Horizontal Gene Transfer from Snakes to Frogs:
Investigation of Geography Dependent Horizontal Transfer Mediated by Parasites

ヘビからカエルへの遺伝子水平伝播：
寄生虫に仲介される脊椎動物間水平伝播の地域依存的発生の実証

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1. Introduction

Horizontal transfer (HT) is a passage of genetic material between organisms through a mechanism other than reproduction. HT is well known in prokaryotes, where it often serves as a driving force for evolution by modifying genome structure, gene content, and/or gene expression pattern in the host genomes (Soucy et al. 2015). Whereas HT has rarely been documented among eukaryotes (Andersson 2005), recent evidence suggests that HTs among multicellular organisms are more common than previously thought (Huang 2013), and that the majority of these HTs correspond to transfers of transposable elements (TEs) (Schaack et al. 2010). Due to the intrinsic ability to transpose within genomes and expand their copy numbers, TEs are the most abundant component of many eukaryotic genomes (e.g., Lander et al. 2001; Schnable et al. 2009). It is also known that TEs are mutagens of the genome and occasionally cause genetic disorders (e.g., Bourque et al. 2018). Thus, understanding the trends of occurrence, and mechanisms of transmission of transposable element HTs is important to understand how potential alien mutagens are acquired from other organisms, how they affect genome evolution, and how they impact fitness of the host organisms (Kazazian and Moran 2017).

Recent large-scale genome analyses have detected a number of HTs of TEs even among multicellular animals (Walsh et al. 2013; Suh et al. 2016; Ivancevic et al. 2018; Zhang et al. 2020). Genomic surveys across the insect taxa have shown a tendency of phylogenetic relatedness and geographical proximity favoring HTs (Peccoud et al. 2017). It has also been suggested that host-parasite interactions facilitate HTs and that parasites may mediate HTs among vertebrates, by the finding of TEs transferred among vertebrates by blood-sucking triatomine bug, ticks, and endoparasitic nematodes (Gilbert et al. 2010; Walsh et al. 2013; Dunemann and Wasmuth 2019). However, since genome-based analyses to date are largely limited by the extent of geographic and taxon sampling, geographic trends and transmission modes of inter-metazoan HT propagations are poorly understood.

Bovine-B (BovB) is a unique LINE retrotransposon initially identified in cattle (*Bos*) (Szemraj et al. 1995) and makes up more than 18% of its genome (Walsh et al. 2013). Subsequent studies have shown that BovB is vertically transmitted in squamates (snakes

and lizards) and that BovB in ruminants originated by HT from snakes (Kordis and Gubensek 1998). Moreover, it has recently been shown that BovB is horizontally transmitted among a wider range of metazoans (Walsh et al. 2013; Ivancevic et al. 2018).

Given the prevalence of amphibians in the diet of snakes (Colston et al. 2010), I hypothesized that BovB HT might also occur between snakes and frogs. Remarkably, the preliminary analyses revealed that the genomes of several Malagasy frogs contain a large number of BovB sequences having quite high nucleotide similarity with those of snakes. The discovery led to the following questions, addressed herein: 1) In which direction did the BovB HT occur (from frog to snake or from snake to frog)? 2) Is the BovB HT between snakes and frogs an isolated (only in Madagascar) or worldwide phenomenon? 3) In what mode did this HT occur, i.e., by direct contact or by mediation of vector organisms?

I addressed the above three questions using a transcontinental and comprehensive taxon sampling comprising 106, 149, and 42 species of snakes, frogs, and their parasites, respectively. I further aimed to understand geographical trends in the frequency of occurrence of inter-vertebrate HTs which to date has remained poorly studied. My analyses showed that, counter-intuitively, BovBs in frogs were derived from those in snakes, indicating recurrent HTs of BovBs from predators (snakes) to their prey (frogs). Furthermore, I demonstrate that there is clear geographical variation in the frequency of occurrence of horizontal transmission of BovB, with Madagascar being a hotspot, which may be mediated by the presence of BovB-carrying parasites in the region. This study provides initial and novel perspectives on the global scale regional pattern of the occurrence of horizontal transmission, and suggests that inter-vertebrate HT has a transmission mode analogous to the infection mode of some vector transmitted endemic diseases, such as malaria (Phillips et al. 2017).

2. Materials and Methods

2.1 Contributions of Collaborators

This study used many animal specimens from multiple regions of the world and applied quite diverged experiment and analytical methods. Therefore, this study could not be completed without the contributions of many collaborators. The specific roles and contribution of the collaborators are specified below.

The specimens used in this study were mainly provided by museums or personal zoological collections (Table 1), with contributions of Dr. Atsushi Kurabayashi (Nagahama Institute of Bio-Science and Technology), Prof. Yoshinori Kumazawa (Nagoya City University), Dr. Zoltán T. Nagy (Royal Belgian Institute of Natural Sciences), Prof. Akira Mori (Kyoto University), Dr. Allen Allison (Bishop Museum), Dr. Stephen C. Donnellan (South Australian Museum), Prof. Hidetoshi Ota (University of Hyogo), Dr. Masaki Hoso (Waseda University), and Prof. Miguel Vences (Braunschweig University of Technology). The parasites and some snakes and frogs in Madagascar were collected by Dr. Ryosuke Kakehashi (Nagahama Institute of Bio-Science and Technology), and collection and exportation permits for Malagasy samples were obtained through the contributions of Dr. Andolalao Rakotoarison (University of Antananarivo) and Prof. Miguel Vences. Partial genome sequencing of Malagasy frogs was performed by Dr. Atsushi Kurabayashi. PCR screening of BovB for the snakes and frogs was mainly due to the contributions of Dr. Atsushi Kurabayashi, Dr. Ryosuke Kakehashi, Mr. Yusuke Sato (Hiroshima University), and Prof. Kazuhiko Ohshima (Nagahama Institute of Bio-Science and Technology). The filtering of sequence reads obtained from multiplex sequencing of PacBio next generation sequencer (NGS) was performed by Dr. Hideaki Mizuno (Independent researcher). Fluorescence *in situ* hybridization (FISH) analysis was conducted by Dr. Hideyuki Tanabe (The Graduate University for Advanced Studies). RNA seq data was provided by Dr. Sven Künzel (Max Planck Institute for Evolutionary Biology). All other experiments/analyses (Sections 2.3–2.5 and 2.7–2.11) and collection of Japanese parasites (Section 2.2) were basically conducted by myself under the support of Dr. Atsushi Kurabayashi, Dr. Ryosuke Kakehashi, Mr. Yusuke Sato, Dr. Nobuaki Furuno (Hiroshima University), Dr. Tetsuya

Yanagida (Yamaguchi University), and Prof. Hiroshi Sato (Yamaguchi University).

2.2 Sampling of Reptiles, Amphibians, and Parasites

A total of 121 individuals of 109 reptile species from 20 snake and 3 lizard families and 167 individuals of 152 amphibian species from 28 frog, 2 salamander and 1 caecilian families were sampled (Table 1). In addition, 97 parasite specimens from 5 animal phyla (Acanthocephala, Annelida, Arthropoda, Nematoda, and Platyhelminthes) were collected from reptiles and amphibians in Madagascar and Japan (Table 1). The sampling in Madagascar for reptiles, frogs, and parasites was conducted under collection and exportation permits issued by the Malagasy authority (No. 215/16-MEEF/SG/DSAP/SCB.Re and 010N-EA01/MG17, respectively). Total DNA was extracted from frozen or ethanol-preserved liver or muscle tissues of reptiles and amphibians, using phenol/chloroform extraction. For parasites, total DNA was extracted from muscles, excluding the digestive tract as much as possible, to rule out possible contamination from ingested host tissue. Most of tissue specimens used were from museum and personal zoological collections (Table 1) but I also took parasites from live frogs and snakes. Also tissue and cell specimens from live snakes and frogs were used for RNA sequencing and FISH experiments. The experiments with live vertebrates were performed under the permissions from the Ethics Committees for Animal Experiments of Nagahama Institute of Bio-Science (# 085) and Hiroshima University (# C16-22 and G17-1).

2.3 Species Identification and Elimination of Contamination

The identification of the species of reptiles, amphibians, and parasites used in this study was confirmed by analysis of partial sequences of mitochondrial cytochrome apoenzyme *b* (Cytb) in reptiles, 16S rRNA in amphibians and nuclear 18S rRNA in parasites. Each fragment was amplified using specific primers (Kocher et al. 1989; Bossuyt and Milinkovitch 2000; Burbrink et al. 2000) (Table 2) and EmeraldAmp PCR Master Mix (Takara Bio Inc., Shiga, Japan). PCR was conducted using the following temperature cycling: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing between 46°C–55°C based on gene-specific gradients for 30 s, and

elongation at 72°C for 1 min, ending with a 7 min elongation step at 72°C. Sequencing of the amplified products was performed using BigDye Terminator v. 3.1 Cycle Sequencing Kits (Applied Biosystems, Foster City, CA). The sequencing reactions were ethanol precipitated and run on an ABI 3100xl automated DNA sequencer (Applied Biosystems). Sequence electropherograms were checked using MEGA X (Kumar et al. 2018) and samples with double peaks were excluded, to remove potential contamination. The species were identified using Megablast search (Zhang et al. 2000) of the National Center for Biotechnology Information (NCBI) databases.

For reptiles, the specimen was identified as the top matched species if the maximum identity was 95% or over. When the maximum identity fell in the range 90%–94%, and Cytb sequence data for the morphologically identified species was not available from the NCBI, the morphologically identified name was labelled with "cf.". When the data of the morphologically identified species was available from the NCBI, the top matched species in the BLAST search was labelled with "cf.". When the maximum identity was 85%–89% or less than 85%, "sp." was labelled in the genus or family of the top matched species. The scientific name was assigned according to Uetz et al. (2020).

For amphibians, the specimen was identified as the top matched species when the identity was 97% or over, while "sp." was added to the top genus match when the identity was less than 97%. The scientific name was assigned according to Frost (2020). For parasites, the specimen was identified as the top species match when the identity was 99.5% or over. When the identity was less than 99.5%, "sp." was added to the taxonomic rank common to the highly matched species.

2.4 BovB Search in Amphibian Genomes

The total DNA of three Malagasy frogs (*Boophis goudotii*, *Mantella betsileo*, and *Mantidactylus betsileanus*) were extracted from fresh livers, as described above. Library construction and sequencing by GS-FLX (Roche, Basel, Switzerland) were outsourced to Eurofins Genomics K.K (Tokyo, Japan), Hokkaido System Science Co. Ltd. (Hokkaido, Japan), and Takara Bio Inc. (Shiga, Japan). Redundant reads and short reads less than 50 bp were eliminated using CDHit 454 (Li and Godzik 2006) and Solexa QA (Cox et al. 2010),

respectively. The BovB sequences were searched using RepeatMasker (Smit et al. 2013–2015), and the percentage of BovB in each genome was calculated.

The genome assemblies of 21 amphibian species available from NCBI as of 2021 were searched using Megablast with BovB_VA as the query. For the six species in which hits were found (*Bufo gargarizans*, *Leptobrachium leishanense*, *Pyxicephalus adspersus*, *Lithobates catesbeianus*, *Rana temporaria*, and *Geotrypetes seraphini*), I then conducted a RepeatMasker search with the same query. The percentage of BovB in the genome of each species was estimated.

2.5 PCR Screening and Multiplex Sequencing

PCR screening for the presence of BovB was conducted using 24 primer combinations, using 4 forward and 6 reverse primers (Table 2). BovB fragments were amplified by standard and touch-down PCR methods using LA taq Hot Start Version (Takara Bio). The temperature cycling of PCR was as follows: for standard PCR, 2 min at 94°C followed by 37 cycles at 94°C for 25 s, 57.5°C for 30 s, 68°C for 3 min, and 72°C for 4 min, and for touch-down PCR, 2 min at 94°C followed by 6 cycles at 94°C for 25 s, 65°C for 30 s, and 68°C for 3 min; 6 cycles at 94°C for 25 s, 62.5°C for 30 s, and 68°C for 3 min; and 25 cycles at 94°C for 25 s, 60°C for 30 s, 68°C for 3 min, and 72°C for 4 min. For the samples in which the candidate band of BovB was amplified, PCR was performed with primers containing 16 bp barcode sequences. The candidate fragments were purified using gel extraction with Qiaex II Gel Extraction Kits (Qiagen, Hilden, Germany), and 120 ng of DNA was collected for each sample. To prevent DNA contamination, the PCR and gel extraction steps were carried out by Dr. Atsushi Kurabayashi, Dr. Ryosuke Kakehashi, and Mr. Yusuke Sato for reptiles and amphibians and myself for parasites, respectively. Because BovB has a multi-locus nature, the PCR fragments amplified from multiple loci are mixed together. Thus normal sequencing method could not be applied for sequencing the PCR products. To sequence the single BovB fragment derived from single locus from the PCR fragment, single-molecule real-time (SMRT) technique (Eid et al. 2009) was applied. The PCR fragments obtained were pooled in a single tube and sequenced in 13 runs of Multiplex-Amplicon analyses using PacBio RS II NGS (Pacific Bioscience, Menlo Park,

CA). The library construction and sequencing were outsourced to the Center of Medical Innovation and Translational Research (CoMIT) of Osaka University, Duke GCB (Sequencing and Genomic Technologies of Duke University), Integral Inc. (Tokyo, Japan), Macrogen Japan Corp. (Tokyo, Japan), and Tomy Digital Biology Inc. (Tokyo, Japan). The raw sequence data generated by PacBio RS II sequencing was assembled in circular consensus sequences (CCSs), and the CCSs were demultiplexed according to their PCR barcodes using the SMRT Portal (Pacific Biosciences). The CCS reads were selected based on the sequence qualities (Quality value ≥ 30), and then a consensus sequence was constructed for each sample with over 50 clean reads. The parameters of the filtering were as follows: (i) contain primer sequences on both ends; (ii) average quality score is over 99%; (iii) sequence similarity with BovB_VA, one of the few full-length consensus sequences of BovB in snakes (Zupunski et al. 2001) (Fig. 1) showing the highest similarities with the BovBs from Malagasy frogs as the snake BovBs reported so far, is more than 70%; and (iv) length falls in the range 1,300–3,500 bp. The original programs to perform this filtering process written by Dr. Hideaki Mizuno are available on GitHub (https://github.com/mizuno-hideaki/horizontal-gene-transfer).

2.6 Fluorescence *in situ* Hybridization

The cells of snakes and frogs were obtained from tissues of each individual and fixed in Carnoy fixative (methanol:acetic acids = 3:1 mixture). The slide spreads were prepared according to the standard procedure and FISH was carried out as described previously (Tanabe et al. 1996, 2021). The BovB_VA sequence cloned in pUC57 (total 5,879 bp) were labelled by nick translation with digoxigenin-11-dUTP (Roche 11093088910) and used as probes. Since BovB_VA could not hybridize with bufonid BovBs due to low sequence similarity (< 80%), I made a specific probe for bufonids. Specifically BovB amplicon from *B. japonicus* was cloned in pCR2.1-TOPO (total ca. 6,700 bp) and used as the probe. Labelled DNA probes were hybridized for 24–36 hours onto slide spreads. After hybridization, the slides were washed and detected with mouse anti-digoxigenin antibody (Sigma D-8156) in the first layer, and successively detected with sheep anti-mouse, Cy3-conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) in the

second layer. The slides were counterstained with DAPI and mounted in Vectashield Antifade (Vector Laboratories, Inc., Burlingame, CA). FISH images were captured and analyzed using a Leica DM5000B fluorescent microscope equipped with a CCD camera and CW4000 image analysis software (Leica Microsystems, Wetzlar, Germany). Dr. Hideyuki Tanabe did the above FISH experiments, excluding the probe preparation.

2.7 Dot Blot Hybridization

For each frog sample used in the dot blot analysis, 500 ng and 50 ng of total DNA were denatured in a solution containing 0.8N NaOH at 95°C and deposited onto a Biodyne B Nylon Membrane (Nihon Pall Ltd., Tokyo, Japan) using a FLE348AA dot blotter (Advantec MFS Inc., Tokyo, Japan). 250 ng and 25 ng of DNA were used in the specimens for which enough DNA was not available (Table 3). The membrane was rinsed in 2x standard saline citrate (SSC), air dried, and baked at 80°C. To enhance the ability to detect BovB elements, I used two probes for hybridization. The first fragment was 526 bp long and corresponded to the 5′ side coding AP endonuclease, amplified in BovB_VA sequence cloned in pUC57 using the primers ME1_Fmod and BovB_VA_1201_Rev (Table 2). The second fragment was 502 bp long and corresponded to the 3′ side coding reverse transcriptase, amplified using BovB_VA_1942_Fow and ME2_Rmod (Table 2). Both PCR products were gel-purified as described above and [a-32P]dCTP-labelled using BcaBEST Labeling Kits (Takara Bio). The membrane was first hybridized with the 5′ side probe using PerfectHyb Hybridization Solution (Toyobo Co. Ltd., Osaka, Japan), washed and exposed for on imaging plate, and scanned using Typhoon 9500 (GE Healthcare, Chicago, IL). It was then stripped and hybridized with the 3′ side probe with the same method.

To approximate the number of BovB copies present in the frog genomes, eight dilutions (2.5, 10, 50, 75, 100, 250, 500, and 1,000 pg) of cloned BovB_VA were blotted onto a membrane. The number of two-probe sequences contained in each dilution was calculated using the formula “copy number = (amount in ng x number/mole)/(length in bp x ng/g x g/mole of bp),” as described and implemented on the website http://cels.uri.edu/gsc/cndna.html. Based on the copy number of each dilution calculated from the integrated density of the signal, I created a standard curve and estimated the total

copy number for each species. To calculate the copy number per haploid genome, I used *C* values taken from the Animal Genome Database (Gregory 2019), and the haploid genome sizes were calculated using the formula (Dolezel et al. 2003) "haploid genome in bp = *C* value x 0.978 x 10^9". When multiple *C* values were given for one species, I used the average of these values. When a *C* value was not available for a species, I used the average within the most closely related taxa, as shown in Pyron and Wiens (2011).

2.8 Molecular Phylogenetic Analyses

I added 74 known BovB sequences (Bao et al. 2015; Ivancevic et al. 2018) to the data from 211 specimens consisting of 121 reptile individuals of 109 species of 100 genera of 23 families, 65 frog individuals of 50 species of 30 genera of 10 families, and 25 parasite individuals of 3 phyla newly obtained in this study. These 285 BovB sequences were aligned using MAFFT with the L-INS-I option (Katoh et al. 2019), and a preliminary ML phylogenetic analysis was conducted using RAxML v. 8.2.10 with the rapid hill-climbing algorithm (Stamatakis 2014) (Fig. 2). The best substitution model was estimated using Kakusan4 (Tanabe 2011), and the independent general time reversible + gamma distribution (GTR+G) substitution model was applied. The supports for the internal branches of reconstructed trees were evaluated using bootstrap percentages (BPs) calculated with 1000 pseudo-replicates. Based on the result, I eliminated the non-squamate type BovB sequences (31 sequences more primitive than the BovB of the anole lizard, *Anolis carolinensis*) and rebuilt a BovB dataset that appear to be vertically transmitted in squamates to perform precise divergence time estimation and ancestral range estimation of the BovB sequences derived from snakes. I also deleted 32 sequences that are almost identical within species. The 222-OTUs dataset was aligned by MAFFT, and phylogenetic trees were inferred using ML and BI. In these analyses, the GTR+G model was selected as the best substitution model by Kakusan4. The ML phylogeny was inferred using RAxML as described above. The BI analysis was performed with MrBayes v. 3.2.6 (Ronquist et al. 2012). Two independent runs of four Markov chains were conducted for 20 million generations, and the tree was sampled every 1000 generations. The convergence of the posterior distribution of model parameters (all parameters reached 200) was checked using Tracer v. 1.7.1 (Rambaut et al. 2018), and

the first 10% of samples were discarded as burn-in. The supports for the internal branches were evaluated using Bayesian posterior probabilities (BPPs). The tree topologies inferred by ML and BI were almost identical with some differences in the branching pattern and the presence of unresolved nodes (polytomies) in the BI tree (Figs. 3 and 4 and Table 4). To verify the vertical transmission of BovB in squamates, I_{cong} index (de Vienne et al. 2007) and normalized Triples metric (Dobson 1975) between the topologies of squamates on the 222-OTUs ML tree and a tree constructed based on orthologous genes (Pyron et al. 2013) were calculated. They were assessed at the species, genus and family levels, and compared to the values among frog topologies (Pyron and Wiens 2011) (Table 5). In the 222-OTUs ML tree (Fig. 3), I recognized the nodes at which phylogenetically distant taxa (above familial level) diverged and the corresponding topology is obviously different from the general phylogenetic hypothesis (Pyron and Wiens 2011; Pyron et al. 2013) as the HT points.

2.9 Divergence Time Estimation

A Bayesian relaxed-clock analysis was performed using BEAST v. 2.6.0 (Bouckaert et al. 2019) to date the occurrence of HT events (Fig. 5). The 222-OTUs ML topology was used as the reference tree. I applied the Yule process (Yule 1924) to describe cladogenesis. MCMC chains were run for 1 billion generations with 1 sampling per every 1000 generations, and the first 10% of generations were discarded as burn-in. The posterior distributions of the model parameters were checked in the same ways as the BI analysis above. Based on the TimeTree database (Kumar et al. 2017), I employed eight calibration points: (A) Henophidia–Caenophidia split, 72–92 million years ago (Ma); (B) Xenodermidae–Pareidae split, 59–89 Ma; (C) most recent common ancestor (MRCA) of Boinae, 43–68 Ma; (D) Loxocemidae–Pythonidae split, 30–68 Ma; (E) MRCA of Lamprophiidae, Prosymnidae, Psammophiidae, Pseudaspididae, and Pseudoxyrhophiidae, 37–51 Ma; (F) MRCA of *Heterixalus*, 15.6–25.7 Ma; (G) MRCA of *Thamnosophis*, 8.06–23.84 Ma; and (H) MRCA of *Bufo*, 11.1–17.8 Ma.

2.10 Ancestral Area Estimation

I defined nine biogeographic areas (Africa, Europe, Madagascar, Oceania, North America,

Central–South America, East Asia, Southeast Asia, and Western–Central–Southern Asia). Each reptile, amphibian, and mammal species was assigned to a location, based on current distribution data from The Reptile Database (Uetz et al. 2020), Amphibian Species of the World (Frost 2020), and Mammal Species of the World (Wilson and Reeder 2005), respectively. For the globally distributed species such as domestic livestock or invasive species, the estimated origins were assigned (Pidancier et al. 2006; Rezaei et al. 2010; Yindee et al. 2010; Pyron and Wallach 2014; Upadhyay et al. 2017; Krinsky 2018), and the collection areas were used for parasites. Ancestral range reconstruction was performed using BioGeoBEARS (Matzke 2013). The maximum number of ancestral areas allowed at each node was set to four. I compared all six models implemented in BioGeoBEARS. The AIC selected the DIVALIKE+J as the best fitting model, and this was subsequently used to infer the most likely geographic history of BovB HT events (Table 6). The availability of connections between areas was unconstrained. The ancestral region with the highest probability at the node at which HT was estimated to have occurred was considered to be the region of occurrence.

2.11 Messenger RNA Sequencing

Tissues from each sample were preserved in RNAlater and frozen at -80°C. Total RNA extraction was carried out using a standard trizol protocol from about 20–100 mg of tissue per specimen (combined or separate skin, muscle or liver). Libraries were barcoded and sequenced on an Illumina NextSeq instrument, in multiple 150 bp or 75 bp paired-end runs (along with other samples of amphibians and reptiles not used for this study) each of which combined 10–14 samples per High-Output NextSeq kit. To assemble the transcriptomes, reads were quality-trimmed and filtered using fastp (Chen et al. 2018) and *de novo* assembled using Trinity v. 2.1.0 (Grabherr et al. 2011) following published protocols (Haas et al. 2013). The expression of BovB was assessed by BLASTN searches (Altschul et al. 1990) with BovB_VA as the query. Prof. Miguel Vences prepared the specimens for the above messenger RNA sequencing (mRNA-Seq) and Dr. Sven Künzel performed mRNA-Seq.

3. Results

3.1 Detection of BovBs in Frog Genomes

The initial screening detected BovB fragments highly similar to those previously found in vipers (sequence similarity > 94%) in the genomes of three frog species from Madagascar (Fig. 1 and Table 1). To investigate the phylogenetic diversity of the taxa involved in HT, and the geographical distribution of BovB-positive frogs, PCR screening of 109 reptile species including 20 of the 30 snake families and three lizard families, and 152 amphibian species from 28 of the 56 frog families was performed (Table 1). BovB PCR products were amplified in all reptiles and 50 frog species (34%). The ratio of BovB-positive frogs varied by geographic region and was highest in Madagascar (91%) (Fisher's exact test with holm correction, $P < 0.05$; Table 7). To rule out false positives due to amplifications of DNA contamination, I performed dot blot analysis and detected intense signals in BovB-positive species (Figs. 6 and 7). FISH analysis with snake BovB sequence as the probe showed clear BovB signals on the frog chromosomes and nuclei (Fig. 8). Partial genome sequencing and dot blot analysis also revealed that BovB sequences are abundant, contributing up to 0.53% of the frog genomes (Table 3 and 8).

I conducted a Megablast search for BovBs in the genome assemblies of 21 amphibian species for which whole genome assemblies were available as of 2021 and obtained hits from five frogs and one caecilian. I detected BovBs in the frogs that belong to the same family as *Bufo gargarizans* (Bufonidae), *Leptobrachium leishanense* (Megophryidae), *Lithobates catesbeianus*, and *Rana temporaria* (Ranidae). This result confirms that the BovB detected in frogs in this study did not originate from field sampling or laboratory or database contamination. For *Pyxicephalus adspersus* (Pyxicephalidae) and *Geotrypetes seraphini* (Dermophiidae), the longest BovB sequences obtained in the RepeatMasker search (Smit et al. 2013–2015) were compared with the BovB sequences used in this study, which were most closely related to the BovB of a boid snake, *Eryx colubrinus* (72%), and a hyrax, *Procavia capensis* (61%). No hits were found in the Megablast search from *Xenopus laevis* and *X. tropicalis*, in which the presence of BovB was previously shown (Ivancevic et al. 2018). My phylogenetic analysis revealed that these two

BovB sequences are more primitive than the BovB of an anole lizard, *Anolis carolinensis* (Fig. 2), indicating that they are different from the squamate-type BovBs focused on in this study.

3.2 Phylogenetic Analyses with BovB Sequences

A phylogenetic analysis of 211 new BovB consensus sequences obtained via SMRT sequencing (Eid et al. 2009), and 74 known BovBs, yielded a phylogenetic tree largely concordant with the ortholog-based phylogenetic relationships at family level for the host squamates (Zheng and Wiens 2016) (Fig. 2). A reduced dataset with 222 OTUs (removing 31 of the non-squamate type BovBs; Figs. 3 and 4), confirms the congruence of the squamate BovB tree with the general host squamate phylogenetic relationships at species, genus, and family levels [tested by I_{cong} index (de Vienne et al. 2007) and Triples metric (Dobson 1975); see Materials and Methods and Table 5]: This suggests that BovBs were primarily transmitted via vertical inheritance in squamates. Some topological consistency was also detected between the BovB tree and established phylogenetic relationships of frogs, but with lower degree of concordance than in squamates (Table 5). This result is consistent with the hypothesis that the BovBs of frogs were only rarely acquired by vertical transmission but rather transmitted from snakes via multiple HT events with a unique transfer direction, from predators to their prey. In the 222-OTUs tree (Fig. 3), the nodes at which different families diverged and the corresponding branch topology differed from the general phylogenetic consensus (Zheng and Wiens 2016; Feng et al. 2017; Hime et al. 2021) were identified as HT points. This procedure led to the identification of at least 54 probable instances of HTs.

In the 222-OTUs tree, frog BovBs were found in various clades (Fig. 3). From the BovB of *Afrotyphlops punctatus*, a representative of the blindsnakes that form the sister group of all other snakes, the monophyletic BovBs of the frog family Bufonidae were derived (Figs. 9 and 10d-I). The clade that comprised primarily the snake superfamily Henophidia also contained BovBs of 15 frog species from 5 families: Dicroglossidae, Mantellidae, Microhylidae, Ranidae, and Rhacophoridae (Fig. 9). In another major clade, comprising mainly the superfamily Caenophidia, BovBs of 30 frog species from 7 families

(Ceratobatrachidae, Hyperoliidae, Mantellidae, Megophryidae, Microhylidae, Ptychadenidae, and Ranidae) were observed; and one Malagasy caenophidian subclade alone, the Pseudoxyrhophiidae, contained BovBs of 26 frog species from 4 families (Hyperoliidae, Mantellidae, Microhylidae, and Ptychadenidae) (Fig. 9).

There were over 10 snakes in which the pattern of BovBs did not reflect the host phylogeny. In seven blindsnakes of the superfamily Typhlopoidea, the BovBs identified were derived from different snake BovB lineages. In addition, two Malagasy boas had BovBs from Malagasy caenophidians (the family Pseudoxyrhophiidae) (Fig. 10d-II, III). The BovBs of the families Colubridae and Viperidae were derived within the clade of the family Elapidae, unlike the phylogeny based on orthologous genes (nodes #26 and 31; see Fig. 9 and Table 4). Moreover, several snake BovBs were derived from the clade of different families (nodes #27, 29, 33, 34, and 35).

Several frog taxa, in which the phylogenetic relationship of BovBs did not reflect the host phylogeny, were also found. In the clade of the snake family Pseudoxyrhophiidae, BovBs of the microhylid frogs belonging to Cophylinae (*Cophyla* and *Plethodontohyla*) form a monophyletic group with that of a mantellid frog, *Gephyromantis boulengeri* (node #45). Similarly, the BovB of a ptychadenid frog, *Ptychadena mascareniensis*, appears in the clade of the *Boophis* frogs of the family Mantellidae (node #54). Moreover, the BovBs of four distantly related frog families (Dicroglossidae, Mantellidae, Microhylidae, and Ranidae) are derived from the clade of Henophidia (primitive snakes).

Several saurian BovBs also emerged in the snake lineages. The BovBs of the Oceanian lizards, including Agamidae and Scincidae, were derived from the common ancestor of Henophidia (node #3). Additionally, the BovBs of a gecko genus, *Gehyra*, and a chameleon, Chamaeleonidae sp., were closely related to that of a snake, *Pareas iwasakii* of the family Pareidae (node #19). The BovB of a Malagasy gecko, *Phelsuma lineata*, was derived from the clade of the Malagasy snake family Pseudoxyrhophiidae (node #48).

The BovBs of ruminants and marsupials each formed a monophyletic group, the former was derived from the common ancestor of Henophidia (node #6) and the latter was closely related with the BovB of *Afrotyphlops punctatus* (Fig. 10d-I).

3.3 Timing and Geographical Area Estimation of BovB HT

The result of divergence time estimation showed that the 54 HTs estimated here have occurred across time between 1.3 and 85 million years ago (Ma) without any age bias. Then I performed geographical area reconstruction and found the occurrence of 54 HTs were widespread across the globe (Fig. 9). Focusing on those 33 HTs inferred among squamates and amphibians over the past 50 million years, i.e., the time after the current continental arrangement formed with the collision of India and Eurasia (Meng et al. 2012) confirmed an uneven geographical distribution. The number of HT events occurring within the past 50 Ma was particularly high in Madagascar with 14 HTs (Fig. 10a), and lower in East Asia and Oceania with five each, and Africa with one HT. In Madagascar the ratio of the number of HTs to the number of species was significantly higher than the ratio in the other six regions, except in Oceania and Western–Central–Southern Asia (binominal test with holm correction, $P < 0.05$). However, the HT frequencies in the latter regions were probably overestimated compared to Madagascar, where many samples were taken within genera in which the inheritance of BovB is vertical. I compared the ratio of the number of HTs to the number of genera and found that the HT frequency in Madagascar was significantly higher than that in any other region (Table 9).

Most of the Madagascan biota comprises descendants from African origin dispersers (Yoder and Nowak 2006), and in frogs, two lineages represented by *Heterixalus* and *Ptychadena mascareniensis*, are known to have migrated from Africa to Madagascar 19–30 Ma and 9.8–22.7 Ma, respectively (Vences et al. 2003; Zimkus et al. 2017). In the course of the geographical analyses, it was shown that the BovB HTs to these frogs occurred in Madagascar. This result is supported by the consistency of the estimated ages of HT events (Table 4) with those of their dispersal and the fact that the closely related frogs indigenous to Africa, *Hyperolius* and *Ptychadena nilotica*, are BovB-negative according to PCR.

3.4 Detection of BovB from Parasites

While the phylogenetic analysis strongly suggests a high proportion of BovB HTs from snakes to frogs, it does not clarify whether the transmission occurred directly or via vector

organisms. I therefore extended the investigation to parasites which could constitute potential vectors of the horizontal transmission of BovBs. A total of 97 individuals of 42 parasite species associated with reptiles and amphibians (Table 1) were collected from Madagascar and East Asia, two regions with different proportions of BovB-positive frogs (91% vs. 23%). These include endoparasites such as nematodes, trematodes, and acanthocephalans, frog-specific intracutaneous mites, as well as more generalist ectoparasites such as leeches and mosquitos. My screening identified BovB PCR products in parasites from three phyla, Annelida, Arthropoda, and Nematoda. To exclude the possibility of DNA contamination from the vertebrate hosts of these parasites, DNA extractions from parasites were performed after eliminating as much of the digestive tract as possible, and an event was not considered to be a HT when the parasite and host BovBs showed more than 98% similarity. The percentage of parasite species collected in Madagascar with a version of BovB different from that of their host (sequence similarity < 98%) and not clearly due to contamination (50%) was significantly higher than that in Japan (2.9%) (Fisher's exact test, $P < 0.01$; Table 7).

The BovBs from the invertebrate parasites were commonly derived from different snake or frog lineages. The BovB of a chigger, Trombiculidae sp. 1, isolated from a mantellid frog, *Blommersia blommersae*, from Madagascar was more closely related to that of a Malagasy snake, *Liophidium torquatum* (sequence similarity 99.8%), than that of its host (96.1%) (Figs. 10d-II and 11a). Similarly, the BovB of a nematode, *Cosmocerca simile*, isolated from the BovB-negative Japanese rhacophorid frog, *Buergeria japonica*, was included in the clade of the snake family Colubridae and was most closely related to a species also occurring in Japan, *Elaphe climacophora* (sequence similarity 100%) (Figs. 10d-IV and 11b). A total of seven nodes in which the parasite BovB were derived from squamates were observed (nodes #5, 16, 20, 24, 30, 37, and 51). The BovB of a tick, *Amblyomma limbatum*, collected from an Oceanian lizard, *Tiliqua rugosa* (reported in Walsh et al. 2013), was monophyletic with that of a ceratobatrachid frog, *Cornufer pelewensis*, also from Oceania (Fig. 10d-V). There were four nodes in which the BovB of snakes, frogs, and parasites formed a clade (nodes #23, 36, 40, and 50). The BovBs of the Malagasy nematodes isolated from the mantellid frogs *Mantidactylus femoralis* and *M.*

betsileanus emerged in the clade of the frog family Hyperoliidae (Figs. 10d-III and 11c). A BovB sequence similar to that from the mantellid frog, *Boophis madagascariensis*, was detected in a Malagasy leech, *Chtonobdella vagans*, collected from humans (Figs. 10d-II and 11d). A total of three nodes were found in which parasite BovB were derived from frog lineages (nodes #42, 52, and 53). The BovB of a bedbug, *Cimex lectularius*, is known to have 80% similarity with those of the elapid, colubrid, and viperid snakes (Ivancevic et al. 2018; Puinongpo et al. 2020). However, this study showed that its BovB is most closely related to that of *Mimophis* cf. *mahfalensis* belonging to the snake family Psammophiidae, with 94% similarity (node #37).

3.5 Survey of BovB Expression in Snakes and Frogs

Novel mRNA-seq data contained BovB fragments in three snakes (*Madatyphlops* sp., *Mimophis mahfalensis*, and *Thamnosophis lateralis*) and seven frogs (*Aglyptodactylus madagascariensis*, *Boophis tephraeomystax*, *Mantella expectata*, *Mt. laevigata*, *Mantidactylus betsileanus*, *Md. multiplicatus*, and *Plethodontohyla notosticta*) collected from Madagascar, but not in a BovB-negative frog from Southeast Asia (*Polypedates otilophus*). In particular, almost full-length BovB sequences with long open reading frames were assembled from two Malagasy snakes (*M. mahfalensis* and *T. lateralis*).

4. Discussion

4.1 Distribution and Timing of BovB Invasions

In this study, phylogenetic analyses confirmed that most BovBs in snakes have been vertically inherited. But this study also showed some discrepancies between the species and BovB phylogenies. For example, although the blindsnakes of the superfamily Typhlopoidea and Malagasy boas should harbor BovBs inherited from their respective ancestors, they were found to have different types of BovBs. These cases are probably due to snake-to-snake horizontal transmission, in which a newly integrated BovB has become dominant over the original BovB. Moreover, within some families (especially Viperidae and Colubridae), BovB sequences reflect little of the general phylogenetic relationships and show low nucleotide divergences, suggesting that HT among snakes may occur more frequently than previously thought (Walsh et al. 2013; Puinongpo et al. 2020).

Similarly, the fact that distantly related frogs shared similar BovB indicates that horizontal transmission also occurred among frogs. Specifically, the BovBs of *Gephyromantis boulengeri* and *Ptychadena mascareniensis* were closely related to the BovBs of *Cophyla* and *Boophis*, respectively. The BovBs of former frogs were probably introduced by HT from the latter frog taxa. For the BovBs of four frog families (Dicroglossidae, Mantellidae, Microhylidae, and Ranidae) derived from the ancestor of Henophidia, the following HT events can be suggested based on the branching ages of each taxa (Kumar et al. 2017) and the parsimony criterion for the HT numbers. First the BovB was transferred from the ancestor of Henophidia (Boidae, Loxocemidae, and Pythonidae) to that of the microhylid subfamilies, Asterophryinae and Dyscophinae (node #8; see Fig. 9 and Table 4). Then BovB HTs have occurred from the ancestor of Dyscophinae to that of Ranidae (*Babina*, *Odorrana*, and *Pelophylax*) (node #9) and a mantellid, *Aglyptodactylus madagascariensis* (node #10), and from the ancestors of Asterophryinae to that of Dicroglossidae (*Euphlyctis*, *Fejrvarya*, and *Hoplobatrachus*) (node #12) and a ranid, *Papurana* sp. (node #13). In this clade, the BovB of the xenopeltid snake, *Xenopeltis unicolor*, which is distributed in Southeast Asia and known to derive from the ancestor of Loxocemidae and Pythonidae, is closely related to that of Asterophryinae. Although

asterophryine taxa are widely distributed in Oceania, the most primitive asterophryine, *Gastrophrynoides*, is distributed in Southeast Asia and is considered that the asterophryine ancestor migrated from Southeast Asia to Oceania 25–48 Ma (Kurabayashi et al. 2011). It is therefore possible that horizontal transfer of BovB occurred there from the ancestor of Asterophryinae to that of *Xenopeltis unicolor* from frogs to snakes (node #11).

Several instances of Sauria (i.e., lizards) BovBs originating from snake lineages were also observed, indicating that the newly integrated BovBs from snakes are more dominant in these lizard lineages than the BovBs that originated in their common ancestors. Furthermore, although the BovB of the Oceanian Agamidae and Scincidae were derived from primitive snakes (node #3), the divergence time of the BovB between the two families (63.8 Ma) is quite recent than the actual divergence time (174 Ma) (Kumar et al. 2017), suggesting multiple occurrences of BovB HTs among Sauria.

Although it is assumed that the BovBs of marsupials are closely related to those of some squamates or ruminants (Walsh et al. 2013; Ivancevic et al. 2018), my phylogeny based on more detailed sampling also showed that BovBs in the marsupials form a monophyletic group with those of a snake, *Afrotyphlops punctatus*, and bufonid frogs (Fig. 10d-I). This indicates that the origin of BovBs in marsupials is more complex than previously thought. Meanwhile, in the present phylogenetic tree, the BovBs of ruminants derived from the ancestor of Henophidia, supporting the result of previous study that the ruminant BovB was originated by HT from primitive snakes (Walsh et al. 2013).

4.2 Potential Parasite Vectors Transferring BovB

This study revealed that several parasites have BovBs differed from their host's BovBs. The BovB of a chigger (Trombiculidae sp. 1) and a nematode (*Cosmocerca simile*) isolated from frogs were most closely related to those of snakes (nodes #30 and 51). Additionally, the BovB of a squamate parasitic tick (*Amblyomma limbatum*) was monophyletic with that of a frog (node #24). These parasites are thus plausible vectors that may have transferred the snake-type BovBs to frogs by moving between hosts. Although it is not known whether these parasite taxa are able to move between snakes and frogs (Table 1), the BovB transmission via these parasites can be explained if 1) they have wider host ranges than

previously known, 2) their ancestors parasitized both snakes and frogs, and/or 3) host switching (from reptiles to frogs) occurred in the parasite ancestral lineages.

While the presence of BovB in a diverse array of parasites leads us to favor the hypothesis of parasite-mediated transmission, the hypothesis of direct transmission remains viable. In fact, one case of BovB HT from a frog lineage to a frog-eating snake lineage was detected in my phylogenetic analyses (node #11), and this finding appears to be a first evidence of direct horizontal transmission of retrotransposon between vertebrates via predation. On the other hand, a scenario of regularly occurring direct HTs from snakes to frogs would require invoking failure of predation attempts, where the escaped prey acquired BovB through direct contact and injury. However, unlike with hard bodied prey such as many squamates, snake teeth can easily penetrate the soft unprotected body of frogs to secure a firm grip. Although comprehensive studies on the topic are rare, it appears unlikely that frogs would commonly escape and survive after a successful snake bite (but see Costa and Trevelin 2020). Furthermore, based on SquamataBase (Grundler 2020), the proportion of snakes known to consume frogs does not differ significantly among regions (chi-square test with holm correction, $P > 0.05$; Table 10), suggesting that direct transmission of BovB does not contribute substantially to the region specific frequency of BovB HTs observed here.

Some of the parasites used in this study had different BovBs among closely related species and within species. The BovBs of three Malagasy chiggers belonging to the family Trombiculidae were derived from the clades of *Boa* snakes (node #16), the chameleon family Chamaeleonidae (node #20) and the snake family Pseudoxyrhophiidae (node #51). Chiggers only suck blood once, as larvae (Mullen and Oconnor 2002), so contamination of DNA from sources other than their hosts is unlikely. I also found that two specimens of the Malagasy nematode, identified based on 18S rRNA sequences as belonging to the same species (*Raillietnema* sp.), have different BovBs, derived from the clade of the hyperoliid frog genus *Heterixalus* (node #42) and the mantellid frog genus *Mantidactylus* (node #52). Since the BovB sequences detected from these chiggers and nematodes differed from those of their hosts (sequence similarity < 98%), the possibility of contamination is low. This evidence indicates that closely related parasites can transport a variety of BovBs to their

hosts through frequent HT incidents or carriage of bacteria and viruses that have been considered to be candidate vectors of HTs among some eukaryotes (Liu et al. 2010; Gilbert et al. 2014; Ortiz et al. 2015).

4.3 Madagascar as a Hotspot of BovB Horizontal Transfer

This study revealed that frequency of BovB HT varied by geographic regions and was significantly higher in Madagascar than in any other regions. This is the first report showing the regional differences in the HT frequency among vertebrates. I also found several plausible parasite vectors; and remarkably, the proportion of BovB positive parasites in Madagascar are significantly higher than in Japan. These results suggest that the frequency of BovB HT correlate with (or affected by) the ratio of BovB-positive parasites in each region. In addition, the finding of the two BovB HTs that appears as if it has occurred as a result of the migration of the frog lineages from Africa to Madagascar exemplify the region-specific occurrence of BovB HT, analogous to malaria infection in humans who migrated to malaria afflicted areas.

Although TEs in genomes are generally transcriptionally silenced by epigenetic regulation (Slotkin and Martienssen 2007), this study found evidence that BovB may be transcribed both in snakes and frogs. In particular, almost full-length mRNA of BovB with long open reading frames were assembled from two Malagasy snakes (*M. mahfalensis* and *T. lateralis*), suggesting that BovB is transcriptionally active. The transcribed BovB mRNA can be taken in by vector organisms and transported to another host; thus, it is possible that horizontal transmission may still be occurring in Madagascar. Since the BovB expression in BovB-positive snakes or frogs outside of Madagascar have not been examined, future studies are needed to reveal if there are regional differences in BovB activity.

4.4 Conclusions

In this study, I analyzed the HT of BovB from snakes to frogs using a comprehensive sampling of snakes, frogs, and their parasites. The results suggest that Madagascar is a hot spot for BovB HTs, in which a variety of parasites mediate HT through host-to-host movement. The observations that leeches infesting humans possess frog-type BovB and that

BovB may be expressed in Malagasy snakes and frogs suggest that the BovB HTs may occur among an even wider range of vertebrate taxa in Madagascar. The finding of these extensive BovB HTs mediated by parasites provides a mechanism for the rapid and broad taxonomic transmission of genetic elements. I also showed that BovB, which originated in frogs by HT from snakes only 27.7 Ma, has accumulated, to constitute 0.53% of the genome of the mantellid frog, *Boophis goudotii* (Fig. 10d-II and Table 8). Mammals are known to be highly susceptible to BovBs introduced from snakes 76–85 Ma, which account for 1.3% of the genome in opossum, 15.2% in sheep, and 18.4% in cattle (Walsh et al. 2013). The worldwide occurrence of BovB HTs revealed in this study highlights the potential for genomic modifications by alien TEs in more diverse vertebrate taxa than previously conceived. In the future, BovB may occupy a position by its proliferation in a genome of host vertebrate similar to L1-LINE, the partner of Alu elements, which comprise 17% of the human genome (Lander et al. 2001).

This study confirmed the proliferation of BovB copies in the frog chromosomes of HT destination and also the BovB clades of closely related frogs (Fig. 9), suggesting that BovB was integrated in the germline of their ancestors, and is then passed on to their descendants. On the other hand, regarding the parasites, the present data do not allow discriminating between two scenarios: 1) the BovBs could be integrated in the parasite genomes and then passed on to their hosts (biological transmission) or 2) the parasites may just carry bacteria and/or viruses whose genomes contain BovB, or cells of a previous BovB-containing host (e.g., blood cells derived from blood sucking: mechanical transmission). Further studies including genome sequencing and FISH analysis of parasites and metagenomic analyses of bacteria and viruses infecting snakes and frogs will shed light on the detailed transmission mode of inter-vertebrate HT mediated by parasites, and on the germline integration of BovB in the host organisms.

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Tables

Table 1. The taxon sampling analyzed in this study.

Taxonomy	Species	Host reptiles or amphibians	Voucher		Distribution	Foward primer	Reverse primer	Cytb	16S rRNA	18S rRNA
Snakes										
Acrochordidae	*Acrochordus* sp.	–	ZCYK_	Aara1	Southeast Asia	BovB_ORF5_FowN	BovB_ORF3_RevN	LC640456	–	–
Atractaspididae	*Aparallactus modestus*	–	RBINS_	18841	Africa	BovB_230F	BovB_3200R	LC640410	–	–
Atractaspididae	*Atractaspis corpulenta*	–	RBINS_	18607	Africa	ME1_Fmod	ME2_Rmod	LC640411	–	–
Boidae	*Acrantophis* cf. *dumerili*	–	FGZC_	0977	Madagascar	ME1_Fmod	ME2_Rmod	LC640433	–	–
Boidae	*Candoia aspera*	–	ZCYK_	Casp1	Oceania	BovB_230F	BovB_3200R	LC640457	–	–
Boidae	*Candoia* cf. *aspera*	–	ZCYK_	Casp2	Oceania	BovB_230F	BovB_3200R	LC640436	–	–
Boidae	*Charina bottae*	–	ZCYK_	Cbot1	North America	BovB_230F	BovB_3200R	LC640379	–	–
Boidae	*Corallus caninus*	–	ZCYK_	Ccan1	Central–South America	ME1_Fmod	ME2_Rmod	LC640380	–	–
Boidae	*Epicrates cenchria*	–	ZCYK_	Ecenc1	Central–South America	BovB_230F	BovB_3200R	LC640381	–	–
Boidae	*Eryx colubrinus*	–	ZCYK_	Ecoll1	Africa	BovB_230F	BovB_3200R	LC640458	–	–
Boidae	*Eryx colubrinus*	–	ZCYK_	Ecoll2	Africa	BovB_230F	BovB_3200R	LC640459	–	–
Boidae	*Sanzinia* cf. *madagascariensis*	–	FGZC_	4524	Madagascar	BovB_230F	BovB_3200R	LC640426	–	–
Colubridae	*Borikenophis portoricensis*	–	MVZ_Herp_	235668	Central–South America	ME1_Fmod	ME2_Rmod	LC640450	–	–
Colubridae	*Calamaria* sp.	–	DCAK_	JPS004	East Asia	ME1_Fmod	ME2_Rmod	LC640360	–	–
Colubridae	*Carphophis vermis*	–	MVZ_Herp_	137556	North America	BovB_230F	BovB_ORF3_RevN	LC640398	–	–
Colubridae	*Coluber constrictor*	–	MVZ_Herp_	223513	North America	ME1_Fmod	ME2_Rmod	LC640399	–	–
Colubridae	*Contia tenuis*	–	MVZ_Herp_	246066	North America	ME1_Fmod	ME2_Rmod	LC640401	–	–
Colubridae	*Elaphe climacophora*	–	ZCCK_	050	East Asia	ME1_Fmod	ME2_Rmod	LC640417	–	–
Colubridae	*Elaphe climacophora*	–	ZCCK_	055	East Asia	ME1_Fmod	ME2_Rmod	LC640418	–	–
Colubridae	*Euprepiophis conspicillata*	–	KUZ_	68828	East Asia	ME1_Fmod	ME2_Rmod	LC640364	–	–
Colubridae	*Gonyosoma boulengeri*	–	MVZ_Herp_	226508	Southeast Asia	BovB_ORF5_FowN	BovB_2820R	LC640447	–	–
Colubridae	*Grayia* sp.	–	RBINS_	18987	Africa	ME1_Fmod	ME2_Rmod	LC640446	–	–
Colubridae	*Hebius pryeri*	–	ZCCK_	116	East Asia	ME1_Fmod	ME2_Rmod	LC640423	–	–
Colubridae	*Hebius vibakari*	–	ZCYK_	Avib1	East Asia	ME1_Fmod	ME2_Rmod	LC640368	–	–
Colubridae	*Helicops* cf. *angulatus*	–	ZCYK_	Hang1	Central–South America	ME1_Fmod	ME2_Rmod	LC640378	–	–
Colubridae	*Imantodes* cf. *cenchoa*	–	MVZ_Herp_	263864	Central–South America	ME1_Fmod	ME2_Rmod	LC640403	–	–
Colubridae	*Lycodon semicarinatus*	–	KUZ_	28044	East Asia	ME1_Fmod	ME2_Rmod	LC640371	–	–
Colubridae	*Nerodia erythrogaster*	–	MVZ_Herp_	246070	North America	ME1_Fmod	ME2_Rmod	LC640402	–	–
Colubridae	*Oligodon* cf. *cyclurus*	–	MVZ_Herp_	224173	Southeast Asia	ME1_Fmod	ME2_Rmod	LC640400	–	–
Colubridae	*Opisthotropis lateralis*	–	ZCYK_	Olat1	East Asia	BovB_ORF5_FowN	ME2_Rmod	LC640383	–	–
Colubridae	*Oxyrhopus* cf. *rhombifer*	–	ZCYK_	Orho1	Central–South America	ME1_Fmod	ME2_Rmod	LC640460	–	–
Colubridae	*Philodryas baroni*	–	ZCYK_	Pbar1	Central–South America	ME1_Fmod	ME2_Rmod	LC640377	–	–
Colubridae	*Pseudoxenodon* sp.	–	MVZ_Herp_	224235	Southeast Asia	BovB_ORF5_FowN, BovB_230F	BovB_3090R	LC640430	–	–
Colubridae	*Ptyas* cf. *semicarinata*	–	KUZ_	28049	East Asia	ME1_Fmod	ME2_Rmod	LC640370	–	–
Colubridae	*Rhabdophis tigrinus*	–	DCAK_	JPS002	East Asia	ME1_Fmod	ME2_Rmod	LC640363	–	–
Colubridae	*Sibynophis* cf. *chinensis*	–	MVZ_Herp_	226584	East Asia	BovB_230F	BovB_3090R	LC640443	–	–
Colubridae	*Thelotornis kirtlandii*	–	UniKis_	CRT_4053	Africa	ME1_Fmod	ME2_Rmod	LC640428	–	–
Colubridae	*Toxicodryas* cf. *blandingii*	–	RBINS_	18995	Africa	ME1_Fmod	ME2_Rmod	LC640397	–	–
Colubridae	*Trimerodytes percarinatus*	–	MVZ_Herp_	226593	Africa	ME1_Fmod	ME2_Rmod	LC640442	–	–
Colubridae	*Xenopholis* cf. *scalaris*	–	MVZ_Herp_	247610	Central–South America	ME1_Fmod	ME2_Rmod	LC640441	–	–
Elapidae	*Bungarus candidus*	–	MVZ_Herp_	258148	Southeast Asia	ME1_Fmod	ME2_Rmod	LC640408	–	–
Elapidae	*Bungarus candidus*	–	ZCYK_	Bmul1	Southeast Asia	ME1_Fmod	ME2_Rmod	LC640386	–	–
Elapidae	*Dendroaspis jamesoni*	–	RBINS_	18790	Africa	BovB_ORF5_FowN, ME1_Fmod	BovB_ORF3_RevN, ME2_Rmod	LC640407	–	–
Elapidae	*Elapsoidea* cf. *guentherii*	–	RBINS_	18649	Africa	ME1_Fmod	ME2_Rmod	LC640451	–	–
Elapidae	*Emydocephalus ijimae*	–	KUZ_	28042	East Asia	BovB_ORF5_FowN	BovB_3090R	LC640367	–	–

Table 1. continued.

Taxonomy	Species	Host reptiles or amphibians	Voucher		Distribution	Foward primer	Reverse primer	Cytb	16S rRNA	18S rRNA
Snakes										
Elapidae	*Furina* sp.	–	ABTC_	55626	Oceania	BovB_230F	BovB_3090R	LC640463	–	–
Elapidae	*Hemiaspis damelii*	–	ABTC_	6524	Oceania	BovB_ORF5_FowN	BovB_3090R	LC640406	–	–
Elapidae	*Hydrophis melanocephalus*	–	NUM_	Az0368	East Asia	BovB_ORF5_FowN	BovB_3090R	LC640369	–	–
Elapidae	*Hydrophis ornatus*	–	KUZ_	21782	East Asia	BovB_ORF5_FowN	BovB_3090R	LC640437	–	–
Elapidae	*Laticauda semifasciata*	–	DCAK_	JPS003	East Asia	BovB_230F	BovB_3200R	LC640427	–	–
Elapidae	*Micropechis* sp.	–	PCMB_	5586	Oceania	BovB_ORF5_FowN	BovB_ORF3_RevN	LC640395	–	–
Elapidae	*Naja melanoleuca*	–	RBINS_	19017	Africa	ME1_Fmod	ME2_Rmod	LC640452	–	–
Elapidae	*Notechis scutatus*	–	ABTC_	56219	Oceania	BovB_ORF5_FowN	BovB_ORF3_RevN	LC640464	–	–
Elapidae	*Parahydrophis mertoni*	–	ABTC_	28239	Oceania	BovB_230F	BovB_3200R	LC640445	–	–
Elapidae	*Simoselaps bertholdi*	–	ABTC_	56525	Oceania	BovB_230F	BovB_3090R	LC640404	–	–
Elapidae	*Sinomicrurus japonicus*	–	ZCYK_	Cjapb1	East Asia	BovB_ORF5_FowN, ME1_Fmod	BovB_ORF3_RevN, ME2_Rmod	LC640366	–	–
Elapidae	*Vermicella intermedia*	–	ABTC_	56627	Oceania	ME1_Fmod	ME2_Rmod	LC640405	–	–
Homalopsidae	*Hypsiscopus plumbea*	–	MVZ_Herp_	230458	East Asia	BovB_230F	BovB_3090R	LC640396	–	–
Lamprophiidae	*Bothrophthalmus lineatus*	–	RBINS_	18996	Africa	ME1_Fmod	ME2_Rmod	LC640409	–	–
Leptotyphlopidae	*Rena humilis*	–	MVZ_	14037	North America	BovB_ORF5_FowN	BovB_ORF3_RevN	LC640372	–	–
Loxocemidae	*Loxocemus bicolor*	–	ZCYK_	Lbic1	Central–South America	ME1_Fmod	ME2_Rmod	LC640373	–	–
Pareidae	*Pareas iwasakii*	–	MHZC_	032	East Asia	BovB_ORF5_FowN	BovB_ORF3_RevN	LC640358	–	–
Pareidae	*Pareas iwasakii*	–	MHZC_	033	East Asia	BovB_ORF5_FowN	BovB_ORF3_RevN	LC640422	–	–
Prosymnidae	*Prosymna* sp.	–	MVZ_Herp_	245380	Africa	BovB_230F	BovB_3090R	LC640444	–	–
Psammophiidae	*Mimophis* cf. *mahfalensis*	–	FGZC_	0842	Madagascar	BovB_230F	BovB_3200R	LC640387	–	–
Psammophiidae	*Psammophis phillipsii*	–	RBINS_	18680	Africa	ME1_Fmod	ME2_Rmod	LC640454	–	–
Pseudaspididae	*Psammodynastes pulverulentus*	–	KUZ_	R88353	East Asia	ME1_Fmod	ME2_Rmod	LC640421	–	–
Pseudaspididae	*Psammodynastes pulverulentus*	–	KUZ_	R88354	East Asia	ME1_Fmod	ME2_Rmod	LC640453	–	–
Pseudaspididae	*Pseudaspis* cf. *cana*	–	ZCCK_	118	Africa	ME1_Fmod	ME2_Rmod	LC640425	–	–
Pseudoxyrhophiidae	*Alluaudina bellyi*	–	FGZC_	0901	Madagascar	BovB_230F	BovB_3200R	LC640439	–	–
Pseudoxyrhophiidae	*Dromicodryas quadrilineatus*	–	ZCMV_	11233	Madagascar	BovB_230F	BovB_3200R	LC640393	–	–
Pseudoxyrhophiidae	*Elapotinus picteti*	–	ZCMV_	02220	Madagascar	BovB_ORF5_FowN	BovB_ORF3_RevN	LC640391	–	–
Pseudoxyrhophiidae	*Ithycyphus* sp.	–	ZCMV_	05467	Madagascar	BovB_230F	BovB_3200R	LC640394	–	–
Pseudoxyrhophiidae	*Langaha* cf. *madagascariensis*	–	ZSM_	567/2001	Madagascar	BovB_230F	BovB_3200R	LC640438	–	–
Pseudoxyrhophiidae	*Leioheterodon geayi*	–	ZSM_	1075/2003	Madagascar	BovB_230F	BovB_3200R	LC640392	–	–
Pseudoxyrhophiidae	*Heteroliodon* sp.	–	ZSM_	548/2000	Madagascar	BovB_230F	BovB_3200R	LC640468	–	–
Pseudoxyrhophiidae	*Liophidium* sp.	–	ZCMV_	02956	Madagascar	BovB_230F	BovB_3200R	LC640435	–	–
Pseudoxyrhophiidae	*Liophidium torquatum*	–	ZCRK_	185	Madagascar	ME1_Fmod	ME2_Rmod	LC640355	–	–
Pseudoxyrhophiidae	*Liophidium torquatum*	–	ZCRK_	182	Madagascar	ME1_Fmod	ME2_Rmod	LC640354	–	–
Pseudoxyrhophiidae	*Liopholidophis sexlineatus*	–	FGZC_	4586	Madagascar	BovB_230F	BovB_3200R	LC640389	–	–
Pseudoxyrhophiidae	*Lycodryas citrinus*	–	FGZC_	0837	Madagascar	BovB_230F	BovB_3200R	LC640390	–	–
Pseudoxyrhophiidae	*Madagascarophis colubrinus*	–	ZSM_	602/2003	Madagascar	BovB_230F	BovB_3200R	AY586246	–	–
Pseudoxyrhophiidae	*Parastenophis betsileanus*	–	FGZC_	0198	Madagascar	BovB_230F	BovB_3200R	LC640388	–	–
Pseudoxyrhophiidae	*Phisalixella variabilis*	–	FGZC_	0526	Madagascar	BovB_230F	BovB_3200R	LC640440	–	–
Pseudoxyrhophiidae	*Thamnosophis* sp.	–	ZCRK_	188	Madagascar	ME1_Fmod	ME2_Rmod	LC640467	–	–
Pseudoxyrhophiidae	*Thamnosophis infrasignatus*	–	ZCRK_	171	Madagascar	ME1_Fmod	ME2_Rmod	LC640353	–	–
Pseudoxyrhophiidae	*Thamnosophis lateralis*	–	FGZC_	2553	Madagascar	ME1_Fmod	ME2_Rmod	LC640434	–	–
Pseudoxyrhophiidae	*Thamnosophis lateralis*	–	ZCRK_	193	Madagascar	ME1_Fmod	ME2_Rmod	LC640356	–	–
Pseudoxyrhophiidae	*Thamnosophis lateralis*	–	ZCRK_	093	Madagascar	ME1_Fmod	ME2_Rmod	LC640357	–	–
Pythonidae	*Antaresia stimsoni*	–	ZCYK_	Achi1	Oceania	BovB_ORF5_FowN	BovB_ORF3_RevN	LC640375	–	–

Table 1. continued.

Taxonomy	Species	Host reptiles or amphibians	Voucher		Distribution	Foward primer	Reverse primer	Cytb	16S rRNA	18S rRNA
Snakes										
Pythonidae	*Bothrochilus* cf. *albertisii*	–	ZCYK_	Lalb1	Oceania	BovB_230F	BovB_3200R	LC640376	–	–
Pythonidae	*Malayopython reticulatus*	–	ZCYK_	Pret1	Southeast Asia	BovB_ORF5_FowN	BovB_ORF3_RevN	LC640374	–	–
Pythonidae	*Morelia spilota*	–	ZCYK_	Mspis1	Oceania	BovB_230F	BovB_3200R	LC640385	–	–
Pythonidae	*Morelia viridis*	–	ZCYK_	Mvir2	Oceania	BovB_230F	BovB_3200R	LC640384	–	–
Pythonidae	*Python regius*	–	ZCYK_	PYT1	Africa	ME1_Fmod	ME2_Rmod	LC640455	–	–
Typhlopidae	*Afrotyphlops* sp.	–	RBINS_	18722	Africa	ME1_Fmod	ME2_Rmod	LC640429	–	–
Typhlopidae	*Afrotyphlops punctatus*	–	MVZ_Herp_	249819	Africa	ME1_Fmod	BovB_ORF3_RevN	LC640432	–	–
Typhlopidae	*Indotyphlops braminus*	–	ZCMV_	02290	Western–Central–Southern Asia	BovB_230F	BovB_3200R	KT316547	–	–
Typhlopidae	*Indotyphlops braminus*	–	DCAK_	JPS001	Western–Central–Southern Asia	BovB_230F	BovB_3200R	LC640465	–	–
Typhlopidae	*Madatyphlops arenarius*	–	ZCMV_	05540	Madagascar	BovB_ORF5_FowN	BovB_3200R	KT316533	–	–
Typhlopidae	*Xerotyphlops vermicularis*	–	MVZ_Herp_	218698	Europe	BovB_230F	BovB_3200R	LC640431	–	–
Typhlopidae	Typhlopidae sp.	–	PCMB_	5526	Oceania	BovB_230F	BovB_3200R	LC640469	–	–
Viperidae	*Bothriechis lateralis*	–	MVZ_Herp_	207372	Central–South America	BovB_230F	BovB_3200R	LC640412	–	–
Viperidae	*Bothrops ammodytoides*	–	MVZ_Herp_	223514	Central–South America	ME1_Fmod	ME2_Rmod	LC640413	–	–
Viperidae	*Cerastes vipera*	–	MVZ_Herp_	235728	Africa	ME1_Fmod	ME2_Rmod	LC640449	–	–
Viperidae	*Cerrophidion godmani*	–	MVZ_Herp_	264333	Central–South America	BovB_ORF5_FowN	BovB_ORF3_RevN	LC640420	–	–
Viperidae	*Crotalus viridis*	–	MVZ_Herp_	128204	North America	BovB_230F	BovB_3200R	LC640448	–	–
Viperidae	*Gloydius blomhoffii*	–	DCAK_	JPS005	East Asia	ME1_Fmod	ME2_Rmod	LC640359	–	–
Viperidae	*Gloydius* cf. *tsushimaensis*	–	ZCCK_	048	East Asia	ME1_Fmod	ME2_Rmod	LC640416	–	–
Viperidae	*Gloydius* cf. *tsushimaensis*	–	ZCCK_	071	East Asia	ME1_Fmod	ME2_Rmod	LC640419	–	–
Viperidae	*Ovophis okinavensis*	–	DCAK_	JPS007	East Asia	ME1_Fmod	ME2_Rmod	LC640362	–	–
Viperidae	*Ovophis okinavensis*	–	ZCCK_	117	East Asia	ME1_Fmod	ME2_Rmod	LC640424	–	–
Viperidae	*Protobothrops flavoviridis*	–	DCAK_	JPS006	East Asia	ME1_Fmod	ME2_Rmod	LC640361	–	–
Viperidae	*Protobothrops mucrosquamatus*	–	MVZ_Herp_	224296	Southeast Asia	ME1_Fmod	ME2_Rmod	LC640414	–	–
Viperidae	*Tropidolaemus* cf. *subannulatus*	–	MVZ_Herp_	253952	Southeast Asia	ME1_Fmod	ME2_Rmod	LC640415	–	–
Xenodermidae	*Achalinus* sp.	–	ZCYK_	Aspi1	East Asia	BovB_ORF5_FowN	BovB_3200R	LC640365	–	–
Xenopeltidae	*Xenopeltis unicolor*	–	ZCYK_	Xuni1	Southeast Asia	BovB_ORF5_FowN	ME2_Rmod	LC640382	–	–
Xenotyphlopidae	*Xenotyphlops grandidieri*	–	FGZC_	1023	Madagascar	BovB_230F	BovB_3200R	KF770846	–	–
Lizards										
Chamaeleonidae	Chamaeleonidae sp.	–	ZCMV_	12236	Madagascar	ME1_Fmod	ME2_Rmod	LC640466	–	–
Gekkonidae	*Phelsuma lineata*	–	ZCMV_	11299	Madagascar	BovB_230F	BovB_3200R	LC640461	–	–
Gerrhosauridae	*Amphiglossus* sp.	–	ZCMV_	02404	Madagascar	ME1_Fmod	ME2_Rmod	LC640462	–	–
Frogs										
Bufonidae	*Bufo bufo*	–	IABHU_	04008	Europe	BovB_ORF5_FowN	BovB_ORF3_RevN	–	LC640489	–
Bufonidae	*Bufo japonicus*	–	DCAK_	JP0001	East Asia	BovB_ORF5_FowN, BovB_230F	BovB_ORF3_RevN, BovB_3090R	–	LC640480	–
Bufonidae	*Duttaphrynus melanostictus*	–	IABHU_	04446	Southeast Asia	BovB_ORF5_FowN	ME2_Rmod	–	LC640494	–
Bufonidae	*Duttaphrynus melanostictus*	–	IABHU_	04998	Southeast Asia	BovB_ORF5_FowN	ME2_Rmod	–	LC640495	–
Bufonidae	*Leptophryne borbonica*	–	ZCYK_	Lbor1	Southeast Asia	BovB_ORF5_FowN	ME2_Rmod	–	LC640631	–
Bufonidae	*Rentapia hosii*	–	ZCYK_	Phos1	Southeast Asia	BovB_ORF5_FowN	BovB_ORF3_RevN	–	LC640474	–
Ceratobatrachidae	*Cornufer pelewensis*	–	IABHU_	19162	Oceania	BovB_230F	BovB_3200R	–	LC640503	–
Dicroglossidae	*Euphlyctis kalasgramensis*	–	IABHU_	02013	Western–Central–Southern Asia	BovB_ORF5_FowN	ME2_Rmod	–	LC640486	–
Dicroglossidae	*Fejervarya orissaensis*	–	IABHU_	02032	Western–Central–Southern Asia	BovB_ORF5_FowN	BovB_ORF3_RevN	–	LC640493	–
Dicroglossidae	*Hoplobatrachus rugulosus*	–	IABHU_	04102	Southeast Asia	BovB_ORF5_FowN	ME2_Rmod	–	LC640504	–
Hyperoliidae	*Heterixalus alboguttatus*	–	IABHU_	06958	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640470	–
Hyperoliidae	*Heterixalus betsileo*	–	UADBA_	AK041208_011	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640481	–

Table 1. continued.

Taxonomy	Species	Host reptiles or amphibians	Voucher		Distribution	Foward primer	Reverse primer	Cytb	16S rRNA	18S rRNA
Frogs										
Hyperoliidae	*Heterixalus betsileo*	–	ZCMV_	15011	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640518	–
Hyperoliidae	*Heterixalus madagascariensis*	–	ZCRK_	175	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640605	–
Hyperoliidae	*Heterixalus madagascariensis*	–	ZCRK_	178	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640514	–
Hyperoliidae	*Heterixalus punctatus*	–	ZCRK_	119	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640600	–
Hyperoliidae	*Heterixalus punctatus*	–	ZCMV_	15008	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640515	–
Hyperoliidae	*Heterixalus punctatus*	–	ZCMV_	15009	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640516	–
Mantellidae	*Aglyptodactylus madagascariensis*	–	IABHU_	06788	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640499	–
Mantellidae	*Blommersia blommersae*	–	ZCRK_	043	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640591	–
Mantellidae	*Blommersia blommersae*	–	ZCMV_	15010	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640517	–
Mantellidae	*Boophis goudotii*	–	ZCAK_	MD0005	Madagascar	BovB_ORF5_FowN, ME1_Fmod	BovB_ORF3_RevN, ME2_Rmod	–	LC640477	–
Mantellidae	*Boophis goudotii*	–	ZCRK_	128	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640511	–
Mantellidae	*Boophis madagascariensis*	–	ZCRK_	163	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640604	–
Mantellidae	*Boophis pyrrhus*	–	ZCRK_	075	Madagascar	BovB_ORF5_FowN	BovB_ORF3_RevN	–	LC640596	–
Mantellidae	*Boophis viridis*	–	ZCMV_	15006	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640617	–
Mantellidae	*Gephyromantis boulengeri*	–	ZCRK_	151	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640602	–
Mantellidae	*Guibemantis bicalcaratus*	–	ZCRK_	144	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640601	–
Mantellidae	*Guibemantis flavobrunneus*	–	ZCMV_	15028	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640507	–
Mantellidae	*Guibemantis tornieri*	–	ZCRK_	071	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640597	–
Mantellidae	*Guibemantis tornieri*	–	ZCRK_	122	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640510	–
Mantellidae	*Mantella betsileo*	–	ZCAK_	MD0001	Madagascar	BovB_230F, ME1_Fmod	BovB_3200R, ME2_Rmod	–	LC640501	–
Mantellidae	*Mantella madagascariensis*	–	IABHU_	06793	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640500	–
Mantellidae	*Mantidactylus betsileanus*	–	ZCRK_	052	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640594	–
Mantellidae	*Mantidactylus betsileanus*	–	ZCRK_	099	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640509	–
Mantellidae	*Mantidactylus betsileanus*	–	ZCMV_	15012	Madagascar	ME1_Fmod	ME2_Rmod	–	MH730073	–
Mantellidae	*Mantidactylus femoralis*	–	ZCRK_	049	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640593	–
Mantellidae	*Mantidactylus femoralis*	–	ZCMV_	15013	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640519	–
Mantellidae	*Mantidactylus lugubris*	–	ZCRK_	046	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640592	–
Mantellidae	*Mantidactylus melanopleura*	–	ZCRK_	087	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640599	–
Megophryidae	*Megophrys nasuta*	–	ZCAK_	SEA0001	Southeast Asia	BovB_230F, ME1_Fmod	BovB_3090R, ME2_Rmod	–	LC640606	–
Megophryidae	*Megophrys nasuta*	–	ZCYK_	Bcar1	Southeast Asia	ME1_Fmod	ME2_Rmod	–	LC640472	–
Megophryidae	*Megophrys nanlingensis*	–	ZCYK_	Xman1	East Asia	BovB_ORF5_FowN	ME2_Rmod	–	LC640471	–
Microhylidae	*Barygenys flavigularis*	–	IABHU_	06597	Oceania	BovB_230F	BovB_3200R	–	LC640497	–
Microhylidae	*Cophixalus cryptotympanum*	–	IABHU_	06602	Oceania	ME1_Fmod	ME2_Rmod	–	AB611880	–
Microhylidae	*Cophyla barbouri*	–	ZCMV_	15015	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640618	–
Microhylidae	*Cophyla tuberifera*	–	ZCRK_	156	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640603	–
Microhylidae	*Cophyla tuberifera*	–	ZCRK_	160	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640512	–
Microhylidae	*Cophyla tuberifera*	–	ZCRK_	166	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640513	–
Microhylidae	*Dyscophus guineti*	–	ZCAK_	MD0010	Madagascar	BovB_230F	BovB_3200R	–	LC640473	–
Microhylidae	*Plethodontohyla inguinalis*	–	UADBA_	AK041208_001	Madagascar	BovB_ORF5_FowN, BovB_230F	BovB_3200R	–	LC640502	–
Microhylidae	*Plethodontohyla mihanika*	–	ZCRK_	084	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640598	–
Microhylidae	*Plethodontohyla notosticta*	–	ZCMV_	15016	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640506	–
Microhylidae	*Plethodontohyla ocellata*	–	ZCYK_	Poce1	Madagascar	BovB_230F	BovB_3090R	–	LC640476	–
Microhylidae	*Scaphiophryne madagascariensis*	–	ZCAK_	MD0009	Madagascar	BovB_230F	BovB_3200R	–	LC640505	–
Ptychadenidae	*Ptychadena mascareniensis*	–	ZCRK_	059	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640508	–
Ptychadenidae	*Ptychadena mascareniensis*	–	ZCRK_	060	Madagascar	ME1_Fmod	ME2_Rmod	–	LC640595	–

Table 1. continued.

Taxonomy	Species	Host reptiles or amphibians	Voucher		Distribution	Foward primer	Reverse primer	Cytb	16S rRNA	18S rRNA
Frogs										
Ranidae	*Babina subaspera*	–	IABHU_	05239	East Asia	BovB_ORF5_FowN	ME2_Rmod	–	LC640488	–
Ranidae	*Papurana* sp.	–	IABHU_	06647	Oceania	ME1_Fmod	ME2_Rmod	–	LC640498	–
Ranidae	*Hylarana* cf. *tytleri*	–	IABHU_	02024	Western–Central–Southern Asia	BovB_230F	BovB_3200R	–	LC640487	–
Ranidae	*Odorrana ishikawae*	–	IABHU_	05275	East Asia	BovB_ORF5_FowN	BovB_3200R	–	AB511282	–
Ranidae	*Odorrana splendida*	–	IABHU_	05140	East Asia	BovB_ORF5_FowN	ME2_Rmod	–	LC640496	–
Ranidae	*Pelophylax bergeri*	–	ZCYK_	Rles1	Europe	BovB_ORF5_FowN	ME2_Rmod	–	LC640475	–
Ranidae	*Pelophylax nigromaculatus*	–	DCAK_	JP0002	East Asia	BovB_ORF5_FowN	ME2_Rmod	–	LC640491	–
Rhacophoridae	*Buergeria buergeri*	–	DCAK_	JP0005	East Asia	BovB_670F	BovB_2460R	–	LC640478	–
Alytidae	*Alytes obstetricans*	–	ZCAK_	EU0001	Europe	–	–	–	LC640555	–
Arthroleptidae	*Leptopelis vermiculatus*	–	ZCAK_	AF0012	Africa	–	–	–	LC640627	–
Bombinatoridae	*Bombina orientalis*	–	DCAK_	EA0002	East Asia	–	–	–	LC640571	–
Brevicipitidae	*Breviceps adspersus*	–	ZCAK_	AF0001	Africa	–	–	–	LC640556	–
Brevicipitidae	*Breviceps mossambicus*	–	ZCAK_	AF0002	Africa	–	–	–	LC640557	–
Bufonidae	*Anaxyrus americanus*	–	IABHU_	04202	North America	–	–	–	LC640490	–
Bufonidae	*Anaxyrus debilis*	–	ZCYK_	Bdeb1	North America	–	–	–	LC640609	–
Bufonidae	*Anaxyrus terrestris*	–	ZCYK_	Bter	North America	–	–	–	LC640575	–
Bufonidae	*Atelopus barbotini*	–	ZCYK_	Aspuh1	Central–South America	–	–	–	LC640608	–
Bufonidae	*Bufo gargarizans*	–	IABHU_	04436	East Asia	–	–	–	LC640620	–
Bufonidae	*Duttaphrynus stomaticus*	–	ZCYK_	Boli	Western–Central–Southern Asia	–	–	–	LC640611	–
Bufonidae	*Ingerophrynus parvus*	–	ZCYK_	Bpar1	Southeast Asia	–	–	–	LC640612	–
Bufonidae	*Melanophryniscus stelzneri*	–	ZCYK_	Mstes1	Central–South America	–	–	–	LC640613	–
Bufonidae	*Rhaebo guttatus*	–	ZCYK_	Bgut1	Central–South America	–	–	–	LC640610	–
Bufonidae	*Rhinella diptycha*	–	IABHU_	04952	Central–South America	–	–	–	LC640544	–
Bufonidae	*Sclerophrys gutturalis*	–	ZCAK_	AF0010	Africa	–	–	–	LC640574	–
Bufonidae	*Strauchbufo raddei*	–	IABHU_	04433	East Asia	–	–	–	LC640543	–
Ceratophryidae	*Ceratophrys cornuta*	–	ZCAK_	SA0006	Central–South America	–	–	–	LC640582	–
Dendrobatidae	*Dendrobates tinctorius*	–	ZCAK_	SA0005	Central–South America	–	–	–	LC640583	–
Dendrobatidae	*Phyllobates aurotaenia*	–	ZCYK_	Paur1	Central–South America	–	–	–	LC640614	–
Dendrobatidae	*Ranitomeya amazonica*	–	ZCAK_	SA0004	Central–South America	–	–	–	LC640584	–
Dicroglossidae	*Fejervarya kawamurai*	–	DCAK_	JP0012	East Asia	–	–	–	LC640568	–
Dicroglossidae	*Fejervarya* sp.	–	IABHU_	03308	Western–Central–Southern Asia	–	–	–	LC640536	–
Dicroglossidae	*Hoplobatrachus occipitalis*	–	IABHU_	20699	Africa	–	–	–	LC640619	–
Dicroglossidae	*Limnonectes* sp.	–	ZCYK_	Rbly	Southeast Asia	–	–	–	LC640524	–
Dicroglossidae	*Nanorana maculosa*	–	ZCYK_	Pmac1	East Asia	–	–	–	LC640523	–
Dicroglossidae	*Occidozyga lima*	–	IABHU_	04104	Southeast Asia	–	–	–	LC640484	–
Dicroglossidae	*Quasipaa spinosa*	–	ZCAK_	EA0004	East Asia	–	–	–	LC640572	–
Hemiphractidae	*Gastrotheca peruana*	–	ZCYK_	Gastsp1	Central–South America	–	–	–	LC640615	–
Hemisotidae	*Hemisus marmoratus*	–	ZCYK_	Hmar1	Africa	–	–	–	LC640526	–
Hylidae	*Boana boans*	–	ZCYK_	Hboa1	Central–South America	–	–	–	LC640616	–
Hylidae	*Dendropsophus melanargyreus*	–	ZCAK_	SA0003	Central–South America	–	–	–	LC640586	–
Hylidae	*Dryophytes cinereus*	–	ZCAK_	NA0004	North America	–	–	–	LC640576	–
Hylidae	*Dryophytes japonicus*	–	IABHU_	06123	East Asia	–	–	–	AB303949	–
Hylidae	*Dryophytes chrysoscelis*	–	ZCYK_	Hver1	North America	–	–	–	LC640624	–
Hylidae	*Hyla chinensis*	–	IABHU_	06164	East Asia	–	–	–	LC640545	–
Hylidae	*Pseudis paradoxa*	–	ZCYK_	Ppara1	Central–South America	–	–	–	LC640521	–

Table 1. continued.

Taxonomy	Species	Host reptiles or amphibians	Voucher		Distribution	Foward primer	Reverse primer	Cytb	16S rRNA	18S rRNA
Frogs										
Hylidae	*Trachycephalus resinifictrix*	–	ZCYK_	Pres1	Central–South America	–	–	–	LC640607	–
Hyperoliidae	*Hyperolius marmoratus*	–	ZCAK_	AF0008	Africa	–	–	–	LC640559	–
Hyperoliidae	*Hyperolius puncticulatus*	–	ZCAK_	AF0009	Africa	–	–	–	LC640560	–
Hyperoliidae	*Phlyctimantis maculatus*	–	ZCAK_	AF0007	Africa	–	–	–	LC640561	–
Leptodactylidae	*Leptodactylus knudseni*	–	ZCAK_	SA0007	Central–South America	–	–	–	LC640485	–
Leptodactylidae	*Pleurodema brachyops*	–	ZCYK_	Pbra1	Central–South America	–	–	–	LC640623	–
Limnodynastidae	*Limnodynastes salmini*	–	ZCYK_	Lsal1	Oceania	–	–	–	LC640626	–
Mantellidae	*Boophis doulioti*	–	ZCMV_	05651	Madagascar	–	–	–	LC640492	–
Mantellidae	*Laliostoma labrosum*	–	FGZC_	3011	Madagascar	–	–	–	LC640573	–
Mantellidae	*Tsingymantis antitra*	–	FGZC_	0531	Madagascar	–	–	–	AY848213	–
Megophryidae	*Leptobrachium hasseltii*	–	ZCYK_	Vlei1	Southeast Asia	–	–	–	LC640621	–
Microhylidae	*Chaperina fusca*	–	ZCYK_	Cfus1	Southeast Asia	–	–	–	LC640529	–
Microhylidae	*Ctenophryne geayi*	–	ZCYK_	Cgea1	Central–South America	–	–	–	LC640530	–
Microhylidae	*Dermatonotus muelleri*	–	ZCYK_	Dmul1	Central–South America	–	–	–	LC640531	–
Microhylidae	*Elachistocleis bicolor*	–	ZCAK_	SA0002	Central–South America	–	–	–	LC640585	–
Microhylidae	*Gastrophryne olivacea*	–	ZCAK_	NA0003	North America	–	–	–	LC640589	–
Microhylidae	*Glyphoglossus guttulatus*	–	ZCAK_	SEA0006	Southeast Asia	–	–	–	LC640579	–
Microhylidae	*Glyphoglossus molossus*	–	ZCAK_	SEA0007	Southeast Asia	–	–	–	LC640625	–
Microhylidae	*Kalophrynus interlineatus*	–	ZCAK_	SEA0017	Southeast Asia	–	–	–	LC640482	–
Microhylidae	*Kalophrynus sinensis*	–	ZCAK_	SEA0003	Southeast Asia	–	–	–	LC640483	–
Microhylidae	*Kaloula pulchra*	–	ZCYK_	Kpul1	Southeast Asia	–	–	–	LC640532	–
Microhylidae	*Microhyla nilphamariensis*	–	IABHU_	02004	Western–Central–Southern Asia	–	–	–	LC640549	–
Microhylidae	*Microhyla okinavensis*	–	DCAK_	JP0015	East Asia	–	–	–	LC640569	–
Microhylidae	*Microhyla mukhlesuri*	–	IABHU_	04103	Southeast Asia	–	–	–	LC640542	–
Microhylidae	*Microhyla pulchra*	–	ZCYK_	Mpul1	Southeast Asia	–	–	–	LC640628	–
Microhylidae	*Phrynomantis bifasciatus*	–	ZCAK_	AF0004	Africa	–	–	–	LC640550	–
Microhylidae	*Phrynomantis microps*	–	ZCAK_	AF0003	Africa	–	–	–	LC640563	–
Pelodryadidae	*Nyctimystes infrafrenatus*	–	IABHU_	06698	Oceania	–	–	–	LC640547	–
Pelodryadidae	*Ranoidea caerulea*	–	ZCYK_	Lcae1	Oceania	–	–	–	LC640520	–
Pelodryadidae	*Ranoidea gracilenta*	–	ZCYK_	Lgra1	Oceania	–	–	–	LC640622	–
Phrynobatrachidae	*Phrynobatrachus acridoides*	–	ZCAK_	AF0006	Africa	–	–	–	LC640562	–
Phyllomedusidae	*Agalychnis callidryas*	–	ZCAK_	SA0001	Central–South America	–	–	–	LC640581	–
Pipidae	*Pipa parva*	–	ZCYK_	Ppar1	Central–South America	–	–	–	LC640533	–
Pipidae	*Xenopus laevis*	–	DCAK_	AF0002	Africa	–	–	–	LC640629	–
Pipidae	*Xenopus tropicalis*	–	DCAK_	AF0001	Africa	–	–	–	LC640630	–
Ptychadenidae	*Hildebrandtia* sp.	–	ZCYK_	Horn1	Africa	–	–	–	LC640522	–
Ptychadenidae	*Ptychadena nilotica*	–	ZCYK_	Pmos1	Africa	–	–	–	LC640525	–
Pyxicephalidae	*Pyxicephalus adspersus*	–	ZCAK_	AF0011	Africa	–	–	–	LC640564	–
Ranidae	*Glandirana rugosa*	–	IABHU_	15117	East Asia	–	–	–	LC640548	–
Ranidae	*Hylarana erythraea*	–	DCAK_	SEA0008	Southeast Asia	–	–	–	LC640590	–
Ranidae	*Hylarana latouchii*	–	IABHU_	03678	East Asia	–	–	–	LC640538	–
Ranidae	*Lithobates catesbeianus*	–	DCAK_	JP0014	North America	–	–	–	LC640479	–
Ranidae	*Lithobates sphenocephalus*	–	ZCAK_	NA0002	North America	–	–	–	LC640577	–
Ranidae	*Papurana volkerjane*	–	IABHU_	06695	Oceania	–	–	–	LC640546	–
Ranidae	*Rana amurensis*	–	IABHU_	03683	East Asia	–	–	–	LC640539	–

Table 1. continued.

Taxonomy	Species	Host reptiles or amphibians	Voucher		Distribution	Foward primer	Reverse primer	Cytb	16S rRNA	18S rRNA
Frogs										
Ranidae	*Rana arvalis*	–	IABHU_	03010	Europe	–	–	–	LC640534	–
Ranidae	*Rana chensinensis*	–	IABHU_	03282	East Asia	–	–	–	LC640535	–
Ranidae	*Rana japonica*	–	DCAK_	JP0010	East Asia	–	–	–	LC640553	–
Ranidae	*Rana kobai*	–	DCAK_	JP0011	East Asia	–	–	–	LC640554	–
Ranidae	*Rana sakuraii*	–	IABHU_	03800	East Asia	–	–	–	LC640541	–
Ranidae	*Rana tagoi*	–	IABHU_	03524	East Asia	–	–	–	LC640537	–
Ranidae	*Rana temporaria*	–	IABHU_	03702	Europe	–	–	–	LC640540	–
Rhacophoridae	*Buergeria japonica*	–	DCAK_	JP0004	East Asia	–	–	–	LC640565	–
Rhacophoridae	*Buergeria robusta*	–	DCAK_	EA0001	East Asia	–	–	–	LC640566	–
Rhacophoridae	*Chiromantis xerampelina*	–	ZCAK_	AF0005	Africa	–	–	–	LC640558	–
Rhacophoridae	*Kurixalus eiffingeri*	–	ZCAK_	JA0020	East Asia	–	–	–	LC640551	–
Rhacophoridae	*Nyctixalus pictus*	–	ZCAK_	SEA0005	Southeast Asia	–	–	–	LC640580	–
Rhacophoridae	*Polypedates leucomystax*	–	ZCYK_	Pleu1	Southeast Asia	–	–	–	LC640527	–
Rhacophoridae	*Zhangixalus prominanus*	–	ZCYK_	Rpro1	Southeast Asia	–	–	–	LC640528	–
Rhacophoridae	*Zhangixalus schlegelii*	–	DCAK_	JP0003	East Asia	–	–	–	LC640570	–
Rhinophrynidae	*Rhinophrynus dorsalis*	–	ZCAK_	CA0001	Central–South America	–	–	–	LC640587	–
Scaphiopodidae	*Scaphiopus holbrookii*	–	ZCAK_	NA0001	North America	–	–	–	LC640578	–
Caecilians										
Typhlonectidae	*Typhlonectes natans*	–	DCAK_	SAG001	Central–South America	–	–	–	LC640588	–
Salamanders										
Hynobiidae	*Hynobius naevius*	–	DCAK_	JPC0002	East Asia	–	–	–	LC640552	–
Salamandridae	*Cynops* sp.	–	DCAK_	JPC0001	East Asia	–	–	–	LC640567	–
Parasites										
Acari	*Amblyomma* sp.	*Gloydius tsushimaensis*	ZCCK_	047	East Asia	ME1_Fmod	ME2_Rmod	–	–	LC624819
Nematoda	Ascarididae sp.	*Ovophis okinavensis*	ZCCK_	088	East Asia	ME1_Fmod	ME2_Rmod	–	–	LC624821
Nematoda	*Cosmocerca simile*	*Buergeria japonica*	ZCCK_	038	East Asia	ME1_Fmod	ME2_Rmod	–	–	LC624826
Nematoda	Rhabditida sp. 1	*Gloydius tsushimaensis*	ZCCK_	057	East Asia	ME1_Fmod	ME2_Rmod	–	–	LC624864
Acanthocephala	*Acanthocephalus* sp.	*Lycodon rufozonatus*	ZCCK_	019	East Asia	–	–	–	–	LC624818
Acanthocephala	*Centrorhynchus conspectus*	*Rana japonica*	ZCCK_	025	East Asia	–	–	–	–	LC624822
Acanthocephala	*Centrorhynchus* sp. 1	*Zhangixalus owstoni*	ZCCK_	059	East Asia	–	–	–	–	LC624823
Acanthocephala	*Centrorhynchus* sp. 1	*Odorrana supranarina*	ZCCK_	068	East Asia	–	–	–	–	LC624824
Acanthocephala	*Centrorhynchus* sp. 2	*Odorrana supranarina*	ZCCK_	069	East Asia	–	–	–	–	LC624825
Acanthocephala	Polymorphidae sp.	*Glandirana rugosa*	ZCCK_	034	East Asia	–	–	–	–	LC624855
Acanthocephala	*Pseudoacanthocephalus* sp. 1	*Zhangixalus arboreus*	ZCCK_	039	East Asia	–	–	–	–	LC624856
Acanthocephala	*Pseudoacanthocephalus* sp. 1	*Bufo japonicus*	ZCCK_	107	East Asia	–	–	–	–	LC624857
Acanthocephala	*Pseudoacanthocephalus* sp. 2	*Buergeria japonica*	ZCCK_	083	East Asia	–	–	–	–	LC624858
Acari	*Tyrophagus* sp.	*Bufo japonicus*	ZCCK_	003	East Asia	–	–	–	–	LC624879
Diptera	*Aedes* sp.	free living	ZCCK_	014	East Asia	–	–	–	–	LC624840
Diptera	*Culex* sp.	human	ZCCK_	015	East Asia	–	–	–	–	LC624839
Hemiptera	*Appasus japonicus*	free living	ZCCK_	024	East Asia	–	–	–	–	LC624820
Hemiptera	*Gerris buenoi*	free living	ZCCK_	011	East Asia	–	–	–	–	LC624842
Hemiptera	*Ranatra chinensis*	free living	ZCCK_	023	East Asia	–	–	–	–	LC624859
Hemiptera	Reduviidae sp. 1	free living	ZCCK_	018	East Asia	–	–	–	–	LC624860
Hemiptera	Reduviidae sp. 2	free living	ZCCK_	022	East Asia	–	–	–	–	LC624861
Hemiptera	Reduviidae sp. 3	free living	ZCCK_	051	East Asia	–	–	–	–	LC624862

Table 1. continued.

Taxonomy	Species	Host reptiles or amphibians	Voucher		Distribution	Foward primer	Reverse primer	Cytb	16S rRNA	18S rRNA
Parasites										
Hemiptera	Reduviidae sp. 4	free living	ZCCK_	085	East Asia	–	–	–	–	LC624863
Hirudinida	*Glossiphonia* sp.	free living	ZCCK_	012	East Asia	–	–	–	–	LC624843
Hirudinida	*Hirudo nipponia*	human	ZCCK_	009	East Asia	–	–	–	–	LC624844
Hirudinida	*Odontobdella blanchardi*	free living	ZCCK_	010	East Asia	–	–	–	–	LC624848
Hirudinida	*Odontobdella blanchardi*	free living	ZCCK_	013	East Asia	–	–	–	–	LC624849
Hirudinida	*Orobdella tsushimensis*	free living	ZCCK_	046	East Asia	–	–	–	–	LC624851
Hirudinida	*Torix tukubana*	*Rana tagoi*	ZCCK_	001	East Asia	–	–	–	–	LC624876
Hirudinida	*Tritetrabdella scandens*	*Echinotriton andersoni*	ZCCK_	093	East Asia	–	–	–	–	LC624878
Nematoda	*Cosmocerca simile*	*Rana japonica*	ZCCK_	027	East Asia	–	–	–	–	LC624827
Nematoda	*Cosmocerca simile*	*Rana japonica*	ZCCK_	028	East Asia	–	–	–	–	LC624828
Nematoda	*Cosmocerca simile*	*Rana japonica*	ZCCK_	032	East Asia	–	–	–	–	LC624829
Nematoda	*Cosmocerca simile*	*Buergeria japonica*	ZCCK_	061	East Asia	–	–	–	–	LC624830
Nematoda	*Cosmocerca simile*	*Odorrana supranarina*	ZCCK_	066	East Asia	–	–	–	–	LC624831
Nematoda	*Cosmocerca simile*	*Odorrana supranarina*	ZCCK_	082	East Asia	–	–	–	–	LC624832
Nematoda	*Cosmocerca simile*	*Buergeria japonica*	ZCCK_	084	East Asia	–	–	–	–	LC624833
Nematoda	*Cosmocerca simile*	*Glandirana rugosa*	ZCCK_	100	East Asia	–	–	–	–	LC624834
Nematoda	*Cosmocerca simile*	*Bufo japonicus*	ZCCK_	102	East Asia	–	–	–	–	LC624835
Nematoda	*Cosmocerca simile*	*Bufo japonicus*	ZCCK_	103	East Asia	–	–	–	–	LC624836
Nematoda	*Cosmocerca simile*	*Bufo japonicus*	ZCCK_	108	East Asia	–	–	–	–	LC624837
Nematoda	*Cosmocercoides* sp.	*Bufo japonicus*	ZCCK_	105	East Asia	–	–	–	–	LC624838
Nematoda	*Oswaldocruzia* sp.	*Zhangixalus arboreus*	ZCCK_	041	East Asia	–	–	–	–	LC624873
Nematoda	*Oswaldocruzia* sp.	*Buergeria japonica*	ZCCK_	063	East Asia	–	–	–	–	LC624874
Nematoda	*Oswaldocruzia* sp.	*Bufo japonicus*	ZCCK_	106	East Asia	–	–	–	–	LC624875
Nematoda	Rhabditidae sp. 1	*Rana japonica*	ZCCK_	026	East Asia	–	–	–	–	LC624865
Nematoda	Rhabditidae sp. 1	*Rana japonica*	ZCCK_	029	East Asia	–	–	–	–	LC624866
Nematoda	Rhabditidae sp. 1	*Rana japonica*	ZCCK_	030	East Asia	–	–	–	–	LC624867
Nematoda	Rhabditidae sp. 1	*Rana japonica*	ZCCK_	031	East Asia	–	–	–	–	LC624868
Nematoda	Rhabditidae sp. 1	*Rana japonica*	ZCCK_	033	East Asia	–	–	–	–	LC624869
Nematoda	Rhabditidae sp. 1	*Glandirana rugosa*	ZCCK_	035	East Asia	–	–	–	–	LC624870
Nematoda	Rhabditidae sp. 1	*Buergeria buergeri*	ZCCK_	096	East Asia	–	–	–	–	LC624871
Nematoda	Rhabditidae sp. 2	*Gloydius blomhoffii*	ZCCK_	044	East Asia	–	–	–	–	LC624872
Nematoda	*Toxocara cati*	unspecified vertebrate	ZCCK_	008	East Asia	–	–	–	–	LC624877
Trematoda	*Diplodiscus japonicus*	*Glandirana rugosa*	ZCCK_	037	East Asia	–	–	–	–	LC624841
Trematoda	*Megalodiscus temperatus*	*Lithobates catesbeianus*	ZCCK_	115	East Asia	–	–	–	–	LC624852
Trematoda	*Mesocoelium* sp.	*Glandirana rugosa*	ZCCK_	036	East Asia	–	–	–	–	LC624854
Trematoda	*Mesocoelium* sp.	*Buergeria japonica*	ZCCK_	060	East Asia	–	–	–	–	LC624845
Trematoda	*Mesocoelium* sp.	*Odorrana supranarina*	ZCCK_	067	East Asia	–	–	–	–	LC624846
Trematoda	*Mesocoelium* sp.	*Hynobius naevius*	ZCCK_	098	East Asia	–	–	–	–	LC624847
Trematoda	Opecoelidae sp.	*Zhangixalus arboreus*	ZCCK_	040	East Asia	–	–	–	–	LC624850
Trematoda	Plagiorchiida sp.	*Elaphe climacophora*	ZCCK_	016	East Asia	–	–	–	–	LC624853
Acari	Trombiculidae sp. 1	*Blommersia blommersae*	ZCRK_	011	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624912
Acari	Trombiculidae sp. 2	*Calumma* sp.	ZCRK_	030	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624913
Acari	Trombiculidae sp. 2	*Calumma gastrotaenia*	ZCRK_	035	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624914
Hirudinida	*Chtonobdella vagans*	human	ZCRK_	115	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624899
Nematoda	*Cosmocerca* sp.	*Liophidium torquatum*	ZCRK_	187	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624897

Table 1. continued.

Taxonomy	Species	Host reptiles or amphibians	Voucher		Distribution	Foward primer	Reverse primer	Cytb	16S rRNA	18S rRNA
Parasites										
Nematoda	Onchocercidae sp.	*Plethodontohyla mihanika*	ZCRK_	086	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624909
Nematoda	*Raillietnema* sp.	*Mantidactylus betsileanus*	ZCRK_	016	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624885
Nematoda	*Raillietnema* sp.	*Mantidactylus femoralis*	ZCRK_	017	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624886
Nematoda	*Raillietnema* sp.	*Plethodontohyla notosticta*	ZCRK_	022	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624887
Nematoda	*Raillietnema* sp.	*Plethodontohyla notosticta*	ZCRK_	023	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624888
Nematoda	*Raillietnema* sp.	*Mantella crocea*	ZCRK_	027	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624889
Nematoda	*Raillietnema* sp.	*Mantidactylus lugubris*	ZCRK_	047	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624890
Nematoda	*Raillietnema* sp.	*Mantidactylus lugubris*	ZCRK_	048	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624891
Nematoda	*Raillietnema* sp.	*Mantidactylus femoralis*	ZCRK_	051	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624892
Nematoda	*Raillietnema* sp.	*Boophis pyrrhus*	ZCRK_	077	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624893
Nematoda	*Raillietnema* sp.	*Mantidactylus betsileanus*	ZCRK_	102	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624894
Nematoda	*Raillietnema* sp.	*Boophis madagascariensis*	ZCRK_	168	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624895
Nematoda	Rhabditida sp. 2	*Cophyla tuberifera*	ZCRK_	158	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624881
Nematoda	Rhabditida sp. 2	*Cophyla tuberifera*	ZCRK_	162	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624883
Nematoda	Rhabditida sp. 2	*Cophyla tuberifera*	ZCRK_	169	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624884
Nematoda	*Rhabditoides* sp.	*Mantidactylus betsileanus*	ZCRK_	101	Madagascar	ME1_Fmod	ME2_Rmod	–	–	LC624911
Diptera	*Anopheles gambiae*	human	ZCRK_	131	Madagascar	–	–	–	–	LC624880
Diptera	*Culex* sp.	human	ZCRK_	181	Madagascar	–	–	–	–	LC624898
Hirudinida	*Chtonobdella vagans*	human	ZCRK_	038	Madagascar	–	–	–	–	LC624900
Hirudinida	*Chtonobdella vagans*	human	ZCRK_	062	Madagascar	–	–	–	–	LC624901
Hirudinida	*Chtonobdella vagans*	human	ZCRK_	063	Madagascar	–	–	–	–	LC624902
Hirudinida	*Chtonobdella vagans*	human	ZCRK_	068	Madagascar	–	–	–	–	LC624903
Hirudinida	*Chtonobdella vagans*	human	ZCRK_	114	Madagascar	–	–	–	–	LC624904
Hirudinida	*Chtonobdella vagans*	human	ZCRK_	116	Madagascar	–	–	–	–	LC624905
Hirudinida	*Chtonobdella vagans*	human	ZCRK_	117	Madagascar	–	–	–	–	LC624906
Hirudinida	*Chtonobdella vagans*	human	ZCRK_	118	Madagascar	–	–	–	–	LC624907
Hirudinida	*Chtonobdella vagans*	human	ZCRK_	130	Madagascar	–	–	–	–	LC624908
Nematoda	*Raillietnema* sp.	*Ptychadena mascareniensis*	ZCRK_	061	Madagascar	–	–	–	–	LC624896
Nematoda	Rhabditida sp. 2	*Cophyla tuberifera*	ZCRK_	159	Madagascar	–	–	–	–	LC624882
Pentastomida	Porocephalidae sp.	*Sanzinia madagascariensis*	ZCRK_	113	Madagascar	–	–	–	–	LC624910
Known BovBs										
Agamidae	*Amphibolurus norrisi*	–	–	–	Oceania	–	–	–	–	–
Agamidae	*Pogona vitticeps*	–	–	–	Oceania	–	–	–	–	–
Boidae	*Boa constrictor*	–	–	–	Central–South America	–	–	–	–	–
Bovidae	*Bison bison*	–	–	–	North America	–	–	–	–	–
Bovidae	*Bubalus bubalis*	–	–	–	Europe, Western–Central–Southern Asia	–	–	–	–	–
Bovidae	*Bos indicus*	–	–	–	Western–Central–Southern Asia	–	–	–	–	–
Bovidae	*Bos mutus*	–	–	–	Western–Central–Southern Asia	–	–	–	–	–
Bovidae	*Bos taurus*	–	–	–	Western–Central–Southern Asia	–	–	–	–	–
Bovidae	*Capra hircus*	–	–	–	Western–Central–Southern Asia	–	–	–	–	–
Bovidae	*Ovis aries*	–	–	–	Europe, Western–Central–Southern Asia	–	–	–	–	–
Bovidae	*Pantholops hodgsonii*	–	–	–	Western–Central–Southern Asia	–	–	–	–	–
Burramyidae	*Burramys parvus*	–	–	–	Oceania	–	–	–	–	–
Cimicidae	*Cimex lectularius*	–	–	–	Western–Central–Southern Asia	–	–	–	–	–
Colubridae	*Natrix tessellata*	–	–	–	Europe	–	–	–	–	–
Dactyloidae	*Anolis carolinensis*	–	–	–	North America	–	–	–	–	–

Table 1. continued.

Taxonomy	Species	Host reptiles or amphibians	Voucher		Distribution	Foward primer	Reverse primer	Cytb	16S rRNA	18S rRNA
Known BovBs										
Dasyuridae	*Antechinus flavipes*	–	–	–	Oceania	–	–	–	–	–
Dasyuridae	*Sarcophilus harrisii*	–	–	–	Oceania	–	–	–	–	–
Didelphidae	*Monodelphis domestica*	–	–	–	Central–South America	–	–	–	–	–
Elapidae	*Ophiophagus hannah*	–	–	–	Southeast Asia	–	–	–	–	–
Elapidae	*Notechis scutatus*	–	–	–	Oceania	–	–	–	–	–
Elapidae	*Hydrophis spiralis*	–	–	–	Western–Central–Southern Asia	–	–	–	–	–
Elapidae	*Walterinnesia aegyptia*	–	–	–	Western–Central–Southern Asia	–	–	–	–	–
Gekkonidae	*Gehyra lazelli*	–	–	–	Oceania	–	–	–	–	–
Gekkonidae	*Gehyra variegata*	–	–	–	Oceania	–	–	–	–	–
Ixodidae	*Amblyomma limbatum*	–	–	–	Oceania	–	–	–	–	–
Ixodidae	*Bothriocroton hydrosauri*	–	–	–	Oceania	–	–	–	–	–
Macropodidae	*Macropus antilopinus*	–	–	–	Oceania	–	–	–	–	–
Macropodidae	*Notamacropus eugenii*	–	–	–	Oceania	–	–	–	–	–
Peramelidae	*Isoodon obesulus*	–	–	–	Oceania	–	–	–	–	–
Peramelidae	*Perameles gunnii*	–	–	–	Oceania	–	–	–	–	–
Petauridae	*Petaurus breviceps*	–	–	–	Oceania	–	–	–	–	–
Pythonidae	*Python molurus*	–	–	–	Western–Central–Southern Asia	–	–	–	–	–
Scincidae	*Ctenotus atlas*	–	–	–	Oceania	–	–	–	–	–
Scincidae	*Egernia stokesii*	–	–	–	Oceania	–	–	–	–	–
Scincidae	*Eremiascincus douglasi*	–	–	–	Oceania	–	–	–	–	–
Scincidae	*Eremiascincus richardsonii*	–	–	–	Oceania	–	–	–	–	–
Scincidae	*Tiliqua rugosa*	–	–	–	Oceania	–	–	–	–	–
Viperidae	*Agkistrodon contortrix*	–	–	–	North America	–	–	–	–	–
Viperidae	*Crotalus horridus*	–	–	–	North America	–	–	–	–	–
Viperidae	*Vipera ammodytes*	–	–	–	Europe	–	–	–	–	–
Viperidae	*Vipera berus*	–	–	–	Europe	–	–	–	–	–
Viperidae	*Echis coloratus*	–	–	–	Western–Central–Southern Asia	–	–	–	–	–
Viperidae	*Bothrops alternatus*	–	–	–	Central–South America	–	–	–	–	–
Acrididae	*Locusta migratoria*	–	–	–	–	–	–	–	–	–
Adinetidae	*Adineta vaga*	–	–	–	–	–	–	–	–	–
Bombycidae	*Bombyx mori*	–	–	–	–	–	–	–	–	–
Buthidae	*Centruroides exilicauda*	–	–	–	–	–	–	–	–	–
Buthidae	*Mesobuthus martensii*	–	–	–	–	–	–	–	–	–
Chrysochloridae	*Chrysochloris asiatica*	–	–	–	–	–	–	–	–	–
Cichlidae	*Oreochromis niloticus*	–	–	–	–	–	–	–	–	–
Cyprinidae	*Danio rerio*	–	–	–	–	–	–	–	–	–
Delphacidae	*Nilaparvata lugens*	–	–	–	–	–	–	–	–	–
Dicroglossidae	*Nanorana parkeri*	–	–	–	East Asia	–	–	–	–	–
Elephantidae	*Loxodonta africana*	–	–	–	–	–	–	–	–	–
Ephemeridae	*Ephemera danica*	–	–	–	–	–	–	–	–	–
Equidae	*Equus caballus*	–	–	–	–	–	–	–	–	–
Equidae	*Equus przewalskii*	–	–	–	–	–	–	–	–	–
Gekkonidae	*Christinus sp.*	–	–	–	Oceania	–	–	–	–	–
Glossiphoniidae	*Helobdella robusta*	–	–	–	–	–	–	–	–	–
Lepisosteidae	*Lepisosteus oculatus*	–	–	–	–	–	–	–	–	–

Table 1. continued.

Taxonomy	Species	Host reptiles or amphibians	Voucher		Distribution	Foward primer	Reverse primer	Cytb	16S rRNA	18S rRNA
Known BovBs										
Macroscelididae	*Elephantulus edwardii*	–	–	–	–	–	–	–	–	–
Ornithorhynchidae	*Ornithorhynchus anatinus*	–	–	–	–	–	–	–	–	–
Orycteropodidae	*Orycteropus afer*	–	–	–	–	–	–	–	–	–
Petromyzontidae	*Lethenteron camtschaticum*	–	–	–	–	–	–	–	–	–
Pipidae	*Xenopus laevis*	–	–	–	Africa	–	–	–	–	–
Pipidae	*Xenopus tropicalis*	–	–	–	Africa	–	–	–	–	–
Procaviidae	*Procavia capensis*	–	–	–	–	–	–	–	–	–
Pythonidae	*Python bivittatus*	–	–	–	Southeast Asia	–	–	–	–	–
Rhinocerotidae	*Ceratotherium simum*	–	–	–	–	–	–	–	–	–
Tachyglossidae	*Tachyglossus aculeatus*	–	–	–	–	–	–	–	–	–
Tenrecidae	*Echinops telfairi*	–	–	–	–	–	–	–	–	–
Trichechidae	*Trichechus manatus*	–	–	–	–	–	–	–	–	–
Vespertilionidae	*Eptesicus fuscus*	–	–	–	–	–	–	–	–	–
Viperidae	*Crotalus mitchellii*	–	–	–	North America	–	–	–	–	–

Voucher abbreviations:
ABTC = Australian Biological Tissue Collection, South Australian Museum
DCAK = DNA Collection of Atsushi Kurabayashi
FGZC = Frank Glaw Zoological Collection
IABHU = Institute for Amphibian Biology, Hiroshima University
KUZ = Zoological Collection of the Kyoto University
MHZC = Masaki Hoso Zoological Collection
MVZ = Museum of Vertebrate Zoology, University of California at Berkeley
NUM = Nagoya University Museum
PCMB = Pacific Center for Molecular Biodiversity
RBINS = Royal Belgian Institute of Natural Sciences
UADBA = University of Antananarivo Department of Animal Biology
UniKis = University of Kisangani
ZCAK = Zoological Collection of Atsushi Kurabayashi
ZCCK = Zoological Collection of Chiaki Kambayashi
ZCMV = Zoological Collection of Miguel Vences
ZCRK = Zoological Collection of Ryosuke Kakehashi
ZCYK = Zoological Collection of Yoshinori Kumazawa
ZSM = Zoologische Staatssammlung München

Table 2. PCR primers used in this study.

Gene	Primer name	Primer sequence (5'→3')	Source
Cytb	L14910	GACCTGTGATMTGAAAACCAYCGTTGT	Burbrink et al. (2000)
	H16064	CTTTGGTTTACAAGAACAATGCTTTA	
	Cytb1	CCATCCAACATCTCAGCATGATGAAA	Kocher et al. (1989)
	Cytb2	CCCTCAGAATGATATTTGTCCTCA	
16S rRNA	16Sar	CGCCTGTTTATCAAAAACAT	Bossuyt and Milinkovitch (2000)
	16Sbr	CGGTCTGAACTCAGATCACG	
18S rRNA	18S_Fow_11_31	TACCTGGTTGATYCTGCCAGT	This study
	18S_Fow_92_109	GAAACYGCGAAYGGCTCA	
	18S_Rev_1252_1272	TTYCCCGTGTTGAGTCAAATT	
	18S_Rev_1692_1714	CGGTGTGTACAAAGGGCAGGGAC	
BovB	ME1_Fmod	ACAGTARTYCAAGYCTAYRCYCCAAC	This study
	BovB_ORF5_FowN	AGRTCGGAAGGTRTCCAATATGCTAC	
	BovB_230F	AGGTCRGAAGRYGYCCAWYATGCTAC	
	BovB_670F	AGTARTCCAAGTCTATGCMCCAAYCA	
	BovB_VA_1942_Fow	GCACTCATTTCTCATGCTAGTAAAGT	
	ME2_Rmod	CWGCAWATCTGAGGTTGTTRAKATTTCT	
	BovB_ORF3_RevN	GTGAYKCCATCCAGCCAYCTCATYCTCTG	
	BovB_2460R	GCCATYAGRGTGGTRTCRTCTGCATAYCT	
	BovB_2820R	TTAYRGTCCARCTYTCRCABCCATACAT	
	BovB_3090R	CYRTCYARCCAYCTCATYCTCTGYCG	
	BovB_3200R	AAGTCGTGTCCGACYCWTCGYRACCCYATG	
	BovB_VA_1201_Rev	ATCTATTTGTCACCTCTACT	

Table 3. The estimation of BovB copy numbers and the genomic coverages in the frog genomes using dot blot analysis.

Family	Species (Voucher)	C-value	5' copy No.	3' copy No.	BovB coverage
Madagascar					
Manteliidae	*Mantella betsileo*	5.30	274.6	1020.9	0.017%
Manteliidae	*Mantella madagascariensis*	5.20	253.0	990.3	0.016%
Manteliidae	*Mantidactylus lugubris*	5.25	57.4	99.5	0.0036%
Manteliidae	*Mantidactylus femoralis* (ZCRK_049)	4.15	44.1	51.9	0.0031%
Manteliidae	*Mantidactylus femoralis* (ZCMV_15013)	4.15	67.7	147.5	0.0048%
Manteliidae	*Mantidactylus betsileanus* (ZCRK_052)	4.65	37.2	57.7	0.0029%
Manteliidae	*Mantidactylus betsileanus* (ZCRK_099)	4.65	35.5	49.2	0.0028%
Manteliidae	*Mantidactylus betsileanus* (ZCMV_15012)	4.65	38.6	130.6	0.003%
Manteliidae	*Mantidactylus melanopleura*	5.17	214.3	996.3	0.014%
Manteliidae	*Guibemantis tornieri* (ZCRK_071)	5.17	795.9	1879.2	0.05%
Manteliidae	*Guibemantis tornieri* (ZCRK_122)	5.17	1243.2	1546.6	0.079%
Manteliidae	*Guibemantis bicalcaratus*	5.17	488.1	1007.8	0.031%
Manteliidae	*Guibemantis flavobrunneus*	5.17	880.5	1543.2	0.056%
Manteliidae	*Gephyromantis boulengeri*	5.17	653.4	1262.2	0.041%
Manteliidae	*Blommersia blommersae* (ZCRK_043)	5.17	96.4	207.6	0.0061%
Manteliidae	*Blommersia blommersae* (ZCMV_15010)	5.17	100.8	471.1	0.0064%
Manteliidae	*Boophis goudotii* (ZCAK_MD0005)	4.20	659.3	1835.9	0.051%
Manteliidae	*Boophis goudotii* (ZCRK_128)	4.20	400.6	1068.3	0.031%
Manteliidae	*Boophis doulioti*	4.26	57.4	76.4	0.0044%
Manteliidae	*Boophis madagascariensis*	3.90	360.8	1082.4	0.03%
Manteliidae	*Boophis pyrrhus*	4.26	419.3	1557.2	0.032%
Manteliidae	*Boohis viridis*	4.26	263.6	1190.1	0.02%
Manteliidae	*Aglyptodactylus madagascariensis*	3.05	61.0	276.7	0.0065%
Ptychadenidae	*Ptychadena mascareniensis* (ZCRK_060)	–	–	–	–
Ptychadenidae	*Ptychadena mascareniensis* (ZCRK_059)	–	–	–	–
Microhylidae	*Plethodontohyla inguinalis*	5.05	297.3	485.9	0.019%
Microhylidae	*Plethodontohyla ocellata*	5.05	571.7	776.8	0.037%
Microhylidae	*Plethodontohyla mihanika*	5.05	45.5	38.9	0.0029%
Microhylidae	*Plethodontohyla notosticta*	5.05	92.5	997.0	0.006%
Microhylidae	*Dyscophus guineti*	4.41	638.7	1796.7	0.047%
Microhylidae	*Cophyla barbouri*	6.30	73.6	617.1	0.0038%
Microhylidae	*Cophyla tuberifera* (ZCRK_156)	5.75	819.6	1072.7	0.0466%
Microhylidae	*Cophyla tuberifera* (ZCRK_160)	5.75	909.7	1168.1	0.052%
Microhylidae	*Cophyla tuberifera* (ZCRK_166)	5.75	920.6	1160.8	0.052%
Microhylidae	*Scaphiophryne madagascariensis*	4.41	257.3	419.6	0.019%
Hyperoliidae	*Heterixalus betsileo* (UADBA_AK041208_011)	4.25	58.8	128.9	0.0045%
Hyperoliidae	*Heterixalus betsileo* (ZCMV_15011)	4.25	41.1	62.5	0.0032%
Hyperoliidae	*Heterixalus madagascariensis* (ZCRK_175)	4.83	105.3	963.5	0.0071%
Hyperoliidae	*Heterixalus madagascariensis* (ZCRK_178)	4.83	113.2	869.1	0.0077%
Hyperoliidae	*Heterixalus punctatus* (ZCRK_119)	4.63	183.6	433.7	0.013%
Hyperoliidae	*Heterixalus punctatus* (ZCMV_15008)	4.63	232.0	418.7	0.016%
Hyperoliidae	*Heterixalus punctatus* (ZCMV_15009)	4.63	220.1	615.6	0.016%
Hyperoliidae	*Heterixalus alboguttatus*	4.63	234.2	634.4	0.017%
Mantellidae	*Tsingymantis antitra*	5.17	419.8	666.9	0.027%
Mantellidae	*Laliostoma labrosum*	3.25	40.3	112.0	0.0041%

Table 3. continued.

Family	Species (Voucher)	C-value	5' copy No.	3' copy No.	BovB coverage
Oceania					
Ranidae	*Papurana* sp.	–	–	–	–
Ceratobatrachidae	*Cornufer pelewensis*	5.90	70.6	101.3	0.0039%
Microhylidae	*Barygenys flavigularis*	4.41	2153.6	2528.4	0.16%
Microhylidae	*Cophixalus cryptotympanum*	4.41	204.6	661.6	0.015%
Ranidae	*Papurana volkerjane*	–	–	–	–
Pelodryadidae	*Nyctimystes infrafrenatus*	–	–	–	–
Pelodryadidae	*Ranoidea caerulea*	–	–	–	–
Pelodryadidae	*Ranoidea gracilenta*	–	–	–	–
Limnodynastidae	*Limnodynastes salmini*	–	–	–	–
Europe					
Ranidae	*Pelophylax bergeri*	6.87	9.6	ND	0.00046%
Bufonidae	*Bufo bufo*	–	–	–	–
Ranidae	*Rana arvalis*	–	–	–	–
Ranidae	*Rana temporaria*	–	–	–	–
Alytidae	*Alytes obstetricans*	–	–	–	–
East Asia					
Rhacophoridae	*Buergeria buergeri*	4.85	568.5	92.0	0.038%
Ranidae	*Odorrana ishikawae*	6.34	499.4	63.2	0.026%
Ranidae	*Odorrana splendida*	6.34	390.1	141.2	0.02%
Ranidae	*Babina subaspera*	6.30	464.5	197.6	0.024%
Ranidae	*Pelophylax nigromaculatus*	5.97	2448.7	295.4	0.13%
Bufonidae	*Bufo japonicus*	–	–	–	–
Bufonidae	*Bufo gargarizans*	–	–	–	–
Bufonidae	*Buergeria japonica*	–	–	–	–
Bufonidae	*Buergeria robusta*	4.85	148.5	5.8	0.01%
Rhacophoridae	*Zhangixalus schlegelii*	–	–	–	–
Rhacophoridae	*Kurixalus eiffingeri* *	–	–	–	–
Dicroglossidae	*Fejervarya kawamurai*	–	–	–	–
Dicroglossidae	*Nanorana maculosa*	–	–	–	–
Dicroglossidae	*Quasipaa spinosa*	–	–	–	–
Ranidae	*Rana amurensis*	–	–	–	–
Ranidae	*Rana chensinensis*	–	–	–	–
Ranidae	*Rana japonica*	5.08	118.5	19.4	0.0076%
Ranidae	*Rana kobai*	5.62	53.2	22.0	0.0031%
Ranidae	*Rana sakuraii*	5.62	39.5	52.2	0.0023%
Ranidae	*Rana tagoi*	5.30	42.6	4.1	0.0026%
Ranidae	*Hylarana latouchii*	–	–	–	–
Ranidae	*Glandirana rugosa*	8.15	60.0	ND	0.0024%
Microhylidae	*Microhyla okinavensis*	–	–	–	–
Hylidae	*Hyla chinensis*	–	–	–	–
Hylidae	*Dryophytes japonicus*	–	–	–	–
Bufonidae	*Strauchbufo raddei*	–	–	–	–
Bombina	*Bombina orientalis*	–	–	–	–
Hynobiidae	*Hynobius naevius*	–	–	–	–
Salamandridae	*Cynops* sp.	–	–	–	–

Table 3. continued.

Family	Species (Voucher)	C-value	5' copy No.	3' copy No.	BovB coverage
Southeast Asia					
Dicroglossidae	*Hoplobatrachus rugulosus*	–	–	–	–
Bufonidae	*Duttaphrynus melanostictus* (IABHU_04446)	–	–	–	–
Bufonidae	*Duttaphrynus melanostictus* (IABHU_04998)	–	–	–	–
Bufonidae	*Rentapia hosii*	–	–	–	–
Bufonidae	*Leptophryne borbonica*	–	–	–	–
Megophryidae	*Megophrys nasuta* (ZCAK_SEA0001)	2.79	516.2	1034.5	0.061%
Megophryidae	*Megophrys nasuta* (ZCYK_Bcar1)	2.79	459.4	1136.6	0.054%
Megophryidae	*Megophrys nanlingensis*	2.79	304.9	1328.6	0.036%
Rhacophoridae	*Zhangixalus prominanus*	–	–	–	–
Rhacophoridae	*Nyctixalus pictus*	–	–	–	–
Rhacophoridae	*Polypedates leucomystax*	–	–	–	–
Dicroglossidae	*Occidozyga lima*	–	–	–	–
Dicroglossidae	*Limnonectes* sp.*	–	–	–	–
Ranidae	*Hylarana erythraea**	–	–	–	–
Microhylidae	*Microhyla mukhlesuri*	–	–	–	–
Microhylidae	*Microhyla pulchra*	–	–	–	–
Microhylidae	*Kalophrynus interlineatus*	4.41	84.2	18.3	0.0062%
Microhylidae	*Kalophrynus sinensis*	4.41	55.0	30.8	0.0041%
Microhylidae	*Glyphoglossus guttulatus*	–	–	–	–
Microhylidae	*Chaperina fusca*	–	–	–	–
Microhylidae	*Glyphoglossus molossus**	–	–	–	–
Microhylidae	*Kaloula pulchra*	–	–	–	–
Bufonidae	*Ingerophrynus parvus*	5.25	ND	50.8	–
Megophryidae	*Leptobrachium hasseltii*	2.89	ND	21.7	–
Central–South America					
Microhylidae	*Ctenophryne geayi*	–	–	–	–
Microhylidae	*Dermatonotus muelleri*	–	–	–	–
Microhylidae	*Elachistocleis bicolor*	–	–	–	–
Dendrobatidae	*Dendrobates tinctorius*	–	–	–	–
Dendrobatidae	*Phyllobates aurotaenia*	–	–	–	–
Dendrobatidae	*Ranitomeya amazonica*	–	–	–	–
Phyllomedusidae	*Agalychnis callidryas*	–	–	–	–
Hylidae	*Dendropsophus melanargyreus*	–	–	–	–
Hylidae	*Boana boans*	–	–	–	–
Hylidae	*Pseudis paradoxa*	–	–	–	–
Hylidae	*Trachycephalus resinifictrix*	–	–	–	–
Leptodactylidae	*Leptodactylus knudseni*	–	–	–	–
Leptodactylidae	*Pleurodema brachyops*	–	–	–	–
Hemiphractidae	*Gastrotheca peruana*	–	–	–	–
Ceratophryidae	*Ceratophrys cornuta*	–	–	–	–
Bufonidae	*Atelopus barbotini**	–	–	–	–
Bufonidae	*Melanophryniscus stelzneri*	–	–	–	–
Bufonidae	*Rhaebo guttatus*	–	–	–	–
Bufonidae	*Rhinella diptycha*	–	–	–	–
Rhinophrynidae	*Rhinophrynus dorsalis*	–	–	–	–
Pipidae	*Pipa parva**	–	–	–	–
Typhlonectidae	*Typhlonectes natans*	–	–	–	–

Table 3. continued.

Family	Species (Voucher)	C-value	5' copy No.	3' copy No.	BovB coverage
North America					
Bufonidae	*Anaxyrus americanus*	–	–	–	–
Ranidae	*Lithobates catesbeianus*	7.59	923.6	175.1	0.04%
Ranidae	*Lithobates sphenocephalus*	6.80	674.1	33.0	0.032%
Microhylidae	*Gastrophryne olivacea*	–	–	–	–
Hylidae	*Dryophytes cinereus*	–	–	–	–
Hylidae	*Dryophytes chrysoscelis*	–	–	–	–
Bufonidae	*Anaxyrus debilis*	–	–	–	–
Bufonidae	*Anaxyrus terrestris*	–	–	–	–
Scaphiopodidae	*Scaphiopus holbrookii*	–	–	–	–
Western–Central–Southern Asia					
Dicroglossidae	*Euphlyctis kalasgramensis*	–	–	–	–
Ranidae	*Hylarana* cf. *tytleri*	3.33	418.3	431.5	0.041%
Dicroglossidae	*Fejervarya orissaensis*	–	–	–	–
Dicroglossidae	*Fejervarya* sp.	–	–	–	–
Microhylidae	*Microhyla nilphamariensis*	–	–	–	–
Bufonidae	*Duttaphrynus stomaticus*	–	–	–	–
Africa					
Rhacophoridae	*Chiromantis xerampelina*	–	–	–	–
Pyxicephalidae	*Pyxicephalus adspersus*	–	–	–	–
Dicroglossidae	*Hoplobatrachus occipitalis*	–	–	–	–
Ptychadenidae	*Ptychadena nilotica*	–	–	–	–
Ptychadenidae	*Hildebrandtia* sp.	–	–	–	–
Phrynobatrachidae	*Phrynobatrachus acridoides* *	–	–	–	–
Microhylidae	*Phrynomantis bifasciatus*	–	–	–	–
Microhylidae	*Phrynomantis microps*	–	–	–	–
Hemisotidae	*Hemisus marmoratus*	–	–	–	–
Brevicipitidae	*Breviceps adspersus*	–	–	–	–
Brevicipitidae	*Breviceps mossambicus*	–	–	–	–
Hyperoliidae	*Hyperolius marmoratus*	–	–	–	–
Hyperoliidae	*Hyperolius puncticulatus*	–	–	–	–
Hyperoliidae	*Phlyctimantis maculatus*	–	–	–	–
Arthroleptidae	*Leptopelis vermiculatus*	–	–	–	–
Bufonidae	*Sclerophrys gutturalis*	–	–	–	–
Pipidae	*Xenopus laevis*	–	–	–	–
Pipidae	*Xenopus tropicalis*	–	–	–	–

For the samples marked with an asterisk, 25 ng and 250 ng of total DNA were used (see Materials and Methods).

Table 4. The nodes of BovB HTs in the ML and BI trees of 222-OTUs.

Node	Date (Ma)	Taxa involved in the HT	HT region	BPs	BPPs (Unresolved: <90)
1	84.9	*Afrotyphlops* or Bufonidae – Marsupialia	–	34	Unresolved (polytomy)
2	63.7	*Afrotyphlops punctatus* – Bufonidae	–	39	Unresolved (polytomy)
3	81.2	Henophidia – Common ancestor of Agamidae or Scincidae	–	12	–
4	63.8	Agamidae – Scincidae	–	91	100
5	20.9	*Ctenotus atlas* – *Bothriocroron hydrosauri*	Oceania	67	81
6	76.0	Henophidia – Bovidae	–	34	98
7	72.1	Henophidia – *Buergeria buergeri*	–	51	–
8	66.8	Henophidia – Microhylidae	–	39	98
9	50.6	Dyscophinae – Ranidae	–	48	99
10	15.8	*Dyscophus guineti* – *Aglyptodactylus madagascariensis*	Madagascar	100	100
11	44.5	Asterophryinae – *Xenopeltis unicolor*	Oceania	56	99
12	17.4	*Cophixalus cryptotympanum* – Dicroglossidae	Oceania	98	96
13	2.3	*Cophixalus cryptotympanum* – *Papurana* sp.	Oceania	100	100
14	6.9	*Loxocemus bicolor* – *Xerotyphlops vermicularis*	Central–South America	100	100
15	1.6	*Morelia viridis* – Typhlopidae sp.	Oceania	100	100
16	22.3	*Boa constrictor* – Trombiculidae sp. 2 (ZCRK_030)	Central–South America	100	100
17	10.5	*Boa constrictor* – *Rena humilis*	Central–South America	100	Unresolved
18	59.4	*Pareas iwasakii* – *Gehyra* or Chamaeleonidae or Trombiculidae	–	39	95
19	42.5	*Gehyra* – Chamaeleonidae or Trombiculidae	Oceania	100	100
20	21.1	Chamaeleonidae sp. – Trombiculidae sp. 2 (ZCRK_035)	Madagascar	100	100
21	66.4	Caenophidia – *Xenotyphlops* or *Amphiglossus*	–	100	100
22	30.9	*Xenotyphlops grandidieri* – *Amphiglossus* sp.	Madagascar	100	100
23	57.2	Caenophidia – *Amblyomma* or *Cornufer*	–	100	100
24	34.6	*Amblyomma limbatum* – *Cornufer pelewensis*	Oceania	100	100
25	36.8	*Hypsiscopus plumbea* – *Megophrys*	East Asia	73	100
26	37.5	Elapidae – Colubridae	East Asia	9	NS
27	11.8	*Helicops* cf. *angulatus* – *Hydrophis spiralis*	Central–South America	16	NS
28	28.8	Colubridae – Elapidae	East Asia	0	Unresolved (polytomy)
29	12.6	*Contia tenuis* – *Ophiophagus hannah*	North America	3	Unresolved (polytomy)
30	1.3	*Elaphe climacophora* – Cosmocerca simile	East Asia	61	97
31	25.8	Elapidae – Viperidae	East Asia	0	–
32	9.0	Viperidae – *Afrotyphlops* sp.	Africa	74	100
33	15.3	*Crotalus horridus* – *Psammodynastes* or *Natrix* or Elapidae	North America	5	94
34	11.8	*Psammodynastes pulverulentus* – *Natrix* or Elapidae	East Asia	7	97
35	8.7	*Natrix tessellata* – Elapidae	Europe	22	96
36	17.2	*Mimophis* cf. *mahfalensis* – *Indotyphlops* or *Hylarana*	Western–Central–Southern Asia	100	100
37	11.2	*Mimophis* cf. *mahfalensis* – *Cimex lectularius*	Western–Central–Southern Asia	84	100
38	9.6	*Indotyphlops braminus* – *Hylarana* cf. *tytleri*	Western–Central–Southern Asia	100	100
39	37.1	Pseudoxyrhophiidae – *Mantella*	Madagascar	96	100
40	29.2	*Alluaudina bellyi* – *Heterixalus* or *Guibemantis*	Madagascar	46	100
41	25.8	*Heterixalus* – *Guibemantis*	Madagascar	43	Unresolved
42	5.7	*Heterixalus betsileo* – Raillietnema sp. (ZCRK_016, 017)	Madagascar	97	Unresolved (polytomy)
43	5.6	*Madagascarophis colubrinus* – *Acrantophis* cf. *dumerili*	Madagascar	78	100
44	30.5	Pseudoxyrhophiidae – Cophylinae	Madagascar	7	Unresolved (polytomy)
45	13.9	*Cophyla* – *Gephyromantis boulengeri*	Madagascar	77	100
46	28.7	Pseudoxyrhophiidae – *Madatyphlops* or *Sanzinia* or *Phelsuma*	Madagascar	6	Unresolved (polytomy)
47	18.1	*Madatyphlops arenarius* – *Sanzinia* or *Phelsuma*	Madagascar	65	100
48	2.5	*Sanzinia* cf. *madagascariensis* – *Phelsuma lineata*	Madagascar	100	100
49	27.7	Pseudoxyrhophiidae – Common ancestor of *Boophis* & *Mantidactylus*	Madagascar	3	Unresolved (polytomy)
50	21.8	Pseudoxyrhophiidae – *Blommersia blommersae*	Madagascar	42	NS
51	2.1	*Liophidium torquatum* – Trombiculidae sp. 1	Madagascar	100	100
52	6.9	*Mantidactylus lugubris* – Raillietnema sp. (ZCRK_047)	Madagascar	100	100
53	6.9	*Boophis madagascariensis* – *Malagabdella vagans*	Madagascar	99	100
54	4.1	*Boophis madagascariensis* – *Ptychadena mascareniensis*	Madagascar	84	100

The node numbers correspond to Fig. 9.

BPs, bootstrap probabilities in ML phylogeny; BPPs, bayesian posterior probabilities in BI phylogeny.

Table 5. The indices of distance between the topologies on the 222-OTUs ML tree and that on a tree constructed based on orthologous genes.

	I_{cong} (P-value)	Triples
squamates		
species	2.4 (2.5×10^{-15})	0.52
genera	2.2 (3.1×10^{-13})	0.53
families	1.7 (1.3×10^{-6})	0.63
frogs		
species	1.6 (1.1×10^{-5})	0.64
genera	1.4 (1.3×10^{-3})	0.64
families	1.4 (5.2×10^{-3})	0.80

The larger the I_{cong} index value and the smaller the triples value, the more congruent the trees are. The p-value smaller than 0.05 indicates that the trees are more congruent than expected by chance.

Table 6. Models and parameters used in the geographic area estimation of HT occurrences.

Model	Parameters	*d*	*e*	*j*	lnL	AIC
DEC	2	0.01	0.01	–	-670.60	1345.21
DEC+J	3	5.5×10^{-5}	1.0×10^{-12}	0.034	-342.96	691.91
DIVALIKE	2	0.01	0.01	–	-642.39	1288.79
DIVALIKE+J	3	7.0×10^{-5}	1.0×10^{-12}	0.034	-342.32	690.63
BAYAREALIKE	2	0.01	0.01	–	-801.79	1607.58
BAYAREALIKE+J	3	4.8×10^{-5}	1.0×10^{-7}	0.034	-346.66	699.33

d, rate of range expansion by adding an area; *e*, rate of range reduction through extirpation in an area; *j*, relative per-event weight of jump dispersal at cladogenesis. lnL, log-likelihood; AIC, Akaike Information Criterion.

Table 7. Frequency of BovB-positive frog and parasite species in each region and comparisons with those in Madagascar.

Region	Frog		Parasite	
	Percentage	P-value (vs. Madagascar)	Percentage	P-value (vs. Madagascar)
Madagascar	91% (29/32)	–	50% (4/8)	–
Western–Central–Southern Asia	50% (3/6)	0.039	–	–
Oceania	44% (4/9)	0.021	–	–
Europe	40% (2/5)	0.045	–	–
Southeast Asia	26% (5/19)	1.6×10^{-5}	–	–
East Asia	23% (7/30)	4.3×10^{-7}	2.9% (1/34)	2.9×10^{-3}
Africa	0% (0/18)	5.2×10^{-10}	–	–
North America	0% (0/9)	3.1×10^{-6}	–	–
Central–South America	0% (0/21)	5.1×10^{-11}	–	–
Average	34% (50/149)	–	12% (5/42)	–

Raw numbers of species used to calculate percentages are in parentheses.

P-value was adjusted using holm correction.

Table 8. The percentage of amphibian genome sequence contributed by BovB.

Family	Species	BovB base count	Genome base count	BovB coverage	Accession number
Mantellidae	*Boophis goudotii*	249,927	47,278,508	0.53%	PRJDB11263
Mantellidae	*Mantella betsileo*	148,950	111,622,769	0.13%	PRJDB11263
Mantellidae	*Mantidactylus betsileanus*	30,072	28,523,085	0.11%	PRJDB11263
Bufonidae	*Bufo gargarizans*	237,696	4,545,465,442	0.0052%	GCA_014858855.1
Megophryidae	*Leptobrachium leishanense*	358,785	3,549,060,464	0.01%	GCA_009667805.1
Pyxicephalidae	*Pyxicephalus adspersus*	81,463	1,563,367,516	0.0052%	GCA_004786255.1
Ranidae	*Lithobates catesbeianus*	656,769	6,250,353,185	0.011%	GCA_002284835.2
Ranidae	*Rana temporaria*	297,767	4,282,724,626	0.007%	GCA_009802015.1
Dermophiidae	*Geotrypetes seraphini*	2,649,618	3,779,430,019	0.07%	GCA_902459505.1
Bufonidae	*Bufo bufo*	No blast hit	5,044,762,059	–	GCA_905171765.1
Bufonidae	*Rhinella marina*	No blast hit	2,551,760,146	–	GCA_900303285.1
Dendrobatidae	*Oophaga pumilio*	No blast hit	3,493,380,730	–	GCA_009801035.1
Dicroglossidae	*Nanorana parkeri*	No blast hit	2,053,867,363	–	GCA_000935625.1
Limnodynastidae	*Limnodynastes dumerilii*	No blast hit	2,378,679,715	–	GCA_011038615.1
Limnodynastidae	*Platyplectrum ornatum*	No blast hit	1,065,311,793	–	GCA_016617825.1
Pipidae	*Xenopus laevis*	No blast hit	2,718,433,805	–	GCA_001663975.1
Pipidae	*Xenopus tropicalis*	No blast hit	1,451,301,209	–	GCA_000004195.4
Scaphiopodidae	*Scaphiopus couchii*	No blast hit	484,148,731	–	GCA_009364435.1
Scaphiopodidae	*Scaphiopus holbrookii*	No blast hit	710,849,620	–	GCA_009364455.1
Scaphiopodidae	*Spea bombifrons*	No blast hit	765,304,928	–	GCA_009364475.1
Scaphiopodidae	*Spea multiplicata*	No blast hit	1,075,972,709	–	GCA_009364415.1
Ambystomatidae	*Ambystoma mexicanum*	No blast hit	32,396,370,977	–	GCA_002915635.2
Rhinatrematidae	*Rhinatrema bivittatum*	No blast hit	5,319,239,201	–	GCA_901001135.1
Siphonopidae	*Microcaecilia unicolor*	No blast hit	4,685,939,420	–	GCA_901765095.1

Table 9. Frequency of horizontal transfer (HT) at both species and genus levels in each geographic region and comparisons with those in Madagascar.

Region	Number of HT occurrence	Number of BovB-positive species	p-value (vs. Madagascar)	Number of BovB-positive genera	p-value (vs. Madagascar)
Madagascar	14	57	–	37	–
Oceania	5	32	0.14	27	5.1×10^{-3}
East Asia	5	53	3.4×10^{-3}	41	3.1×10^{-4}
Central–South America	3	36	1.1×10^{-3}	35	6.3×10^{-6}
Western–Central–Southern Asia	2	12	0.11	11	8.9×10^{-3}
North America	2	20	4.8×10^{-3}	14	1.0×10^{-3}
Africa	1	36	2.8×10^{-9}	31	2.8×10^{-11}
Europe	1	9	0.013	7	1.0×10^{-3}
Southeast Asia	0	32	$< 1.8 \times 10^{-15}$	29	$< 1.8 \times 10^{-15}$

The numbers of reptile and amphibian taxa and the numbers of HT occurrences among them within the past 50 Ma were summarized. P-value was adjusted using holm correction.

Table 10. Frequency of snake species known to consume frogs in each region and comparisons with those in Madagascar.

Region	Percentage	P-value (vs. Madagascar)
Madagascar	39% (11/28)	–
Oceania	45% (13/29)	1.00
East Asia	49% (36/74)	1.00
Central–South America	37% (163/435)	1.00
Western–Central–Southern Asia	24% (29/120)	1.00
North America	34% (39/115)	1.00
Africa	43% (55/129)	1.00
Europe	21% (4/19)	1.00
Southeast Asia	23% (18/77)	1.00
Average	36% (368/1026)	–

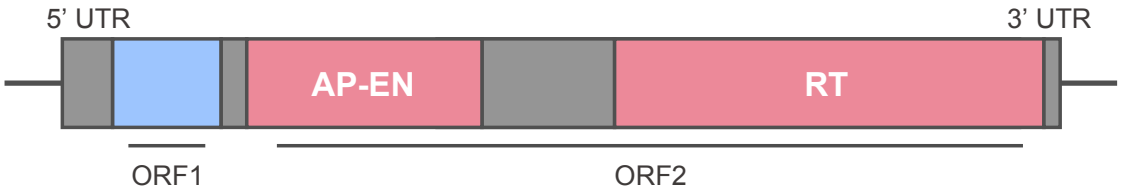
Raw numbers of species used to calculate percentages are in parentheses.
P-value was adjusted using holm correction.

Figures

Figure 1.

Structure and amino acid sequence of BovB_VA. a, The structure of BovB_VA, the full-length consensus sequence of BovB in the nose-horned viper (*Vipera ammodytes*). AP-EN, AP endonuclease; RT, reverse transcriptase; UTR, untranslated region; ORF, open reading frame. b, The amino acid sequence of ORF2. The locations of the probes used for dot blot analysis are highlighted in orange. Modified from Zupunski et al. (2001).

a



b

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      RRNGVAFTVNKRVEKAILGYNPQNDRMISVRIQGKPFNITVVQVYAPTTSAEEDEIDRFCE
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RT    WKTQQWPQDWKRSVYIPIPKRGSAKECSNYRTIALISHASKVMLKILQARLQQYVDRELP
      EVQAGFRRGRGTRDQIANIRWLMEKAREFQKNIYFCFIDYAKAFDCVDHNKLWQVLKEM
      GVPDHLICLLRNLYAGQEATVRTGHGTTDWFKIGKGVRQGCILLPCLFNLYAEHIMRKAGL
      DESKVGIKIAGRNINNLRYADDTTLMAESEEELKSLLLRVKKESAKLGLKLNIKKTKIMASNP
      LNSWQIDGEEMEVVTDFIFLGSKITADGDCSQEIKRRTLLRRKAMANLDSTLKSRDITLSTK
      VRIVKAMVFPVAMYGSESWTIKKAERQRIEAFELWCWRRLLRVPWTARRSNRSVLEEINP
      DCSLEGQILKLKLKYFGHLMRRKDSLEKSLMLGKIEGNKRMGRQRMRVLDGVTEAVGVS
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```

Figure 2.

ML tree of 285 BovB sequences. The ML phylogeny reconstructed from the 211 BovB sequences obtained in this study plus 74 known sequences.

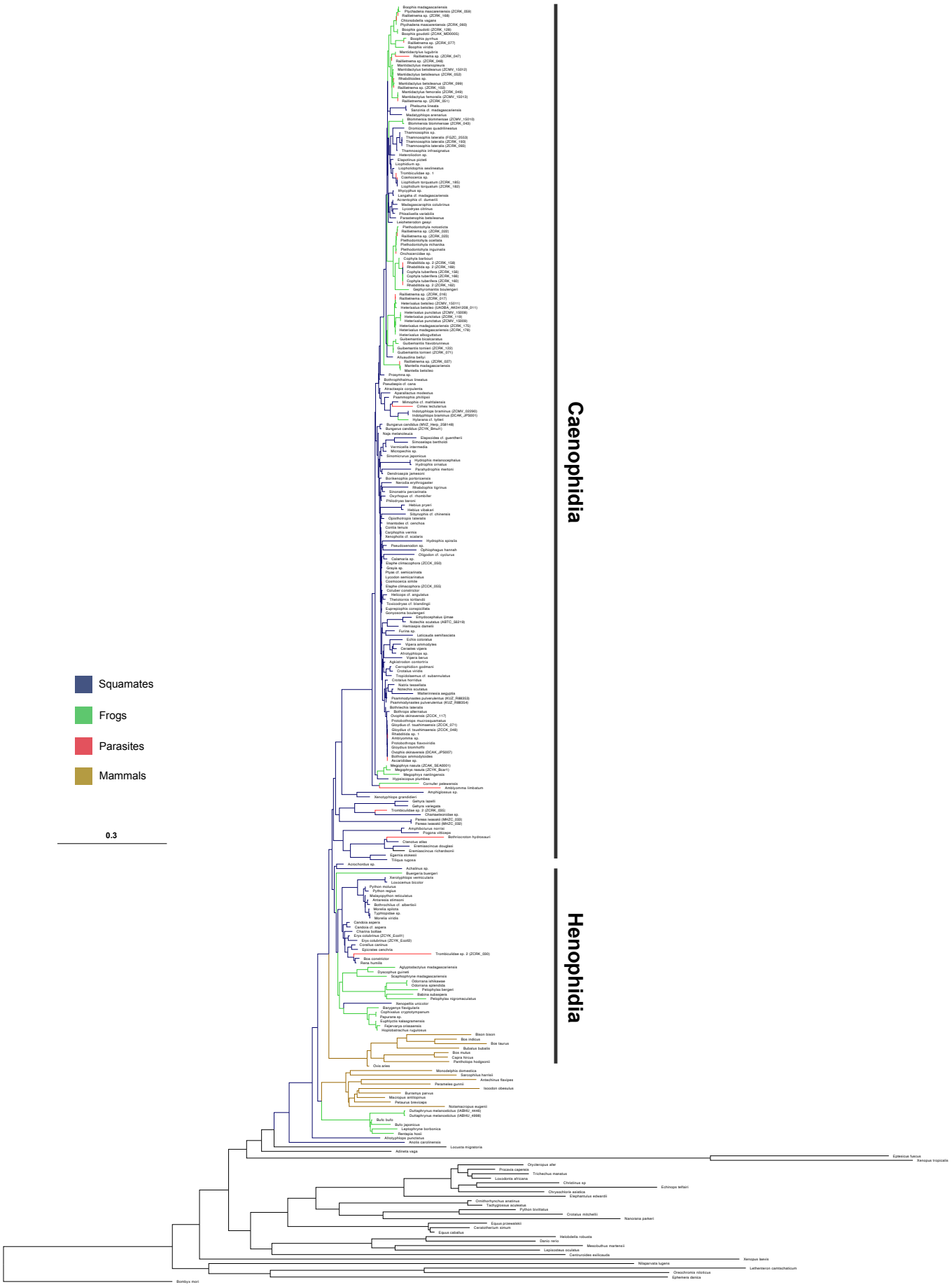

Caenophidia
Henophidia
Squamates
Frogs
Parasites
Mammals
0.3

Figure 3.

ML analysis results of 222-OTUs BovB sequences. The ML phylogeny of the 222 BovB sequences reconstructed by RAxML program (see the main text) is shown.

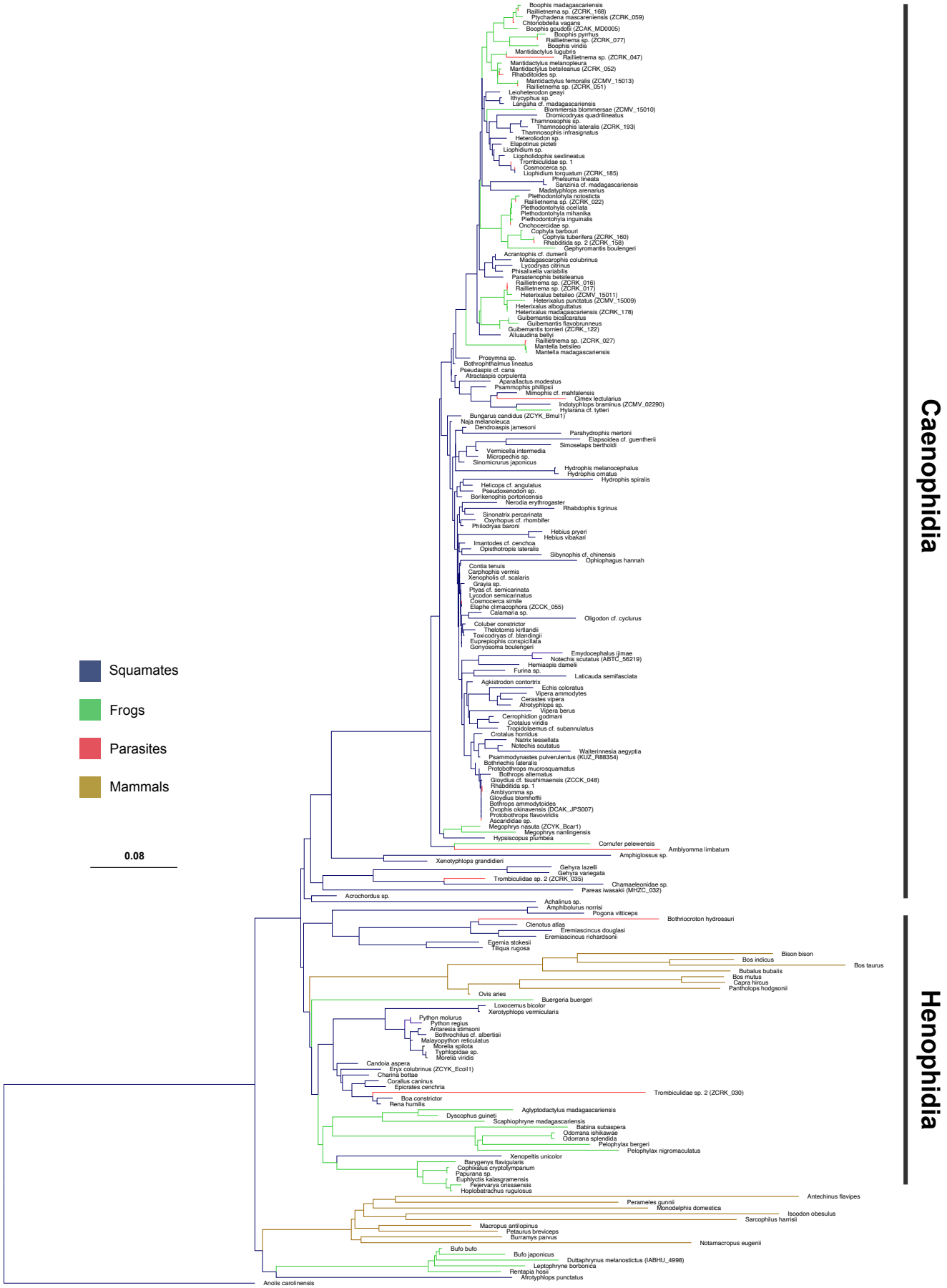
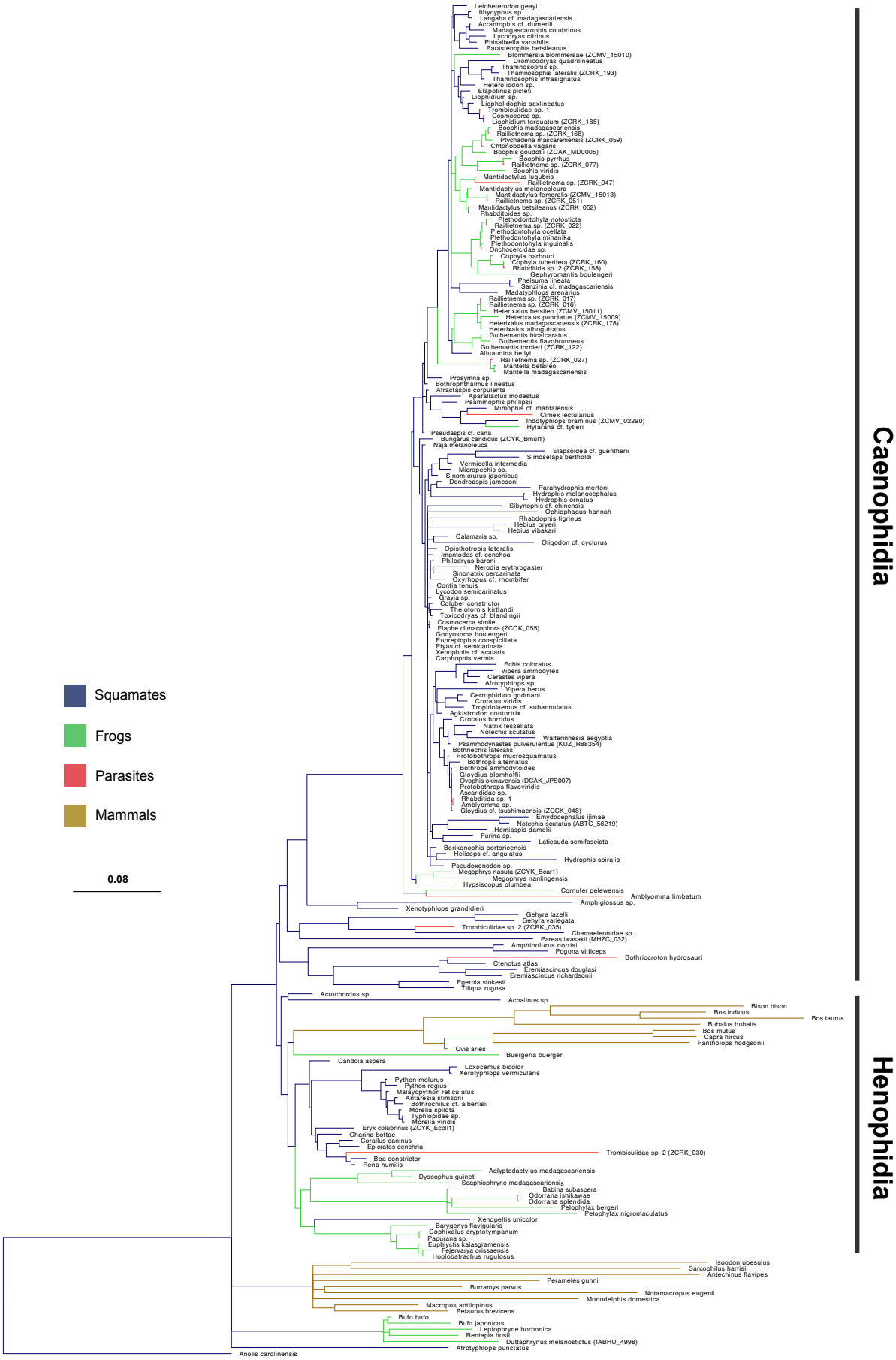

Caenophidia
Henophidia
Squamates
Frogs
Parasites
Mammals
0.08
Boophis madagascariensis
Raillietnema sp. (ZCRK_168)
Phychadena mascareniensis (ZCRK_059)
Chtoniobdella vagans
Boophis goudotii (ZCAK_MD0005)
Boophis pyrrhus
Raillietnema sp. (ZCRK_077)
Boophis viridis
Mantidactylus lugubris
Raillietnema sp. (ZCRK_047)
Mantidactylus melanopleura
Mantidactylus betsileanus (ZCRK_052)
Rhabditoides sp.
Mantidactylus femoralis (ZCMV_15013)
Raillietnema sp. (ZCRK_051)
Leioheterodon geayi
Ithycyphus sp.
Langaha cf. madagascariensis
Blommersia blommersae (ZCMV_15010)
Dromicodryas quadrilineatus
Thamnosophis sp.
Thamnosophis lateralis (ZCRK_193)
Thamnosophis infrasignatus
Heterolidon sp.
Elapotinus picteti
Liophidium sp.
Liopholidophis sexlineatus
Trombiculidae sp. 1
Cosmocerca sp.
Liophidium torquatum (ZCRK_185)
Pheluma lineata
Sanzinia cf. madagascariensis
Madatyphlops arenarius
Plethodontohyla notosticta
Raillietnema sp. (ZCRK_022)
Plethodontohyla ocellata
Plethodontohyla mihanika
Plethodontohyla inguinalis
Onchocercidae sp.
Cophyla barbouri
Cophyla tuberifera (ZCRK_160)
Rhabditida sp. 2 (ZCRK_158)
Gephyromantis boulengeri
Acrantophis cf. dumerili
Madagascarophis colubrinus
Lycodryas citrinus
Phisalixella variabilis
Parastenophis betsileanus
Raillietnema sp. (ZCRK_016)
Raillietnema sp. (ZCRK_017)
Heterixalus betsileo (ZCMV_15011)
Heterixalus punctatus (ZCMV_15009)
Heterixalus alboguttatus
Heterixalus madagascariensis (ZCRK_178)
Guibemantis bicalcaratus
Guibemantis flavobrunneus
Guibemantis tornieri (ZCRK_122)
Alluaudina bellyi
Raillietnema sp. (ZCRK_027)
Mantella betsileo
Mantella madagascariensis
Prosymna sp.
Bothrophthalmus lineatus
Pseudaspis cf. cana
Atractaspis corpulenta
Aparallactus modestus
Psammophis phillipsii
Mimophis cf. mahfalensis
Cimex lectularius
Indotyphlops braminus (ZCMV_02290)
Hylarana cf. tytleri
Bungarus candidus (ZCYK_Bmul1)
Naja melanoleuca
Dendroaspis jamesoni
Parahydrophis mertoni
Elapsoidea cf. guentherii
Simoselaps bertholdi
Vermicella intermedia
Micropechis sp.
Sinomicrurus japonicus
Hydrophis melanocephalus
Hydrophis ornatus
Hydrophis spiralis
Helicops cf. angulatus
Pseudoxenodon sp.
Borikenophis portoricensis
Nerodia erythrogaster
Rhabdophis tigrinus
Sinonatrix percarinata
Oxyrhopus cf. rhombifer
Philodryas baroni
Hebius pryeri
Hebius vibakari
Imantodes cf. cenchoa
Opisthotropis lateralis
Sibynophis cf. chinensis
Ophiophagus hannah
Contia tenuis
Carphophis vermis
Xenopholis cf. scalaris
Grayia sp.
Ptyas cf. semicarinata
Lycodon semicarinatus
Cosmocerca simile
Elaphe climacophora (ZCCK_055)
Calamaria sp.
Oligodon cf. cyclurus
Coluber constrictor
Thelotornis kirtlandii
Toxicodryas cf. blandingii
Euprepiophis conspicillata
Gonyosoma boulengeri
Emydocephalus ijimae
Notechis scutatus (ABTC_56219)
Hemiaspis damelii
Furina sp.
Laticauda semifasciata
Agkistrodon contortrix
Echis coloratus
Vipera ammodytes
Cerastes vipera
Afrotyphlops sp.
Vipera berus
Cerrophidion godmani
Crotalus viridis
Tropidolaemus cf. subannulatus
Crotalus horridus
Natrix tessellata
Notechis scutatus
Walterinnesia aegyptia
Psammodynastes pulverulentus (KUZ_R88354)
Bothriechis lateralis
Protobothrops mucrosquamatus
Bothrops alternatus
Gloydius cf. tsushimaensis (ZCCK_048)
Rhabditida sp. 1
Amblyomma sp.
Gloydius blomhoffii
Bothrops ammodytoides
Ovophis okinavensis (DCAK_JPS007)
Protobothrops flavoviridis
Ascarididae sp.
Megophrys nasuta (ZCYK_Bcar1)
Megophrys nanlingensis
Hypsiscopus plumbea
Cornufer pelewensis
Amblyomma limbatum
Amphiglossus sp.
Xenotyphlops grandidieri
Gehyra lazelli
Gehyra variegata
Trombiculidae sp. 2 (ZCRK_035)
Chamaeleonidae sp.
Pareas iwasakii (MHZC_032)
Acrochordus sp.
Achalinus sp.
Amphiblurus norrisi
Pogona vitticeps
Bothriocroton hydrosauri
Ctenotus atlas
Eremiascincus douglasi
Eremiascincus richardsonii
Egernia stokesii
Tiliqua rugosa
Bison bison
Bos indicus
Bos taurus
Bubalus bubalis
Bos mutus
Capra hircus
Pantholops hodgsonii
Ovis aries
Buergeria buergeri
Loxocemus bicolor
Xerotyphlops vermicularis
Python molurus
Python regius
Antaresia stimsoni
Bothrochilus cf. albertisii
Malayopython reticulatus
Morelia spilota
Typhlopidae sp.
Morelia viridis
Candoia aspera
Eryx colubrinus (ZCYK_Ecol1)
Charina bottae
Corallus caninus
Epicrates cenchria
Trombiculidae sp. 2 (ZCRK_030)
Boa constrictor
Rena humilis
Aglyptodactylus madagascariensis
Dyscophus guineti
Scaphiophryne madagascariensis
Babina subaspera
Odorrana ishikawae
Odorrana splendida
Pelophylax bergeri
Pelophylax nigromaculatus
Xenopeltis unicolor
Barygenys flavigularis
Cophixalus cryptotympanum
Papurana sp.
Euphlyctis kalasgramensis
Fejervarya orissaensis
Hoplobatrachus ruguliosus
Antechinus flavipes
Perameles gunnii
Monodelphis domestica
Isoodon obesulus
Sarcophilus harrisii
Macropus antilopinus
Petaurus breviceps
Burramys parvus
Notamacropus eugenii
Bufo bufo
Bufo japonicus
Duttaphrynus melanostictus (IABHU_4998)
Leptophryne borbonica
Rentapia hosii
Afrotyphlops punctatus
Anolis carolinensis

Figure 4.

BI analysis result of 222-OTUs BovB sequences. The BI phylogeny estimated using the BovB sequences inferred by MrBayes software (see the main text) is shown.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Dromicodryas quadrilineatus
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Thamnosophis infrasignatus
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Cosmocerca sp.
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Ptychadena mascareniensis (ZCRK_059)
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Boophis goudotii (ZCAK_MD0005)
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Raillietnema sp. (ZCRK_077)
Boophis viridis
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Mantidactylus melanopleura
Mantidactylus femoralis (ZCMV_15013)
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Mantidactylus betsileanus (ZCRK_052)
Rhabditoides sp.
Plethodontohyla notosticta
Raillietnema sp. (ZCRK_022)
Plethodontohyla ocellata
Plethodontohyla mihanika
Plethodontohyla inguinalis
Onchocercidae sp.
Cophyla barbouri
Cophyla tuberifera (ZCRK_160)
Rhabditida sp. 2 (ZCRK_158)
Gephyromantis boulengeri
Phelsuma lineata
Sanzinia cf. madagascariensis
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Aparallactus modestus
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Calamaria sp.
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Sinonatrix percarinata
Oxyrhopus cf. rhombifer
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Lycodon semicarinatus
Grayia sp.
Coluber constrictor
Thelotornis kirtlandii
Toxicodryas cf. blandingii
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Afrotyphlops sp.
Vipera berus
Cerrophidion godmani
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Rhabditida sp. 1
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Furina sp.
Laticauda semifasciata
Borikenophis portoricensis
Helicops cf. angulatus
Hydrophis spiralis
Pseudoxenodon sp.
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Megophrys nanlingensis
Hypsiscopus plumbea
Cornufer pelewensis
Amblyomma limbatum
Amphiglossus sp.
Xenotyphlops grandidieri
Gehyra lazelli
Gehyra variegata
Trombiculidae sp. 2 (ZCRK_035)
Chamaeleonidae sp.
Pareas iwasakii (MHZC_032)
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Pogona vitticeps
Bothriocroton hydrosauri
Ctenotus atlas
Eremiascincus douglasi
Eremiascincus richardsonii
Egernia stokesii
Tiliqua rugosa
Acrochordus sp.
Achalinus sp.
Bison bison
Bos indicus
Bos taurus
Bubalus bubalis
Bos mutus
Capra hircus
Pantholops hodgsonii
Ovis aries
Buergeria buergeri
Candoia aspera
Loxocemus bicolor
Xerotyphlops vermicularis
Python molurus
Python regius
Malayopython reticulatus
Antaresia stimsoni
Bothrochilus cf. albertisii
Morelia spilota
Typhlopidae sp.
Morelia viridis
Eryx colubrinus (ZCYK_Ecol1)
Charina bottae
Corallus caninus
Epicrates cenchria
Trombiculidae sp. 2 (ZCRK_030)
Boa constrictor
Rena humilis
Aglyptodactylus madagascariensis
Dyscophus guineti
Scaphiophryne madagascariensis
Babina subaspera
Odorrana ishikawae
Odorrana splendida
Pelophylax bergeri
Pelophylax nigromaculatus
Xenopeltis unicolor
Barygenys flavigularis
Cophixalus cryptotympanum
Papurana sp.
Euphlyctis kalasgramensis
Fejervarya orissaensis
Hoplobatrachus rugulosus
Isoodon obesulus
Sarcophilus harrisii
Antechinus flavipes
Perameles gunnii
Burramys parvus
Notamacropus eugenii
Monodelphis domestica
Macropus antilopinus
Petaurus breviceps
Bufo bufo
Bufo japonicus
Leptophryne borbonica
Rentapia hosii
Duttaphrynus melanostictus (IABHU_4998)
Afrotyphlops punctatus
Anolis carolinensis

Figure 5.

Time tree of squamate-type BovBs. The time tree with mean node age inferred based on the 222-OTUs ML topology by BEAST software (see the main text) is shown. The 95% confidence intervals of the divergence ages were depicted as blue bars at each node.

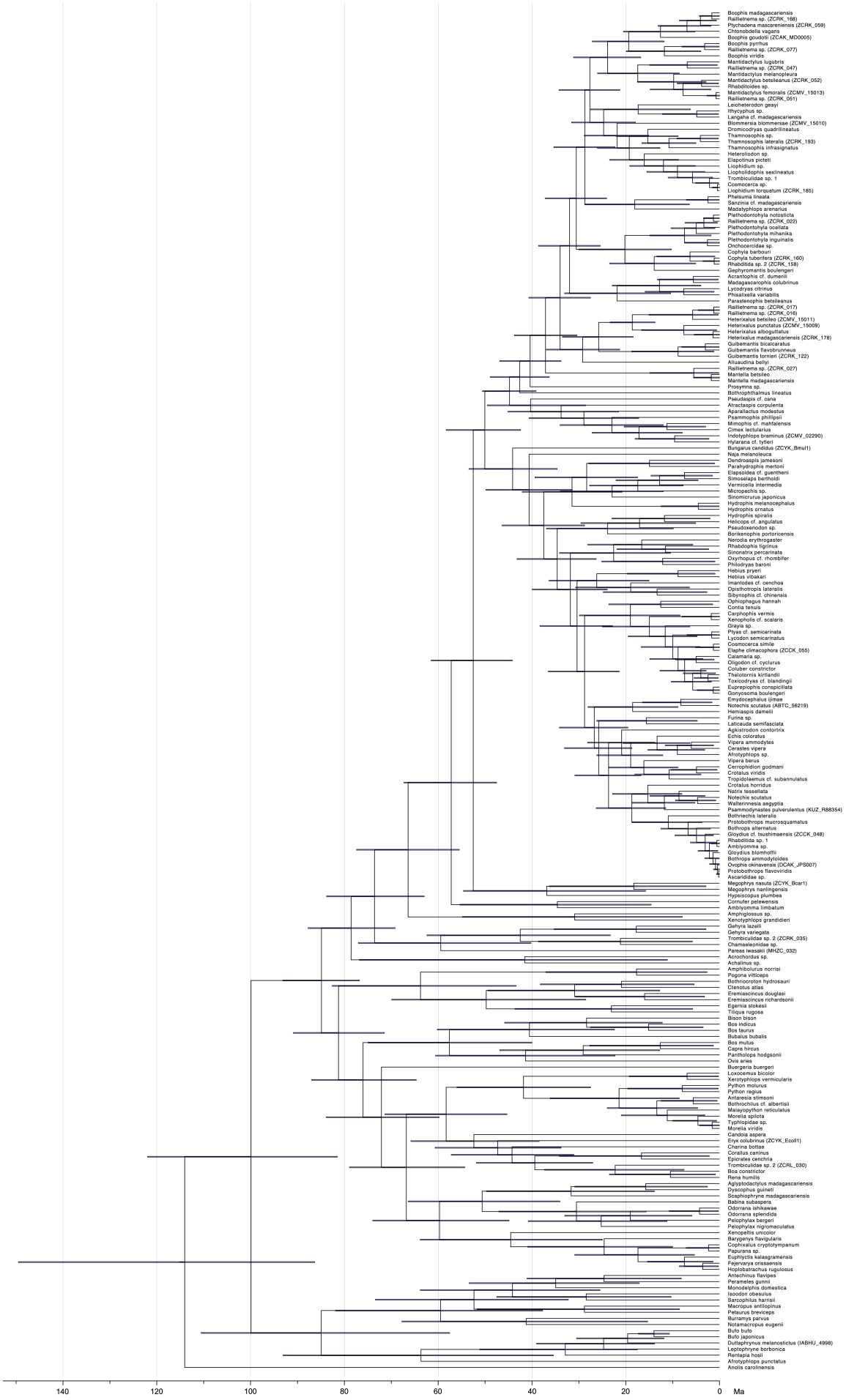
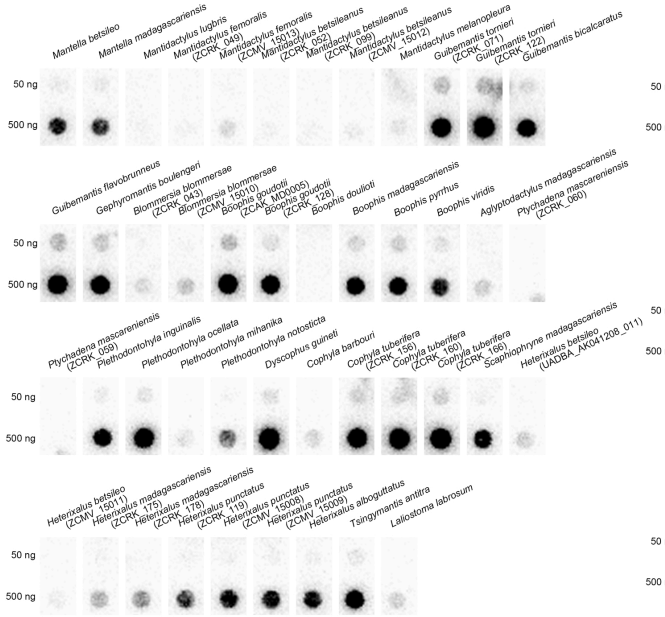

Boophis madagascariensis
Raillietnema sp. (ZCRK_168)
Ptychadena mascareniensis (ZCRK_059)
Chiromobdella vagans
Boophis goudotii (ZCAK_MD0005)
Boophis pyrrhus
Raillietnema sp. (ZCRK_077)
Boophis viridis
Mantidactylus lugubris
Raillietnema sp. (ZCRK_047)
Mantidactylus melanopleura
Mantidactylus betsileanus (ZCRK_052)
Rhabditoides sp.
Mantidactylus femoralis (ZCMV_15013)
Raillietnema sp. (ZCRK_051)
Leioheterodon geayi
Ithycyphus sp.
Langaha cf. madagascariensis
Blommersia blommersae (ZCMV_15010)
Dromicodryas quadrilineatus
Thamnosophis sp.
Thamnosophis lateralis (ZCRK_193)
Thamnosophis infrasignatus
Heterolidon sp.
Elapotinus picteti
Liophidium sp.
Liopholidophis sexlineatus
Trombiculidae sp. 1
Cosmocerca sp.
Liophidium torquatum (ZCRK_185)
Phelsuma linaata
Sanzinia cf. madagascariensis
Madatyphlops arenarius
Plethodontohyla notosticta
Raillietnema sp. (ZCRK_022)
Plethodontohyla ocellata
Plethodontohyla mihanika
Plethodontohyla inguinalis
Onchocercidae sp.
Cophyla barbouri
Cophyla tuberifera (ZCRK_160)
Rhabditida sp. 2 (ZCRK_158)
Gephyromantis boulengeri
Acrantophis cf. dumerilii
Madagascarophis colubrinus
Lycodryas citrinus
Phisalixella variabilis
Parastenophis betsileanus
Raillietnema sp. (ZCRK_017)
Raillietnema sp. (ZCRK_016)
Heterixalus betsileo (ZCMV_15011)
Heterixalus punctatus (ZCMV_15009)
Heterixalus alboguttatus
Heterixalus madagascariensis (ZCRK_178)
Guibemantis bicalcaratus
Guibemantis flavobrunneus
Guibemantis tornieri (ZCRK_122)
Aliuaudina baliyi
Raillietnema sp. (ZCRK_027)
Mantella betsileo
Mantella madagascariensis
Prosymna sp.
Bothrophthalmus lineatus
Pseudaspis cf. cana
Atractaspis corpulenta
Aparallactus modestus
Psammophis phillipsii
Mimophis cf. mahfalensis
Cimex lectularius
Indotyphlops braminus (ZCMV_02290)
Hylarana cf. tytleri
Bungarus candidus (ZCYK_Bmul1)
Naja melanoleuca
Dendroaspis jamesoni
Parahydrophis mertoni
Elapsoidea cf. guentherii
Simoselaps bertholdi
Vermicella intermedia
Micropechis sp.
Sinomicrurus japonicus
Hydrophis melanocephalus
Hydrophis ornatus
Hydrophis spiralis
Hellcops cf. angulatus
Pseudoxenodon sp.
Borikenophis portoricensis
Nerodia erythrogaster
Rhabdophis tigrinus
Sinonatrix percarinata
Oxyrhopus cf. rhombifer
Philodryas baroni
Hebius pryeri
Hebius vibakari
Imantodes cf. cenchoa
Opisthotropis lateralis
Sibynophis cf. chinensis
Ophiophagus hannah
Contia tenuis
Carphophis vermis
Xenopholis cf. scalaris
Grayia sp.
Ptyas cf. semicarinata
Lycodon semicarinatus
Cosmocerca simile
Elaphe climacophora (ZCCK_055)
Calamaria sp.
Oligodon cf. cyclurus
Coluber constrictor
Thelotornis kirtlandii
Toxicodryas cf. blandingii
Euprepiophis conspicillata
Gonyosoma boulengeri
Emydocephalus ijimae
Notechis scutatus (ABTC_56219)
Hemiaspis damelii
Furina sp.
Laticauda semifasciata
Agkistrodon contortrix
Echis coloratus
Vipera ammodytes
Cerastes vipera
Afrotyphlops sp.
Vipera berus
Cerrophidion godmani
Crotalus viridis
Tropidolaemus cf. subannulatus
Crotalus horridus
Natrix tessellata
Notechis scutatus
Walterinnesia aegyptia
Psammodynastes pulverulentus (KUZ_R88354)
Bothriechis lateralis
Protobothrops mucrosquamatus
Bothrops alternatus
Glyptus cf. tsushimaensis (ZCCK_048)
Rhabditida sp. 1
Ambilyomma sp.
Glyptus blomhoffii
Bothrops ammodytoides
Oxyphis okinawensis (DCAK_JPS007)
Protobothrops flavoviridis
Ascarididae sp.
Megophrys nasuta (ZCYK_Bcar1)
Megophrys nanlingensis
Hypsiscopus plumbea
Cornufer pelewensis
Amblyomma limbatum
Amphiglossus sp.
Xenotyphlops grandidieri
Gehyra lazaili
Gehyra variegata
Trombiculidae sp. 2 (ZCRK_035)
Chamaeleonidae sp.
Pareas iwasakii (MHZC_032)
Acrochordus sp.
Achalinus sp.
Amphibolurus norrisi
Pogona vitticeps
Bothriocroton hydrosauri
Ctenotus atlas
Eremiascincus douglasi
Eremiascincus richardsonii
Egernia stokesii
Tiliqua rugosa
Bison bison
Bos indicus
Bos taurus
Bubalus bubalis
Bos mutus
Capra hircus
Pantholops hodgsonii
Ovis aries
Buergeria buergeri
Loxocemus bicolor
Xenotyphlops vermicularis
Python molurus
Python regius
Antaresia stimsoni
Bothrochilus cf. albertisii
Malayopython reticulatus
Morelia spilota
Typhlopidae sp.
Morelia viridis
Candoia aspera
Eryx colubrinus (ZCYK_Ecol1)
Charina bottae
Coralus caninus
Epicrates cenchria
Trombiculidae sp. 2 (ZCRL_030)
Boa constrictor
Rena humilis
Aglyptodactylus madagascariensis
Dyscophus guineti
Scaphiophryne madagascariensis
Babina subaspera
Odorrana ishikawae
Odorrana splendida
Pelophylax bergeri
Pelophylax nigromaculatus
Xenopeltis unicolor
Barygenys flavigularis
Cophixalus cryptotympanum
Papurana sp.
Euphlyctis kalasgramensis
Fejervarya orissaensis
Hoplobatrachus rugulosus
Antechinus flavipes
Perameles gunnii
Monodelphis domestica
Isoodon obesulus
Sarcophilus harrisii
Macropus antilopinus
Petaurus breviceps
Burramys parvus
Notamacropus eugenii
Bufo bufo
Bufo japonicus
Duttaphrynus melanostictus (IABHU_4998)
Leptophryne borbonica
Rantapia hosii
Afrotyphlops punctatus
Anolis carolinensis
140
120
100
80
60
40
20
0
Ma

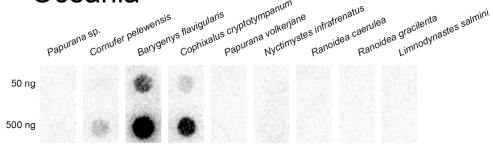
Figure 6.

Dot blot hybridization with AP endonuclease sequence probe. BovB was detected in frog genomes by dot blot analysis using the 5' side partial sequences of BovB_VA corresponding to AP endonuclease region (526 bp) as a probe.

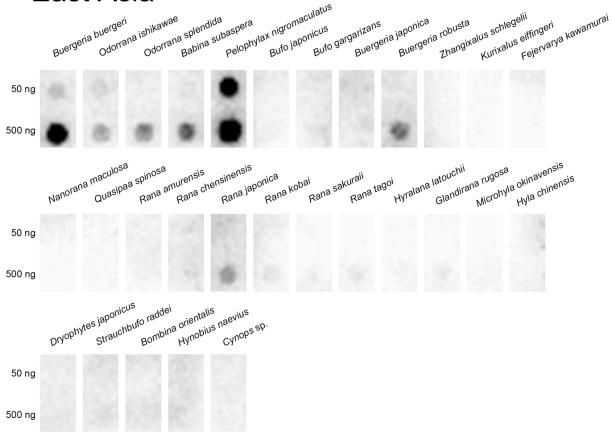
Madagascar



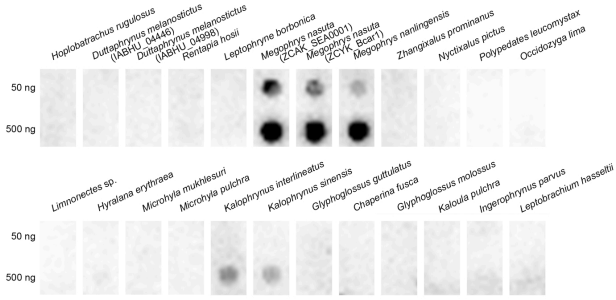
Oceania



East Asia

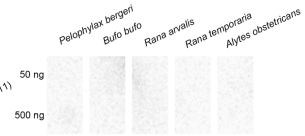


Southeast Asia

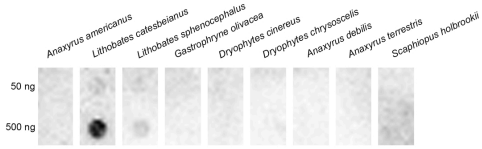


Western–Central–Southern Asia

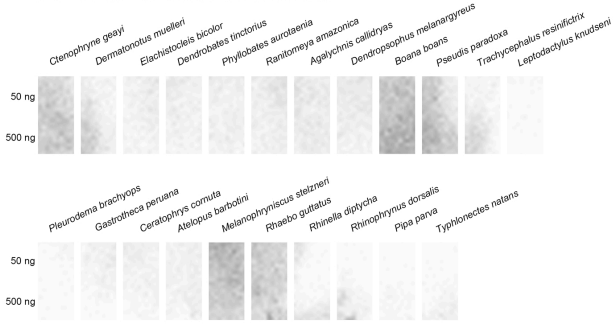
Europe



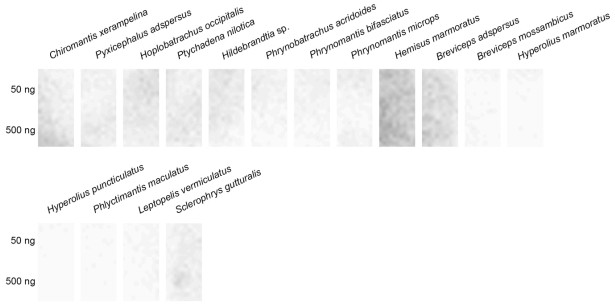
North America



Central–South America



Africa



Standard

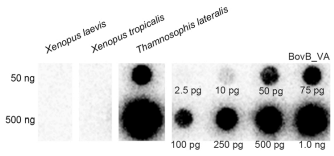
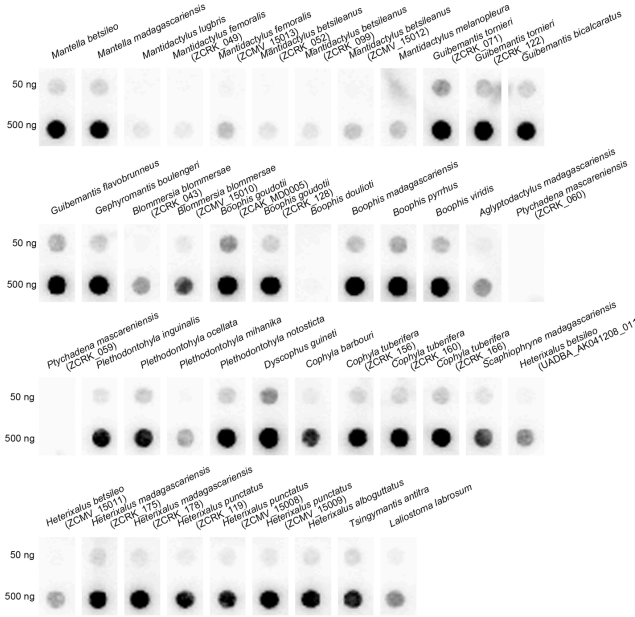


Figure 7.

Dot blot hybridization with reverse transcriptase sequence probe. BovB was detected in frog genomes using the 3' side partial sequences of BovB_VA corresponding to reverse transcriptase region (502 bp) as a probe.

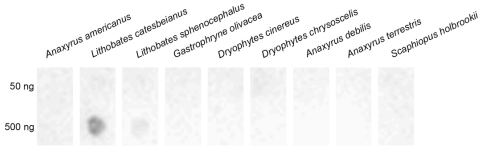
Madagascar



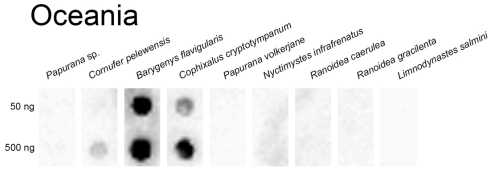
Western–Central–Southern Asia

Europe

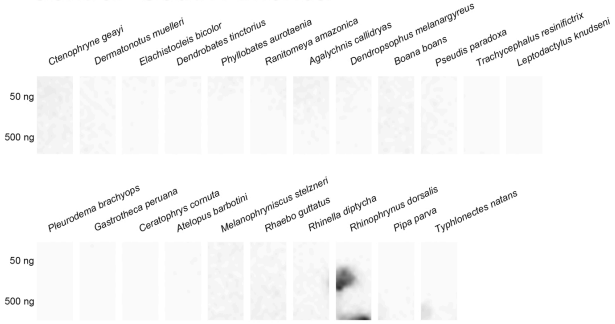
North America



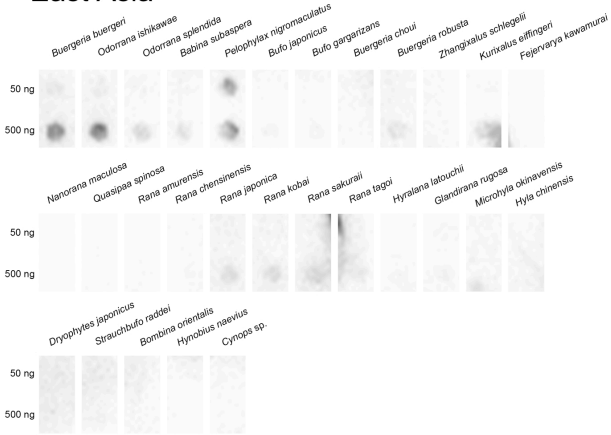
Oceania



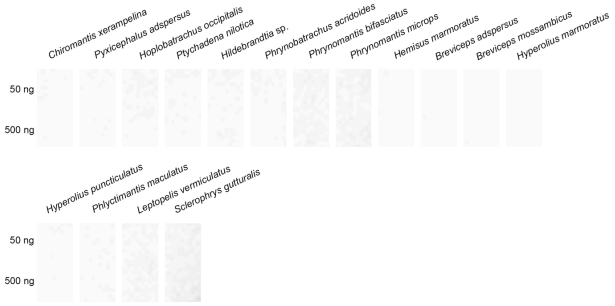
Central–South America



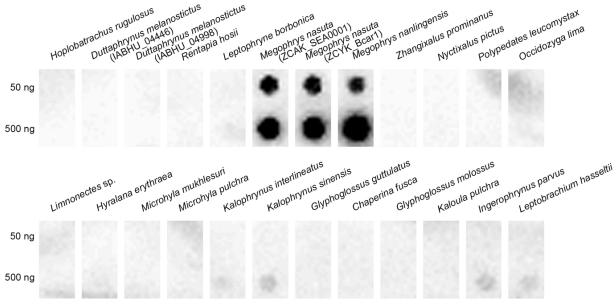
East Asia



Africa



Southeast Asia



Standard

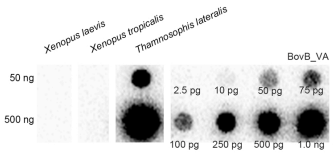


Figure 8.

The FISH visualization of BovBs. BovB sequences were detected on the chromosomes or nuclei of snake and frog species. The nuclei and chromosomes were counterstained with DAPI. a, *Elaphe quadrivirgata*; b, *Mantella betsileo*; c, *Bufo japonicus*; d, *Xenopus laevis*; e, *Xenopus tropicalis*; f, *Buergeria japonica*. BovB was detected using full BovB_VA sequence cloned in *E. coli* pUC57 vector (a, b, d–f) and the BovB amplicon of *B. japonicus* cloned in pCR2.1-TOPO vector (c) as probes.

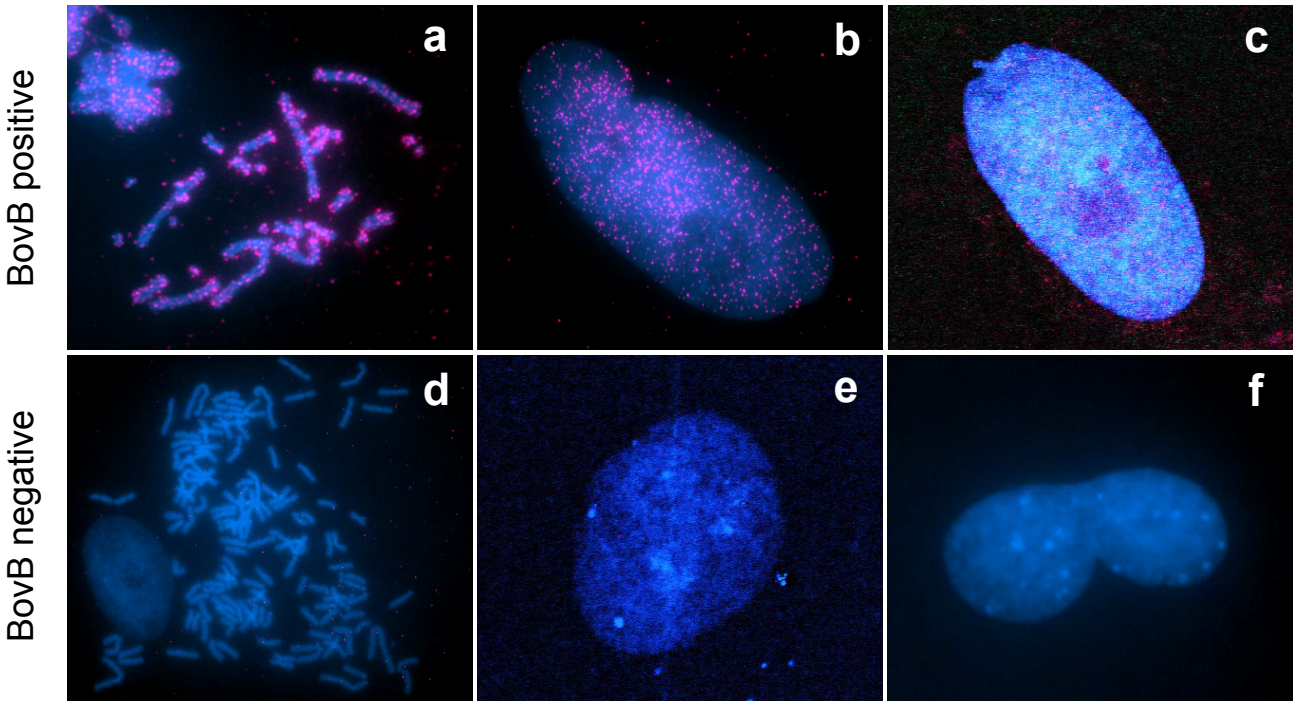

BovB positive
BovB negative
a
b
c
d
e
f

Figure 9.

Estimation of ancestral areas of HTs. The areas where the BovB HTs had occurred were estimated based on the 222-OTUs chronogram using the DIVALIKE+J model implemented in BioGeoBEARS software (see the main text). The pie charts at each node represent the set of possible ancestral areas, and the colour is associated with the area on the map. The node numbers correspond to Table 4.

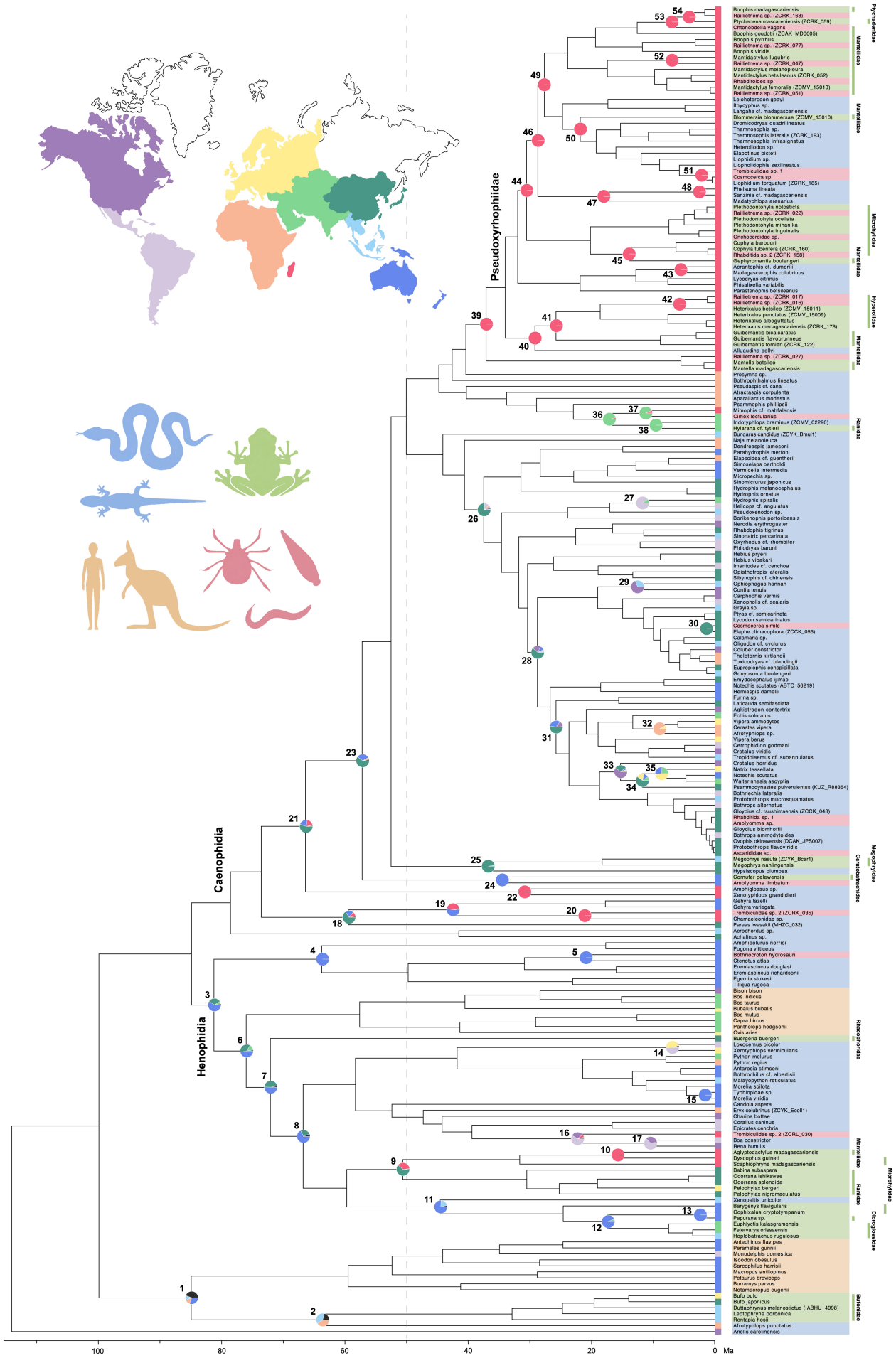

Pseudoxyrhophiidae
Caenophidia
Henophidia
Boophis madagascariensis
Raillietnema sp. (ZCRK_168)
Ptychadena mascareniensis (ZCRK_059)
Chlonobdella vagans
Boophis goudotii (ZCAK_MD0005)
Boophis pyrrhus
Raillietnema sp. (ZCRK_077)
Boophis viridis
Mantidactylus lugubris
Raillietnema sp. (ZCRK_047)
Mantidactylus melanopleura
Mantidactylus betsileanus (ZCRK_052)
Rhabdoloides sp.
Mantidactylus femoralis (ZCMV_15013)
Raillietnema sp. (ZCRK_051)
Leioheterodon geayi
Ithycyphus sp.
Langaha cf. madagascariensis
Blommersia blommersae (ZCMV_15010)
Dromicodryas quadrilineatus
Thamnosophis sp.
Thamnosophis lateralis (ZCRK_193)
Thamnosophis infrasignatus
Heteroliodon sp.
Etapotinus picteti
Liophidium sp.
Lisphaldophis sexlineatus
Trombiculidae sp. 1
Cosmocerca sp.
Liophidium torquatum (ZCRK_185)
Phelsuma lineata
Sanzinia cf. madagascariensis
Madatyphlops arenarius
Plethodontohyla notosticta
Raillietnema sp. (ZCRK_022)
Plethodontohyla ocellata
Plethodontohyla mihanika
Plethodontohyla inguinalis
Onchocercidae sp.
Cophyla barbouri
Cophyla tuberifera (ZCRK_160)
Rhabditida sp. 2 (ZCRK_158)
Gephyromantis boulengeri
Acrantophis cf. dumerilii
Madagascarophis colubrinus
Lycodryas citrinus
Phisalixella variabilis
Parastenophis betsileanus
Raillietnema sp. (ZCRK_017)
Raillietnema sp. (ZCRK_016)
Heterixalus betsileo (ZCMV_15011)
Heterixalus punctatus (ZCMV_15009)
Heterixalus alboguttatus
Heterixalus madagascariensis (ZCRK_178)
Guibemantis bicalcaratus
Guibemantis flavobrunneus
Guibemantis tornieri (ZCRK_122)
Aliaudina bellyi
Raillietnema sp. (ZCRK_027)
Mantella betsileo
Mantella madagascariensis
Piosymna sp.
Bothrophthalmus lineatus
Pseudaspis cf. cana
Atractaspis corpulenta
Aparallactus modestus
Psammophis phillipsii
Mimophis cf. mahfalensis
Cimex lectularius
Indotyphlops braminus (ZCMV_02290)
Hylarana cf. tytleri
Bungarus candidus (ZCYK_Bmul1)
Naja melanoleuca
Dendroaspis jamesoni
Parahydrophis mertoni
Elapsoidea cf. guentherii
Simoselaps bertholdi
Vermicella intermedia
Micropechis sp.
Sinomicrurus japonicus
Hydrophis melanocephalus
Hydrophis ornatus
Hydrophis spiralis
Helicops cf. angulatus
Pseudoxenodon sp.
Borikenophis portoricensis
Nerodia erythrogaster
Rhabdophis tigrinus
Sinonatrix percarinata
Oxyrhopus cf. rhombifer
Philodryas baroni
Hebius pryeri
Hebius vibakari
Imantodes cf. cenchoa
Opisthotropis lateralis
Sibynophis cf. chinensis
Ophiophagus hannah
Contia tenuis
Carphophis vermis
Xenopholis cf. scalaris
Grayia sp.
Ptyas cf. semicarinata
Lycodon semicarinatus
Cosmocerca simile
Elaphe climacophora (ZCCK_055)
Calamaria sp.
Oligodon cf. cyclurus
Coluber constrictor
Thelotornis kirtlandii
Toxicodryas cf. blandingii
Euprepiophis conspicillata
Gonyosoma boulengeri
Emydocephalus ijimae
Notechis scutatus (ABTC_56219)
Hemiaspis damelii
Furina sp.
Laticauda semifasciata
Agkistrodon contortrix
Echis coloratus
Vipera ammodytes
Cerastes vipera
Afrotyphlops sp.
Vipera berus
Cerrophidion godmani
Crotalus viridis
Tropidolaemus cf. subannulatus
Crotalus horridus
Natrix tessellata
Notechis scutatus
Waterinnesia aegyptia
Psammodynastes pulverulentus (KUZ_R88354)
Bothriechis lateralis
Protobothrops mucrosquamatus
Bothrops alternatus
Gloydius cf. tsushimaensis (ZCCK_048)
Rhabditida sp. 1
Amblyomma sp.
Gloydius blomhoffii
Bothrops ammodytoides
Ovophis okinavensis (DCAK_JPS007)
Protobothrops flavoviridis
Ascarididae sp.
Megophrys nasuta (ZCYK_Bcar1)
Megophrys nanlingensis
Hypsiscopus plumbea
Cornufer pelewensis
Amblyomma limbatum
Amphiglossus sp.
Xenotyphlops grandidieri
Gehyra lazelli
Gehyra variegata
Trombiculidae sp. 2 (ZCRK_035)
Chamaeleonidae sp.
Pareas iwasakii (MHZC_032)
Acrochordus sp.
Achalinus sp.
Amphibolurus norrisi
Pogona vitticeps
Bothriocroton hydrosauri
Otenotus atlas
Eremiascincus douglasi
Eremiascincus richardsonii
Egernia stokesii
Tiliqua rugosa
Bison bison
Bos indicus
Bos taurus
Bubalus bubalis
Bos mutus
Capra hircus
Panthotops hodgsonii
Ovis aries
Buergeria buergeri
Loxocemus bicolor
Xerotyphlops vermicularis
Python molurus
Python regius
Antaresia stimsoni
Bothrochilus cf. albertisii
Malayopython reticulatus
Morelia spilota
Typhlopidae sp.
Morelia viridis
Candoia aspera
Eryx colubrinus (ZCYK_Ecol1)
Charina bottae
Corallus caninus
Epicrates cenchria
Trombiculidae sp. 2 (ZCRL_030)
Boa constrictor
Rena humilis
Aglyptodactylus madagascariensis
Dyscophus guineti
Scaphiophryne madagascariensis
Babina subaspera
Odorrana ishikawae
Odorrana splendida
Pelophylax bergeri
Pelophylax nigromaculatus
Xenopeltis unicolor
Barygenys flavigularis
Cophixalus cryptotympanum
Papurana sp.
Euphlyctis kalasgramensis
Fejervarya orissaensis
Hoplobatrachus rugulosus
Antechinus flavipes
Perameles gunnii
Monodelphis domestica
Isoodon obesulus
Sarcophilus harrisii
Macropus antilopinus
Petaurus breviceps
Burramys parvus
Notamacropus eugenii
Bufo bufo
Bufo japonicus
Duttaphrynus melanostictus (IABHU_4998)
Leptophryne borbonica
Rentapia hosii
Afrotyphlops punctatus
Anolis carolinensis
Ptychadenidae
Mantellidae
Microhylidae
Hyperoliidae
Ranidae
Megophryidae
Ceratobatrachidae
Rhacophoridae
Dicroglossidae
Bufonidae
100
80
60
40
20
0 Ma

Figure 10.

Phylogeny, ages, and geographic distribution of horizontal transfers (HTs) of squamate-type BovB. a) The nine biogeographic areas defined in this study. Numbers at each region on the map indicate the numbers of HTs among reptiles and amphibians occurring within the past 50 Ma (33 of total 54 HTs). b) The animal taxa surveyed (reptile, blue; frog, green; mammal, orange; and parasite, red). c) The time tree of BovBs from 222 OTUs with HT occurrence geographic region estimated by BioGeoBEARS. Each tip of the tree is color-coded according to distribution within nine world regions (left, the color code is the same with a) and taxa (right = b). Pie charts on nodes represent the relative probabilities of occurrence areas for the 54 possible HTs. Reconstructions resulting in more than two possible regions are shown in dark grey. The compartments marked with Roman numerals correspond to those in d. d) The topologies showing remarkable HTs. Numbers at nodes indicate divergence time (Ma). The blindsnakes and Malagasy boas are labelled by capital letters in parentheses (T - Typhlopidae and B - Boidae, respectively). The grey colored animal symbols represent the hosts of BovB-positive parasites. The arrows with small letters correspond to those in Fig. 11.

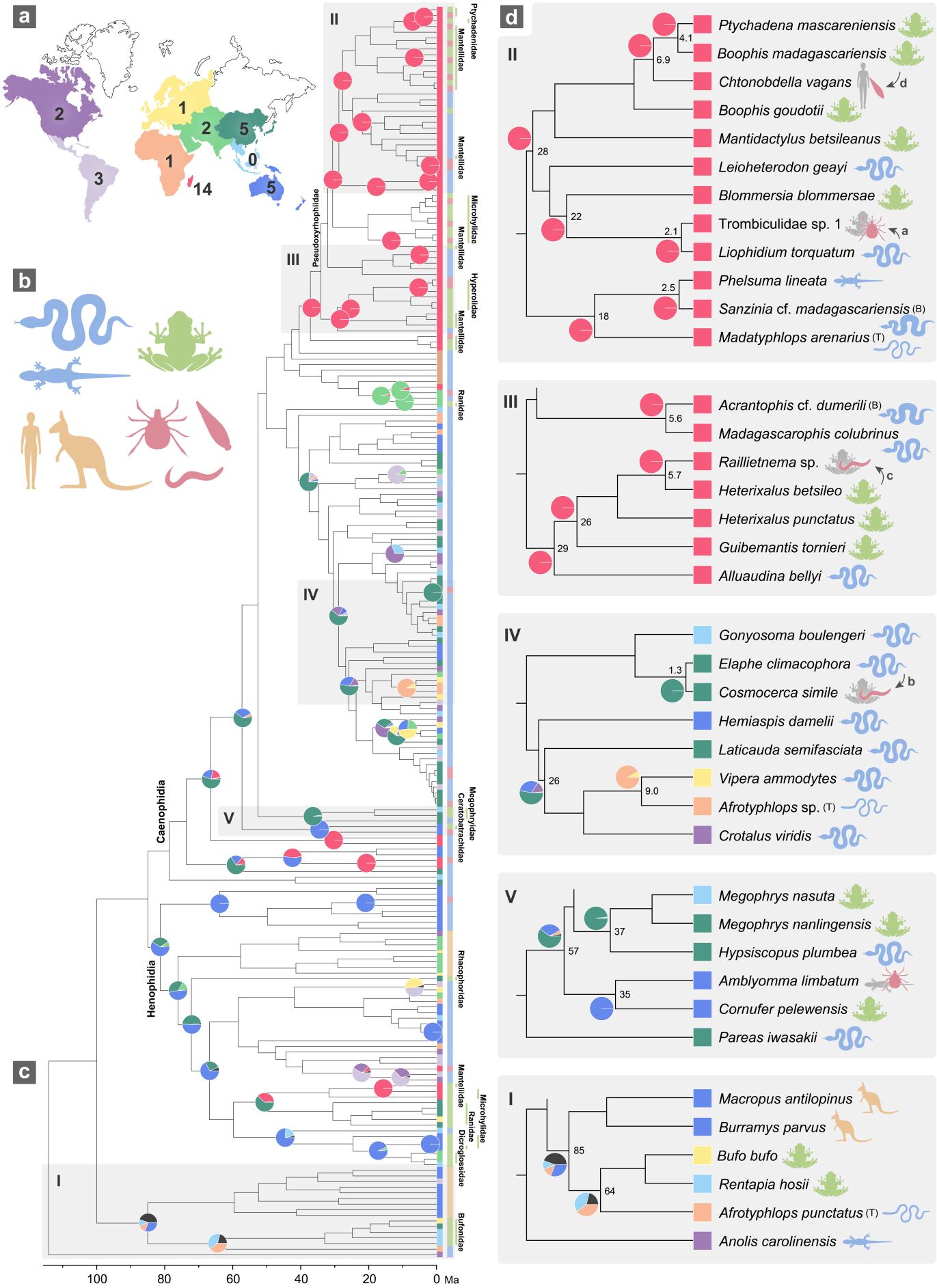
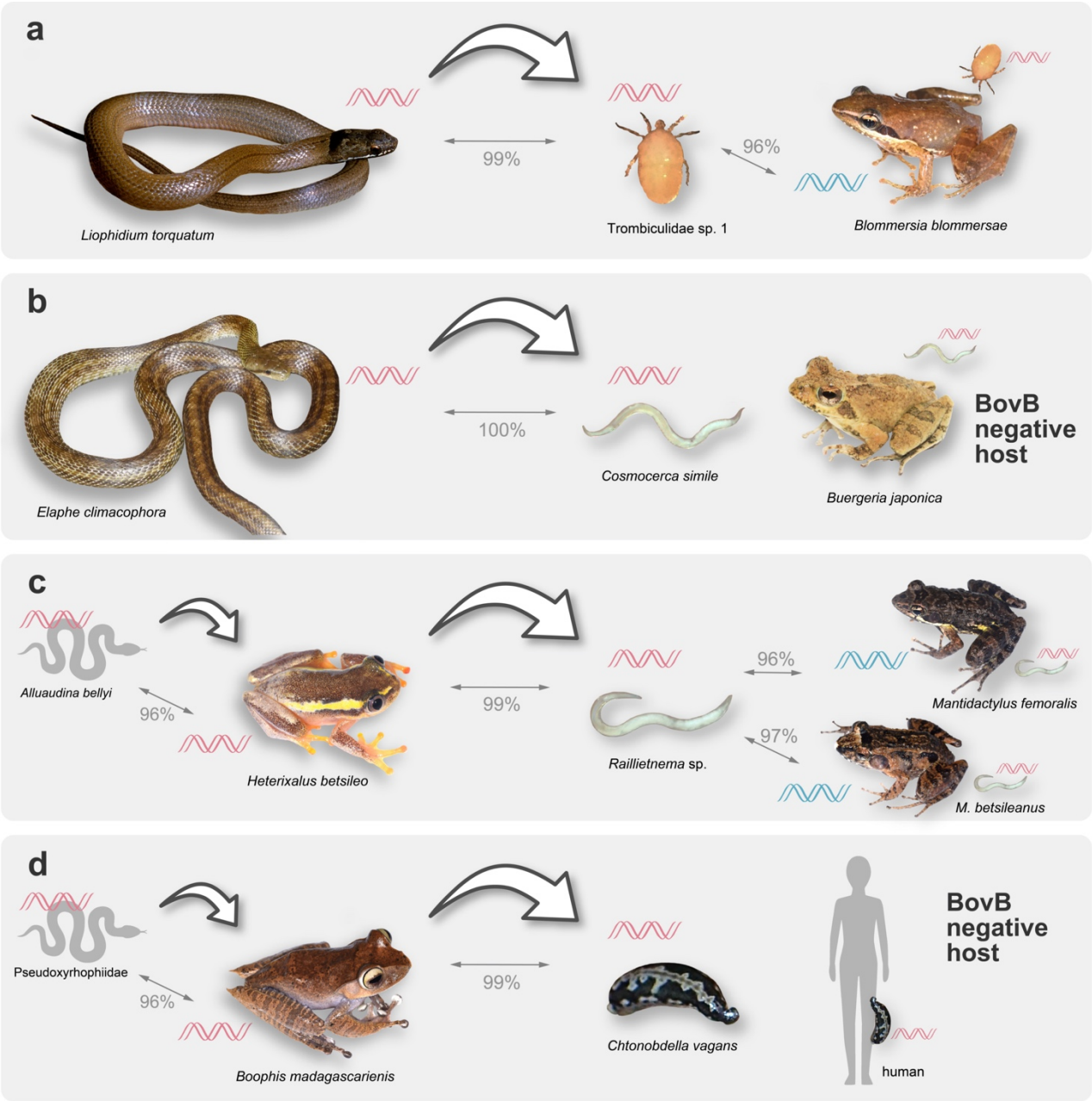

a
2
3
1
1
2
5
0
14
5
b
c
I
II
III
IV
V
Pseudoxyrhophiidae
Caenophidia
Henophidia
Ptychadenidae
Mantellidae
Mantellidae
Microhylidae
Mantellidae
Hyperoliidae
Mantellidae
Ranidae
Megophryidae
Ceratobatrachidae
Rhacophoridae
Mantellidae
Microhylidae
Ranidae
Dicroglossidae
Bufonidae
100
80
60
40
20
0 Ma
d
Ptychadena mascareniensis
Boophis madagascariensis
Chtonobdella vagans
Boophis goudotii
Mantidactylus betsileanus
Leioheterodon geayi
Blommersia blommersae
Trombiculidae sp. 1
Liophidium torquatum
Phelsuma lineata
Sanzinia cf. madagascariensis (B)
Madatyphlops arenarius (T)
4.1
6.9
28
22
2.1
2.5
18
Acrantophis cf. dumerili (B)
Madagascarophis colubrinus
Raillietnema sp.
Heterixalus betsileo
Heterixalus punctatus
Guibemantis tornieri
Alluaudina bellyi
5.6
5.7
26
29
Gonyosoma boulengeri
Elaphe climacophora
Cosmocerca simile
Hemiaspis damelii
Laticauda semifasciata
Vipera ammodytes
Afrotyphlops sp. (T)
Crotalus viridis
1.3
26
9.0
Megophrys nasuta
Megophrys nanlingensis
Hypsiscopus plumbea
Amblyomma limbatum
Cornufer pelewensis
Pareas iwasakii
37
57
35
Macropus antilopinus
Burramys parvus
Bufo bufo
Rentapia hosii
Afrotyphlops punctatus (T)
Anolis carolinensis
85
64

Figure 11.

Transmission pathways of snake BovBs via parasites. The representatives of horizontal transfers (HTs) of snake BovBs via parasites are shown. The thick and thin arrows show the direction of HT and the similarities of BovB sequences between taxa, respectively.


a
Liophidium torquatum
99%
Trombiculidae sp. 1
96%
Blommersia blommersae
b
Elaphe climacophora
100%
Cosmocerca simile
Buergeria japonica
BovB negative host
c
Alluaudina bellyi
96%
Heterixalus betsileo
99%
Raillietnema sp.
96%
Mantidactylus femoralis
97%
M. betsileanus
d
Pseudoxyrhophiidae
96%
Boophis madagascarienis
99%
Chtonobdella vagans
human
BovB negative host