



## **Appendix A. Primer sequences used for DNA amplification and sequencing**

All primers are given in 5'-3' direction. Primers marked with an asterisk were newly developed for this paper.

### ***Cytochrome b***

F-Primer 1 (*I*): CBJ10933 - TAT GTT CTA CCA TGA GGA CAA ATA TC

F-Primer 2 (*I*): Cytb-a - CCA TGA GGA CAA ATA TCA TTY TGR GG

\*F-Primer 3: MVZ15L-mod - AAC TWA TGG CCC MCA CMA TMC GWA A

R-Primer 1 (*I*): Cytb-c - CTA CTG GTT GTC CTC CGA TTC ATG T

\*R-Primer 2: CytbAR-H-mod - TAW ARG GRT CYT CKA CTG GTT G

### ***Tyrosinase (exon 1)***

F-Primer 1 (*I*): Tyr-1b - AGG TCC TCY TRA GGA AGG AAT G

F-Primer 2 (*I*): Tyr-1d - TCC TCC GTG GGC ACC CAR TTC CC

F-Primer 3 (*I*): Tyr-1a - AGG TCC TCT TRA GCA AGG AAT G

\*F-Primer 4: Tyr-F40 - AAR GAR TGY TGY CCI GTI TGG

\*F-Primer 5: Tyr - Fx3 - ACT GGC CCA YTG THT TYT ACA AC

\*F-Primer 6: Tyr - Fx4 - YTG GCC YWY TGT NTT YTA YAA C

R-Primer 1 (*I*): Tyr-1g - TGC TGG CRT CTC TCC ART CCC A

R-Primer 2 (*I*): Tyr-1e - GAG AAG AAA GAW GCT GGG CTG AG

\*R-Primer 3: Tyr-SPA - GAI GAG AAR AAR GAI GCT GGG CT

***Rhodopsin (exon 1)***

\*F-Primer 1: Rhod-ma - AAC GGA ACA GAA GGY CC

F-Primer 2 (1): Rhod-1a - ACC ATG AAC GGA ACA GAA GGY CC

\*R-Primer 1: Rhod-md - GTA GCG AAG AAR CCT TC

R-Primer 2 (1): Rhod-1d - GTA GCG AAG AAR CCT TCA AMG TA

R-Primer 3: Rhod-1c - CCA AGG GTA GCG AAG AAR CCT TC

***12S rRNA & tRNA<sub>Val</sub>***

F-Primer 1 (2): 12SAL - AAA CTG GGA TTA GAT ACC CCA CTA T

R-Primer 1 (2): 12SBH - GAG GGT GAC GGG CGG TGT GT

R-Primer 2: 16SR3 - TTT CAT CTT TCC CTT GCG GTA C

***16S rRNA (5' fragment)***

F-Primer (3): 16SL3 - AGC AAA GAH YWW ACC TCG TAC CTT TTG CAT

R-Primer (3): 16SAH - ATG TTT TTG ATA AAC AGG CG

***16S rRNA (3' fragment)***

F-Primer (2): 16SAL - CGC CTG TTT ATC AAA AAC AT

R-Primer (2): 16SBH - CCG GTC TGA ACT CAG ATC ACG T

## Appendix B. Voucher specimens and Genbank accession numbers

Voucher specimens are deposited in the herpetological collections of the Museo Regionale di Scienze Naturali, Torino (MRSN), Université d'Antananarivo, Département de Biologie Animale (UADBA), Laboratorio de Biogeografía, Universidad de los Andes, Mérida, Venezuela (ULABG), Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn (ZFMK), Zoologische Staatssammlung München (ZSM). Some UADBA numbers are preliminary fieldnumbers of F. Glaw and M. Vences (UADBA-FG/MV and UADBA-MV), some MRSN numbers are preliminary fieldnumbers of F. Andreone and J. E. Randrianirina (MRSN-FAZC and MRSN-RJS).

Asterisks mark sequences newly obtained in this study.

### *Mantellidae*

*Aglyptodactylus madagascariensis*: AF249068 (cytochrome *b*), AF249103 (rhodopsin), AF249166 (tyrosinase), AF249007 (12S and tRNA<sub>Val</sub>), AY341678\* (ZSM 183/2002, Tolagnaro, Madagascar) (16S [5' fragment]), AF249036 (16S [3' fragment]).

*Boophis* new species (Comoros): AY341733\* (cytochrome *b*), AY341796\* (rhodopsin), AY341752\* (tyrosinase), AY341610\* (12S and tRNA<sub>Val</sub>), AY341667\* (16S [5' fragment]), AY341716\* (16S [3' fragment]); all sequences from specimen ZSM 658/2000 (Tsingoni, Mayotte).

*Boophis boehmei*: AY341798\* (rhodopsin), AY341612\* (12S and tRNA<sub>Val</sub>), AY341669\* (16S [5' fragment]), AY341717\* (16S [3' fragment]); all sequences from specimen UADBA-MV 2001.1205 (Andasibe, Madagascar).

*Boophis doulioti*: AY341792\* (rhodopsin), AY341608\* (12S and tRNA<sub>Val</sub>), AY341663\* (16S [5' fragment]), AF215334 (ZFMK 66690, Kirindy, Madagascar) (16S [3' fragment]); all other sequences from specimen ZSM 185/2002 (Nahampoana, Madagascar).

*Boophis goudoti*: AY341797\* (rhodopsin), AY341611\* (12S and tRNA<sub>Val</sub>), AY341668\* (16S [5' fragment]), AJ315917 (not preserved; Col des Tapias, Madagascar) (16S [3' fragment]); all other sequences from specimen UADBA-MV 2001.557 (Andringitra, Madagascar).

*Boophis idae*: AY341795\* (rhodopsin), AY341609\* (12S and tRNA<sub>Val</sub>), AY341666\* (16S [5' fragment]), AY341715\* (16S [3' fragment]); all sequences from specimen ZSM 45/2002 (Andasibe, Madagascar).

*Boophis luteus*: AY341800\* (rhodopsin), AY341614\* (12S and tRNA<sub>Val</sub>), AY341671\* (16S [5' fragment]), AJ315916 (UADBA-FG/MV 2000.063, Andasibe, Madagascar) (16S [3' fragment]); all sequences from specimen UADBA-FG/MV 2000.63 (Andasibe, Madagascar).

*Boophis marojezensis*: AY341803\* (rhodopsin), AY341617\* (12S and tRNA<sub>Val</sub>), AY341674\* (16S [5' fragment]), AJ315923 (ZSM 326/2000, Vohidrazana, Madagascar) (16S [3' fragment]); all other sequences from specimen ZSM 189/2002 (Vohidrazana, Madagascar).

*Boophis microtypanum*: AY341799\* (rhodopsin), AY341613\* (12S and tRNA<sub>Val</sub>), AY341670\* (16S [5' fragment]), AJ315918 (16S [3' fragment]); all sequences from specimen ZSM 393/2000 (Col des Tapias, Madagascar).

*Boophis occidentalis*: AY341806\* (rhodopsin), AY341620\* (12S and tRNA<sub>Val</sub>), AY341677\* (16S [5' fragment]), AY341720\* (16S [3' fragment]); all sequences from specimen ZSM 44/2002 (Antoetra, Madagascar).

*Boophis rappiodes*: AY341804\* (rhodopsin), AY341618\* (12S and tRNA<sub>Val</sub>), AY341675\* (16S [5' fragment]), AJ314815 (16S [3' fragment]); all sequences from specimen ZSM 347/2000 (Andasibe, Madagascar).

*Boophis sibilans*: AY341801\* (rhodopsin), AY341615\* (12S and tRNA<sub>Val</sub>), AY341672\* (16S [5' fragment]), AY341718\* (16S [3' fragment]); all sequences from specimen ZSM 39/2002 (Andasibe, Madagascar).

*Boophis tephraeomystax*: AF249070 (cytochrome *b*), AY341793\* (rhodopsin), AF249168 (tyrosinase), AF249009 (12S and tRNA<sub>Val</sub>), AY341664\* (16S [5' fragment]), AJ312116 (16S [3' fragment]); all sequences from specimen UADBA-FG/MV 2000.379 (Sambava, Madagascar).

*Boophis vittatus*: AY341802\* (rhodopsin), AY341616\* (12S and tRNA<sub>Val</sub>), AY341673\* (16S [5' fragment]), AY341719\* (16S [3' fragment]); all sequences from specimen UADBA-FG/MV 2000.82 (Tsaratanana, Madagascar).

*Boophis viridis*: AY341805\* (rhodopsin), AY341619\* (12S and tRNA<sub>Val</sub>), AY341676\* (16S [5' fragment]), AJ314818 (16S [3' fragment]); all sequences from specimen ZSM 338/2000 (Andasibe, Madagascar).

*Boophis xerophilus*: AY341794\* (rhodopsin), AF249008 (12S and tRNA<sub>Val</sub>), AY341665\* (16S [5' fragment]), AF215335 (16S [3' fragment]); all sequences from specimen ZFMK 66705 (Kirindy, Madagascar).

*Laliostoma labrosum*: AF249096 (cytochrome *b*), AF249106 (rhodopsin), AF249169 (tyrosinase), AF249010 (12S and tRNA<sub>Val</sub>), AY341679\* (UADBA-MV 2001.289, Ankarafantsika, Madagascar) (16S [5' fragment]), AF249037 (16S [3' fragment]).

*Mantella laevigata*: AY263277 (rhodopsin), AY341607\* (12S and tRNA<sub>Val</sub>), AJ438538 (16S [5' fragment]), AF215279 (16S [3' fragment]).

*Mantella madagascariensis*: AF249076 (cytochrome *b*), AF249101 (rhodopsin), AF249164 (tyrosinase), AF249005 (12S and tRNA<sub>Val</sub>), AJ438892 (16S [5' fragment]), AF249049 (16S [3' fragment]).

*Mantidactylus* new species (Comoros): AY341731\* (cytochrome *b*), AY323742\* (rhodopsin), AY341750\* (tyrosinase), AY341585\* (12S and tRNA<sub>Val</sub>), AY341639\* (16S [5' fragment]), AY330888\* (16S [3' fragment]); all sequences from specimen ZSM 652/2000 (Mont Combani, Mayotte).

*Mantidactylus albolineatus*: AY341766\* (rhodopsin), AY341580\* (12S and tRNA<sub>Val</sub>), AY341635\* (16S [5' fragment]), AY341701\* (16S [3' fragment]); all sequences from specimen ZSM 250/2002 (Andasibe, Madagascar).

*Mantidactylus ambreensis*: AY341788\* (rhodopsin), AY341603\* (12S and tRNA<sub>Val</sub>), AY341659\* (16S [5' fragment]), AY324822\* (ZSM 492/2000, Montagne d'Ambre) (16S [3' fragment]); all other sequences from specimen ZSM 634/2001 (Tsaratanana, Madagascar).

*Mantidactylus asper*: AY341783\* (rhodopsin), AY341598\* (12S and tRNA<sub>Val</sub>), AY341653\* (16S [5' fragment]), AJ314802 (16S [3' fragment]); all sequences from specimen UADBA-FG/MV 2000.17 (Mandraka, Madagascar).

*Mantidactylus biporus*: AY341784\* (rhodopsin), AY341599\* (12S and tRNA<sub>Val</sub>), AY341655\* (16S [5' fragment]), AF215322 (ZFMK 70481, Masoala) (16S [3' fragment]); all other sequences from specimen ZSM 122/2002 (Andranofotsy, Madagascar).

*Mantidactylus blommersae*: AY341770\* (rhodopsin), AY341584\* (12S and tRNA<sub>Val</sub>), AY341638\* (16S [5' fragment]), AF317688 (16S [3' fragment]); all sequences from specimen UADBA-FG/MV 2000.65 (Andasibe, Madagascar).

*Mantidactylus charlotteae*: AY341790\* (rhodopsin), AY341605\* (12S and tRNA<sub>Val</sub>), AY341661\* (16S [5' fragment]), AY341713\* (16S [3' fragment]); all sequences from specimen ZSM 127/2002 (Andranofotsy, Madagascar).

*Mantidactylus depressiceps*: AY341775\* (rhodopsin), AY341590\* (12S and tRNA<sub>Val</sub>), AY341645\* (16S [5' fragment]), AF215326 (ZFMK 60131, Andasibe) (16S [3' fragment]); all other sequences from specimen ZSM 688/2001 (Andasibe, Madagascar).

*Mantidactylus domerguei*: AY341768\* (rhodopsin), AY341582\* (12S and tRNA<sub>Val</sub>), AY341636\* (16S [5' fragment]), AF317689 (16S [3' fragment]); all sequences from specimen ZSM 353/2000 (Mantaoa, Madagascar).

*Mantidactylus massorum*: AY341776\* (rhodopsin), AY341591\* (12S and tRNA<sub>Val</sub>), AY341646\* (16S [5' fragment]), AY341705\* (16S [3' fragment]); all sequences from specimen MRSN-FAZC 6737 (Ambolokopatrika, Madagascar).

*Mantidactylus grandidieri*: AY341789\* (rhodopsin), AY341604\* (12S and tRNA<sub>Val</sub>), AY341660\* (16S [5' fragment]), AY341712\* (16S [3' fragment]); all sequences from specimen UADBA-MV 2001.1201 (Andasibe, Madagascar).

*Mantidactylus grandisonae*: AY341771\* (rhodopsin), AY341640\* (16S [5' fragment]), AF215315 (16S [3' fragment]); all sequences from specimen ZFMK 66669 (Ambato, Madagascar).

*Mantidactylus granulatus*: AY341779\* (rhodopsin), AY341594\* (12S and tRNA<sub>Val</sub>), AY341649\* (16S [5' fragment]), AJ314794 (ZSM 645/2001, Tsaratanana) (16S [3' fragment]); all other sequences from specimen MRSN-FAZC 8011 (Nosy Be, Madagascar).

*Mantidactylus horridus*: AY341781\* (rhodopsin), AY341596\* (12S and tRNA<sub>Val</sub>), AY341651\* (16S [5' fragment]), AY341708\* (16S [3' fragment]); all sequences from specimen UADBA 10002 (Tsaratanana, Madagascar).

*Mantidactylus kely*: AY341769\* (rhodopsin), AY341583\* (12S and tRNA<sub>Val</sub>), AY341637\* (16S [5' fragment]), AF317690 (16S [3' fragment]); all sequences from specimen ZSM 363/2000 (Ambatolampy, Madagascar).

*Mantidactylus liber*: AY341774\* (rhodopsin), AY341589\* (12S and tRNA<sub>Val</sub>), AY341644\* (16S [5' fragment]), AJ314801 (16S [3' fragment]); all sequences from specimen ZSM 491/2000 (Montagne d'Ambre, Madagascar).

*Mantidactylus lugubris*: AY341785\* (rhodopsin), AY341600\* (12S and tRNA<sub>Val</sub>), AY341656\* (16S [5' fragment]), AY341710\* (16S [3' fragment]); all sequences from specimen ZSM 166/2002 (Mantady, Madagascar).

*Mantidactylus* new species (aff. *lugubris*): AY341786\* (rhodopsin), AY341601\* (12S and tRNA<sub>Val</sub>), AY341657\* (16S [5' fragment]), AY341711\* (16S [3' fragment]); all sequences from specimen ZSM 171/2002 (Mantady, Madagascar).

*Mantidactylus madinika*: AY341772\* (rhodopsin), AY341587\* (12S and tRNA<sub>Val</sub>), AY341642\* (16S [5' fragment]), AY341703\* (16S [3' fragment]); all sequences from one paratype specimen from Antsirrasira, Madagascar.

*Mantidactylus femoralis*: AY341787\* (rhodopsin), AY341602\* (12S and tRNA<sub>Val</sub>), AY341658\* (16S [5' fragment]), AY324812\* (16S [3' fragment]); all sequences from specimen UADBA-MV 2001.1277 (Andasibe, Madagascar).

*Mantidactylus opiparis*: AY341791\* (rhodopsin), AY341606\* (12S and tRNA<sub>Val</sub>), AY341662\* (16S [5' fragment]), AY341714\* (16S [3' fragment]); all sequences from specimen UADBA-MV 2001.1069 (Marolambo region, Madagascar).

*Mantidactylus peraccae*: AY341777\* (rhodopsin), AY341592\* (12S and tRNA<sub>Val</sub>), AY341647\* (16S [5' fragment]), AY341706\* (16S [3' fragment]); all sequences from specimen MRSN-RJS 109 (Tsaratanana, Madagascar).

*Mantidactylus redimitus*: AY341778\* (rhodopsin), AY341593\* (12S and tRNA<sub>Val</sub>), AY341648\* (16S [5' fragment]), AY341707\* (16S [3' fragment]); all sequences from specimen ZSM 152/2002 (Vohidrazana, Madagascar).

*Mantidactylus sculpturatus*: AY341782\* (rhodopsin), AY341597\* (12S and tRNA<sub>Val</sub>), AY341652\* (16S [5' fragment]), AY341709\* (16S [3' fragment]); all sequences from specimen ZSM 95/2002 (Mantady, Madagascar).

*Mantidactylus striatus*: AY341780\* (rhodopsin), AY341595\* (12S and tRNA<sub>Val</sub>), AY341650\* (16S [5' fragment]), AJ314796\* (16S [3' fragment]).

*Mantidactylus* aff. *ulcerosus*: AF249102\* (rhodopsin), AF249006\* (12S and tRNA<sub>Val</sub>), AY341654\* (16S [5' fragment]), AF215319 (16S [3' fragment]); all sequences from specimen ZFMK 66659 (Ambato, Madagascar).

*Mantidactylus wittei*: AY341732\* (cytochrome *b*), AY323743\* (rhodopsin), AY341751\* (tyrosinase), AY341586\* (12S and tRNA<sub>Val</sub>), AY341641\* (16S [5' fragment]), AF317691 (UADBA-FG/MV 2000.123, Ambanja) (16S [3' fragment]); all other sequences from specimen ZSM 405/2000 (Benavony, Madagascar).

## ***Ranidae***

*Hoplobatrachus crassus*: AF249090 (cytochrome *b*), AF249109 (rhodopsin), AF249172 (tyrosinase), AF249013 (12S and tRNA<sub>Val</sub>), AY341688\* (16S [5' fragment]), AY014375 (16S [3' fragment]).

*Hoplobatrachus occipitalis*: AJ564733\* (cytochrome *b*), AJ564730\* (rhodopsin), AJ564729\* (tyrosinase), AJ564734\* (12S and tRNA<sub>Val</sub>), AY341689\* (16S [5' fragment]), AY014373 (16S [3' fragment]); all sequences from specimen ZFMK-WB 02 (Mauritania).

*Indirana* cf. *leptodactyla*: AF215392 (16S [5' fragment]), AY341686\* (16S [3' fragment]); sequences from specimen ZFMK uncatalogued (Ooty, India).

*Indirana* sp. (aff. *leptodactyla*): AF249080 (cytochrome *b*), AF249123 (rhodopsin), AF249186 (tyrosinase), AF249027 (12S and tRNA<sub>Val</sub>).

*Nyctibatrachus major*: AF249084 (cytochrome *b*), AF249113 (rhodopsin), AF249176 (tyrosinase), AF249017 (12S and tRNA<sub>Val</sub>), AY341687\* (16S [5' fragment]), AF215397 (16S [3' fragment]).

*Petropedetes parkeri*: AY341813\* (rhodopsin), AY341757\* (tyrosinase), AY341628\* (12S and tRNA<sub>Val</sub>), AY341694\* (16S [5' fragment]), AY341724\* (16S [3' fragment]); all sequences from a specimen from Nlonako (Cameroon).

*Petropedetes* sp. (aff. *parkeri*): AY341738\* (cytochrome *b*); sequence from a specimen from Cameroon.

*Ptychadena mascareniensis*: AY341734\* (cytochrome *b*), AY341809\* (rhodopsin), AY341753\* (tyrosinase), AY341624\* (12S and tRNA<sub>Val</sub>), AY341690\* (16S [5' fragment]); all sequences from specimen ZSM 258/2002 (Nahampoana, Madagascar).

*Rana temporalis*: AF249083 (cytochrome *b*), AF249118 (rhodopsin), AF249181 (tyrosinase), AF249022 (12S and tRNA<sub>Val</sub>), AY341683\* (16S [5' fragment]), AF215390 (16S [3' fragment]).

*Rana temporaria*: AF249078 (cytochrome *b*), AF249119 (rhodopsin), AF249182 (tyrosinase), AF249023 (12S and tRNA<sub>Val</sub>), AY341684\* (16S [5' fragment]), AF249048 (16S [3' fragment]).

*Rana cretensis*: AY148010 (rhodopsin).

*Rana cerigensis*: AY148009 (rhodopsin).

### ***Rhacophoridae***

*Polypedates cruciger*: AF249089 (cytochrome *b*), AF249124 (rhodopsin), AF249187 (tyrosinase), AF249028 (12S and tRNA<sub>Val</sub>), AY341685\* (16S [5' fragment]), AF215357 (16S [3' fragment]).

### ***Astylosternidae***

*Astylosternus diadematus*: AY341735\* (cytochrome *b*), AY341810\* (rhodopsin), AY341754\* (tyrosinase), AY341625\* (12S and tRNA<sub>Val</sub>), AY341691\* (16S [5' fragment]), AY341723\* (16S [3' fragment]); all sequences from a specimen from Nlonako (Cameroon).

### ***Arthroleptidae***

*Arthroleptis variabilis*: AY341737\* (cytochrome *b*), AY341812\* (rhodopsin), AY341756\* (tyrosinase), AY341627\* (12S and tRNA<sub>Val</sub>), AY341693\* (16S [5' fragment]), AF124107 (ZFMK 68794, Cameroon) (16S [3' fragment]); all other sequences from a specimen from Nlonako (Cameroon).

### ***Hyperoliidae***

*Leptopelis natalensis*: AY341736\* (cytochrome *b*), AY341811\* (rhodopsin), AY341755\* (tyrosinase), AY341626\* (12S and tRNA<sub>Val</sub>), AY341692\* (16S [5' fragment]), AF215448 (16S [3' fragment]); all sequences from specimen ZFMK 68785 (Mtunzini, South Africa).

*Hyperolius* cf. *viridiflavus*: AF249066 (cytochrome *b*), AF249098 (rhodopsin), AF249161 (tyrosinase), AF249002 (12S and tRNA<sub>Val</sub>).

*Hyperolius viridiflavus*: AY341695\* (16S [5' fragment]), AF215440 (16S [3' fragment]); sequences from specimen ZFMK 66726 (Barberton, South Africa).

*Tachycnemis seychellensis*: AY341739\* (cytochrome *b*), AY341814\* (rhodopsin), AY341758\* (tyrosinase), AY341629\* (12S and tRNA<sub>Val</sub>), AY341696\* (16S [5' fragment]), AF215451 (16S [3' fragment]).

*Heterixalus tricolor*: AY341740\* (cytochrome *b*), AY323741\* (rhodopsin), AY341759\* (tyrosinase), AY341630\* (12S and tRNA<sub>Val</sub>), AY341697\* (16S [5' fragment]), AY341725\* (16S [3' fragment]); all sequences from specimen ZSM 700/2001 (Ankarafantsika, Madagascar).

### ***Bufonidae***

*Bufo melanostictus*: AF249082 (cytochrome *b*), AF249097 (rhodopsin), AF249001 (12S and tRNA<sub>Val</sub>), AF249061 (16S [3' fragment]).

*Bufo asper*: AY263257 (16S [5' fragment]).

### ***Leptodactylidae***

*Leptodactylus fuscus*: AY341741\* (cytochrome *b*), AY323746\* (rhodopsin), AY341760\* (tyrosinase), AY341631\* (12S and tRNA<sub>Val</sub>), AY263262\* (16S [5' fragment]), AY263226\* (16S [3' fragment]); all sequences from specimen ULABG 4591 (Canaima, Venezuela).

### ***Sooglossidae***

*Nesomantis thomasseti*: AY341742\* (cytochrome *b*), AY323744\* (rhodopsin), AY341761\* (tyrosinase), AY341632\* (12S and tRNA<sub>Val</sub>), AY341698\* (16S [5' fragment]), AY330889\* (16S [3' fragment]).

### ***Pipidae***

*Pipa parva*: AY341743\* (cytochrome *b*), AY323734\* (rhodopsin), AY341762\* (tyrosinase), AY341633\* (12S and tRNA<sub>Val</sub>), AY341699\* (16S [5' fragment]), AY333690\* (16S [3' fragment]).

*Xenopus laevis*: M10217 (cytochrome *b*), S62229 (rhodopsin), AY341764\* (tyrosinase), M10217 (12S and tRNA<sub>Val</sub>), M10217 (16S [5' fragment]), AY341727\* (16S [3' fragment]).

*Hymenochirus boettgeri*: AY341744\* (cytochrome *b*), AY323735\* (rhodopsin), AY341763\* (tyrosinase), AY341634\* (12S and tRNA<sub>Val</sub>), AY341700\* (16S [5' fragment]), AY341726\* (16S [3' fragment]).

### ***Pelobatidae***

*Pelobates cultripes*: AY323736\* (rhodopsin).

*Pelobates varaldi*: AY341815\* (rhodopsin).

### ***Discoglossidae***

*Alytes muletensis*: AY341728\* (cytochrome *b*), AY323731\* (rhodopsin), AY341747\* (tyrosinase), AY341621\* (12S and tRNA<sub>Val</sub>), AY341680\* (16S [5' fragment]), AF224729 (16S [3' fragment]); all sequences from a specimen preserved in the ZFMK (Mallorca, Spain).

*Alytes maurus*: AY341816\* (rhodopsin).

*Alytes dickhillenii*: AY341817\* (rhodopsin).

### ***Urodela***

*Ambystoma mexicanum*: AY341745\* (cytochrome *b*), U36574 (rhodopsin), Y10947 (12S and tRNA<sub>Val</sub>), Y10947 (16S [5' fragment]), Y10947 (16S [3' fragment]).

*Hynobius kimurae*: AY341746 (cytochrome *b*), AY341765\* (tyrosinase).

### Appendix C. Substitution models used in Maximum Likelihood Phylogenetic analysis

We tested the goodness-of-fit of nested substitution models for homogeneous data partitions of ingroup taxa by a hierarchical likelihood ratio test (HLRT). Modeltest (4) version 3.06 was used to calculate the test statistic  $\delta = 2 \log \Lambda$  with  $\Lambda$  being the ratio of the likelihood of the null model divided by the likelihood of the alternative model (5). The best fitting model was used for Maximum Likelihood phylogenetic analyses using PAUP\* (6).

Before applying the HLRT and any phylogenetic analysis, we excluded all gapped and hypervariable regions of the rRNA and tRNA genes. Rather than trying to fit these regions (which mainly correspond to loops in the tertiary structure of the rRNA molecules) into secondary structure models (7), we preferred to exclude all sections in which homology was not immediately obvious, as well as all gapped positions. Additionally, we excluded the third positions of cytochrome *b* which are known to be fully saturated at the level of anuran families (8).

The following substitution models were selected by the hierarchical likelihood ratio test (HLRT) as implemented in Modeltest:

For the among-Mantellidae dataset of the concatenated rhodopsin, 12S rRNA, 16SrRNA and tRNA<sub>Val</sub> sequences, a GTR+I+G substitution model (-lnL = 26025.7305) with empirical base frequencies (freqA = 0.3551; freqC = 0.2199; freqG = 0.1644; freqT = 0.2607) and substitution rates ([A-C] = 3.7878; [A-G] = 10.5790; [A-T] = 5.7768; [C-G] = 1.1797; [C-T] = 29.2325; [G-T] = 1), a proportion of invariable sites of 0.4042 and a gamma distribution shape parameter of 0.6582.

For the higher-level relationship data set of the concatenated rhodopsin, tyrosinase, cytochrome *b*, 12SrRNA, 16S rRNA, and tRNA<sub>Val</sub> sequences, a TrN+I+G substitution model (-lnL = 30463.0156), with empirical base frequencies (freqA = 0.3371; freqC = 0.2527; freqG = 0.1497; freqT = 0.2606) and substitution rates ([A-G] = 3.3874; [C-T] = 5.2170; all other rates = 1), a proportion of invariable sites of 0.2851, and a gamma distribution shape parameter of 0.6848.

For the higher-level relationship data set of nuclear genes only (rhodopsin, tyrosinase), a HKY+I+G substitution model ( $-\ln L = 8573.2109$ ) with empirical base frequencies (freqA = 0.2261; freqC = 0.3242; freqG = 0.2048; freqT = 0.2449), a transition/transversion ratio of 2.1689, a proportion of invariable sites of 0.3788, and a gamma distribution shape parameter of 1.25.

For the higher-level relationship data set of rRNA and tRNA genes only (12S rRNA, 16S rRNA, tRNA<sub>Val</sub>), a GTR+I+G substitution model ( $-\ln L = 17708.6992$ ) with empirical base frequencies (freqA = 0.3763; freqC = 0.2075; freqG = 0.1800; freqT = 0.2362) and substitution rates ([A-C] = 3.8266; [A-G] = 7.5824; [A-T] = 5.2028; [C-G] = 0.6641; [C-T] = 24.8576; [G-T] = 1), a proportion of invariable sites of 0.1657, and a gamma distribution shape parameter of 0.5400.

## **Appendix D. Details of Bayesian phylogenetic analysis**

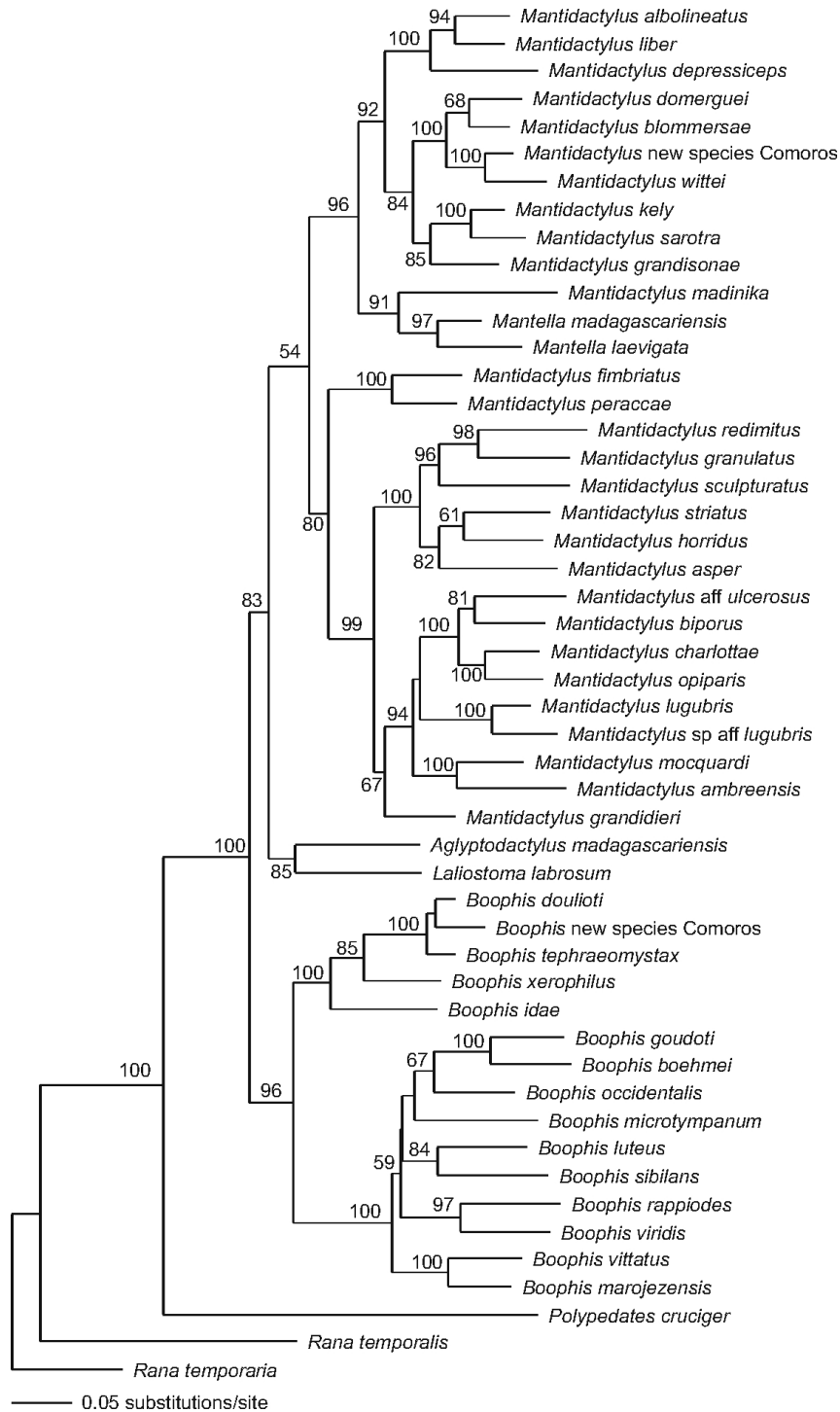
Bayesian phylogenetic analyses were performed using the program MrBayes, version 2.01 (9). The analysis consisted of Maximum Likelihood (ML) comparisons of trees in which the tree topology and ML parameters were permuted using a Markov chain Monte Carlo method with Metropolis-coupling (MCMCMC) (9), and sampled periodically according to the Metropolis-Hastings algorithm. Because Modeltest (4) suggested complex substitution models (GTR+I+G; see previous section), we set the ML parameters in MrBayes as follows: "lset nst = 6" (the GTR model), "rates = invgamma" (site-specific rate variation, with some invariant sites and other sites with a gamma distribution), and "basefreq = estimate" (proportion of different base categories estimated from the data).

All our analyses employed one cold chain and three incrementally heated chains. We run 500,000 generations in the higher-level phylogeny (Fig. 5) and 300,000 generations in the mantellid phylogeny (fig. 4). Trees were sampled every ten generations. The initial set of generations needed before convergence on stable likelihood values (burnin) was set at 6-7% based on own empirical evaluation.

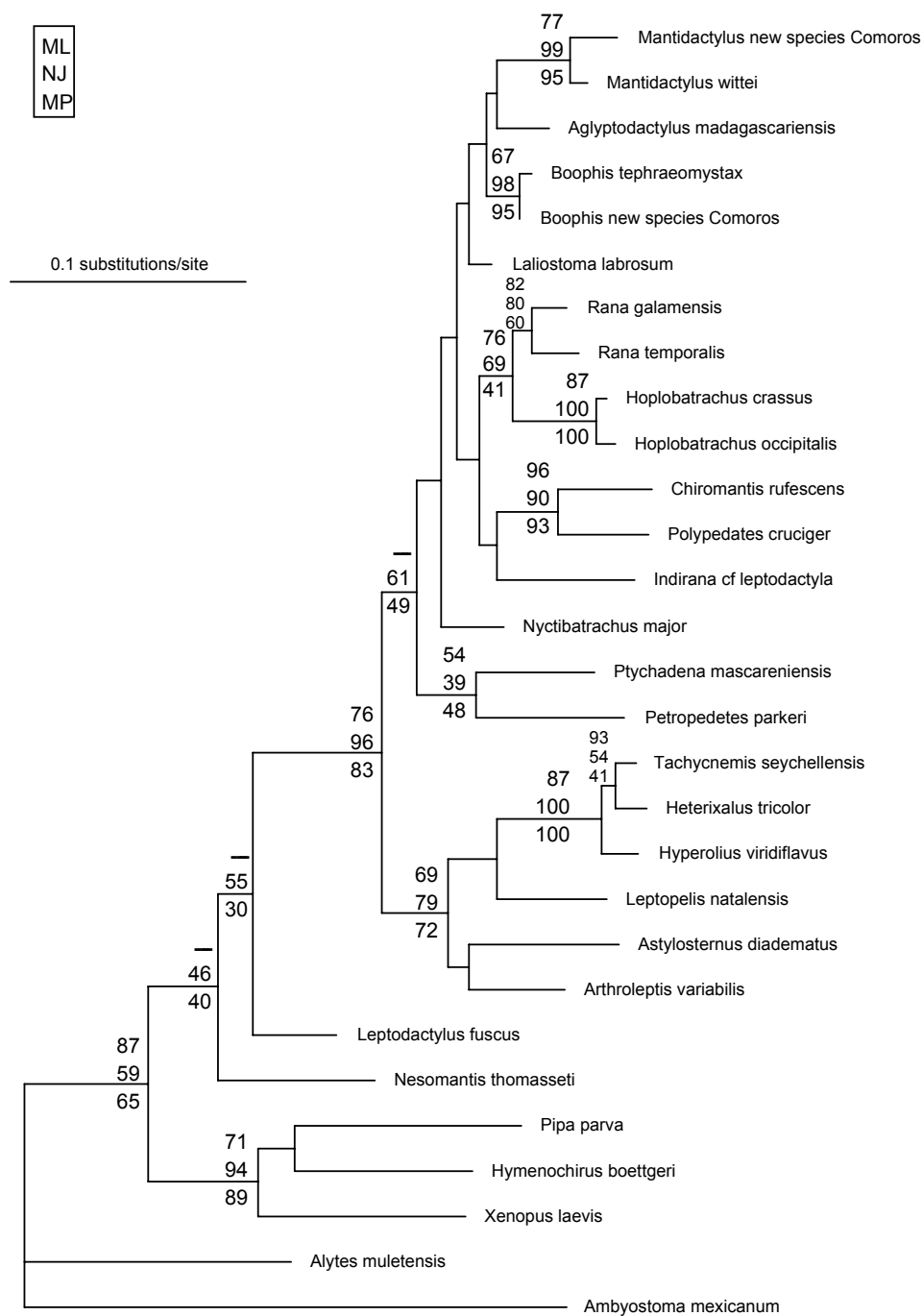
## Appendix E. Separate analysis of gene fragments

The following figures show the results of additional analyses of the two data sets. For the Mantellidae data set, we present the maximum likelihood (ML) phylogram with maximum parsimony (MP) bootstrap values. For the higher-level relationship data set, we show (a) a ML analysis of nuclear genes (rhodopsin and tyrosinase) at the amino acid level, (b) a ML analysis of nuclear genes only at the amino acid level, but using *Alytes muletensis* (Discoglossidae) as outgroup instead of a salamander, (c) a ML analysis of nuclear genes only at the nucleotide level, and (d) a ML analysis based on ribosomal genes only (12SrRNA, 16SrRNA, tRNA<sub>Val</sub>). Bootstrap values are shown for ML, MP and Neighbor-joining (NJ).

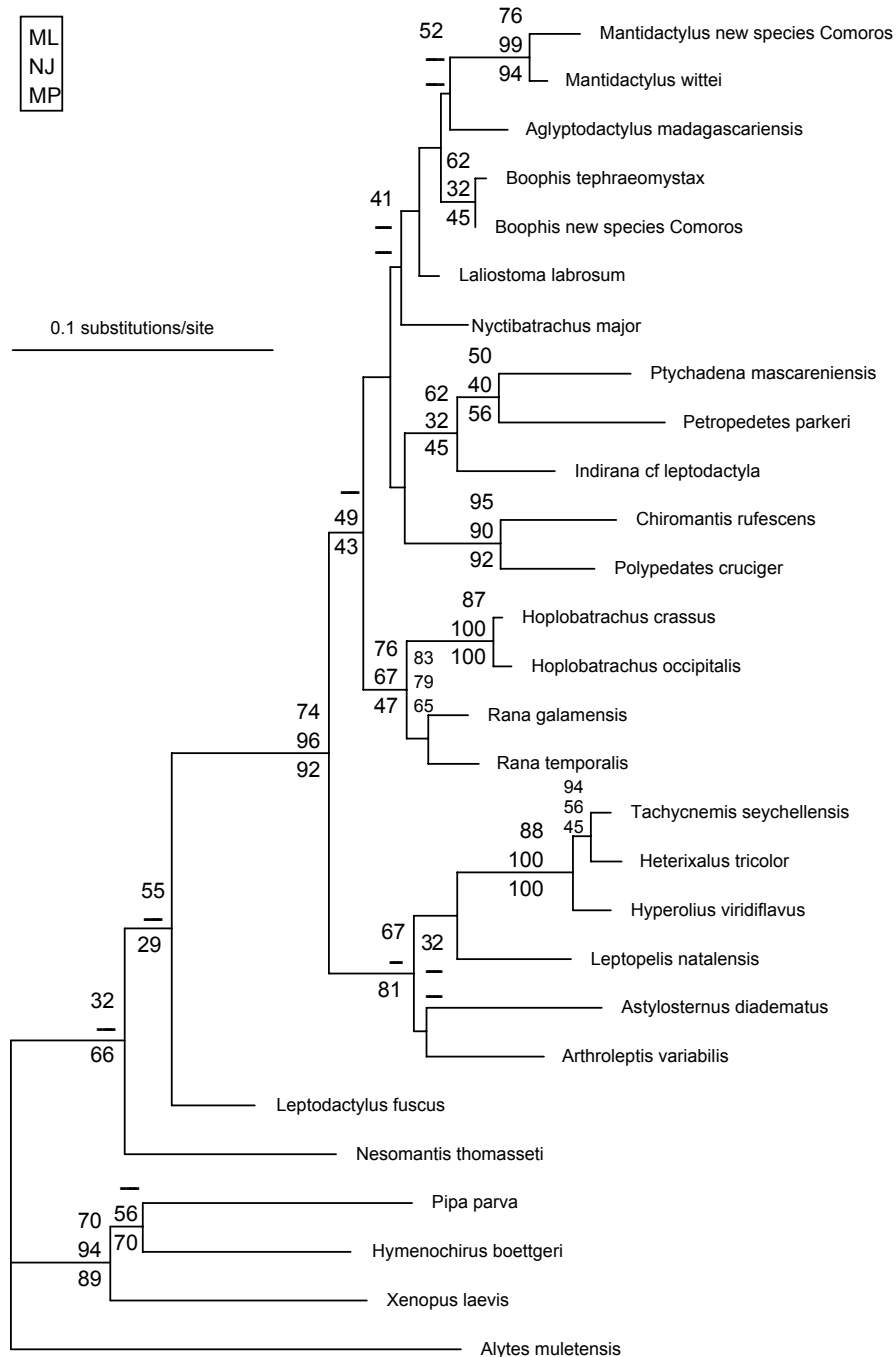
**Fig. 4.** ML phylogram showing relationships among the Mantellidae, obtained by heuristic searches in PAUP\* using settings suggested by Modeltest. The numbers are bootstrap values in percent under MP (2000 replicates).



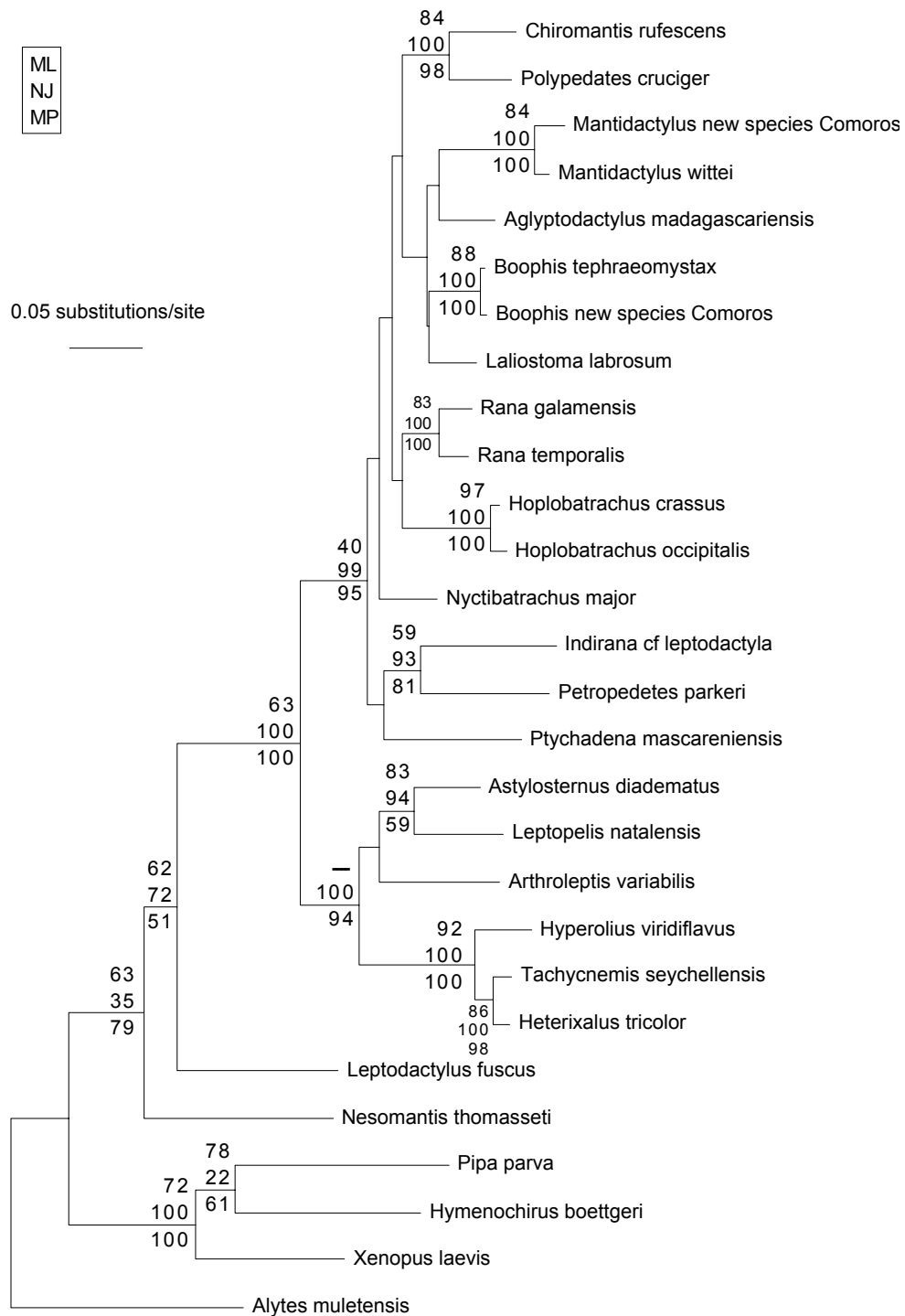
**Fig. 5.** ML phylogram showing higher-level relationships among Gondwanan frogs. Based on amino acid sequences of nuclear genes (rhodopsin and tyrosinase; VT substitution model (10)). The numbers are quartet puzzling values in percent under ML (10,000 puzzling steps, obtained using Tree-Puzzle (11)), bootstrap values under MP (2000 replicates; obtained using PAUP\*, with 10 random addition sequence replicates) and NJ (obtained using Mega (12), under minimum evolution criterion using an empirically determined gamma distribution shape parameter with eight rate categories).



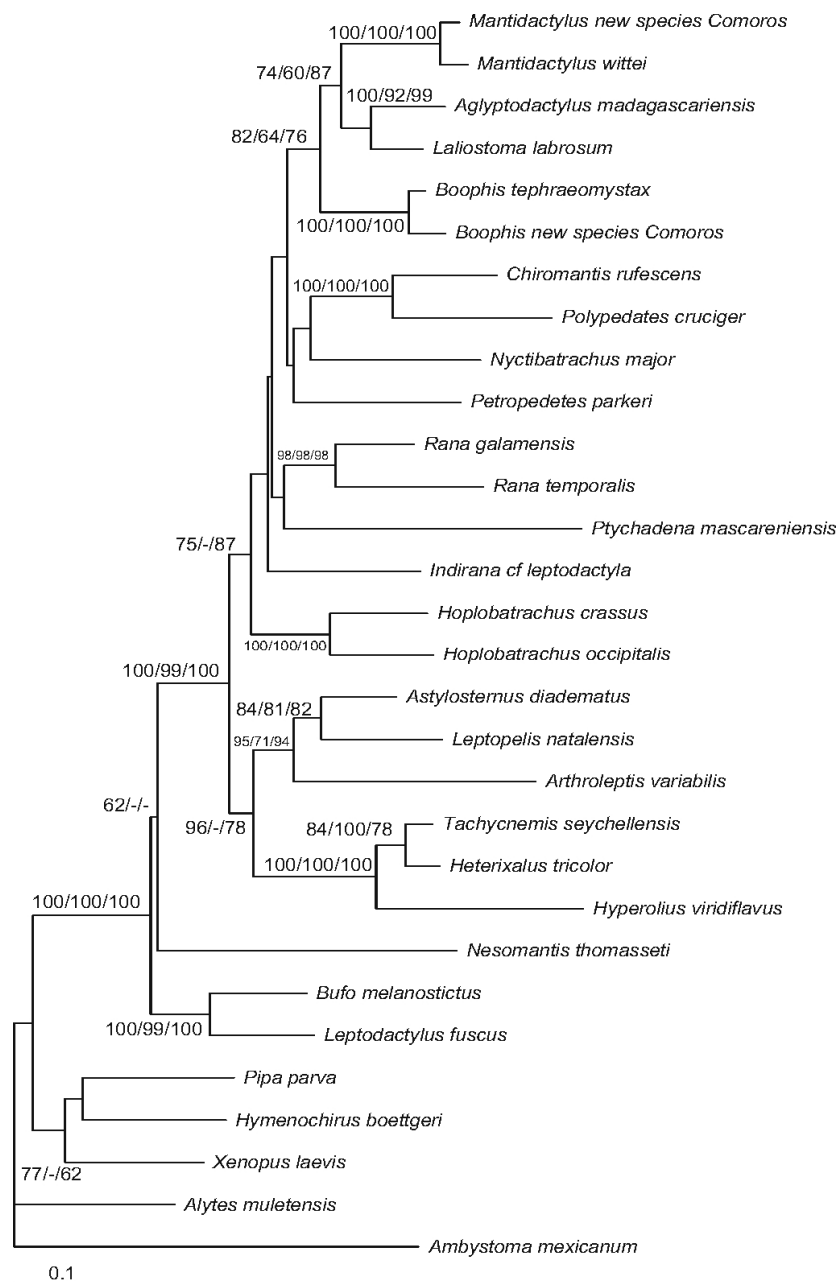
**Fig. 6.** ML phylogram showing higher-level relationships among Gondwanan frogs. The numbers are bootstrap values in percent under ML, MP, and NJ. Based on amino acid sequences of nuclear genes (rhodopsin and tyrosinase). Because of the high divergence of the salamander sequences, this separate tree was calculated using the basal discoglossid frog *Alytes muletensis* as outgroup. See legend of Fig. 5 for further details of analysis.



**Fig. 7.** ML phylogram showing higher-level relationships among Gondwanan frogs. The numbers are bootstrap values in percent under ML, MP and NJ. Based on nucleotide sequences of nuclear genes (rhodopsin and tyrosinase). See legend of Fig. 5 for further details of analysis.



**Fig. 8.** ML phylogram showing higher-level relationships among Gondwanan frogs. Obtained by heuristic searches in PAUP\* with TBR branch-swapping and 10 random sequence addition replicates, under the substitution model proposed by Modeltest. The numbers are bootstrap values in percent under ML (100 replicates), Maximum Parsimony (2000 replicates), and Neighbor-joining (2000 replicates). Based on nucleotide sequences of ribosomal genes (12SrRNA, 16SrRNA, tRNA<sub>Val</sub>) only. Although the ML tree did not place *Nesomantis* (Sooglossidae) as sister group of all Neobatrachia, such a topology was suggested by the MP and NJ bootstrap consensus trees (66% and 63% support), in agreement with the trees based on nuclear genes (see Figs. 5-7).



## Appendix F. Testing alternative topologies with Shimodaira-Hasegawa tests

Non-parametric likelihood ratio tests (Shimodaira-Hasegawa tests - SH tests) (13) as implemented in PAUP\* (6), were used to test alternative topologies (chosen *a priori*) in the phylogenetic trees obtained by Maximum Likelihood searches. This is the only statistically valid method currently available for multiple topology testing (14). We run SH tests with 2000 bootstrap replicates and full optimization settings. The following tables show the topologies tested, their likelihood scores, the difference in likelihood score as compared to the best tree, and the significance of this difference.

In the mantellid phylogeny, we tested alternative positions of the two Comoroan species, keeping the overall topology unchanged. The results, summarized in Table 1, show that the general position of these taxa is statistically significant. The only topologies that could not be excluded were (a) permutations of the position of *Mantidactylus* sp. (Comoros) relative to *M. wittei*, *M. domerguei* and *M. blommersae*, and (b) of *Boophis* sp. (Comoros) relative to *B. tephraeomystax*, *B. doulioti* and *B. xerophilus*. Considering any of these species other than *M. wittei* and *B. tephraeomystax* as sister taxa of the Comoroan species would result in higher genetic divergences of the Comoroan taxa, thus reinforcing the molecular clock calculations performed by suggesting even younger origins of the lineages studied. In contrast, hypotheses placing the Comoroan taxa as sister groups to each other, or as basal to their respective lineages (subgenera or species groups) could be significantly rejected. This provides evidence that the Comoroan taxa are deeply nested within their clades, and strongly confirms their double independent origin. Although the Comoroan *Mantidactylus* had previously been included in *Mantidactylus granulatus*, a relationship to this species or its relatives (*M. redimitus*, *M. sculpturatus*) could also be excluded with high significance.

In the taxa set used to assess higher-level relationships, the SH-tests significantly excluded relationships between the two Seychellean taxa *Tachycnemis* and *Nesomantis*, confirming the independent origin of the two Seychellean frog lineages. In contrast, the arrangement of the different mantellid subfamilies was not sufficiently clarified, and also several alternative positions of *Nesomantis* could not be significantly excluded, indicating that our data set is insufficient for resolving satisfyingly the deep splits among the main frog lineages.

**Table 1.** Results of SH-tests in the Mantellidae data set. *M. sp.* and *B. sp.* refer to the *Mantidactylus* and *Boophis* species from Mayotte (Comoros). Significances of  $P < 0.05$  are marked with an asterisk.

Tree	Description of ree permutation	Likelihood (-lnL)	Diff (-lnL)	Significance
A0	Best ML tree	26013.35215	---	---
A1	<i>M. sp.</i> sister to <i>M. domerguei</i> / <i>M. blommersae</i>	26042.78229	29.43014	0.5185
A2	<i>M. sp.</i> sister to <i>M. wittei</i> / <i>M. domerguei</i> / <i>M. blommersae</i>	26042.78229	29.43014	0.5185
A3	<i>M. sp.</i> sister to <i>M. domerguei</i>	26074.46334	61.11119	0.0730
A4	<i>M. sp.</i> sister to <i>M. blommersae</i>	26074.84296	61.49082	0.0695
A5	<i>M. sp.</i> sister to <i>M. grandisonae</i> / <i>M. kely</i> / <i>M. sarotra</i>	26090.12128	76.76914	0.0160*
A6	<i>M. sp.</i> sister to <i>M. grandisonae</i>	26108.24787	94.89572	0.0045*
A7	<i>M. sp.</i> sister to <i>M. kely</i> / <i>M. sarotra</i>	26108.24787	94.89572	0.0045*
A8	<i>M. sp.</i> sister to <i>M. kely</i>	26187.82919	174.47704	0.0000*
A9	<i>M. sp.</i> sister to <i>M. sarotra</i>	26187.82919	174.47704	0.0000*
A10	<i>M. sp.</i> sister to all <i>Blommersia</i>	26090.12128	76.76914	0.0160*
A11	<i>M. sp.</i> sister to <i>M. madinika</i> / <i>Mantella</i> spp.	26164.94468	151.59254	0.0000*
A12	<i>M. sp.</i> sister to <i>M. madinika</i>	26205.35170	191.99955	0.0000*
A13	<i>M. sp.</i> sister to species of <i>Guibemantis</i> / <i>Pandanusicola</i>	26133.00009	119.64794	0.0005*
A14	<i>M. sp.</i> sister to species of <i>Guibemantis</i> / <i>Pandanusicola</i>	26188.96063	175.60848	0.0000*
A15	<i>M. sp.</i> sister to species of <i>Guibemantis</i> / <i>Pandanusicola</i>	26188.96063	175.60848	0.0000*
A16	<i>M. sp.</i> sister to <i>M. granulatus</i>	26381.78107	368.42892	0.0000*
A17	<i>M. sp.</i> sister to <i>M. redimitus</i>	26381.78107	368.42892	0.0000*
A18	<i>M. sp.</i> sister to <i>M. granulatus</i> / <i>M. redimitus</i>	26346.10407	332.75192	0.0000*
A19	<i>M. sp.</i> sister to <i>M. sculpturatus</i> / <i>M. granulatus</i> / <i>M. redimitus</i>	26338.20727	324.85512	0.0000*
A20	<i>B. sp.</i> sister to <i>M. sp.</i>	26465.21993	451.86778	0.0000*
A21	<i>M. sp.</i> sister to <i>B. sp.</i>	26443.05343	429.70129	0.0000*
A22	<i>B. sp.</i> sister to <i>B. tephraeomystax</i> / <i>B. doulioti</i>	26014.00790	0.65576	0.9875
A23	<i>B. sp.</i> sister to <i>B. tephraeomystax</i>	26016.30986	2.95772	0.9785
A24	<i>B. sp.</i> sister to <i>B. xerophilus</i>	26068.06433	54.71218	0.1380
A25	<i>B. sp.</i> sister to <i>B. doulioti</i> / <i>B. tephraeomystax</i> / <i>B. xerophilus</i>	26069.01388	55.66174	0.1245
A26	<i>B. sp.</i> sister to <i>B. idae</i>	26117.88218	104.53003	0.0035*
A27	<i>B. sp.</i> sister to all pond breeding <i>Boophis</i>	26117.88218	104.53003	0.0035*
A28	<i>B. sp.</i> sister to all <i>Boophis</i>	26173.48306	160.13091	0.0000*
A29	<i>B. sp.</i> sister to all brook breeding <i>Boophis</i>	26173.48306	160.13091	0.0000*

**Table 2.** Results of SH-tests in the higher-level relationship data set. Significances of  $P < 0.05$  are marked with an asterisk.

Tree	Description of ree permutation	Likelihood (-lnL)	Diff (-lnL)	Significance
B0	Best ML tree	30444.99194	---	---
B1	Different position of <i>Nesomantis</i>	30447.52743	2.53548	0.940
B2	Different position of <i>Nesomantis</i>	30508.07683	63.08489	0.085
B3	Different position of <i>Nesomantis</i>	30510.36233	65.37039	0.077
B4	Different position of <i>Nesomantis</i>	30516.91704	71.92510	0.060
B5	<i>Nesomantis</i> sister to Ranoidea	30449.36063	4.36869	0.935
B6	<i>Nesomantis</i> sister to Ranidei	30509.67657	64.68463	0.044*
B7	<i>Nesomantis</i> sister to Arthroleptoidei	30508.79564	63.80370	0.050
B8	<i>Nesomantis</i> sister to <i>Tachycnemis</i>	30644.78662	199.79468	0.000*
B9	<i>Tachycnemis</i> sister to <i>Nesomantis</i>	31004.34740	559.35546	0.000*
B10	Rhacophoridae sister of Mantellidae	30451.63068	6.63874	0.847
B11	Rhacophoridae sister of Mantellidae	30450.20593	5.21398	0.859
B12	Different arrangement of mantellid subfamilies	30448.63226	3.64031	0.942
B13	Different arrangement of mantellid subfamilies	30452.15834	7.16640	0.891
B14	Different arrangement of mantellid subfamilies	30489.00288	44.01094	0.217

**Table 3. (next page).** Topologies of trees included in Shimodaira-Hasegawa non-parametric likelihood ratio tests (dataset: Mantellidae). Taxa: 1, *Polypedates cruciger*; 2, *Mantidactylus depressiceps*; 3, *Mantidactylus albolineatus*; 4, *Mantidactylus liber*; 5, *Mantidactylus domerguei*; 6, *Mantidactylus blommersae*; 7, *Mantidactylus* new species Comoros; 8, *Mantidactylus wittei*; 9, *Mantidactylus grandisonae*; 10, *Mantidactylus kely*; 11, *Mantidactylus sarotra*; 12, *Mantidactylus madinika*; 13, *Mantella madagascariensis*; 14, *Mantella laevigata*; 15, *Mantidactylus fimbriatus*; 16, *Mantidactylus peraccae*; 17, *Mantidactylus sculpturatus*; 18, *Mantidactylus redimitus*; 19, *Mantidactylus granulatus*; 20, *Mantidactylus asper*; 21, *Mantidactylus striatus*; 22, *Mantidactylus horridus*; 23, *Mantidactylus grandidieri*; 24, *Mantidactylus aff ulcerosus*; 25, *Mantidactylus biporus*; 26, *Mantidactylus charlotteae*; 27, *Mantidactylus opiparis*; 28, *Mantidactylus lugubris*; 29, *Mantidactylus* sp aff *lugubris*; 30, *Mantidactylus mocquardi*; 31, *Mantidactylus ambreensis*; 32, *Aglyptodactylus madagascariensis*; 33, *Laliostoma labrosum*; 34, *Boophis idae*; 35, *Boophis xerophilus*; 36, *Boophis tephraeomystax*; 37, *Boophis doulioti*; 38, *Boophis* new species Comoros; 39, *Boophis microtypanum*; 40, *Boophis occidentalis*; 41, *Boophis goudoti*; 42, *Boophis boehmei*; 43, *Boophis luteus*; 44, *Boophis sibilans*; 45, *Boophis rappiodes*; 46, *Boophis viridis*; 47, *Boophis vittatus*; 48, *Boophis marojezensis*; 49, *Rana temporaria*; 50, *Rana temporalis*.



- A22 (1, (((((2, (3,4)), ((5,6), (7,8)), (9, (10,11))), (12, (13,14))), (15,16), ((17, (18,19)), (20, (21,22))), (23, (((24,25), (26,27)), (28,29)), (30,31))))), (32,33)), ((34, (35, (38, (36,37))))), (((39, (40, (41,42))), (43,44)), (45,46)), (47,48))), (49,50))
- A23 (1, (((((2, (3,4)), ((5,6), (7,8)), (9, (10,11))), (12, (13,14))), (15,16), ((17, (18,19)), (20, (21,22))), (23, (((24,25), (26,27)), (28,29)), (30,31))))), (32,33)), ((34, (35, (37, (36,38))))), (((39, (40, (41,42))), (43,44)), (45,46)), (47,48))), (49,50))
- A24 (1, (((((2, (3,4)), ((5,6), (7,8)), (9, (10,11))), (12, (13,14))), (15,16), ((17, (18,19)), (20, (21,22))), (23, (((24,25), (26,27)), (28,29)), (30,31))))), (32,33)), ((34, (35,38), (36,37))), (((39, (40, (41,42))), (43,44)), (45,46)), (47,48))), (49,50))
- A25 (1, (((((2, (3,4)), ((5,6), (7,8)), (9, (10,11))), (12, (13,14))), (15,16), ((17, (18,19)), (20, (21,22))), (23, (((24,25), (26,27)), (28,29)), (30,31))))), (32,33)), ((34, (38, (35, (36,37))))), (((39, (40, (41,42))), (43,44)), (45,46)), (47,48))), (49,50))
- A26 (1, (((((2, (3,4)), ((5,6), (7,8)), (9, (10,11))), (12, (13,14))), (15,16), ((17, (18,19)), (20, (21,22))), (23, (((24,25), (26,27)), (28,29)), (30,31))))), (32,33)), ((34,38), (35, (36,37))), (((39, (40, (41,42))), (43,44)), (45,46)), (47,48))), (49,50))
- A27 (1, (((((2, (3,4)), ((5,6), (7,8)), (9, (10,11))), (12, (13,14))), (15,16), ((17, (18,19)), (20, (21,22))), (23, (((24,25), (26,27)), (28,29)), (30,31))))), (32,33)), ((38, (34, (35, (36,37))))), (((39, (40, (41,42))), (43,44)), (45,46)), (47,48))), (49,50))
- A28 (1, (((((2, (3,4)), ((5,6), (7,8)), (9, (10,11))), (12, (13,14))), (15,16), ((17, (18,19)), (20, (21,22))), (23, (((24,25), (26,27)), (28,29)), (30,31))))), (32,33)), ((38, (34, (35, (36,37))))), (((39, (40, (41,42))), (43,44)), (45,46)), (47,48))), (49,50))
- A29 (1, (((((2, (3,4)), ((5,6), (7,8)), (9, (10,11))), (12, (13,14))), (15,16), ((17, (18,19)), (20, (21,22))), (23, (((24,25), (26,27)), (28,29)), (30,31))))), (32,33)), ((34, (35, (36,37))))), ((38, (((39, (40, (41,42))), (43,44)), (45,46)), (47,48))), (49,50))
-

**Table 4.** Topologies of trees included in Shimodaira-Hasegawa non-parametric likelihood ratio tests (dataset: higher-level rfelections). Taxa: 1, *Ahytes muletensis*; 2, *Chiromantis rufescens*; 3, *Rana galamensis*; 4, *Mantidactylus* new species Comoros; 5, *Mantidactylus wittei*; 6, *Boophis tephraeomystax*; 7, *Boophis* new species Comoros; 8, *Aglyptodactylus madagascariensis*; 9, *Laliostoma labrosum*; 10, *Rana temporalis*; 11, *Polypedates cruciger*; 12, *Indirana cf leptodactyla*; 13, *Nyctibatrachus major*; 14, *Hoplobatrachus crassus*; 15, *Hoplobatrachus occipitalis*; 16, *Ptychadena mascareniensis*; 17, *Astylosternus diadematus*; 18, *Leptopelis natalensis*; 19, *Arthroleptis variabilis*; 20, *Petropedetes parkeri*; 21, *Hyperolius viridiflavus*; 22, *Tachycnemis seychellensis*; 23, *Heterixalus tricolor*; 24, *Bufo melanostictus*; 25, *Leptodactylus fuscus*; 26, *Nesomantis thomasseti*; 27, *Pipa parva*; 28, *Hymenochirus boettgeri*; 29, *Xenopus laevis*; 30, *Ambystoma mexicanum*

Tree	Topology
B0	((1, (((((((((2,11),13), (3,10)), (4,5), (6,7), (8,9))), (12, (16,20))), (14,15)), ((17,18),19), (21, (22,23))), (24,25)),26), ((27,28),29))),30)
B1	(30, (1, (((((((((13, (2,11)), (3,10)), (4,5), (6,7), (8,9))), (12, (16,20))), (14,15)), (19, (17,18)), (21, (22,23))), (26, (24,25))), (29, (27,28))))))
B2	(30, (1, (26, (((((((((13, (2,11)), (3,10)), (4,5), (6,7), (8,9))), (12, (16,20))), (14,15)), (19, (17,18)), (21, (22,23))), (24,25))), (29, (27,28))))))
B3	(30, (1, (((((((((13, (2,11)), (3,10)), (4,5), (6,7), (8,9))), (12, (16,20))), (14,15)), (19, (17,18)), (21, (22,23))), (24,25))), (26, (29, (27,28))))))
B4	(30, ((1,26), (((((((((13, (2,11)), (3,10)), (4,5), (6,7), (8,9))), (12, (16,20))), (14,15)), (19, (17,18)), (21, (22,23))), (24,25))), (29, (27,28))))))
B5	(30, (1, (((26, (((((((((13, (2,11)), (3,10)), (4,5), (6,7), (8,9))), (12, (16,20))), (14,15)), (19, (17,18)), (21, (22,23))), (24,25))), (29, (27,28))))))
B6	(30, (1, (((26, (((((((((13, (2,11)), (3,10)), (4,5), (6,7), (8,9))), (12, (16,20))), (14,15)), (19, (17,18)), (21, (22,23))), (24,25))), (29, (27,28))))))
B7	(30, (1, (((((((((13, (2,11)), (3,10)), (4,5), (6,7), (8,9))), (12, (16,20))), (14,15)), (19, (17,18)), (21, (22,23))), (24,25))), (29, (27,28))))))
B8	(30, (1, (((((((((13, (2,11)), (3,10)), (4,5), (6,7), (8,9))), (12, (16,20))), (14,15)), (19, (17,18)), (21, (22,23))), (24,25))), (29, (27,28))))))
B9	(30, (1, (((((((((13, (2,11)), (3,10)), (4,5), (6,7), (8,9))), (12, (16,20))), (14,15)), (19, (17,18)), (21, (22,23))), (24,25))), (29, (27,28))))))
B10	(30, (1, (26, (((((((((2,11), (4,5), (6,7), (8,9))), (13, (3,10))), (12, (16,20))), (14,15)), (19, (17,18)), (21, (22,23))), (24,25))), (29, (27,28))))))
B11	(30, (1, (26, (((((((((13, (2,11)), (3,10)), (4,5), (6,7), (8,9))), (3,10))), (12, (16,20))), (14,15)), (19, (17,18)), (21, (22,23))), (24,25))), (29, (27,28))))))
B12	(30, (1, (26, (((((((((13, (2,11)), (3,10)), (4,5), (8,9)), (6,7))), (12, (16,20))), (14,15)), (19, (17,18)), (21, (22,23))), (24,25))), (29, (27,28))))))
B13	(30, (1, (26, (((((((((13, (2,11)), (3,10)), (4,5), (6,7)), (8,9))), (12, (16,20))), (14,15)), (19, (17,18)), (21, (22,23))), (24,25))), (29, (27,28))))))
B14	(30, (1, (26, (((((((((13, (2,11)), (3,10)), (4,5), (6,7), (8,9))), (12, (16,20))), (14,15)), (17,19), (18, (21, (22,23))), (24,25))), (29, (27,28))))))

## Appendix G. Estimates of divergence times

Since our results indicated younger ages of most anuran cladogenetic events within the Anura as compared to previous publications, we followed a multiple conservative approach, to avoid any methodological error that could mask our results. This implied (a) using the oldest available geological dating of the origin of Mayotte for calibration of the split between the Comoroan *Boophis* and *Mantidactylus* from their Malagasy sister groups (8.7 MYr ago), (b) usage of an extremely old age estimate of separation between salamanders and frogs (370 million years, the age of the first tetrapod fossils) (15), (c) usage of the oldest age estimate of the breakup of Africa and South America (101 million years) (16) to calibrate the vicariance between the fully freshwater aquatic *Hymenochirus* (Africa) and *Pipa* (South America) (17).

We followed two separate approaches to estimate the ages of the relevant splits in our phylogeny:

(i) non-parametric rate smoothing using the program r8s (written by M. J. Sanderson) including the calculation of confidence intervals; as input we used a phylogram with the topology obtained by ML searches of the complete (mitochondrial and nuclear) dataset but with branch lengths based on the nuclear gene sequences only.

(ii) Regression of pairwise divergences between taxa in the rhodopsin gene, using additional vicariant species pairs from the Mediterranean area for calibration, and calculation of prediction confidence intervals (18).

### 7.1 Non Parametric Rate Smoothing

Maximum Likelihood (ML) heuristic searches were carried out with PAUP\* (6) under the substitution model proposed by Modeltest (4), with a random sequence addition sequence (10 replicates). The obtained tree was saved without branch lengths, and loaded again after excluding of all mitochondrial sequences and constraining to ML settings proposed by MODELTEST for the nuclear dataset alone.

The obtained phylogram was saved to a treefile including branch lengths, and submitted to non parametric rate smoothing (19) using the program r8s, written by M. J. Sanderson, on a Pentium III computer with Linux 2.2.18 partition. We preferred NPRS over penalized likelihood (PL) (20) following again a conservative approach, since it usually produces more extended confidence intervals. NPRS calculations were carried out with the Powell algorithm, fixing the age of the root at 370 or 250 MYA,

the ages of the splits between the two *Boophis* and the two *Mantidactylus* at 8.7 MYA, and the age of the split between *Pipa* and *Hymenochirus* at 101 MYA. Confidence intervals were calculated using a cutoff value of 4.0. However, due to the lack of crossover points, confidence intervals could not be calculated for the young dispersal events but only for the older splits in the cladogram.

	Root fixed at 250 MYr ago	Root fixed at 370 MYr ago
<b>Fixed ages used for calibration</b>		
Root	250 MYr ago	370 MYr ago
Pipidae	101 MYr ago	101 MYr ago
<i>Boophis</i> (Mayotte-Madagascar)	8.7 MYr ago	8.7 MYr ago
<i>Mantidactylus</i> (Mayotte-Madagascar)	8.7 MYr ago	8.7 MYr ago
<b>Age estimate</b>		
<i>Hyperolius-Heterixalus</i>	22.05 MYr ago	26.55
<i>Heterixalus-Tachycnemis</i>	11.28 MYr ago	13.57
<i>Hoplobatrachus occipitalis</i> - <i>H. crassus</i>	8.94 MYr ago	10.29
<i>Rana galamensis</i> - <i>R. temporalis</i>	23.03 MYr ago	26.44
<i>Polypedates</i> - <i>Chiromantis</i>	28.37 MYr ago	32.70
<b>Age estimate and confidence intervals</b>		
<i>Nesomantis</i> - Neobatrachia	150.50 (118.07-180.56) MYr ago	194.40 (127.61-273.17) MYr ago
Hyloidea-Ranoidea	137.57 (105.63-168.39) MYr ago	176.16 (111.13-255.81) MYr ago
Ranidei-Arthroleptoidei	92.71 (66.73-126.75) MYr ago	113.63 (70.88-113.68) MYr ago
Basal splits within Ranidei	62.79 (41.78-96.99) MYr ago	73.34 (44.96-149.52) MYr ago

## 7.2 Regression analysis of pairwise Rhodopsin divergences

The calibration of the phylogram submitted to NPRS (see above) was based on three different splits that could be geologically dated (two colonizations of the Comoros, and the vicariance split between the African and South American pipids). To confirm these calibrations with independent data, we sequenced homologous rhodopsin fragments in three species pairs of the Mediterranean region which are likely to have been separated by vicariance at the end of the Messinian salinity crisis of the Mediterranean Sea, 5.2-5.3 MYr ago (21, 22): *Rana cretensis* (Crete) vs. *Rana cerigensis* (Rhodos), *Pelobates cultripes* (Spain) vs. *Pelobates varaldii* (Morocco), *Alytes dickhillenii* (Spain) vs. *Alytes maurus* (Morocco). The two *Rana* species belong to the European green frogs (or water frogs), which have been successfully used to calibrate a protein clock based on geologically dated Mediterranean sea barriers (21), making it highly probable that the assumed age reflects their biogeographic history. The two *Pelobates* and *Alytes* species, respectively, are sister species within their genera (22), and their separation is likely to have occurred at the opening of the strait of Gibraltar.

The 1-2 substitutions which were consistently found between the species of these pairs correspond well to the 5 substitutions between the Comoroan and Malagasy *Mantidactylus* species, indicating a rhodopsin substitution rate of 0.1-0.3 substitutions (0.03-0.1%) per lineage per MYr. This agrees with the rate calculated using the ancient split between *Xenopus* and *Pipa* (0.2 substitutions per lineage per MYr, corresponding to 0.06%). It also agrees with the placement of the diapsid/synapsid split at 310 MYr ago (23) as exemplified by the *Homo/Gallus* divergence of 62 substitutions (0.1 substitutions or 0.03% per lineage per MYr).

Since the amount of substitutions between the two *Rana* (three substitutions) and *Hoplobatrachus* (three substitutions), as well as between the hyperoliids *Heterixalus*, *Tachycnemis* and *Hyperolius* (6-9 substitutions) were in a similar order of magnitude as in the Mediterranean and Comoran taxa used for calibration, it is highly improbable (and could clearly be excluded by 95% confidence intervals) that these divergences are due to ancient (Mesozoic) vicariance. Such vicariance, for the three hyperoliids, would imply a dramatic three to ten-fold decrease of substitution rate (down to 0.01% per lineage per MYr) for which there is no indication in the branch lengths of the phylograms (see section 5).

---

Calibration	Pairwise divergences	Age
-------------	----------------------	-----

---

	(# substitutions)	(MYr ago)
<i>Pelobates varaldi</i> - <i>P. cultripipes</i>	2	5.3
<i>Alytes maurus</i> - <i>A. dickhillenii</i>	1	5.3
<i>Rana cretensis</i> - <i>R. cerigensis</i>	1	5.3
<i>Mantidactylus wittei</i> - <i>M. sp.</i> (Mayotte)	5	8.7
<i>Xenopus</i> - <i>Pipa</i>	37	101

Age estimate	Pairwise divergences (# substitutions)	Age_mean (MYr ago)	Age_min (MYr ago)	Age_max (MYr ago)
<i>Chiromantis</i> - <i>Polypedates</i>	16	43.1471	36.3026	50.7792
<i>Rana temporalis</i> - <i>R. galamensis</i>	3	7.9301	3.9599	12.6879
<i>Hoplobatrachus occidentalis</i> - <i>H. crassus</i>	3	7.9301	3.9599	12.6879
<i>Tachycnemis</i> - <i>Heterixalus</i>	6	16.0571	11.4236	21.4782
<i>Heterixalus</i> - <i>Hyperolius</i>	9	24.1841	18.8873	30.2685
<i>Nesomantis</i> - Neobatrachia Min	41	110.8721	98.5001	124.0317
<i>Nesomantis</i> - Neobatrachia Max	49	132.5441	118.4033	147.4725
Neobatrachia Min	33	89.2001	78.5969	100.5909
Neobatrachia Max	51	137.9621	123.3791	153.3327
Ranoidea Min	29	78.3641	68.6453	88.8705
Ranoidea Max	42	113.5811	100.988	126.9618
Ranidei Max	33	89.2001	78.5969	100.5909
Mantellidae-Rhacophoridae Min	10	26.8931	21.3752	33.1986
Mantellidae-Rhacophoridae Max	16	43.1471	36.3026	50.7792









