carcinosoma\_newlini","Megalograptus\_ohioensis","Mixopterus\_kiaeri","Orcanopterus\_manitoulinensis","Adelophthalmus\_sievertsi","Nanahughmilleria\_norvegica","Hughmilleria\_socialis","Slimonia\_acuminata","Erettopterus\_bilobus","Pterygotus\_anglicus","Strobilopterus\_proteus","Dolichopterus\_macrocheirus","Brachyopterus\_stubblefieldi","Rhenopterus\_diensti","Parastylonurus\_ornatus","Laurieipterus\_elegans","Kokomopterus\_longicaudatus","Hardieopterus\_macrophthalmus","Drepanopterus\_pentlandicus","Hibbertopterus\_scouleri","Megarachne\_servinei","Centruroides\_vittatus","Androctonus\_australis","Palaeophonus\_nuncius","Proscorpius\_osborni","Caddo\_agilis","Leiobunum\_rotundum","Gonyleptes\_horridus","Sclerobunus\_robustus","Chileogovea\_oedipus","Cyphophthalmus\_duricorius","Allothrombium\_simoni","Amblyomma\_americanum","Neocarus\_texanus","Cryptocellus\_goodnighti","Ricinoides\_atewa","Poliochera\_punctulata","Anthracomartus\_hindi","Eophrynus\_prestvicii","Gilboarachne\_griersoni","Palaeocharinus\_sp","Plesiosiro\_madeleyi","Mastigoproctus\_giganteus","Thelyphonus\_caudatus","Hubbardia\_pentapeltis","Schizomus\_crassicaudatus","Phrynus\_marginemaculatus","Charinus\_victori","Permarachne\_novokshonovi","Attercopus\_filmbriunguis","Liphistius\_malayanus","Aphonopelma\_hentzi","Hypochilus\_pococki","Cupienius\_getazi","Eukoenenia\_mirabilis","Prokoenenia\_wheeleri","Mesotarbus\_peteri","Neobisium\_maritimum","Chelifer\_cancroides","Eremocosta\_striata","Galeodes\_armeniacus","Chasmataspis\_laurencii","Loganamaraspis\_dunlopi","Achanarraspis\_reedi","Heteroaspis\_stoermeri","Octoberaspis\_ushakovi","Diploaspis\_casteri","Diploaspis\_muelleri","Dvulikiaspis\_menneri","Euproops\_anthrax","Euproops\_mariae","Euproops\_rotundatus","Anacontium\_carpenteri","Anacontium\_carpenteri","Pringlia\_birtwelli","Bellinurus\_lunatus","Bellinurus\_arcuatus","Bellinurus\_reginae","Bellinurus\_truemanii","Bellinurus\_truemanii","Bellinurus\_bellulus","Bellinurus\_trilobitoides","Kasibelinurus\_randalli","Limulus\_coffini","Limulus\_darwini","Limulus\_woodwardi","Lunataspis\_aurora","Mesolimulus\_crespelli","Mesolimulus\_sp","Paleolimulus\_fuchsbergensis","Paleolimulus\_longispinus","Paleolimulus\_woodae","Panduralimulus\_babcocki","Pringlia\_birtwelli","Paleolimulus\_longispinus","Casterolimulus\_kletti","Crenatolimulus\_paluxyensis","Dubbolimulus\_peetae","Carcinoscorpius\_rotundicauda","Tachypleus\_gigas","Tachypleus\_decheni","Tachypleus\_syriacus","Tarracolimulus\_rieki","Vaderlimulus\_tricki","Austrolimulus\_fletcheri","Bellinuroopsis\_rossicus","Heterolimulus\_gadeai","Kasibelinurus\_amoricum","Limulitella\_bronni","Limulitella\_henkeli","Limulitella\_tejraensis","Limulitella\_vicensis","Psammolimulus\_gottingensis","Rolfeia\_fouldenensis","Victalimulus\_mcqueeni","Weinbergina\_opitzi","Xaniopyramis\_linseyi","Yunnanolimulus\_luopingensis","Valloisella\_lievinensis"))

Pruned\_tree\_noDekatriata=drop.tip(Xipho\_tree, c("Eurypterus\_tetragonophthalmus","Hoplitaspis\_hiawathai","Alanops\_magnifica","Tasmaniolimulus\_patersoni","Paleolimulus\_jakovlevi","Palaeocharinus\_sp\_","Madagascan\_limulid","Mesolimulus\_sibiricus","Mesolimulus\_tafraoutensis","Moravurus\_rehori","Liomesaspis\_laevis","Stilpnocephalus\_pontebbanus","Pickettia\_carteri","Fuxianhuia\_protensa","Emeraldella\_brocki","Olenoides\_serratus","Sydneyia\_inexpectans","Leanchoilia\_illecebrosa","Alalcomenaeus\_cambricus","Yohoia\_tenuis","Pycnogonum\_litorale","Haliestes\_dasos","Palaeoisopus\_problematicus","Offacolus\_kingi","Dibasterium\_durgae","Anderella\_parva","Camanchia\_grovensis","Venustulus\_waukeshaensis","Legrandella\_lombardi","Bembicosoma\_pomphicus","Bunodes\_lunula","Limuloides\_limuloides","Pasternakevia\_podolica","Cyamocephalus\_loganensis","Pseudoniscus\_roosevelti","Winneshiekia\_youngae","Houia\_yueya","Stoermeropterus\_conicus","Moselopterus\_ancylotelson","Onychopterella\_kokomoensis","Erieopterus\_macrophthalmus","Carcinosoma\_newlini","Megalograptus\_ohioensis","Mixopterus\_kiaeri","Orcanopterus\_manitoulinensis","Adelophthalmus\_sievertsi","Nanahughmilleria\_norvegica","Hughmilleria\_socialis","Slimonia\_acuminata","Erettopterus\_bilobus","Pterygotus\_anglicus","Strobilopterus\_proteus","Dolichopterus\_macrocheirus","Brachyopterus\_stubblefieldi","Rhenopterus\_diensti","Parastylonurus\_ornatus","Laurieipterus\_elegans","Kokomopterus\_longicaudatus","Hardieopterus\_macrophthalmus","Drepanopterus\_pentlandicus","Hibbertopterus\_scouleri","Megarachne\_servinei","Centruroides\_vittatus","Androctonus\_australis","Palaeophonus\_nuncius","Proscorpius\_osborni","Caddo\_agilis","Leiobunum\_rotundum","Gonyleptes\_horridus","Sclerobunus\_robustus","Chileogovea\_oedipus","Cyphophthalmus\_duricorius","Allothrombium\_simoni","Amblyomma\_americanum","Neocarus\_texanus","Cryptocellus\_goodnighti","Ricinoides\_atewa","Poliochera\_punctulata","Anthracomartus\_hindi","Eophrynus\_prestvicii","Gilboarachne\_griersoni","Palaeocharinus\_sp","Plesiosiro\_madeleyi","Mastigoproctus\_giganteus","Thelyphonus\_caudatus","Hubbardia\_pentapeltis","Schizomus\_crassicaudatus","Phrynus\_marginemaculatus","Charinus\_victori","Permarachne\_novokshonovi","Attercopus\_filmbriunguis","Liphistius\_malayanus","Aphonopelma\_hentzi","Hypochilus\_pococki","Cupienius\_getazi","Eukoenenia\_mirabilis","Prokoenenia\_wheeleri","Mesotarbus\_peteri","Neobisium\_maritimum","Chelifer\_cancroides","Eremocosta\_striata","Galeodes\_armeniacus","Chasmataspis\_laurencii","Loganamaraspis\_dunlopi","Achanarraspis\_reedi","Heteroaspis\_stoermeri","Octoberaspis\_ushakovi","Diploaspis\_casteri","Diploaspis\_muelleri","Dvulikiaspis\_menneri","Euproops\_anthrax","Euproops\_mariae","Euproops\_rotundatus","Anacontium\_carpenteri","Anacontium\_carpenteri","Pringlia\_birtwelli","Bellinurus\_lunatus","Bellinurus\_arcuatus","Bellinurus\_reginae","Bellinurus\_truemanii","Bellinurus\_truemanii","Bellinurus\_bellulus","Bellinurus\_trilobitoides","Kasibelinurus\_randalli","Limulus\_coffini","Limulus\_darwini","Limulus\_woodwardi","Lunataspis\_aurora","Mesolimulus\_crespelli","Mesolimulus\_sp","Paleolimulus\_fuchsbergensis","Paleolimulus\_longispinus","Paleolimulus\_woodae","Panduralimulus\_babcocki","Pringlia\_birtwelli","Paleolimulus\_longispinus","Casterolimulus\_kletti","Crenatolimulus\_paluxyensis","Dubbolimulus\_peetae","Carcinoscorpius\_rotundicauda","Tachypleus\_gigas","Tachypleus\_decheni","Tachypleus\_syriacus","Tarracolimulus\_rieki","Vaderlimulus\_tricki","Austrolimulus\_fletcheri","Bellinuroopsis\_rossicus","Heterolimulus\_gadeai","Kasibelinurus\_amoricum","Limulitella\_bronni","Limulitella\_henkeli","Limulitella\_tejraensis","Limulitella\_vicensis","Psammolimulus\_gottingensis","Rolfeia\_fouldenensis","Victalimulus\_mcqueeni","Weinbergina\_opitzi","Xaniopyramis\_linseyi","Yunnanolimulus\_luopingensis","Valloisella\_lievinensis"))

**The following script has been used for the analyses of both the all-euchelicerates and xiphosurids-only trees and data sets. Here is the script with the file name of xiphosurid-only data.**

writeNexus(Pruned\_tree\_noDekatriata, file="Pruned\_tree\_noDekatriata")

###Upload the environmental variables and slops

Slops\_envi<- read.csv(file="xipho\_slopsLog\_frontiers\_noDekatriata.csv", header = TRUE, sep = ",", row.names = 1)

###Ancestral environment for target species

habitat <- Slops\_envi$Habitat

names(habitat) <- Pruned\_tree\_noDekatriata$tip.label

pi<-setNames(c(0.99,0.01),c("marine","marginal"))

###Environment has been settled as marine a priori pi= at the root of the tree

Pruned\_mapped\_tree\_noDekatriata <- make.simmap(Pruned\_tree\_noDekatriata, habitat, pi=c(0,1), model="ARD", nsim=1000)

###To obtain the pie chart in figure 6 and 7

countSimmap(Pruned\_mapped\_tree)

###For the following analysis we calculate the ancestral state of environmental factors with only 1 simulation assuming marine environment at the root of the tree

Pruned\_mapped\_tree\_noDekatriata <- make.simmap(Pruned\_tree\_noDekatriata, habitat, pi=c(0,1), model="ARD", nsim=1)

simmap\_summary<-summary(Pruned\_mapped\_tree\_noDekatriata)

cols<-setNames(c("lightblue","lightcoral"),c("marine","marginal"))

simmap\_summary\_plot<-plot(simmap\_summary, fsize=0.6, ftype="i", colors=cols, ylim=c(-2, Ntip(Pruned\_tree\_noDekatriata)))

legend("topleft",c("marine","marginal"),pch=21,pt.bg=cols,pt.cex=2)

pL\_pW\_slop<- Slops\_envi$pL\_pW\_slop

names(pL\_pW\_slop) <- Slops\_envi$species

pL\_pW\_slop\_contMap <- contMap(Pruned\_tree\_noDekatriata, pL\_pW\_slop)

MLreconstruction <- ace(pL\_pW\_slop, Pruned\_tree\_noDekatriata, type="continuous", method="ML")

nodelabels(adj = -0.2, frame = "n", cex = 0.8, font = 2)

###Fitting a multivariate Brownian motion process

xipho\_traits <- (as.matrix(Slops\_envi[,c("pL\_pW\_slop")]))

fit\_BM1 <- mvBM(Pruned\_mapped\_tree\_noDekatriata, xipho\_traits, model="BM1")

fit\_BM1$sigma

###Correlations between the traits

cor2pcor(fit\_BM1$sigma)

###Fit a multi-rate Brownian motion

fit\_BMM <- mvBM(Pruned\_mapped\_tree\_noDekatriata, xipho\_traits, model="BMM")

###compare a multi-rate Brownian motion with a multivariate Brownian motion

results <- list(bm1=fit\_BM1, bmm=fit\_BMM)

results

aicw(results)

LRT(fit\_BMM, fit\_BM1)