



Cryptic infection of a broad taxonomic and geographic diversity of tadpoles by Perkinsea protists

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Edited by Francisco J. Ayala, University of California, Irvine, CA, and approved July 6, 2015 (received for review January 5, 2015)

The decline of amphibian populations, particularly frogs, is often cited as an example in support of the claim that Earth is undergoing its sixth mass extinction event. Amphibians seem to be particularly sensitive to emerging diseases (e.g., fungal and viral pathogens), yet the diversity and geographic distribution of infectious agents are only starting to be investigated. Recent work has linked a previously undescribed protist with mass-mortality events in the United States, in which infected frog tadpoles have an abnormally enlarged yellowish liver filled with protist cells of a presumed parasite. Phylogenetic analyses revealed that this infectious agent was affiliated with the Perkinsea: a parasitic group within the alveolates exemplified by *Perkinsus* sp., a “marine” protist responsible for mass-mortality events in commercial shellfish populations. Using small subunit (SSU) ribosomal DNA (rDNA) sequencing, we developed a targeted PCR protocol for preferentially sampling a clade of the Perkinsea. We tested this protocol on freshwater environmental DNA, revealing a wide diversity of Perkinsea lineages in these environments. Then, we used the same protocol to test for Perkinsea-like lineages in livers of 182 tadpoles from multiple families of frogs. We identified a distinct Perkinsea clade, encompassing a low level of SSU rDNA variation different from the lineage previously associated with tadpole mass-mortality events. Members of this clade were present in 38 tadpoles sampled from 14 distinct genera/phylotypes, from five countries across three continents. These data provide, to our knowledge, the first evidence that Perkinsea-like protists infect tadpoles across a wide taxonomic range of frogs in tropical and temperate environments, including oceanic islands.

frog decline | emerging disease | parasite | alveolates | molecular diversity

It is widely recognized that amphibians are among the most threatened animal groups: for example, in 2008, 32% of species were listed as “threatened or extinct” and 42% were listed as in decline (www.iucnredlist.org/initiatives/amphibians/analysis; accessed October 29, 2014) (1, 2). The main causes of amphibian decline have been identified as habitat loss, environmental change, and the introduction of nonnative species (e.g., refs. 3–6). Emerging infectious diseases have also been shown to play a key role in many amphibian declines: for example, the chytrid fungal pathogen *Batrachochytrium dendrobatidis* has caused mass mortality events (MMEs) in Australia, in Europe, and across the Americas (e.g., refs. 7–9). MMEs have also been associated with infection by *Ranavirus* in, for example, the United Kingdom (UK), United States (US), and Canada (10, 11). Recent work has linked local MMEs in the United States with the infection of larval frogs (tadpoles) of the genera *Lithobates* and *Acris* by a protist (*SI Appendix*, Fig. S1) (12, 13). In 2006, histological examinations of tadpole tissues revealed the presence of thousands of small

spherical cells preferentially infecting livers of tadpoles of the Southern Leopard Frog (*Lithobates sphenocephalus*, formerly *Rana sphenocephala*) sampled from an MME in Georgia (United States) (12). Small subunit (SSU) ribosomal DNA (rDNA) PCR and direct-amplicon sequencing, combined with phylogenetic tree reconstruction, showed that a lineage of protists closely related to *Perkinsus*, a parasite of marine bivalves (14), was the likely infectious agent (12).

Perkinsea alveolates were first described as being affiliated with the Apicomplexa (14, 15), which includes important human pathogens such as *Toxoplasma gondii* and *Plasmodium* spp. (the causative agents of malaria). Phylogenetic analysis has shown that Perkinsea are a deeply divergent sister-group of dinoflagellate alveolates (16). Only three representative groups of Perkinsea were previously described morphologically and taxonomically: *Perkinsus* spp., parasites of marine bivalves (e.g., oysters and clams), *Parvilucifera* spp., parasites of dinoflagellates, and *Rastromonas subtilis* (previously *Cryptophagus subtilis*), parasites of cryptophyte algae (17–20). However, environmental sequence

Significance

Amphibians are among the most threatened animal groups. Population declines and extinctions have been linked, in part, to emerging infectious diseases. One such emerging disease has been attributed to Perkinsea-like protists causing mass mortality events in the United States. Using molecular methods, we evaluated the diversity of Perkinsea parasites in livers sampled from a wide taxonomic collection of tadpoles from six countries across three continents. We discovered a previously unidentified phylogenetically distinct infectious agent of tadpole livers present in a broad range of frogs from both tropical and temperate sites and across all sampled continents. These data demonstrate the high prevalence and global distribution of this infectious protist.

Author contributions: A.C. and T.A.R. designed research; A.C. and D.J.G. performed research; D.J.G., M.J., A.K.D., and G.B.B.-S. contributed new reagents/analytic tools; A.C., G.L., F.M., T.M.D.-B., and T.A.R. analyzed data; A.C., D.J.G., M.J.Y., M.W., and T.A.R. wrote the paper; and D.J.G., M.J., M.J.Y., A.K.D., T.M.D.-B., G.B.B.-S., and M.W. collected field samples.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database. For a list of accession numbers, see *SI Appendix*.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1500163112/-DCSupplemental.

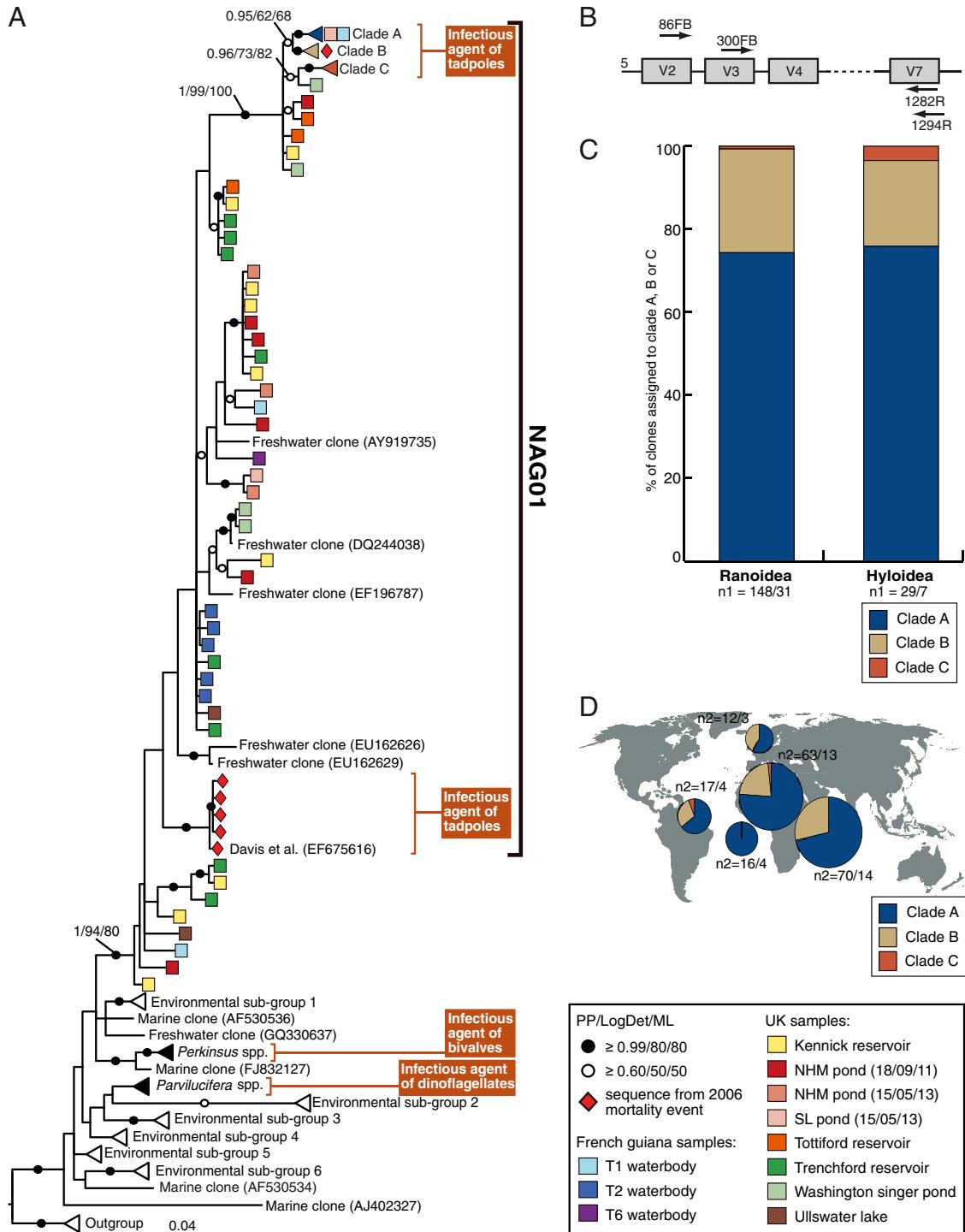


Fig. 1. (A) Phylogenetic tree of *Perkinsus* SSU rDNA sequences focusing on the NAG01 group that includes two separate phylogenetic groups recovered from tadpole liver tissue samples. The phylogeny is estimated from a masked alignment consisting of 292 taxa and 776 characters. Bayesian posterior probability (6,000 samples from 2,000,000 MCMC generations), LogDet distance bootstrap (1,000 pseudoreplicates), and maximum likelihood bootstrap (1,000 pseudoreplicates) values are added to each node using the following convention: support values are summarized by black circles when all are equal to or greater than 0.9/80%/80%, and white circles when the topology support is less but equal to or greater than 0.6/50%/50%. Five sequences of *Amoebophrya* sp. were used as outgroup. Each square represents one environmental operational taxonomic unit (OTU), and the provenance of the OTUs is indicated by colored boxes (see key for the detail of sample provenance) (*SI Appendix, Table S2* provides more details on the environments sampled). A red diamond indicates the individual clone sequences from the *L. sphaenocephalus* 2006 mass mortality event in Georgia (United States) (12). A subset of the published environmental sequences have been reduced to representative triangles (see *SI Appendix, Table S10* for detail of each environmental clade). (B) Representation of the V4 hyper-variable region of the template SSU rDNA and the relative position of the different primers used in this study (not to scale). (C) Histogram representing the percentage of clones per clade A, B, and C within each host superfamily from infected tadpoles. n_1 represents the number of total clones sequenced per host superfamily/number of infected tadpoles per host superfamily. (D) Geographical distribution of clade A, B, and C. Pie charts represent the proportion of clones per clade A, B, and C in each of the five geographical locations where NAG01 was detected (United Kingdom, French Guiana, São Tomé, Cameroon, and Tanzania). n_2 represents the number of clones sequenced per geographical location/number of infected tadpoles per geographical location.

analysis has considerably expanded the known diversity of Perkinsea-like organisms (21–25). The putative causal agent of the *L. sphenocephalus* MME lies within a clade (monophyletic group) of this environmental sequence diversity (Fig. 1A) (12) named here, for convenience, Novel Alveolate Group 01 (NAG01).

In this study, we developed SSU rDNA primers that preferentially target the NAG01 group. We used these PCR primers for targeted screening of NAG01 diversity in both tropical and temperate freshwater environments, demonstrating the efficacy of this PCR protocol and expanding the diversity of Perkinsea-like sequences sampled from freshwater environments. Using the same protocol, we also detected NAG01 from livers of a wide taxonomic diversity of tadpoles from five countries across three continents. Within our study sample, we found evidence of infection by lineages different from the Perkinsea-like *L. sphenocephalus* pathogen identified by Davis et al. (12), suggesting that multiple Perkinsea lineages infect tadpoles.

Results and Discussion

Development of a Targeted PCR Assay for Perkinsea-Like Infection of Frog Tissue. Culture-independent environmental DNA methods can be useful for detecting microbial lineages from the environment, including from the tissues of plants and animals (e.g., refs. 26–28). To investigate the prevalence and diversity of Perkinsea-like parasites infecting tadpoles, we designed two independent sets of PCR primers targeting ~800 base pairs (bp) of the small subunit (SSU) rRNA-encoding gene, including the V4 variable region (29) (see Fig. 1B and *SI Appendix*, Table S1 for primers used in this study). The primers were designed to amplify the rDNA sequences of the wider NAG01 group (Fig. 1A), including the previously sampled SSU rDNA sequence of the Perkinsea-like infectious agent of the Southern Leopard Frog (12). To investigate the specificity of these two pairs of primers, we performed PCR on 10 environmental DNA samples from three tropical (French Guiana) and seven temperate (United Kingdom) planktonic freshwater samples (*SI Appendix*, Table S2). A total of 248 clones were sampled from the UK temperate water masses and 60 clones from the French Guiana water masses (*SI Appendix*, Table S2). Among these 308 clones, sequences from 240 clustered together into 46 nonidentical sample-specific NAG01 sequences (*SI Appendix*, Table S2 and S3) that were included in the phylogenetic analysis (Fig. 1A). The remaining 68 clones were non-NAG01 sequences encompassing a mixed assemblage of Fungi, Cryptophyta, and nematode sequences. Based on these results, our PCR protocol was judged as adequate for preferentially targeted environmental clone library analyses of NAG01, including the previously identified Perkinsea-like *L. sphenocephalus* infectious agent and seven environmental DNA sequences present in the GenBank database that were recovered from freshwater planktonic samples (30–32) (*SI Appendix*, Table S4).

Investigating the Global Prevalence of NAG01 Infections in Tadpoles. Using the same PCR protocol used to detect NAG01 from freshwater environmental samples, we screened for NAG01 sequences in DNA extractions from liver samples dissected from 182 ethanol-preserved tadpoles. We sampled tadpoles from French Guiana (80 individuals from 8 sampling localities), Cameroon (37 from 14), Tanzania (15 from 1), the island of São Tomé (4 from 1), the United Kingdom (40 from 5), and the Czech Republic (6 from 3), of which 38 (21%) were PCR-positive for NAG01. See *SI Appendix*, Table S5 for more details, including the following: GPS location, sampling date, and description of environment where the tadpoles were found. For each positive sample, the PCR was repeated three times, and the amplicons were pooled. Each PCR product was checked on a 1% agarose gel for the presence of a single band of ~800 bp. The PCR product was then purified, cloned, and sequenced. We sequenced

six clones using M13F primers from each PCR-positive liver sample, resulting in 228 sequences. Based on preliminary sequence analysis of the forward reads, all NAG01 SSU sequences that were unique were double-strand sequenced. This sequencing effort encompassed a minimum of four clones per tadpole liver DNA clone library even though most libraries included fewer nonidentical clones (*SI Appendix*, Table S6).

Phylogenetic analysis demonstrated that all 177 sample-specific, unique NAG01 sequences recovered from tadpole livers form three discrete strongly supported and closely related clades (labeled clades A, B, and C in Fig. 1A). Clades A, B, and C do not correspond directly with host taxon or geographic origin (Figs. 1C and D and 2), a similar result to that described for *Perkinsus* sp. (33) parasites of marine bivalves. Interestingly, 21 of the 38 (55%) NAG01-positive tadpole liver samples yielded sequences from both clade A and B (Fig. 2), suggesting that these livers harbored representatives from different nonclonal strains/species or that there is intranuclear SSU rDNA variation within NAG01 genomes sampled. Indeed, there is less than 3% nucleotide variation among clade A, B, and C sequences (*SI Appendix*, Fig. S2), consistent with intranuclear variation such as that observed in SSU-5.8S-LSU paralogues and pseudogenes. For example, variant copies of the rRNA gene are known to occur in alveolates (34, 35) with SSU rRNA gene paralogues, with 11% difference (36) transcribed at different stages of *Plasmodium* spp. life cycle (37).

It is possible that the NAG01 DNA detected could have arisen from contamination from the environment and/or gastrointestinal tissue in the liver samples. However, this possibility was judged unlikely because all of the NAG01 SSU sequences detected from tadpole liver sequences grouped into one discrete phylogenetic subgroup whereas the primers used were capable of detecting a wide diversity of Perkinsea-like sequences from environmental DNA samples (Fig. 1A). To further test for cases of environmental contamination, DNA was extracted from tail samples (muscle and fin) taken from the same 182 tadpoles as a control to identify possible sources of nontissue-specific PCR detection or environmental contamination of NAG01 sequences. Although infections of an unknown alveolate-like parasite have been identified in the muscles of adult frogs (38), the tadpoles sampled here showed no evidence of disease progression so this experimental approach was judged as an adequate control to identify cases of environmental and wider animal tissue contamination. All controls were negative for the two primer-paired NAG01-specific PCR protocols, suggesting that detection of NAG01 was not an artifact of environmental contamination but instead a tissue-specific signal consistent with infection of a Perkinsea-like protist associated with the liver of these tadpoles. Interestingly, these liver-derived NAG01 sequences are closely related to some of the sequences recovered from filtered plankton environmental DNA sample sequences, suggesting that this protist group is found both associated with tadpoles and either as free living stage or associated with additional microbial hosts. Indeed, experimental manipulations have shown that infection of putative members of the NAG01 clade likely occur through ingestion of spores and/or zoospores from the watercolumn (39).

Diversity of Perkinsea Parasites Recovered from Liver Tissues from United States Mass Mortality Event. Sequences generated from the tadpole livers sampled here are from a different NAG01 Perkinsea lineage from that detected in liver tissues of *L. sphenocephalus* tadpoles sampled from an MME in Georgia (United States) in 2006 (Fig. 1A). Only formalin-fixed, paraffin-embedded liver samples were available from this event (12). We successfully performed two DNA extractions from a historical sample. The standard NAG01 primers failed to generate an amplicon from both DNA samples, most likely because the template DNA was highly fragmented. Consequently, we targeted a shorter template using

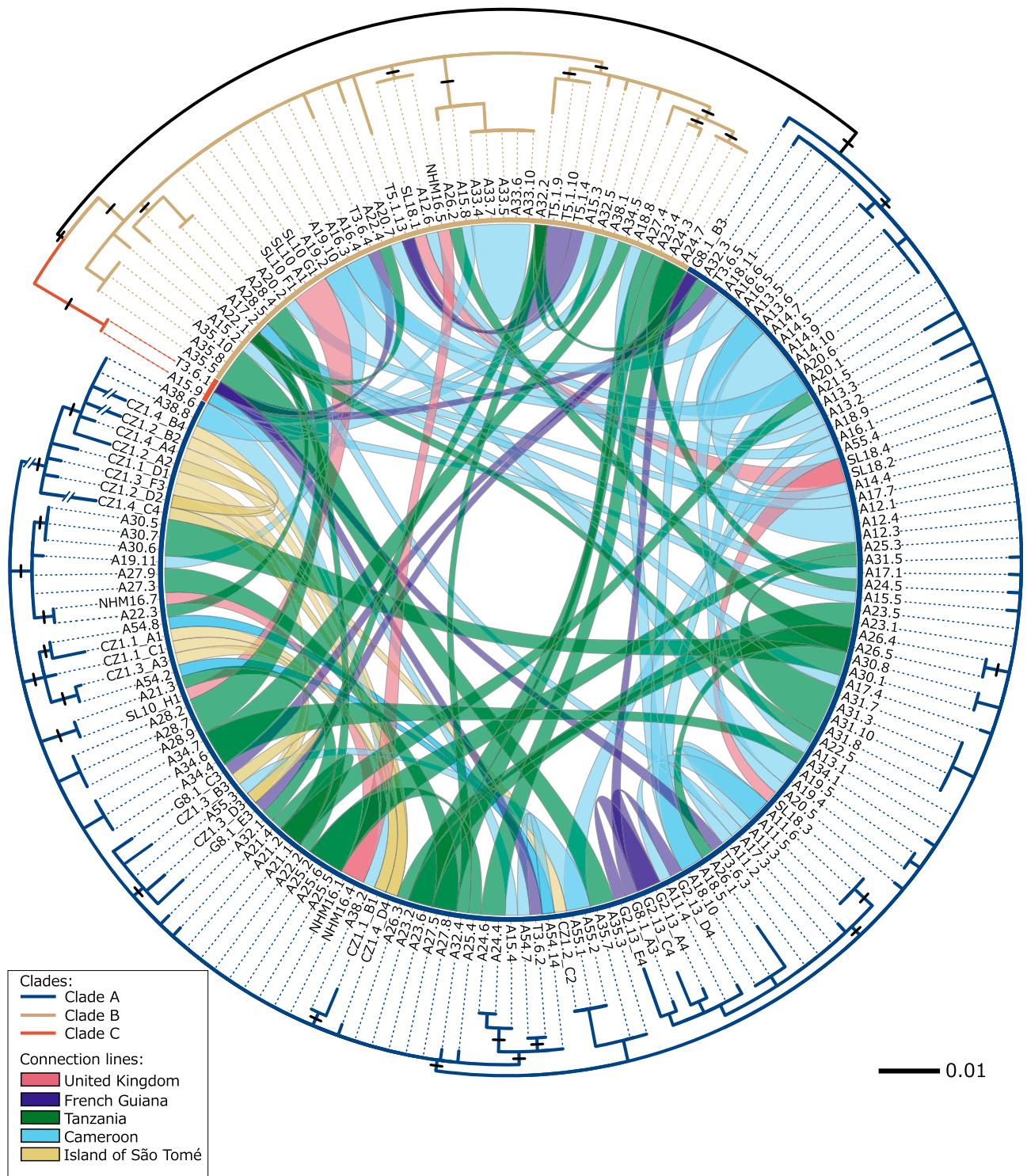


Fig. 2. A maximum likelihood phylogenetic circle tree (inverted) showing clades A, B, and C and demonstrating the provenance of NAG01 phylotypes detected in tadpole liver tissue. The RAxML phylogeny is estimated from a masked alignment consisting of 177 NAG01 sequences and 806 characters from infected tadpoles. Branches proportionally shortened by 1/2 are labeled with a double-sashed line. Each clone sequence detected from the same liver sample is connected across the central circle. The colors of the connected lines were defined by the geographical location of tadpoles sampled (see the key). Black lines on the branches mark sequence variation confirmed across multiple samples and therefore cannot be the product of PCR error during clone library construction.

the 300F-B forward primer in combination with a eukaryotic general reverse primer 600R (*SI Appendix, Table S1*), and we amplified 414 bp of the SSU rDNA. PCR reactions were repeated three times for both DNA samples, and amplicons were pooled

and cloned separately for the two DNA samples. In total, across the two DNA samples, we sequenced 45 clones in the forward and reverse direction. Phylogenetic analysis revealed that 44 clones represented four unique sequences that are closely related to

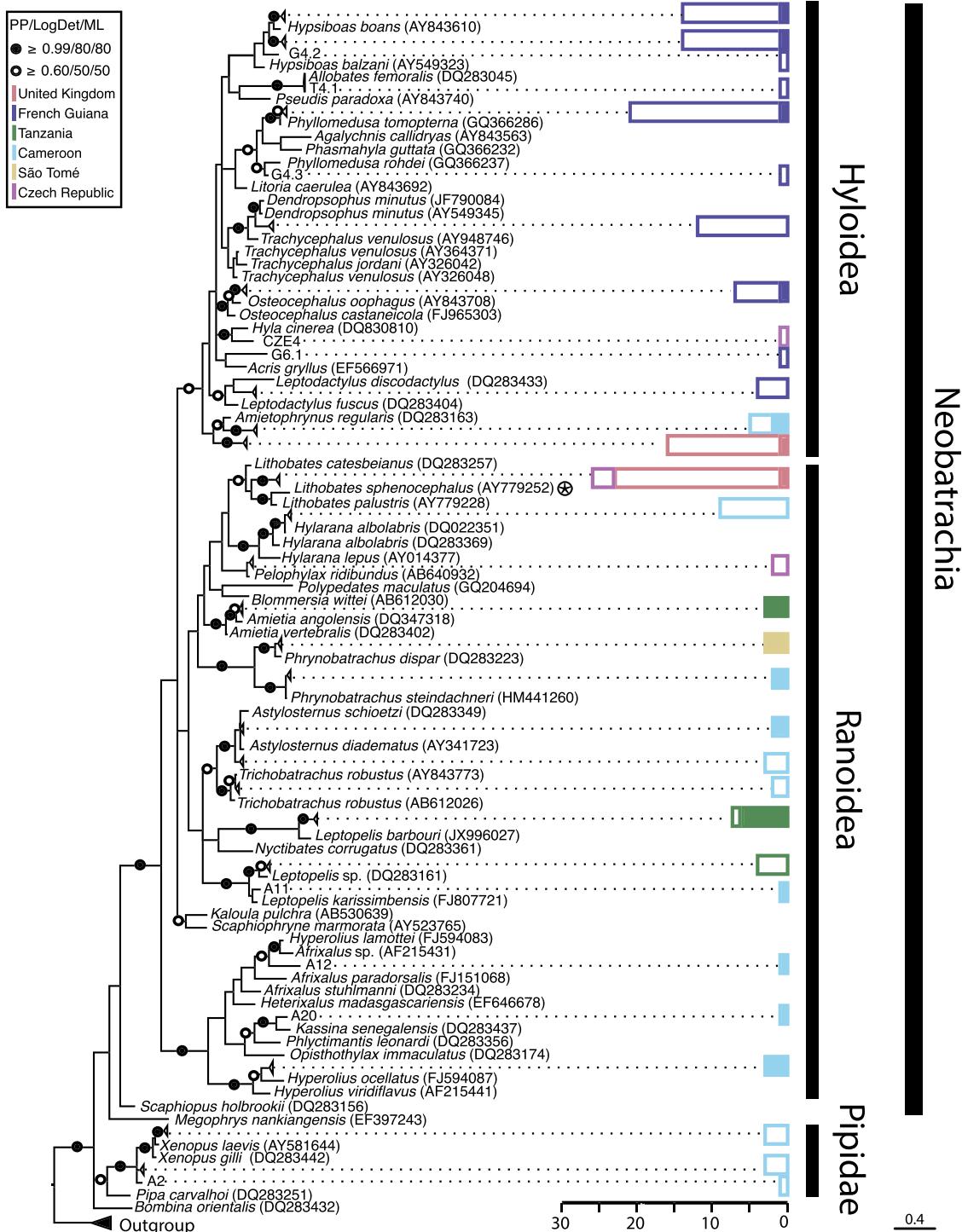


Fig. 3. Bayesian 16S rDNA phylogenetic tree of tadpole diversity sampled in this study, with histograms showing prevalence of NAG01 detection. The phylogeny is inferred from a masked alignment consisting of 247 taxa and 440 characters. Bayesian posterior probability (6,000 samples from 2,000,000 MCMCMC generations), LogDet distance bootstrap (1,000 replicates), and maximum likelihood bootstrap (1,000 replicates). Support values are summarized by black circles when all are equal to or greater than 0.9/80%/80%, and a white circle when topology support is weaker but all values are equal to or greater than 0.6/50%/50%. Sequences of *Ambystoma* sp. and *Pleurodeles* sp. (salamanders) were used as outgroup. Some frog species with multiple nonidentical 16S rDNA sequences recorded in GenBank are retained. The color-coded histogram represents the number of NAG01-negative tadpole samples (uncolored bars) and the number of NAG01-positive samples (colored bars). Each color corresponds to the tadpole's country of origin as detailed in the key. The superfamily and suborder of the tadpoles tested is indicated on the histogram. The circled star indicates the host species described by Davis et al. (12) during the 2006 mortality event.

the published sequence of the *L. sphenocephalus* infectious agent (EF675616) and one unique sequence closely related to NAG01 clade B (Fig. 1A).

Barcode Sequencing Reveals a Wide Host–Taxon Diversity and Biogeography for NAG01. Precise taxonomic identification of tadpoles using morphological characters can be difficult, especially in

geographic regions with high species diversity. We amplified a mitochondrial 16S rDNA barcode, shown to be effective for higher taxonomic assignment of amphibians (40, 41), for all 182 sampled tadpoles. The majority ($n = 175$) of the tadpoles sampled are members of the Neobatrachia (which comprises >95% of extant frogs) whereas the other 7 are tadpoles of the Pipidae: i.e., *Xenopus* (Fig. 3). We note that, although the pipid tadpoles sampled all tested negative, increased sampling is required to more confidently determine the presence/prevalence of this protist in this group of frogs.

NAG01 DNA was detected in tadpoles of two of the largest groups of neobatrachians, with 6% of sampled Hyloidea ($n = 102$) infected and 42% of Ranoidea ($n = 73$) infected (Fig. 3). In separate studies, a Perkinsea-like parasite linked to local mortality events was detected using histology in six species of Ranoidea (*L. sphenocephalus*, *Lithobates capito*, *Lithobates sevostus*, *Lithobates catesbeianus*, *Lithobates heckscheri*, and *Lithobates sylvaticus*) and one species of Hyloidea (*Acris gryllus*) (www.nwhc.usgs.gov/publications/quarterly_reports; accessed October 29, 2014) (SI Appendix, Fig. S1). Similar dissection-based approaches have also shown related infectious agents present in a wide diversity of ranids, as well as *A. gryllus*, *Hyla femoralis*, *Hyla gratiosa*, *Pseudacris ornata*, and *Gastrophryne carolinensis* (39). In the absence of molecular data, it is not clear whether these infections were of protists from NAG01 clades A, B, or C reported here, the *L. sphenocephalus* parasite detected by Davis et al. (12), or a lineage not yet sampled for DNA analysis. However, the NAG01 sequences from tadpole livers detected in this study were recovered in five of the six countries sampled, including both tropical and temperate environments and an oceanic island. Mean prevalence estimate per country and 95% confidence intervals (CIs) using the Jeffreys method were as follows: 3% (four of 80, 95% CI of 0.4–34%) in French Guiana (eight sampling events); 55.2% (13 of 37, 11–78% CI) in Cameroon (14 sampling events); 93.3% (14 of 15, 72–99% CI) in Tanzania (one sampling event); 100% (four of four, 73–100% CI) on the Island of São Tomé (one sampling event); and 9% (three of 40, 1–40% CI) in the United Kingdom (five sampling events). Taken together, these data suggest a high prevalence and broad spatial distribution of infection by a specific subclade of NAG01 Perkinsea-like protists in neobatrachian tadpole populations specifically of the superfamily Ranoidea. Information connecting the putative phylogeny inferred taxonomy of the host tadpole to the presence of the NAG01 sequence type is given in SI Appendix, Table S5 to supplement the results shown in Fig. 3.

NAG01 Perkinsea-Like Protist and Disease. All our tadpole field samples were preserved in ethanol, and, as such, we could not attempt to purify NAG01 clade A, B, or C into culture. Infection by Perkinsea-like parasites identified using histological and/or dissection microscopy techniques have been reported as visible from Gosner life cycle stages 24–42 (39, 42). In some cases, the disease phenotype of tadpoles infected by Perkinsea-like parasites has previously been described as bloated, lethargic, and showing cutaneous hemorrhages (12, 13, 42, 43) although we note that these symptoms are not diagnostic for Perkinsea disease of tadpoles, because other diseases can cause similar pathologies. The majority of the 38 infected tadpoles analyzed in this study were sampled from early Gosner stages, with the majority being of, or close to, Gosner stage 25, with one at Gosner stage 42 (SI Appendix, Table S5). The infected tadpoles showed no gross morphological symptoms of disease. A more precise definition of the disease in the cases of Perkinsea infection is infiltration of the liver and other visceral organs by large numbers of Perkinsea-like organisms (12, 38). Indications of tissue level disease in samples showing molecular evidence for the presence of NAG01 were sought through histology of liver with H&E staining of 5-μm sections from representative samples

(two from French Guiana, three from Cameroon, and three from Tanzania; for examples, see SI Appendix, Fig. S3). These data showed no identifiable tissue damage consistent with disease and no cells attributable to the NAG01 microbes detected using molecular methods, demonstrating that these infectious agents are either a small cellular form and/or badly preserved in histological sections, or, alternatively, that the population of NAG01 is very low, suggesting a low infection intensity in these tadpoles. Thus, we cannot confirm that the infectious protists identified here are pathogenic, either because (i) the tadpoles were all sampled in an early phase of disease progression and/or the infectious protists are currently dormant, (ii) NAG01 clades A, B, or C detected in this study and that branch in a different part of the phylogenetic tree to previously reported disease-causing Perkinsea of tadpoles (Fig. 14 and ref. 12) have a limited or absent disease pathology, and/or (iii) disease is caused only in association with other infectious pathogens such as *Ranavirus* (43) or other forms of host stress (44). Other parasitic Perkinsea, such as *Perkinsus sensu stricto* (parasites of bivalves), are also widely geographically distributed, but infection and catastrophic host population MMEs are localized, with pathogenicity related in part to abiotic factors (33, 45–47).

These data demonstrate that the NAG01 protists detected in tadpole livers in this study (i) are not a ubiquitous agent or contaminant but instead are liver-associated, (ii) represent a closely related phylogenetically distinct subgroup within NAG01, (iii) lie within the phylum Perkinsea, for which all known taxa are potential parasites (e.g., refs. 18, 48, and 49), and (iv) are prevalent in a range of tadpole developmental stages (Gosner stages 25–42) (SI Appendix, Table S5). Although frog MMEs associated with a Perkinsea-like parasite have been recorded only in the United States, our results demonstrate that a greater diversity of Perkinsea-like protist infections of tadpoles are widespread. There are increasing efforts to monitor the health of wild amphibian populations (50–52), with justifiable focus on fungal chytrid pathogens and *Ranavirus*, both of which have been identified as causing disease in adult frogs (6–8, 10). Further studies on the etiology of tadpole (and other larval amphibian) infections are necessary to understand the impact of these protists on amphibian populations and to inform conservation planning.

Materials and Methods

Developing Group-Specific Primers. Based on a multiple sequence alignment of available Perkinsea SSU rDNA sequences (SI Appendix, Tables S4 and S7) assembled using ARB (53), sets of “NAG01-specific primers” were designed to recover a central portion of the SSU rRNA-encoding gene, including the variable V4 region (Fig. 1B and SI Appendix, Table S1). Specificity of the PCR primers was checked first in silico by submitting sequences to the National Center for Biotechnology Information (NCBI) nonredundant (nr) DNA database (using Primer-BLAST—May 2014) and SILVA (TestProbe search—May 2014) databases (54) and, second, in situ using an environmental DNA clone library approach.

Environmental DNA Sampling of Freshwater Environments. Water samples from the surface of the watercolumn were collected from multiple freshwater environments in the United Kingdom and French Guiana (details of each sample are given in SI Appendix, Table S2). For the sampling in French Guiana, water samples were prefiltered through 10-μm polycarbonate filters (Merck Millipore), and the filtrate was then serially filtered through 5-μm and then 2-μm polycarbonate filters to collect size-specific subsections of the microbial community. This process was conducted until each filter became saturated (the volume of water filtered for each sample is reported in SI Appendix, Table S2). Saturated filters were then submerged in RNAlifeguard (MoBio) and then stored at –20 °C for 2 weeks before being transported back to the United Kingdom at ambient temperatures and then finally stored at –80 °C. The UK samples were processed in a similar manner but were serially passed through 20-μm and then 2-μm or 0.2-μm filters and transformed directly to storage at –80 °C. DNA extraction was performed using the PowerWater DNA Isolation Kit (MoBio) using the protocol recommended by the manufacturer. Details of filtration size for positive detection of NAG01 are

given in *SI Appendix, Table S2* and indicate the presence of this group in both the 0.2- to 20- μ m and 2- to 5- μ m size fraction samples.

Sampling of Tadpole Tissue for NAG01 Molecular Screening. A total of 182 ethanol-preserved tadpoles were examined: Details of the phylogenetic diversity sampled are given in Fig. 3, and the highest BLAST hit for each sample in the NCBI nr database (accessed October 2014) is given in *SI Appendix, Table S8*. *SI Appendix, Table S5* provides details of the environmental provenance of the tadpoles. Tadpoles preserved whole in ethanol were dissected using sterile tools. A piece of liver of each tadpole was removed (taking care not to pierce the gut) and placed in a fresh tube of ethanol. Concurrently, a similarly sized piece of tail was excised and placed in a separate tube of ethanol. Total DNA was extracted from all tadpole tissue samples using the Blood and Tissue DNeasy extraction kit (Qiagen) following the manufacturer's protocol, with an overnight lysis and incubation step.

SSU rDNA Clone Libraries from Environmental DNA and Tadpole Liver DNA. Environmental DNA and tadpole liver and tadpole tail DNA extractions were used as a source template to construct NAG01-specific SSU rDNA gene clone libraries. Two sets of primers were used, including two NAG01-specific forward primers paired with two general eukaryotic reverse primers (Fig. 1B). For every PCR, we included a negative control (distilled H₂O). All PCR amplification reactions were performed in 25 μ L of total volume containing 8 ng of DNA and PCR MasterMix (Promega). Cycling reactions were as follows: 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C, and 120 s at 72 °C, with an additional 10-min extension at 72 °C. For each template, three independent PCR reactions were performed, mixed together, purified using the Wizard SV Gel and PCR Clean-Up System kit (Promega), and cloned using the Stratagene PCR cloning kit (Stratagene) according to the manufacturer's instructions. Clones were blue/white screened, and a subset was selected for PCR using M13F and M13R primers, which flank the vector insertion site. Clones showing the presence of an insert of the correct size were sequenced using M13F primers. For each environmental clone library, a minimum of 20 clones was sequenced. All of the tadpole tail samples were PCR-negative. For each tadpole liver sample with a positive PCR result, we sequenced six clones using the M13F primer. All M13F NAG01 SSU sequences that were unique in one or more position were double-strand sequenced.

Mitochondrial Encoded 16S SSU rRNA Gene Barcoding of Tadpoles. Tadpole liver DNA extracts were subject to PCR using the 16Sar-F and 16Sar-R primers (40), which amplify an ~600-bp region of the 16S SSU rRNA-encoding gene. PCR amplification reactions were performed in 50 μ L of total volume containing 8 ng of DNA and PCR MasterMix (Promega). Cycling reactions were as follows: 2 min at 95 °C, followed by 25 cycles of 30 s at 95 °C, 30 s at 55 °C, and 120 s at 72 °C, with an additional 10-min extension at 72 °C. PCR products were checked on 1% agarose gel and purified using the Wizard SV Gel and PCR Clean-up System kit (Promega).

DNA Extraction and SSU rDNA Clone Library Construction from Formalin-Fixed, Paraffin-Embedded Liver Tissue from the United States Southern Leopard Frog MMEs. Frozen and/or ethanol-preserved tadpole tissue from the 2006 mortality event (12) was unavailable due to theft of copper wiring from the freezers of the M.J.Y. laboratory, resulting in loss of these samples. Thus, only formalin-fixed and paraffin-embedded tissues used for microscopy sections were available for molecular analysis. Two liver sections were excised from the paraffin block using sterile scalpel blades. Each section was incubated with xylene at 50 °C for 3 min until the paraffin had dissolved. After this process, the liver section still constituted a compact tissue aggregate. The xylene solution was removed by pipetting, and the tissue sample was washed twice using pure ethanol and dried for 15 min at 37 °C. DNA was then extracted using the DNeasy Blood and Tissue kit (Qiagen) protocol with an overnight incubation in 100 μ L of lysis buffer at 55 °C. The DNA extractions were conducted twice on the two different formalin-fixed, paraffin-embedded liver samples.

The DNA extractions were checked using a 2100 Bio-analyzer (Agilent Technologies) demonstrating highly fragmented template DNA (similar to ancient DNA samples) with an average fragment size of ~160 bp. We thus amended our PCR protocol to target a shorter amplicon using the forward NAG01-specific 300F-B primer with the eukaryotic general reverse primer 600R (see *SI Appendix, Table S1* for details). The PCR amplification reaction was performed in 25 μ L of total volume of PCR MasterMix (Promega) again with an additional negative control reaction (distilled H₂O). Cycling reactions were as follows: 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 59 °C, and 120 s at 72 °C, with an additional 10-min extension at 72 °C. For

each clone library, three independent PCRs were completed, mixed together, and cloned. Two clone libraries were constructed using the Stratagene cloning kit (Stratagene) according to the manufacturer's instructions. Forty-five independent clones with insertions of appropriate size were selected and sequenced in both directions using M13F and M13R primers.

Sequencing and Assembly. All sequencing was performed externally by Beckman Coulter Genomics. The final sets of sequences were trimmed to regions of high sequencing quality; vector sequences were removed, sequence reads were assembled into a contiguous sequence using Sequencher (Genecodes), and ambiguous sites were corrected.

Multiple Sequence Alignment and Phylogenetic Analysis. Our NAG01 clone library sequencing resulted in 177 sample-specific unique clones from the tadpole liver samples (*SI Appendix, Table S6*), 46 sample-specific unique clones from the environmental DNA samples, and five sample-specific unique clones from formalin-fixed, paraffin-embedded liver samples (see *SI Appendix, Table S3* for details). These sequences were assembled into a multiple sequence alignment with 59 Perkinsea-like sequences and *Amoeboaphrya* sp. SSU rDNA outgroup sequences (HQ658161, HM483395, HM483394, AY208894, and AF472555) recovered from the NCBI nr database (accessed May 2014; see *SI Appendix, Tables S4 and S7*). The sequences were aligned using MUSCLE (55), available via the graphical multiple sequence alignment viewer Seaview v4.2.12 (56), using default settings. The alignment was then checked and masked manually in Seaview, resulting in a data matrix of 292 sequences and 776 alignment positions. We note that the alignment included some partial database sequences to best sample the diversity of sequences sampled previously. However, Perkinsea-like sequences with the major central portion of the sequence absent were excluded from the final analysis (e.g., EUY162621, EUY162622, and EUY162623). This data matrix contained 487 variable alignment positions (excluding alignment positions with gaps) and 571 parsimony informative sites (including gaps).

All Perkinsea SSU rDNA sequences generated as a part of this study have been deposited in GenBank (see *SI Appendix, Tables S3 and S6* for details). The Perkinsea alignment is available in the Seaview (56) Mase format with the alignment mask information retained and is available at doi 10.5281/zenodo.12712.

The 182 tadpole 16S SSU rDNA sequences were aligned with a collection of frog 16S SSU rDNA sequences (*SI Appendix, Table S9*) using the same approach described for the NAG01 sequences and using MUSCLE (55) via Seaview v4.2.12 (56). All tadpole 16S SSU rDNA sequences generated in this study were BLASTn searched against the NCBI nr database (accessed May 2014), and the most similarly named frog sequence based on sequence similarity was noted (*SI Appendix, Table S8*). Preliminary phylogenies were compared with published trees (57), and additional frog 16S SSU rDNA sequences representing intermediate branches (arbitrarily selected) were added to the phylogeny. Sequences of Caudata (salamanders) were chosen as outgroup for the phylogeny: *Pleurodeles waltli* (DQ283445), *Pleurodeles nebulosus* (DQ092266), *Ambystoma mexicanum* (EF107170), and *Ambystoma tigrinum* (DQ283407). The alignment was then checked and masked manually in Seaview, resulting in a data matrix of 247 sequences and 440 alignment positions with 225 variable alignment positions (excluding alignment positions with gaps) and with 232 parsimony informative sites (including sites with gaps in the masked data matrix).

All frog SSU rDNA sequences generated as apart of this study have been deposited in GenBank (see *SI Appendix, Table S5* for details). The amphibian alignment is available in the Seaview (56) Mase format with the mask information retained and is available at doi 10.5281/zenodo.12712.

The best-fitting nucleotide substitution model for each alignment was determined using the information criterion and likelihood ratio tests implemented in Modelgenerator v0.85 (58). For Perkinsea and amphibian alignments, GTR+ Γ and GTR+I+ Γ models were selected, respectively. The α parameters for the Γ distributions were 0.38 and 0.30, respectively, with eight discrete rate categories whereas the I parameter for the amphibian alignment was 0.28. These parameters, where possible, were input into a Bayesian analysis using MrBayes v3.1.2 (59): i.e., lset, nst = rates = gamma (or invgamma in the case of the amphibian alignment), and, in both cases, we included the covariation parameter search. Two independent runs of four Metropolis-coupled (MC) Markov chain Monte Carlo (MCMC) chains (with a heat parameter of 2) were run for 2,000,000 generations. Trees were sampled every 250 generations. In both analyses, the MCMC searches had converged within the first 25% of the generations sampled; as such, the first quarter of the search results were discarded (as the burnin). Convergence between the runs and burn-in were assessed using Tracer v1.6 (tree.bio.ed.ac.uk/software/tracer). The consensus

topologies and posterior probabilities of each node were then calculated from the remaining sampled trees.

Support for the Bayesian tree topology was evaluated using two bootstrap methods and the Bayesian posterior probabilities from the MrBayes runs. Bootstrap support values were estimated using (i) RAxML v8.0.3 (60) with 1,000 pseudoreplicates and (ii) LogDet distance analysis with 1,000 pseudoreplicates using a BioNJ search method (available through Seaview v4.2.1). This second bootstrap analysis was conducted for comparison because it uses a method that minimizes artifacts arising from biases in base composition across the alignment (61).

To further investigate the phylogenetic diversity of the NAG01 sequences recovered from the tadpole livers, we resampled the alignment mask specifically focusing on sequences recovered from clades A, B, and C with the aim of maximizing unambiguously aligned sites. This analysis excluded all environmental sequences and retained only tadpole-associated *Perkinsea* sequences from clades A, B, and C. This new alignment resulted in a data matrix of 177 sequences and 806 alignment positions. This data matrix was analyzed with Modelgenerator, selecting the GTR + Γ (α parameter of 1.18) using the standard Akaike information criterion. The phylogeny was then estimated using RAxML v8.0.3 (60) using the GTR + Γ substitution model. For this analysis, we aimed to calculate a single tree that best displays the phylogenetic relationships of the NAG01 sequences sampled from tadpole livers. This alignment encompassed little sequence variation, and tree searches did not result in a consensus tree with consistently high/moderate bootstrap support values. This phylogenetic analysis therefore primarily serves to demonstrate the distribution of different SSU types across the different tissue samples and not to present a resolved phylogeny. To display codetection of NAG01 clades A, B, and C from specific liver samples, we used the Circos tool. Fig. 2 was created using Inkscape (<https://inkscape.org/en/>), Circos (62), and the Interactive Tree of Life (iTOL) (itol.embl.de) (63). Connections highlighting phylogenetically disparate clones from the same frog were plotted and color-coded by geographic sampling location (see key in Fig. 2). This plot was then combined with an inverted circular tree generated by iTOL using Inkscape.

Histology of Representative Tadpole Tissue Samples. For eight samples, half of the liver that was not used for DNA extraction was stored in 100% ethanol at 4 °C. Each liver was then embedded in paraffin wax (Sigma-Aldrich) and cut

into serial sections 5 μm thick using a Shandon tissue processor (Thermo Electron Corporation). Sections were collected onto glass slides and stained with hematoxylin and eosin staining methods (Thermo Fisher). The sections of each slide were mounted using Histomount (National Diagnostics). Sections were examined by light microscopy (Microscope Olympus IX73) for the presence of putative parasites, and digital images were obtained using the Infinity 3 camera (Lumenera Corporation).

ACKNOWLEDGMENTS. D.J.G., T.M.D.-B., and M.W. thank Marcel Kouete, Solo Ndeme, Gespo Albowede, and many local people, especially in Mamfe Division. For access to Tanzanian material, we thank Michele Menegon and Simon Loader. We thank Hendrik Müller for tadpole expertise and David Blackburn for assistance in identifying *Xenopus* life cycle stages. We thank Caroline Ware of the Natural History Museum Wildlife Garden for assistance with sampling and Anke Lange of Biosciences at the University of Exeter for assistance in histological analysis. For permits and approvals for fieldwork in French Guiana, G.B.B.-S., D.J.G., and M.W. thank Myrian Virevare (Directions de l'Environnement de l'Aménagement et du Logement) and Le Comité Scientifique Régional du Patrimoine Naturel. Additionally, for assisting our fieldwork, we thank Elodie Courtoise, Antoine Fouquet, Philippe Gaucher, Fausto Starace and family, and Jeannot and Odette (Camp Patawa). F.M. is a joint Natural History Museum, London and University College London PhD student. This research was funded primarily by the United Kingdom Department for Environment, Food and Rural Affairs, through the Systematics and Taxonomy scheme run by the Linnean Society of London and the Systematics Association UK (awarded to T.A.R.), and by fellowship awards (to A.C.). A.C. is supported by Marie Curie Intra-European Fellowship Grant FP7-PEOPLE-2011-IEF-299815 PARAFROGS and European Molecular Biology Organization (EMBO) Long-Term Fellowship ATL-1069-2011. T.A.R. is an EMBO Young Investigator and is supported by research grants from the Gordon and Betty Moore Foundation (Grant GBMF3307), the Natural Environment Research Council, the Leverhume Trust, the Biotechnology and Biological Sciences Research Council, the Canadian Institute for Advanced Research, and the Royal Society. Cameroon fieldwork was permitted by the Ministry of Forestry & Wildlife (no. 5032) funded by the US Fish & Wildlife's Wildlife Without Borders—Amphibians in Decline scheme, the Royal Geographical Society, and the Zoological Society of London's Evolutionarily Distinct and Globally Endangered Fellowship scheme. M.J. was supported by Czech Science Foundation Grant P506/10/2330 and the Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic (institutional support RVO: 60077344).

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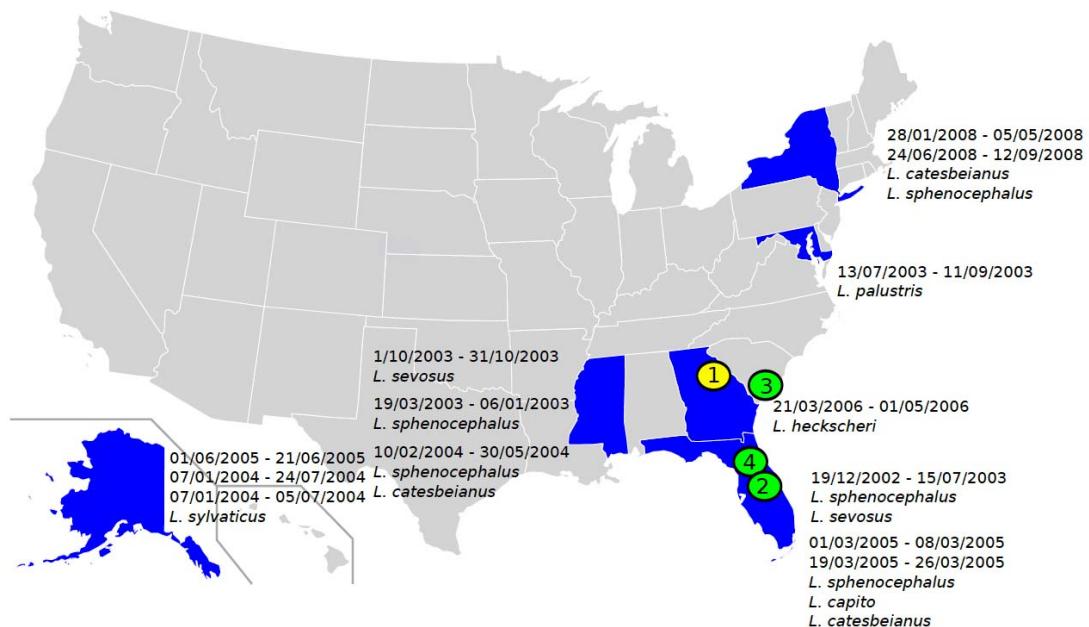
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Supplementary Materials for ‘Cryptic infection of a broad taxonomic and geographic diversity of tadpoles by Perkinsea protists’ by Chambouvet et al.

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Supplementary Figure S1: Geographic distribution of previously reported detections of Perkinsus-like parasites in the USA.

States coloured in blue are where a mortality diagnosis has been attributed to *Perkinsus*-like parasites (data available at http://www.nwhc.usgs.gov/publications/quaterly_reports). Circles represented the mortality events described in peer-reviewed scientific publications. The yellow circle represents mortality events where molecular data has been used to confirm the presence of the *Perkinsus*-like parasite. The green circles represent the sites of mortality events attributed to *Perkinsus*-like parasites using microscopy but where no molecular data was published. The number inside each circles correspond to the table below with details of each mortality event/publication.

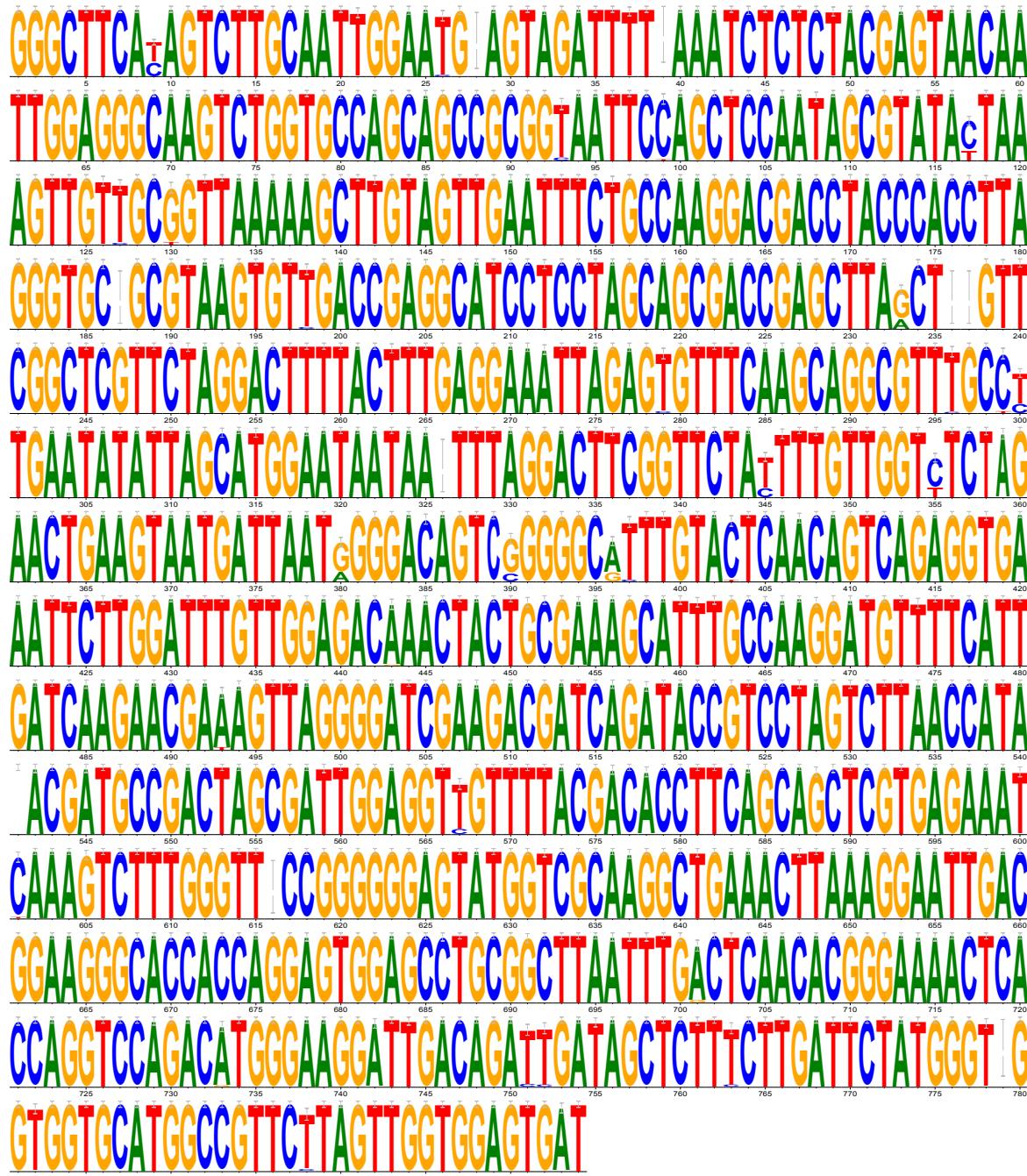


Number in circles	States	Location	GPS point	Dates	Host species	Reference
1	GA	Georgia Whitehall forest	33°53'05"N 83°21'28"W	April / May 2006	<i>L. sphenocephalus</i>	(1)
2	FL	Blue pond	29°42'40.3"N 81°59'36.6"W	Jan. 2006	<i>L. sphenocephalus</i>	(2)
	FL	Perkinsus pond	30°3'38.6"N 84°23'45.8"N	Dec. 2002, Feb. 2003, May 2003	<i>L. sphenocephalus,</i> <i>A. gryllus</i>	
	FL	Withlacoochee State forest-croom15	28°35'52.9"N 82°14'59.4"W	March 2005	<i>L. capito, L. catesbeianus, L. sphenocephalus</i>	
	FL	Withlacoochee State forest-croom16	28°35'51.1"N 82°14'56.4"W	March 2005	<i>L. capito, L. catesbeianus, L. sphenocephalus</i>	
3	SC	Savannah river site	33°18'59.4"N 81°32'21"W	Jan. 2006	<i>L. sphenocephalus</i>	(3)
4	FL	Pebbe lake	29°49'31.4"N 81°57'12.6"W	April 2011	<i>L. capito, L. catesbeianus, L. sphenocephalus</i>	(4)

Supplementary Figure S2: similarity between SSU rDNA sequences from clades A, B and C identified in this study.

(A) Graphical representation of sequence polymorphisms across the SSU rDNA sequence sampled in clusters A, B and C (<http://weblogo.berkeley.edu>). Gaps indicate rare nucleotide insertions. **(B)** Identity percentage between the three clusters A, B, C and the published sequence of the previously sampled frog *Perkinsus* (EF675616 – (1)). Data reported in Tables B was calculated using the SIAS website (available at <http://imed.med.ucm.es/Tools/sias.html>).

A.

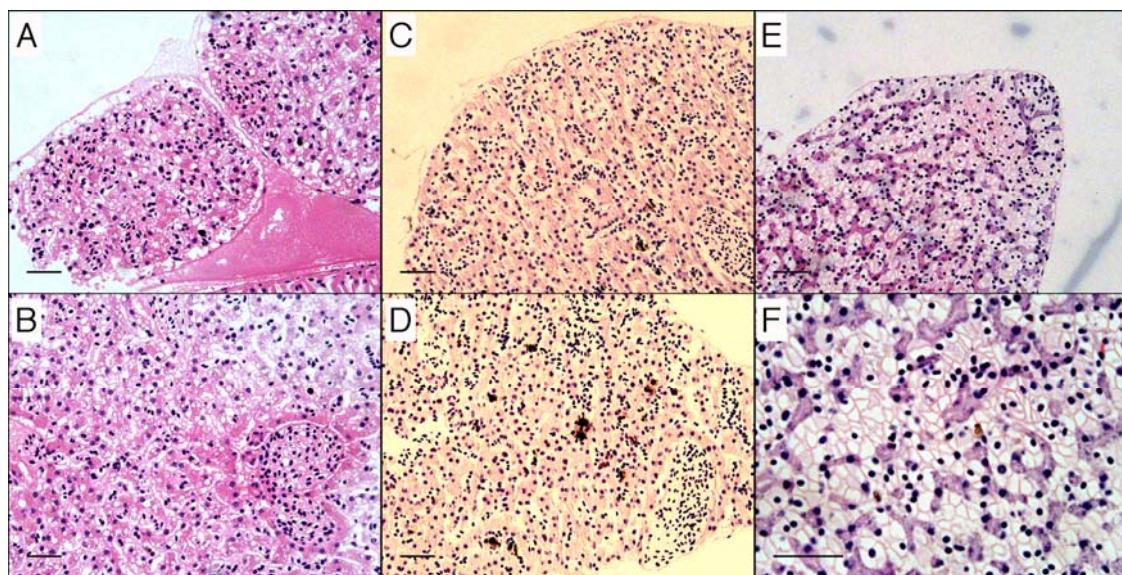


B.

	Clade A	Clade B	Clade C	EF675616
Clade A	100%	98.64%	97.52%	92.21%
Clade B		100%	97.89%	92.58%
Clade C			100%	91.96%
EF675616				100%

Supplementary Figure S3: Transverse sections (5 μ m thick) of liver tissue sample stained with H&E method.

(A-B) represent A11 liver sample from Cameroon (Africa), (C-D) from A35 liver sample from Tanzania (Africa) and (E-F) from T5.1 liver sample from Fr. Guiana (sections from five further samples not shown). Digital image was obtained using x 10 objective (A-E) and x 20 objective (F). Scale bar represent 10 μ m.



Supplementary Table S1: Primers used in this study.

Primer name	Primer sequence (5'-3')	Specificity	Reference
86F-B	CTTGCCYGGCGATRGAYCT	NAG01 Cluster	This study
300F-B	GGGCTTCAYAGTCTTGCAAT	NAG01 Cluster	This study
1294 R	CCAGRACATCTAAGGGCATCA	eukaryotes	This study
1282 R	TAAGGGCATCACWGACCTGTTAT	eukaryotes	This study
600R	GAATTTCACCTCTGACSGTTTG	eukaryotes	This study
16Sar-F	CGCCTGTTTATCAAAACAT	vertebrates	(5)
16Sar-R	CCGGTCTGAACTCAGATCACGT	vertebrates	(5)

Supplementary Table S2: List of freshwater environments sampled in this study.

Name	Locations	Country	GPS	Date	Size	Volume	Description	Number of clones	Number of NAG01 clones
				dd/mm/yyyy	fraction	filtered (μm)			
Kennick reservoir	Dartmoor National Park (Devon)	UK	N50°39'00" W3°41'24"	26/01/2010	0.2-20	0.5	Large reservoir	21	16
Washington Singer Pond	Exeter University	UK	N50°43'58.8" W3°32'13.2"	03/11/2009	2-20	0.6	Small permanent pond	65	61
				29/11/2009	2-20	0.6		15	13
Tottiford reservoir	Dartmoor National Park (Devon)	UK	N50°37'55.2" W3°40'58.799"	26/01/2010	0.2-20	0.4	Large reservoir	42	31
Trenchford reservoir	Dartmoor National Park (Devon)	UK	N50°37'48" W3°41'16.8"	26/01/2010	2-20	1	Large reservoir	24	22
NHM pond	Natural History Museum, London	UK	N51°29'45" W0°10'42"	18/09/2011	0.2-20	1	Small permanent pond	53	30

				15/05/2013	2-5	1		10	5
SL	Sevenoaks pond London	UK	N51°16'14.5" E0°11'22.1"	15/05/2013	2-5	1	Small pond	10	9
Ullswater lake	Lake District National Park (Cumbria)	UK	N54°34'34" W2°53'09"	April/July 2010	0.2-10	1	Lake- Oligo/mesotrophic	8	7
T1	Near Roura	Fr. Guiana	N4°38'34" W52°17'57"	April 2013	2-5	1	Seasonal pond	20	15
T2	Near Patawa	Fr. Guiana	N4°31'45.8" W52°09'05.6"	April 2013	2-5	0.55	Permanent pond	20	13
T6	Patawa-Kaw	Fr. Guiana	N4°31'23.8" W52°6'36.8"	April 2013	2-5	1	Small seasonal pond	20	18

Supplementary Table S3: Details of the environmental sequences included in the phylogenetic analysis shown in figure 1.

Sequence name	Name	Location	Country	Date dd/mm/yyyy	NCBI accession number
BIB1_A2	Kennick reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122562
Bib1_E2	Kennick reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122557
Bib1_E3	Kennick reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122534
BIB1_F2	Kennick reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122523
BIB1_G1	Kennick reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122546
BIB1-D1	Kennick reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122521
BiB1-D3	Kennick reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122539
BIB3_B11	Washington Singer Pond	Exeter University	UK	03/11/2009	KP122563
BIB3_B9	Washington Singer Pond	Exeter University	UK	03/11/2009	KP122559
BIB3_D3	Washington Singer Pond	Exeter University	UK	03/11/2009	KP122529
BIB3_H8	Washington Singer Pond	Exeter University	UK	29/10/2009	KP122530
BIB4_A10	NHM pond	Natural History Museum, London	UK	18/09/2011	KP122537
BIB4_D12	NHM pond	Natural History Museum, London	UK	18/09/2011	KP122540
BIB4_E12	NHM pond	Natural History Museum, London	UK	18/09/2011	KP122536
BIB4_F10	NHM pond	Natural History Museum, London	UK	18/09/2011	KP122520

BIB4_G2	Ullswater lake	Lake District National Park (Cumbria)	UK	2010	KP122551
BIB4_G4	NHM pond	Natural History Museum, London	UK	18/09/2011	KP122528
BIB4_G7	Ullswater lake	Lake District National Park (Cumbria)	UK	2010	KP122525
BIB4_H10	NHM pond	Natural History Museum, London	UK	18/09/2011	KP122552
BIB5_A11	Trenchford reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122555
BIB5_A4	Tottiford reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122558
BIB5_B10	Trenchford reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122524
BIB5_B12	Trenchford reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122544
BIB5_B5	Tottiford reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122560
BIB5_C10	Trenchford reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122538
BIB5_C12	Trenchford reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122556
BIB5_C6	Tottiford reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122561
BIB5_D12	Trenchford reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122522
BIB5_F1	Tottiford reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122554
BIB5_F12	Trenchford reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122553
BIB5_F9	Kennick reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122527

BIB5_H9	Kennick reservoir	Dartmoor National Park (Devon)	UK	26/01/2010	KP122535
BIB6_A8	SL	Sevenoaks pond London	UK	15/05/2013	KP122564
BIB6_B2	NHM pond	Natural History Museum, London	UK	15/05/2013	KP122533
BIB6_C3	NHM pond	Natural History Museum, London	UK	15/05/2013	KP122532
BIB6_E1	NHM pond	Natural History Museum, London	UK	15/05/2013	KP122542
BIB6_F7	SL	Sevenoaks pond London	UK	15/05/2013	KP122531
G1_2013_A1	T2	Near Patawa	Fr. Guiana	April 2013	KP122545
G1_2013_A2	T2	Near Patawa	Fr. Guiana	April 2013	KP122548
G1_2013_G1	T2	Near Patawa	Fr. Guiana	April 2013	KP122547
G1_2013_H1	T2	Near Patawa	Fr. Guiana	April 2013	KP122549
G1_2013_H2	T2	Near Patawa	Fr. Guiana	April 2013	KP122550
G2013_T1_A7	T1	Near Roura	Fr. Guiana	April 2013	KP122543
G2013_T1_E5	T1	Near Roura	Fr. Guiana	April 2013	KP122565
G2013_T1_H5	T1	Near Roura	Fr. Guiana	April 2013	KP122526
G2013_T6_F11	T6	Patawa-Kaw	Fr. Guiana	April 2013	KP122541
Liver_mortality event_clone_85.1c	University of Georgia's Whitehall Forest pond	Athens, GA	USA	2003	KP122566

Liver_mortality	University of	Athens, GA	USA	2003	KP122615
event_clone_85.2c	Georgia's Whitehall Forest pond				
Liver_mortality	University of	Athens, GA	USA	2003	KP122567
event_clone_85.3c	Georgia's Whitehall Forest pond				
Liver_mortality	University of	Athens, GA	USA	2003	KP122568
event_clone_85.4c	Georgia's Whitehall Forest pond				
Liver_mortality	University of	Athens, GA	USA	2003	KP122569
event_clone_85.5c	Georgia's Whitehall Forest pond				

Supplementary Table S4: GenBank environmental 18S rDNA sequences belonging to *Perkinsea* (Alveolata) used in phylogenetic analysis reported in Figure 1.

Sequences sampled from marine environments are highlighted in grey. Database searched May 2014.

Accession no.	Environmental clone	Environment	Location	Sampled environment	Reference
EF196787	B91	Lake Bourget	Alps, France	Mesotrophic lake	(6)
DQ244038	PAB5AU2004	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(7)
EU162625	PAG5SP2005	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(8)
EU162626	PAC11SP2005	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(8)
EU162628	PAD1SP2005	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(8)
EU162627	PAD12SP2005	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(8)

EU162629	PAA8SP2005	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(8)
DQ244020	PAB11AU2004	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(8)
DQ244037	PAF7AU2004	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(7)
DQ244021	PAD10AU2004	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(7)
DQ244035	PAD7AU2004	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(7)
DQ244034	PAG2AU2004	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(7)
EF527175	SA2_2G9	Framvaren Fjord	South-western Norway	Axonic/sulfidic waters	(9)
EU162624	PAA9SP2005	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(8)

EU162630	PAC8SP2005	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(8)
HQ191345	PA2009C9	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(10)
AJ402327	OLI1001	Equatorial Pacific Ocean	Pacific Ocean	Marine waters	(11)
AY642744	A31	Lake Aydat	Massif Central, France	Eutrophic lake	(12)
AY642737	A20	Lake Aydat	Massif Central, France	Eutrophic lake	(12)
EF526795	NA2_4B10	Framvaren Fjord	Norway	Anoxic marine waters	(9)
EF526831	NA1_3C6	Framvaren Fjord	Norway	Anoxic marine waters	(9)
EF526760	NA2_2D10	Framvaren Fjord	Norway	Anoxic marine waters	(9)

DQ103802	M2_18C03	Mariager Fjord	Danish east coast	Anoxic marine waters	(13)
AF530536	AT4-98	Hydrothermal sediment	Hydrothermal vents, Mid-Atlantic Ridge	Extreme environment	(14)
GQ330637	PR2_3E_17	Praz Rodet peat bog	Switzerland	Acidic extreme waters	(15)
FJ832127	BW-dinocclone29	Ship ballast water	Singapore ballast water	Marine waters	Park <i>et al.</i> unpublished
DQ455739	BB01-172.24w	Estuary	USA: Barnegat Bay, NJ	Marine waters	Lim <i>et al.</i> unpublished
AY919720	LG10-12	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(16)
AY919735	LG15-08	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(16)
AY919809	LG36-11	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(16)

AY919712	LG09-02	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(16)
AY919821	LG53-06	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(16)
AY919820	LG50-10	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(16)
AY919736	LG15-10	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(16)
AF530534	IN242	Microcolonizers	Hydrothermal vents, Mid-Atlantic Ridge	Extreme environment	(14)
DQ244027	PAA22AU2004	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(7)
DQ244031	PAF8AU2004	Lake Pavin	Massif Central, France	Oligomesotrophic lake	(7)
AY919769	LG25-01	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(16)

GU290070	TKR07E.24	Lake Tanganyika	East Africa	Tarbe <i>et al.</i> unpublished
AY919682	LG02-01	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake (16)
JN090897	KRL01E37	Lake Karla	Greece	Lake (17)
JN090873	KRL01E13	Lake Karla	Greece	Lake (17)
AF530535	AT2-6	Microcolonizers	Hydrothermal vents, Mid-Atlantic Ridge	Extreme environment (14)
EU162621*	PAA10SP2005	Lake Pavin	Massif Central, France	Oligomesotrophic lake (8)
EU162622*	PAB8SP2005	Lake Pavin	Massif Central, France	Oligomesotrophic lake (8)
EU162623*	PAB88SP2005	Lake Pavin	Massif Central, France	Oligomesotrophic lake (8)

* Excluded as central portion of the database sequence was absent

Supplementary Table S5: Detail of tadpoles tested in this study and the corresponding NCBI accession number of the 16S rRNA gene sequence.

Individual tadpoles highlighted in grey are positive for PCR detection of the NAG01 protist. Life stage was determined using the Gosner's table (18) except the tadpoles from A1 to A7 where life stages (labelled with an '*') was identified using the Nieuwkoop and Faber table (19). The column headed "Tadpole BLAST iD" reports the taxonomy of the closest GenBank, please see Table S8 for further details.

Location	Date	Environment	GPS coordinate	Locality	Altitude (m)	Sampling event / locality	Tadpole ID	Tadpole BLAST ID	Life stage	NCBI accession number
Fr. Guiana	04/2013	T1	N4°38'34" W52°17'57"	Fourgassie junction, near Roura	240	FG1	G1.1	<i>Leptodactylus knudseni</i>	40	KP122399
Fr. Guiana	04/2013	T1	N4°38'34" W52°17'57"	Fourgassie junction, near Roura	240	FG1	G1.2	<i>Leptodactylus knudseni</i>	34/35	KP122400
Fr. Guiana	04/2013	T1	N4°38'34" W52°17'57"	Fourgassie junction, near Roura	240	FG1	G1.3	<i>Leptodactylus knudseni</i>	40	KP122401
Fr. Guiana	04/2013	T1	N4°38'34" W52°17'57"	Fourgassie junction, near Roura	240	FG1	G1.4	<i>Leptodactylus knudseni</i>	31	KP122402
Fr. Guiana	04/2013	T1	N4°38'34" W52°17'57"	Fourgassie junction, near Roura	240	FG1	G1.5	<i>Phyllomedusa tomopterna</i>	29	KP122403

Fr. Guiana	14/04/20 13	T2	N4°31'45.8" W52°09'05.6"	Near Patawa	309	FG2	G2.1	<i>Phyllomedusa tomopterna</i>	30	KP122405
Fr. Guiana	14/04/20 13	T2	N4°31'45.8" W52°09'05.6"	Near Patawa	309	FG2	G2.2	<i>Phyllomedusa tomopterna</i>	29	KP122406
Fr. Guiana	14/04/20 13	T2	N4°31'45.8" W52°09'05.6"	Near Patawa	309	FG2	G2.3	<i>Phyllomedusa tomopterna</i>	29	KP122407
Fr. Guiana	14/04/20 13	T2	N4°31'45.8" W52°09'05.6"	Near Patawa	309	FG2	G2.4	<i>Phyllomedusa tomopterna</i>	26	KP122408
Fr. Guiana	14/04/20 13	T2	N4°31'45.8" W52°09'05.6"	Near Patawa	309	FG2	G2.5	<i>Phyllomedusa tomopterna</i>	37	KP122409
Fr. Guiana	14/04/20 13	T2	N4°31'45.8" W52°09'05.6"	Near Patawa	309	FG2	G2.6	<i>Phyllomedusa tomopterna</i>	36	KP122410
Fr. Guiana	14/04/20 13	T2	N4°31'45.8" W52°09'05.6"	Near Patawa	309	FG2	G2.7	<i>Phyllomedusa tomopterna</i>	38	KP122411
Fr. Guiana	14/04/20 13	T2	N4°31'45.8" W52°09'05.6"	Near Patawa	309	FG2	G2.8	<i>Phyllomedusa tomopterna</i>	25	KP122412

Fr. Guiana	14/04/20 13	T2	N4°31'45.8" W52°09'05.6"	Near Patawa	309	FG2	G2.9	<i>Phyllomedusa tomopterna</i>	27	KP122404
Fr. Guiana	14/04/20 13	T2	N4°31'45.8" W52°09'05.6"	Near Patawa	309	FG2	G2.10	<i>Phyllomedusa tomopterna</i>	26	KP122413
Fr. Guiana	14/04/20 13	T2	N4°31'45.8" W52°09'05.6"	Near Patawa	309	FG2	G2.11	<i>Phyllomedusa tomopterna</i>	25	KP122414
Fr. Guiana	14/04/20 13	T2	N4°31'45.8" W52°09'05.6"	Near Patawa	309	FG2	G2.12	<i>Phyllomedusa tomopterna</i>	28/29	KP122415
Fr. Guiana	14/04/20 13	T2	N4°31'45.8" W52°09'05.6"	Near Patawa	309	FG2	G2.13	<i>Phyllomedusa tomopterna</i>	28/29	KP122416
Fr. Guiana	04/2013	T3	N4°32'36.9" W52°9'08.1"	Patawa	177	FG3	G3.1	<i>Hypsiboas geographicus</i>	32	KP122417
Fr. Guiana	04/2013	T3	N4°32'36.9" W52°9'08.1"	Patawa	177	FG3	G3.2	<i>Hypsiboas geographicus</i>	25	KP122418
Fr. Guiana	04/2013	T3	N4°32'36.9" W52°9'08.1"	Patawa	177	FG3	G3.3	<i>Hypsiboas geographicus</i>	33	KP122419

Fr. Guiana	18/04/20 13	T4	N5°16'41.2" W54°11'57.8"	Apatou-St Laurent	98	FG4	G4.1	<i>Phyllomedusa tomopterna</i>	35	KP122420
Fr. Guiana	18/04/20 13	T4	N5°16'41.2" W54°11'57.8"	Apatou-St Laurent	98	FG4	G4.2	<i>Hypsiboas crepitans</i>	35	KP122427
Fr. Guiana	18/04/20 13	T4	N5°16'41.2" W54°11'57.8"	Apatou-St Laurent	98	FG4	G4.3	<i>Phyllomedusa hypochondrialis</i>	38	KP122428
Fr. Guiana	18/04/20 13	T4	N5°16'41.2" W54°11'57.8"	Apatou-St Laurent	98	FG4	G4.4	<i>Dendropsophus (Hyla) minutus (minuta)</i>	39	KP122439
Fr. Guiana	18/04/20 13	T4	N5°16'41.2" W54°11'57.8"	Apatou-St Laurent	98	FG4	G4.5	<i>Dendropsophus (Hyla) minutus (minuta)</i>	30/31	KP122440
Fr. Guiana	18/04/20 13	T5	N5°27'42.1" W53°53'11.1"	Near St Laurent	45	FG5	G5.1	<i>Dendropsophus (Hyla) minutus (minuta)</i>	35	KP122429
Fr. Guiana	18/04/20 13	T5	N5°27'42.1" W53°53'11.1"	Near St Laurent	45	FG5	G5.2	<i>Dendropsophus (Hyla) minutus (minuta)</i>	39	KP122430
Fr. Guiana	18/04/20 13	T5	N5°27'42.1" W53°53'11.1"	Near St Laurent	45	FG5	G5.3	<i>Dendropsophus (Hyla) minutus (minuta)</i>	37	KP122431

Fr. Guiana	18/04/20 13	T5	N5°27'42.1" W53°53'11.1"	Near St Laurent	45	FG5	G5.4	<i>Dendropsophus (Hyla) minutus (minuta)</i>	31	KP122432
Fr. Guiana	18/04/20 13	T5	N5°27'42.1" W53°53'11.1"	Near St Laurent	45	FG5	G5.5	<i>Dendropsophus (Hyla) minutus (minuta)</i>	29/30	KP122433
Fr. Guiana	18/04/20 13	T5	N5°27'42.1" W53°53'11.1"	Near St Laurent	45	FG5	G5.6	<i>Dendropsophus (Hyla) minutus (minuta)</i>	26/27	KP122434
Fr. Guiana	18/04/20 13	T5	N5°27'42.1" W53°53'11.1"	Near St Laurent	45	FG5	G5.7	<i>Dendropsophus (Hyla) minutus (minuta)</i>	41	KP122435
Fr. Guiana	18/04/20 13	T5	N5°27'42.1" W53°53'11.1"	Near St Laurent	45	FG5	G5.8	<i>Dendropsophus (Hyla) minutus (minuta)</i>	34	KP122436
Fr. Guiana	18/04/20 13	T5	N5°27'42.1" W53°53'11.1"	Near St Laurent	45	FG5	G5.9	<i>Dendropsophus (Hyla) minutus (minuta)</i>	41	KP122437
Fr. Guiana	18/04/20 13	T5	N5°27'42.1" W53°53'11.1"	Near St Laurent	45	FG5	G5.10	<i>Dendropsophus (Hyla) minutus (minuta)</i>	31	KP122438
Fr. Guiana	23/04/20 13	T6	N4°31'23.8" W52°6'36.8"	Patawa-Kaw	262	FG6	G6.1	<i>Scinax elaeochroa</i>	36/37	KP122441

Fr. Guiana	23/04/20 13	T6	N4°31'23.8" W52°6'36.8"	Patawa-Kaw	262	FG6	G6.2	<i>Phyllomedusa tomopterna</i>	41	KP122421
Fr. Guiana	23/04/20 13	T6	N4°31'23.8" W52°6'36.8"	Patawa-Kaw	262	FG6	G6.3	<i>Phyllomedusa tomopterna</i>	36	KP122422
Fr. Guiana	23/04/20 13	T6	N4°31'23.8" W52°6'36.8"	Patawa-Kaw	262	FG6	G6.4	<i>Phyllomedusa tomopterna</i>	29/30	KP122423
Fr. Guiana	23/04/20 13	T6	N4°31'23.8" W52°6'36.8"	Patawa-Kaw	262	FG6	G6.5	<i>Phyllomedusa tomopterna</i>	31	KP122424
Fr. Guiana	23/04/20 13	T6	N4°31'23.8" W52°6'36.8"	Patawa-Kaw	262	FG6	G6.6	<i>Phyllomedusa tomopterna</i>	38	KP122425
Fr. Guiana	23/04/20 13	T6	N4°31'23.8" W52°6'36.8"	Patawa-Kaw	262	FG6	G6.7	<i>Phyllomedusa tomopterna</i>	32/33	KP122426
Fr. Guiana	26/04/20 13	T8	N4°32'30" W52°09'00"	Patawa	175	FG7	G8.1	<i>Hypsiboas geographicus</i>	37/38	KP122442
Fr. Guiana	26/04/20 13	T8	N4°32'30" W52°09'00"	Patawa	175	FG7	G8.2	<i>Hypsiboas geographicus</i>	40	KP122450

Fr. Guiana	26/04/20 13	T8	N4°32'30" W52°09'00"	Patawa	175	FG7	G8.3	<i>Hypsiboas geographicus</i>	40	KP122443
Fr. Guiana	26/04/20 13	T8	N4°32'30" W52°09'00"	Patawa	175	FG7	G8.4	<i>Hypsiboas geographicus</i>	40	KP122444
Fr. Guiana	26/04/20 13	T8	N4°32'30" W52°09'00"	Patawa	175	FG7	G8.5	<i>Hypsiboas geographicus</i>	38/39	KP122445
Fr. Guiana	26/04/20 13	T8	N4°32'30" W52°09'00"	Patawa	175	FG7	G8.6	<i>Hypsiboas geographicus</i>	37/38	KP122446
Fr. Guiana	26/04/20 13	T8	N4°32'30" W52°09'00"	Patawa	175	FG7	G8.7	<i>Hypsiboas geographicus</i>	37/38	KP122447
Fr. Guiana	26/04/20 13	T8	N4°32'30" W52°09'00"	Patawa	175	FG7	G8.8	<i>Hypsiboas geographicus</i>	38/39	KP122448
Fr. Guiana	26/04/20 13	T8	N4°32'30" W52°09'00"	Patawa	175	FG7	G8.9	<i>Hypsiboas geographicus</i>	38/39	KP122449
Fr. Guiana	26/04/20 13	T8	N4°32'30" W52°09'00"	Patawa	175	FG7	G8.10	<i>Hypsiboas geographicus</i>	38/39	KP122451

Fr. Guiana	26/04/20 13	T8	N4°32'30" W52°09'00"	Patawa	175	FG7	G8.11	<i>Hypsiboas geographicus</i>	38/39	KP122452
Fr. Guiana	17/04/20 10	T1 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T1.1	<i>Hypsiboas geographicus</i>	42	KP122373
Fr. Guiana	17/04/20 10	T3 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T3.1	<i>Hypsiboas geographicus</i>	25	KP122374
Fr. Guiana	17/04/20 10	T3 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T3.3	<i>Hypsiboas geographicus</i>	25	KP122375
Fr. Guiana	17/04/20 10	T3 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T3.4	<i>Hypsiboas geographicus</i>	25	KP122376
Fr. Guiana	17/04/20 10	T3 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T3.5	<i>Hypsiboas geographicus</i>	25	KP122377
Fr. Guiana	17/04/20 10	T3 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T3.6	<i>Hypsiboas geographicus</i>	25	KP122378
Fr. Guiana	17/04/20 10	T3 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T3.7	<i>Hypsiboas geographicus</i>	25	KP122379

Fr. Guiana	17/04/20 10	T3 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T3.8	<i>Hypsiboas geographicus</i>	25	KP122380
Fr. Guiana	17/04/20 10	T3 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T3.9	<i>Hypsiboas geographicus</i>	25	KP122381
Fr. Guiana	17/04/20 10	T3 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T3.10	<i>Hypsiboas geographicus</i>	25	KP122390
Fr. Guiana	17/04/20 10	T3 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T3.11	<i>Hypsiboas geographicus</i>	25	KP122391
Fr. Guiana	17/04/20 10	T3 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T3.12	<i>Hypsiboas geographicus</i>	25	KP122392
Fr. Guiana	17/04/20 10	T3 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T3.13	<i>Hypsiboas geographicus</i>	25	KP122393
Fr. Guiana	17/04/20 10	T3 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T3.14	<i>Hypsiboas geographicus</i>	25	KP122397
Fr. Guiana	17/04/20 10	T4 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T4.1	<i>Allobates femoralis</i>	28/29	KP122382

Fr. Guiana	17/04/20 10	T5 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T5.1	<i>Hyla helenae</i>	27	KP122383
Fr. Guiana	17/04/20 10	T5 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T5.2	<i>Hyla helenae</i>	25	KP122384
Fr. Guiana	17/04/20 10	T5 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T5.4	<i>Hyla helenae</i>	25	KP122385
Fr. Guiana	17/04/20 10	T5 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T5.5	<i>Hyla helenae</i>	25	KP122386
Fr. Guiana	17/04/20 10	T5 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T5.6	<i>Hyla helenae</i>	25	KP122398
Fr. Guiana	17/04/20 10	T5 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T5.8	<i>Hyla helenae</i>	25	KP122394
Fr. Guiana	17/04/20 10	T5 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T5.9	<i>Hyla helenae</i>	25	KP122395
Fr. Guiana	17/04/20 10	T6 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T6.2	<i>Phyllomedusa tomopterna</i>	26/27	KP122387

Fr. Guiana	17/04/20 10	T6 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T6.3	<i>Phyllomedusa tomopterna</i>	26/27	KP122388
Fr. Guiana	17/04/20 10	T6 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T6.4	<i>Phyllomedusa tomopterna</i>	26/27	KP122389
Fr. Guiana	17/04/20 10	T6 - Patawa	N04°32'45" W52°09'01"	Small pool in drying out, rocky stream	220	FG8	T6.5	<i>Phyllomedusa tomopterna</i>	26/27	KP122396
Cameroon	18/09/20 10	Near Tombel, SWP	N4°42'9.5" E9°38'20.1"	Marsh wetland surrounded by cocoa plantations	347	CM1	A1	<i>Xenopus tropicalis</i>	66*	KP122359
Cameroon	18/09/20 10	Near Tombel, SWP	N4°42'9.5" E9°38'20.1"	Marsh wetland surrounded by cocoa plantations	347	CM1	A2	<i>Xenopus tropicalis</i>	66*	KP122358
Cameroon	18/09/20 10	Near Tombel, SWP	N4°42'9.5" E9°38'20.1"	Marsh wetland surrounded by cocoa plantations	347	CM1	A3	<i>Xenopus tropicalis</i>	64*	KP122365
Cameroon	18/09/20 10	Near Tombel, SWP	N4°42'9.5" E9°38'20.1"	Marsh wetland surrounded by cocoa plantations	347	CM1	A4	<i>Xenopus tropicalis</i>	64*	KP122366
Cameroon	27/07/20 10	Lake Oku	N6°12'3.3" E10°27'2.6"	Lake	2225	CM2	A5	<i>Xenopus tropicalis</i>	53*	KP122367

Cameroon	28/07/20 10	Lake Oku	N6°12'3.3" E10°27'2.6"	Lake	2225	CM2	A6	<i>Xenopus tropicalis</i>	52*	KP122368
Cameroon	29/07/20 10	Lake Oku	N6°12'3.3" E10°27'2.6"	Lake	2225	CM2	A7	<i>Xenopus tropicalis</i>	55*	KP122369
Cameroon	25/05/20 09	Lake Oku	N6°12'3.3" E10°27'2.6"	Lake	2225	CM3	A8	<i>Astylosternus schioetzi</i>	27	KP122370
Cameroon	17/04/20 09	Lake Oku	N6°12'3.3" E10°27'2.6"	Lake	2225	CM4	A9	<i>Astylosternus schioetzi</i>	41	KP122371
Cameroon	17/04/20 09	Lake Oku	N6°12'3.3" E10°27'2.6"	Lake	2225	CM4	A10	<i>Astylosternus schioetzi</i>	26	KP122372
Cameroon	31/05/20 09	Ngashie-Oku	N6°14'19.9" E10°30'19.3"	Stream in small holder agriculture and eucalyptus plantation	1956	CM5	A13	<i>Astylosternus schioetzi</i>	25	KP122352
Cameroon	31/05/20 09	Ngashie-Oku	N6°14'19.9" E10°30'19.3"	Stream in small-holder agriculture and eucalyptus plantation	1956	CM5	A16	<i>Astylosternus schioetzi</i>	ND	KP122354
Cameroon	18/05/20 09	Mt. Oku	N6° 10'36.6" E10°30'1.8"	Stream in forest-grassland transition	2751	CM6	A14	<i>Phrynobatrachus steindachneri</i>	37/40	KP122353

Cameroon	26/08/20 06	Anyafou ma, NWR	N6°13'57.8" E10°25'13.5"	Stream in primary mountain forest	2481	CM7	A15	<i>Phrynobatrachus</i> sp.	31/34	KP122338
Cameroon	24/11/20 08	Near Buea, Mt Cameroon	N4°10'30.6" E9°12'15.6"	Small stream in degraded forest	1840	CM8	A18	<i>Hyperolius riggenbachi</i>	25	KP122356
Cameroon	21/05/20 09	Elemigh ong, NWR	N6°12'58.0" E10°23'37.6"	Abandoned fish pond	1793	CM9	A19	<i>Hyperolius riggenbachi</i>	27/28	KP122357
Cameroon	21/05/20 09	Elemigh ong, NWR	N6°12'58.0" E10°23'37.6"	Abandoned fish pond	1793	CM9	A20	<i>Kassina maculosa</i>	27/28	KP122364
Cameroon	21/05/20 09	Elemigh ong, NWR	N6°12'58.0" E10°23'37.6"	Abandoned fish pond	1793	CM9	A17	<i>Hyperolius riggenbachi</i>	25/26	KP122355
Cameroon	20- 22/05/20 09	Elemigh ong, NWR	N6°12'58" E10°23'37.6"	Abandoned fish pond	1793	CM9	A11	<i>Leptopelis vermiculatus</i>	37	KP122350
Cameroon	20- 22/05/20 09	Elemigh ong, NWR	N6°12'58" E10°23'37.6"	Abandoned fish pond	1793	CM9	A12	<i>Hyperolius lamottei</i>	38	KP122351
Cameroon	14/04/20 12	Kakpeny i, Mamfe	N6°14'41.3" E9°31'31.6"	Small river next to village, c. 3m wide	420	CM10	A38	<i>Trichobatrachus robustus</i>	39/40	KP122507

Cameroon	14/04/20 12	Kakpenyi, Mamfe	N6°14'41.3" E9°31'31.6"	Small river next to village, c. 3m wide	420	CM10	A48	<i>Trichobatrachus robustus</i>	39/40	KP122508
Cameroon	14/04/20 12	Kakpenyi, Mamfe	N6°14'41.3" E9°31'31.6"	Small river next to village, c. 3m wide	420	CM10	A46	<i>Trichobatrachus robustus</i>	42	KP122506
Cameroon	16/04/20 12	Makamune, Mamfe	N6°9'25.5" E9°31'1.6"	River in agricultural landscape, c. 5m wide	515	CM11	A53	<i>Hylarana (Amnirana) albolarbris</i>	25	KP122519
Cameroon	18/04/20 12	Tinta, Mamfe	N6°16'57.2" E9°30'47.7"	River in agricultural landscape	640	CM12	A47	<i>Hylarana (Amnirana) albolarbris</i>	Unknown	KP122503
Cameroon	18/04/20 12	Tinta, Mamfe	N6°16'57.2" E9°30'47.7"	River in agricultural landscape, c. 5m wide	640	CM12	A49	<i>Hylarana (Amnirana) albolarbris</i>	Unknown	KP122504
Cameroon	18/04/20 12	Tinta, Mamfe	N6°16'57.2" E9°30'47.7"	River in agricultural landscape, c. 5m wide	640	CM12	A50	<i>Hylarana (Amnirana) albolarbris</i>	Unknown	KP122505
Cameroon	13/04/20 12	Ote, Mamfe	N6°10'6.1" E9°30'25.5"	Very small stream in mixed forest/agriculture	164	CM13	A40	<i>Amietophrynu (Bufo) regularis</i>	27/28	KP122510
Cameroon	13/04/20 12	Ote, Mamfe	N6°10'6.1" E9°30'25.5"	Very small stream in mixed forest/agriculture	164	CM13	A52	<i>Amietophrynu (Bufo) regularis</i>	32/33	KP122511

Cameroon	13/04/20 12	Ote, Mamfe	N6°10'6.1" E9°30'25.5"	Very small stream in mixed forest/agricul ture	164	CM13	A54	<i>Amietophrynu</i> s (<i>Bufo</i>) <i>regularis</i>	25	KP122512
Cameroon	13/04/20 12	Ote, Mamfe	N6°10'6.1" E9°30'25.5"	Very small stream in mixed forest/agricul ture	164	CM13	A55	<i>Amietophrynu</i> s (<i>Bufo</i>) <i>regularis</i>	36	KP122509
Cameroon	13/04/20 12	Ote, Mamfe	N6°10'6.1" E9°30'25.5"	Very small stream in mixed forest/agricul ture	164	CM13	A56	<i>Amietophrynu</i> s (<i>Bufo</i>) <i>regularis</i>	29	KP122513
Cameroon	16/04/20 12	Makamu ne, Mamfe	N6°9'25.5" E9°31'1.6"	River in agricultural landscape, c. 5m wide	515	CM14	A57	<i>Hylarana</i> (<i>Amnirana</i>) <i>albolabris</i>	39	KP122518
Cameroon	16/04/20 12	Makamu ne, Mamfe	N6°9'25.5" E9°31'1.6"	River in agricultural landscape, c. 5m wide	515	CM14	A58	<i>Hylarana</i> (<i>Amnirana</i>) <i>albolabris</i>	37	KP122517
Cameroon	16/04/20 12	Makamu ne, Mamfe	N6°9'25.5" E9°31'1.6"	River in agricultural landscape, c. 5m wide	515	CM14	A59	<i>Hylarana</i> (<i>Amnirana</i>) <i>albolabris</i>	25	KP122516
Cameroon	16/04/20 12	Makamu ne, Mamfe	N6°9'25.5" E9°31'1.6"	River in agricultural landscape, c. 5m wide	515	CM14	A60	<i>Hylarana</i> (<i>Amnirana</i>) <i>albolabris</i>	39	KP122515
Cameroon	16/04/20 12	Makamu ne, Mamfe	N6°9'25.5" E9°31'1.6"	River in agricultural landscape, c. 5m wide	515	CM14	A61	<i>Hylarana</i> (<i>Amnirana</i>) <i>albolabris</i>	25	KP122514

Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A21	<i>Amietia (Afrana) angolensis</i>	25	KP122339
Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A22	<i>Amietia (Afrana) angolensis</i>	25	KP122363
Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A23	<i>Amietia (Afrana) angolensis</i>	25	KP122340
Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A24	<i>Amietia (Afrana) angolensis</i>	25	KP122341
Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A25	<i>Leptopelis barbouri</i>	25	KP122342
Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A26	<i>Leptopelis barbouri</i>	25	KP122361
Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A27	<i>Leptopelis barbouri</i>	25	KP122343
Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A28	<i>Leptopelis barbouri</i>	42	KP122362

Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A29	<i>Leptopelis barbouri</i>	41	KP122344
Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A30	<i>Leptopelis barbouri</i>	41	KP122360
Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A31	<i>Leptopelis</i> sp.	25/26	KP122345
Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A32	<i>Leptopelis</i> sp.	25/26	KP122346
Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A33	<i>Leptopelis</i> sp.	25/26	KP122347
Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A34	<i>Leptopelis</i> sp.	25/26	KP122348
Tanzania	winter 1998	Udzung wa Kihanga	S8°22'24" E35°58'43"	Stream mountain, rain forest	1648	TZ1	A35	<i>Leptopelis barbouri</i>	28/29	KP122349
São Tomé	30- 31/10/20 11	Bom Sucesso,	N00°17'19.44" E06°36'44.88"	Puddle on the road in front of botanic gardens	1153	ST1	CZ1.1	<i>Phrynobatrachus dispar</i>	<30	KP122497

São Tomé	30- 31/10/20 11	Bom Sucesso	N00°17'19.44" E06°36'44.88"	Puddle on the road in front of botanic gardens	1153	ST1	CZ1.2	<i>Phrynobatrachus dispar</i>	<28	KP122498
São Tomé	30- 31/10/20 11	Bom Sucesso	N00°17'19.44" E06°36'44.88"	Puddle on the road in front of botanic gardens	1153	ST1	CZ1.3	<i>Phrynobatrachus dispar</i>	<30	KP122499
São Tomé	30- 31/10/20 11	Bom Sucesso	N00°17'19.44" E06°36'44.88"	Puddle on the road in front of botanic gardens	1153	ST1	CZ1.4	<i>Phrynobatrachus dispar</i>	<28	KP122500
Czech Rep.	2003- 2005	Šnejdlík pond, Vrbensk é rybníky, Č. Budějovi ce	N49°00'20" E14°25'04"		381	CZ1	CZ2	<i>Pelophylax (Rana) ridibundus</i>	<39	KP122502
Czech Rep.	2003- 2005	Šnejdlík pond, Vrbensk é rybníky, Č. Budějovi ce	N49°00'20" E14°25'04"		381	CZ1	CZ3	<i>Pelophylax (Rana) ridibundus</i>	<25	KP122501
Czech Rep.	25/06/20 04	Zajěčí potok, Brno	N49°14'15" E16°36'23"		300	CZ2	NemE1	<i>Rana temporaria</i>	ND	KP122493
Czech Rep.	25/06/20 04	Zajěčí potok, Brno	N49°14'15" E16°36'23"		300	CZ2	NemE2	<i>Rana temporaria</i>	ND	KP122494

Czech Rep.	25/06/20 04	Zajěčí potok, Brno	N49°14'15" E16°36'23"	300	CZ2	NemE3	<i>Rana temporaria</i>	ND	KP122495
Czech Rep.	15/07/20 04	Zajěčí potok, Brno	N49°14'15" E16°36'23"	300	CZ3	NemE4	<i>Hyla arborea</i>	ND	KP122496
UK	2/05/201 3	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK1	NHM1	<i>Bufo bufo</i>	29/30	KP122453
UK	2/05/201 3	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK1	NHM2	<i>Bufo bufo</i>	29/30	KP122454
UK	2/05/201 3	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK1	NHM3	<i>Bufo bufo</i>	29/30	KP122455
UK	2/05/201 3	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK1	NHM4	<i>Bufo bufo</i>	29/30	KP122456
UK	2/05/201 3	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK1	NHM5	<i>Rana temporaria</i>	29/30	KP122469

UK	2/05/2013	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK1	NHM6	<i>Rana temporaria</i>	29/30	KP122470
UK	2/05/2013	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK1	NHM7	<i>Rana temporaria</i>	29/30	KP122471
UK	2/05/2013	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK1	NHM8	<i>Rana temporaria</i>	29/30	KP122472
UK	2/05/2013	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK1	NHM9	<i>Bufo bufo</i>	29/30	KP122457
UK	15/05/2013	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK2	NHM16	<i>Bufo bufo</i>	30/31	KP122458
UK	15/05/2013	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK2	NHM17	<i>Bufo bufo</i>	30/31	KP122459
UK	15/05/2013	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK2	NHM18	<i>Bufo bufo</i>	30/31	KP122460

UK

UK	15/05/20 13	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK2	NHM19	<i>Bufo bufo</i>	30/31	KP122461
UK	15/05/20 13	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK2	NHM20	<i>Bufo bufo</i>	30/31	KP122462
UK	15/05/20 13	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK2	NHM21	<i>Bufo bufo</i>	30/31	KP122463
UK	15/05/20 13	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK2	NHM22	<i>Bufo bufo</i>	30/31	KP122464
UK	15/05/20 13	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK2	NHM23	<i>Bufo bufo</i>	30/31	KP122465
UK	15/05/20 13	NHM pond, Cromwel l road, London, UK	N51°29'45" W0°10'42"	17	UK2	NHM24	<i>Bufo bufo</i>	30/31	KP122466

UK	15/05/2013	NHM pond, Cromwell road, London, UK	N51°29'45" W0°10'42"	17	UK2	NHM25	<i>Bufo bufo</i>	30/31	KP122467
UK	15/05/2013	NHM pond, Cromwell road, London, UK	N51°29'45" W0°10'42"	17	UK2	NHM26	<i>Bufo bufo</i>	30/31	KP122468
UK	1/05/2013	Sevenoaks pond London, UK	N51°16'14.5" E0°11'22.1"	184	UK3	SL1	<i>Rana temporaria</i>	24	KP122474
UK	1/05/2013	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK3	SL2	<i>Rana temporaria</i>	24	KP122475
UK	1/05/2013	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK3	SL3	<i>Rana temporaria</i>	24	KP122476
UK	1/05/2013	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK3	SL4	<i>Rana temporaria</i>	24	KP122477
UK	1/05/2013	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK3	SL5	<i>Rana temporaria</i>	24	KP122478
UK	1/05/2013	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK3	SL6	<i>Rana temporaria</i>	24	KP122479

UK	1/05/2013	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK3	SL7	<i>Rana temporaria</i>	33	KP122480
UK	15/05/2013	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK4	SL8	<i>Rana temporaria</i>	32	KP122481
UK	15/05/2013	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK4	SL9	<i>Rana temporaria</i>	32	KP122482
UK	15/05/2013	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK4	SL10	<i>Rana temporaria</i>	30	KP122473
UK	15/05/2013	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK4	SL11	<i>Rana temporaria</i>	31	KP122483
UK	08/07/2013	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK5	SL12	<i>Rana temporaria</i>	37	KP122484
UK	08/07/2013	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK5	SL13	<i>Rana temporaria</i>	34	KP122485
UK	08/07/2013	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK5	SL14	<i>Rana temporaria</i>	38	KP122486

UK	08/07/20 13	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK5	SL15	<i>Rana temporaria</i>	32	KP122487
UK	08/07/20 13	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK5	SL16	<i>Rana temporaria</i>	38	KP122488
UK	08/07/20 13	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK5	SL17	<i>Rana temporaria</i>	32	KP122489
UK	08/07/20 13	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK5	SL18	<i>Rana temporaria</i>	30	KP122490
UK	08/07/20 13	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK5	SL19	<i>Rana temporaria</i>	31	KP122491
UK	08/07/20 13	Sevenoaks pond, Kent, UK	N51°16'14.5" E0°11'22.1"	184	UK5	SL20	<i>Rana temporaria</i>	39	KP122492

Supplementary Table S6: Label information for each NAG01 targeted clone library sequence recovered from the frog liver sampled.

Country	Year	Frog sample number	Clones double strand sequenced	NCBI accession number
Cameroon	2009	A11	A11.2	KP122697
			A11.3	KP122709
			A11.4	KP122632
			A11.5	KP122706
			A11.6	KP122704
Cameroon	2009	A12	A12.1	KP122715
			A12.3	KP122633
			A12.4	KP122634
			A12.6	KP122584
Cameroon	2009	A13	A13.1	KP122618
			A13.2	KP122648
			A13.3	KP122626
			A13.5	KP122730
			A13.6	KP122638
Cameroon	2009	A14	A14.7	KP122667
			A14.4	KP122635
			A14.9	KP122619
			A14.10	KP122700
			A14.5	KP122692
Cameroon	2006	A15	A15.8	KP122605
			A15.9	KP122570
			A15.2	KP122599
			A15.3	KP122582
			A15.4	KP122642

Cameroon	2009	A16	A15.5 A16.6 A16.1 A16.3 A16.4 A16.5	KP122643 KP122629 KP122649 KP122575 KP122574 KP122650
Cameroon	2009	A17	A17.7 A17.4 A17.1 A17.2 A17.3	KP122724 KP122726 KP122639 KP122573 KP122651
Cameroon	2008	A18	A18.11 A18.8 A18.9 A18.5 A18.10	KP122646 KP122592 KP122711 KP122658 KP122659
Cameroon	2009	A19	A19.11 A19.2 A19.5 A19.4 A19.10	KP122729 KP122572 KP122660 KP122661 KP122590
Cameroon	2009	A20	A20.6 A20.7 A20.1 A20.2 A20.5	KP122622 KP122600 KP122662 KP122588 KP122663
Cameroon	2012	A38	A38.1 A38.2	KP122586 KP122628

			A38.6	KP122744
			A38.8	KP122743
Cameroon	2012	A55	A55.1	KP122675
			A55.2	KP122674
			A55.3	KP122676
			A55.4	KP122693
Cameroon	2012	A54	A54.2	KP122733
			A54.7	KP122677
			A54.8	KP122735
			A54.14	KP122678
Tanzania	1998	A21	A21.1	KP122637
			A21.2	KP122654
			A21.3	KP122684
			A21.4	KP122656
			A21.5	KP122617
Tanzania	1998	A22	A22.1	KP122601
			A22.2	KP122680
			A22.3	KP122623
			A22.4	KP122580
			A22.5	KP122721
Tanzania	1998	A23	A23.1	KP122699
			A23.2	KP122719
			A23.4	KP122596
			A23.5	KP122686
			A23.6	KP122717
Tanzania	1998	A24	A24.4	KP122640
			A24.3	KP122595
			A24.5	KP122723

Tanzania	1998	A25	A24.6	KP122641
			A24.7	KP122594
			A25.2	KP122625
			A25.3	KP122666
			A25.4	KP122620
			A25.5	KP122687
			A25.6	KP122630
Tanzania	1998	A26	A26.1	KP122657
			A26.2	KP122606
			A26.3	KP122645
			A26.5	KP122689
			A26.4	KP122688
Tanzania	1998	A27	A27.3	KP122691
			A27.4	KP122597
			A27.5	KP122728
			A27.8	KP122631
			A27.9	KP122710
Tanzania	1998	A28	A28.2	KP122621
			A28.4	KP122603
			A28.5	KP122602
			A28.7	KP122636
			A28.9	KP122644
Tanzania	1998	A30	A30.1	KP122701
			A30.5	KP122670
			A30.6	KP122669
			A30.7	KP122668
			A30.8	KP122702
Tanzania	1998	A31	A31.3	KP122653

Tanzania	1998	A32	A31.5	KP122683
			A31.7	KP122679
			A31.8	KP122652
			A31.10	KP122665
Tanzania	1998	A32	A32.1	KP122690
			A32.2	KP122614
			A32.3	KP122703
			A32.4	KP122698
			A32.5	KP122579
Tanzania	1998	A33	A33.5	KP122607
			A33.4	KP122608
			A33.6	KP122609
			A33.7	KP122610
			A33.10	KP122611
Tanzania	1998	A34	A34.1	KP122713
			A34.4	KP122694
			A34.5	KP122593
			A34.6	KP122695
			A34.7	KP122696
Tanzania	1998	A35	A35.3	KP122672
			A35.5	KP122578
			A35.7	KP122671
			A35.8	KP122576
			A35.10	KP122577
Fr. Guiana	2010	T3.6	T3.6.1	KP122571
			T3.6.2	KP122712
			T3.6.3	KP122681
			T3.6.4	KP122598

			T3.6.5	KP122655
Fr. Guiana	2010	T5.1	T5.1.4	KP122581
			T5.1.13	KP122604
			T5.1.9	KP122612
			T5.1.10	KP122613
Fr. Guiana	2013	G2.13	G2.13_A4	KP122725
			G2.13_C4	KP122727
			G2.13_D4	KP122747
			G2.13_E4	KP122714
Fr. Guiana	2013	G8.1	G8.1_A3	KP122647
			G8.1_B3	KP122616
			G8.1_C3	KP122746
			G8.1_E3	KP122682
São Tomé	2011	CZ1.1	CZ1.1_A1	KP122731
			CZ1.1_B1	KP122627
			CZ1.1_C1	KP122734
			CZ1.1_D1	KP122740
São Tomé	2011	CZ1.2	CZ1.2_A2	KP122738
			CZ1.2_B2	KP122736
			CZ1.2_C2	KP122673
			CZ1.2_D2	KP122737
São Tomé	2011	CZ1.3	CZ1.3_A3	KP122732
			CZ1.3_B3	KP122708
			CZ1.3_D3	KP122707
			CZ1.3_F3	KP122745
São Tomé	2011	CZ1.4	CZ1.4_A4	KP122741
			CZ1.4_B4	KP122739
			CZ1.4_C4	KP122742

London, UK	2013	SL10	CZ1.4_D4 SL10_A1 SL10_F1 SL10_G1 SL10_H1	KP122747 KP122591 KP122589 KP122587 KP122685
London, UK	2013	SL18	SL18.1 SL18.2 SL18.3 SL18.4	KP122585 KP122722 KP122705 KP122716
NHM pond, London, UK	2013	NHM16	NHM16.1 NHM16.4 NHM16.5 NHM16.7	KP122720 KP122718 KP122583 KP122624

Supplementary Table S7: Details of published 18S rDNA sequences of known taxa used in phylogenetic analysis reported in Figure 1.

Accession no.	Class	Order	Genus	Species	Strain/Clone	Reference
EF675616	Perkinsea	Perkinsida	*	*	<i>Lithobates sphenocephalus</i> pathogen	(1)
AF126013	Perkinsea	Perkinsida	<i>Perkinsus</i>	<i>marinus</i>	Isolate P1	(20)
AF497479	Perkinsea	Perkinsida	<i>Perkinsus</i>	<i>marinus</i>	TXsc	(21)
AY486141	Perkinsea	Perkinsida	<i>Perkinsus</i>	<i>mediterraneus</i>	isolate 4	(22)
AF509333	Perkinsea	Perkinsida	<i>Perkinsus</i>	<i>atlanticus</i>	ALG1	(23)
AF140295	Perkinsea	Perkinsida	<i>Perkinsus</i>	<i>atlanticus</i>	Galicia	(24)
AY486139	Perkinsea	Perkinsida	<i>Perkinsus</i>	<i>mediterraneus</i>	isolate 1	(22)
AF252288	Perkinsea	Perkinsida	<i>Perkinsus</i>	*	CCA2001	(25)
AY305326	Perkinsea	Perkinsida	<i>Perkinsus</i>	<i>andrewsi</i>	ATCC 50807	(26)
AF042707	Perkinsea	Perkinsida	<i>Perkinsus</i>	sp.	G117	(20)
AY487831	Perkinsea	Perkinsida	<i>Perkinsus</i>	<i>mediterraneus</i>	Isolate 5	(22)
AF102171	Perkinsea	Perkinsida	<i>Perkinsus</i>	<i>andrewsi</i>		(25)
AF133909	Perkinsea	Perkinsida	<i>Parvilucifera</i>	<i>infectans</i>		(27)
FJ424512	Perkinsea	Perkinsida	<i>Parvilucifera</i>	<i>prorocentri</i>		(28)
KF359483	Perkinsea	Perkinsida	<i>Parvilucifera</i>	<i>rostrata</i>		(29)

EU502912	Perkinsea	Perkinsida	<i>Parvilucifera</i>	<i>sinerae</i>		(30)
HM483394	Syndiniales	Amoebophryaceae	<i>Amoebophrya</i>	sp.	ex <i>Gymnodinium instriatum</i>	(31)
HM483395	Syndiniales	Amoebophryaceae	<i>Amoebophrya</i>	sp.	ex <i>Akashiwo sanguineum</i>	(31)
HQ658161	Syndiniales	Amoebophryaceae	<i>Amoebophrya</i>	sp.	RCC1626	(32)
AF472555	Syndiniales	Amoebophryaceae	<i>Amoebophrya</i>	sp.	ex <i>Akashiwo sanguineum</i>	(33)
AY208894	Syndiniales	Amoebophryaceae	<i>Amoebophrya</i>	sp.	ex <i>Scrippsiella sp.</i>	John <i>et al.</i> unpublished

Supplementary Table S8: Table of first hit of the BLASTn results of the amphibian 16S sequence using Mega BLAST.
 (NCBI- accessed October 2014).

Tadpole Id	Query Id	% Identity	Length	E value	Accession number	NCBI definition
A15	16S_A15	100	516	0	FJ769103	<i>Phrynobatrachus</i> sp. BMZ-2009 voucher MCZ
A21	16S_A21	97.83	554	0	DQ347318	<i>Afrana angolensis</i> isolate 0992
A23	16S_A23	97.65	554	0	DQ347318	<i>Afrana angolensis</i> isolate 0992
A24	16S_A24	97.65	554	0	DQ347318	<i>Afrana angolensis</i> isolate 0992
A25	16S_A25	92.52	535	0	JX996027	<i>Leptopelis barbouri</i> voucher CAS:168472
A27	16S_A27	92.52	535	0	JX996027	<i>Leptopelis barbouri</i> voucher CAS:168472
A29	16S_A29	92.52	535	0	JX996027	<i>Leptopelis barbouri</i> voucher CAS:168472
A31	16S_A31	97.84	555	0	DQ283161	<i>Leptopelis</i> sp. AMNH A168408
A32	16S_A32	97.84	555	0	DQ283161	<i>Leptopelis</i> sp. AMNH A168408
A33	16S_A33	97.84	555	0	DQ283161	<i>Leptopelis</i> sp. AMNH A168408
A34	16S_A34	97.84	555	0	DQ283161	<i>Leptopelis</i> sp. AMNH A168408
A35	16S_A35	92.51	534	0	JX996027	<i>Leptopelis barbouri</i> voucher CAS:168472
A11	16S_A11	95.67	554	0	JX564875	<i>Leptopelis vermiculatus</i>
A12	16S_A12	92.12	533	0	FJ594083	<i>Hyperolius lamottei</i>
A13	16S_A13	96.95	557	0	DQ283349	<i>Astylosternus schioetzi</i> voucher UTA 52398
A14	16S_A14	99.63	537	0	HM441260	<i>Phrynobatrachus steindachneri</i> voucher MCZ A-138080
A16	16S_A16	97.13	557	0	DQ283349	<i>Astylosternus schioetzi</i> voucher UTA-52398
A17	16S_A17	100	503	0	GU443976	<i>Hyperolius riggenbachi</i> isolate MVZ234752
A18	16S_A18	100	503	0	GU443976	<i>Hyperolius riggenbachi</i> isolate MVZ234752
A19	16S_A19	100	503	0	GU443976	<i>Hyperolius riggenbachi</i> isolate MVZ234752
A2	16S_A2	98.45	582	0	AY789013	<i>Xenopus tropicalis</i>
A1	16S_A1	98.45	582	0	AY789013	<i>Xenopus tropicalis</i>
A30	16S_A30	92.52	535	0	JX996027	<i>Leptopelis barbouri</i> voucher CAS:168472
A26	16S_A26	92.52	535	0	JX996027	<i>Leptopelis barbouri</i> voucher CAS:168472
A28	16S_A28	92.51	534	0	JX996027	<i>Leptopelis barbouri</i> voucher CAS:168472
A22	16S_A22	97.83	554	0	DQ347318	<i>Afrana angolensis</i> isolate 0992
A20	16S_A20	98.67	527	0	KF178891	<i>Kassina maculosa</i> voucher MCZ:A-147846

A3	16S_A3	98.45	582	0	AY789013	<i>Xenopus tropicalis</i>
A4	16S_A4	98.45	582	0	AY789013	<i>Xenopus tropicalis</i>
A5	16S_A5	98.11	581	0	DQ283363	<i>Xenopus (Silurana) tropicalis</i> voucher UTA A47158
A6	16S_A6	98.11	581	0	DQ283363	<i>Xenopus (Silurana) tropicalis</i> voucher UTA A47158
A7	16S_A7	98.11	581	0	DQ283363	<i>Xenopus (Silurana) tropicalis</i> voucher UTA A47158
A8	16S_A8	96.98	530	0	AF124108	<i>Astylosternus schioetzi</i>
A9	16S_A9	96.98	530	0	AF124108	<i>Astylosternus schioetzi</i>
A10	16S_A10	96.98	530	0	AF124108	<i>Astylosternus schioetzi</i>
T1.1	16S_T1.1	99.64	560	0	AY843628	<i>Hypsiboas geographicus</i>
T3.1	16S_T3.1	99.64	560	0	AY843628	<i>Hypsiboas geographicus</i>
T3.3	16S_T3.3	99.64	560	0	AY843628	<i>Hypsiboas geographicus</i>
T3.4	16S_T3.4	99.64	560	0	AY843628	<i>Hypsiboas geographicus</i>
T3.5	16S_T3.5	99.64	560	0	AY843628	<i>Hypsiboas geographicus</i>
T3.6	16S_T3.6	99.64	560	0	AY843628	<i>Hypsiboas geographicus</i>
T3.7	16S_T3.7	99.64	560	0	AY843628	<i>Hypsiboas geographicus</i>
T3.8	16S_T3.8	99.64	560	0	AY843628	<i>Hypsiboas geographicus</i>
T3.9	16S_T3.9	99.64	560	0	AY843628	<i>Hypsiboas geographicus</i>
T4.1	16S_T4.1	100	560	0	DQ502246	<i>Allobates femoralis</i> isolate 1325
T5.1	16S_T5.1	99.83	572	0	KF002053	<i>Hyla helenae</i> voucher MHNLS 20151
T5.2	16S_T5.2	100	571	0	KF002053	<i>Hyla helenae</i> voucher MHNLS 20151
T5.4	16S_T5.4	99.83	572	0	KF002053	<i>Hyla helenae</i> voucher MHNLS 20151
T5.5	16S_T5.5	100	571	0	KF002053	<i>Hyla helenae</i> voucher MHNLS 20151
T6.2	16S_T6.2	99.47	568	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
T6.3	16S_T6.3	99.47	568	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
T6.4	16S_T6.4	99.47	568	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
T3.10	T3.10_16S	99.46	560	0	AY843628	<i>Hypsiboas geographicus</i>
T3.11	T3.11_16S	99.46	560	0	AY843628	<i>Hypsiboas geographicus</i>
T3.12	T3.12_16S	99.64	560	0	AY843628	<i>Hypsiboas geographicus</i>
T3.13	T3.13_16S	99.64	560	0	AY843628	<i>Hypsiboas geographicus</i>
T5.8	T5.8_16S	100	571	0	KF002053	<i>Hyla helenae</i> voucher MHNLS 20151
T5.9	T5.9_16S	100	571	0	KF002053	<i>Hyla helenae</i> voucher MHNLS 20151
T6.5	T6.5_16S	98.94	568	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451

T3.14	T3.14_16S	99.64	560	0	AY843628	<i>Hypsiboas geographicus</i>
T5.6	T5.6_16S	100	571	0	KF002053	<i>Hyla helenae</i> voucher MHNLS 20151
G1.1	G1.1_16S	96.07	585	0	EF632056	<i>Leptodactylus knudseni</i> voucher QCAZ 13244
G1.2	G1.2_16S	96.07	585	0	EF632056	<i>Leptodactylus knudseni</i> voucher QCAZ 13244
G1.3	G1.3_16S	96.07	585	0	EF632056	<i>Leptodactylus knudseni</i> voucher QCAZ 13244
G1.4	G1.4_16S	96.07	585	0	EF632056	<i>Leptodactylus knudseni</i> voucher QCAZ 13244
G1.5	G1.5_16S	98.13	587	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G2.9	G2.9_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G2.1	G2.1_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G2.2	G2.2_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G2.3	G2.3_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G2.4	G2.4_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G2.5	G2.5_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G2.6	G2.6_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G2.7	G2.7_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G2.8	G2.8_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G2.10	G2.10_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G2.11	G2.11_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G2.12	G2.12_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G2.13	G2.13_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G3.1	G3.1_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
G3.2	G3.2_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
G3.3	G3.3_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
G4.1	G4.1_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G6.2	G6.2_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G6.3	G6.3_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G6.4	G6.4_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G6.5	G6.5_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G6.6	G6.6_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G6.7	G6.7_16S	98.29	586	0	GQ366286	<i>Phyllomedusa tomopterna</i> voucher CFBH 2451
G4.2	G4.2_16S	99.64	559	0	JN970655	<i>Hypsiboas crepitans</i> isolate ROM_44089
G4.3	G4.3_16S	99.66	584	0	AY843724	<i>Phyllomedusa hypochondrialis</i>

G5.1	G5.1_16S	93.41	592	0	AY549345	<i>Hyla minuta</i> voucher MACN 33799
G5.2	G5.2_16S	93.41	592	0	AY549345	<i>Hyla minuta</i> voucher MACN 33799
G5.3	G5.3_16S	93.41	592	0	AY549345	<i>Hyla minuta</i> voucher MACN 33799
G5.4	G5.4_16S	93.41	592	0	AY549345	<i>Hyla minuta</i> voucher MACN 33799
G5.5	G5.5_16S	93.41	592	0	AY549345	<i>Hyla minuta</i> voucher MACN 33799
G5.6	G5.6_16S	93.41	592	0	AY549345	<i>Hyla minuta</i> voucher MACN 33799
G5.7	G5.7_16S	93.41	592	0	AY549345	<i>Hyla minuta</i> voucher MACN 33799
G5.8	G5.8_16S	93.41	592	0	AY549345	<i>Hyla minuta</i> voucher MACN 33799
G5.9	G5.9_16S	93.41	592	0	AY549345	<i>Hyla minuta</i> voucher MACN 33799
G5.10	G5.10_16S	93.41	592	0	AY549345	<i>Hyla minuta</i> voucher MACN 33799
G4.4	G4.4_16S	93.41	592	0	AY549345	<i>Hyla minuta</i> voucher MACN 33799
G4.5	G4.5_16S	93.41	592	0	AY549345	<i>Hyla minuta</i> voucher MACN 33799
G6.1	G6.1_16S	90.13	598	0	AY843757	<i>Scinax elaeochroa</i>
G8.1	G8.1_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
G8.3	G8.3_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
G8.4	G8.4_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
G8.5	G8.5_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
G8.6	G8.6_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
G8.7	G8.7_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
G8.8	G8.8_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
G8.9	G8.9_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
G8.2	G8.2_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
G8.10	G8.10_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
G8.11	G8.11_16S	95.82	550	0	JF790119	<i>Hypsiboas geographicus</i> A isolate AS0175
NHM1	NHM1_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM2	NHM2_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM3	NHM3_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM4	NHM4_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM9	NHM9_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM16	NHM16_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM17	NHM17_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM18	NHM18_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu

NHM19	NHM19_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM20	NHM20_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM21	NHM21_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM22	NHM22_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM23	NHM23_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM24	NHM24_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM25	NHM25_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM26	NHM26_16S	99.5	596	0	AY840246	<i>Bufo bufo</i> isolate karasu
NHM5	NHM5_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
NHM6	NHM6_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
NHM7	NHM7_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
NHM8	NHM8_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL10	SL10_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL1	SL1_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL2	SL2_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL3	SL3_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL4	SL4_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL5	SL5_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL6	SL6_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL7	SL7_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL8	SL8_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL9	SL9_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL11	SL11_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL12	SL12_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL13	SL13_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL14	SL14_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL15	SL15_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL16	SL16_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL17	SL17_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL18	SL18_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL19	SL19_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000
SL20	SL20_16S	99.65	572	0	DQ283129	<i>Rana temporaria</i> voucher ZSM 762/2000

CZE1	16S_CZE1	99.46	554	0	AB685766	<i>Rana temporaria</i> voucher: KUHE:28259
CZE2	16S_CZE2	100	554	0	AB685766	<i>Rana temporaria</i> voucher: KUHE:28259
CZE3	16S_CZE3	100	554	0	AB685766	<i>Rana temporaria</i> voucher: KUHE:28259
CZE4	16S_CZE4	99.65	576	0	AY843601	<i>Hyla arborea</i>
CZ11	16S_CZ11	99.11	560	0	DQ283223	<i>Phrynobatrachus dispar</i> voucher CAS 218995
CZ12	16S_CZ12	98.94	567	0	DQ283223	<i>Phrynobatrachus dispar</i> voucher CAS 218995
CZ13	16S_CZ13	98.94	567	0	DQ283223	<i>Phrynobatrachus dispar</i> voucher CAS 218995
CZ14	16S_CZ14	99.12	566	0	DQ283223	<i>Phrynobatrachus dispar</i> voucher CAS 218995
CZ3	16S_CZ3	99.66	583	0	JN627423	<i>Rana ridibundus</i> isolate RAFA-02
CZ2	16S_CZ2	99.66	583	0	JN627423	<i>Rana ridibundus</i> isolate RAFA-02
A47	16S_A47	99.43	528	0	DQ022351	<i>Amnirana albolabris</i>
A49	16S_A49	99.43	528	0	DQ022351	<i>Amnirana albolabris</i>
A50	16S_A50	99.43	528	0	DQ022351	<i>Amnirana albolabris</i>
A46	16S_A46	99.47	571	0	AY843773	<i>Trichobatrachus robustus</i>
A38	16S_A38	99.47	571	0	AY843773	<i>Trichobatrachus robustus</i>
A48	16S_A48	99.47	571	0	AY843773	<i>Trichobatrachus robustus</i>
A55	16S_A55	98.02	606	0	GU183858	<i>Bufo cf. regularis</i> FB-2010 voucher MW 6140
A40	16S_A40	98.02	606	0	GU183858	<i>Bufo cf. regularis</i> FB-2010 voucher MW 6140
A52	16S_A52	98.02	606	0	GU183858	<i>Bufo cf. regularis</i> FB-2010 voucher MW 6140
A54	16S_A54	98.02	606	0	GU183858	<i>Bufo cf. regularis</i> FB-2010 voucher MW 6140
A56	16S_A56	98.02	606	0	GU183858	<i>Bufo cf. regularis</i> FB-2010 voucher MW 6140
A61	16S_A61	99.43	528	0	DQ022351	<i>Amnirana albolabris</i>
A60	16S_A60	99.43	528	0	DQ022351	<i>Amnirana albolabris</i>
A59	16S_A59	99.43	528	0	DQ022351	<i>Amnirana albolabris</i>
A58	16S_A58	99.43	528	0	DQ022351	<i>Amnirana albolabris</i>
A57	16S_A57	99.43	528	0	DQ022351	<i>Amnirana albolabris</i>
A53	16S_A53	99.43	528	0	DQ022351	<i>Amnirana albolabris</i>

Supplementary Table S9: Details of published 16S rDNA sequences of known amphibian taxa used in the phylogenetic analysis shown in Figure 3.

Accession no.	Order	Sub-order	Super family	Family	Genus	Species	Strains	Reference
AY843773	Anura	Neobatrachia	Ranoidea	Arthroleptidae	<i>Trichobatrachus</i>	<i>robustus</i>	DPL 3932	(34)
HM441260	Anura	Neobatrachia	Ranoidea	Phrynobatrachidae	<i>Phrynobatrachus</i>	<i>steindachneri</i>	MCZ A-138080	Blackburn, DC unpublished
DQ347318	Anura	Neobatrachia	Ranoidea	Pyxicephalidae	<i>Afrana</i> <i>(Amietia)</i>	<i>angolensis</i>	isolate 0992	(35)
DQ283402	Anura	Neobatrachia	Ranoidea	Pyxicephalidae	<i>Afrana</i> <i>(Amietia)</i>	<i>vertebralis</i>	AMNH A144977	(36)
DQ283349	Anura	Neobatrachia	Ranoidea	Arthroleptidae	<i>Astylosternus</i>	<i>schioetzi</i>	UTA 52398	(36)
AY523765	Anura	Neobatrachia	Ranoidea	Microhylidae	<i>Scaphiophryne</i>	<i>marmorata</i>	VUB 0540	(37)

DQ283161	Anura	Neobatrachia	Ranoidea	Arthroleptidae	<i>Leptopelis</i>	sp.	AMNH A168408	(36)
FJ807721	Anura	Neobatrachia	Ranoidea	Arthroleptidae	<i>Leptopelis</i>	<i>karissimbensis</i>	ZFMK:87996	Hoelting, M <i>et al.</i> unpublished
DQ283437	Anura	Neobatrachia	Ranoidea	Hyperoliidae	<i>Kassina</i>	<i>senegalensis</i>	RdS 803	(36)
DQ283356	Anura	Neobatrachia	Ranoidea	Hyperoliidae	<i>Phlyctimantis</i>	<i>leonardi</i>	DPL 4058	(36)
DQ283174	Anura	Neobatrachia	Ranoidea	Hyperoliidae	<i>Opisthothylax</i>	<i>immaculatus</i>	DPL 3968	(36)
EF646678	Anura	Neobatrachia	Ranoidea	Hyperoliidae	<i>Heterixalus</i>	<i>madagascariensis</i>	ZSM 569/2000	(38)
AB612026	Anura	Neobatrachia	Ranoidea	Arthroleptidae	<i>Trichobatrachus</i>	<i>robustus</i>	taxon:11109 6	(39)
AY341723	Anura	Neobatrachia	Ranoidea	Arthroleptidae	<i>Astylosternus</i>	<i>diadematus</i>	taxon:25228 6	(40)

FJ594083	Anura	Neobatrachia	Ranoidea	Hyperoliidae	<i>Hyperolius lamottei</i>		taxon:64348 9	(41)
AF215431	Anura	Neobatrachia	Ranoidea	Hyperoliidae	<i>Afrixalus</i>	sp.	MV-1999	(42)
DQ283234	Anura	Neobatrachia	Ranoidea	Hyperoliidae	<i>Afrixalus</i>	<i>Stuhlmanni</i> (<i>pygmaeus</i>)	CAS 214837	(36)
FJ151068	Anura	Neobatrachia	Ranoidea	Hyperoliidae	<i>Afrixalus</i>	<i>paradorsalis</i>	AMCC:1 17589	(43)
GQ366232	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Phasmahyla</i>	<i>guttata</i>	MNRJ 41688	(44)
GQ366237	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Phyllomedusa</i>	<i>rohdei</i>	CFBHT 93	(44)
AY843708	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Osteocephalus</i>	<i>oophagus</i>	MNHN 2001.0828	(34)
FJ965303	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Osteocephalus</i>	<i>castaneicola</i>	NMP6V 73820	(45)

AY326042	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Trachycephalus jordani</i>	DCC 2917	(46)
AY326048	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Trachycephalus venulosus</i>	DCC 3069	(46)
AY948746	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Trachycephalus venulosus</i> (<i>Phrynohyas</i>)	VUB 0987	(47)
AY364371	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Trachycephalus venulosus</i> (<i>Phrynohyas</i>)	taxon:21380 3	(48)
AY549323	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Hypsiboas balzani</i> (<i>Hyla</i>)	DLR 41193	(49)
DQ283442	Anura	*	Pipidae	Xenopodinae	<i>Xenopus gilli</i>	AMNH A153027	(36)
AB612030	Anura	Neobatrachia	Ranoidea	Mantellinae	<i>Blommersia wittei</i>	ZSM:D48/20 00	(39)
AB530639	Anura	Neobatrachia	Ranoidea	Microhylidae	<i>Kaloula pulchra</i>	taxon:16793 4	(50)

DQ283251	Anura	*	Pipidae	Pipinae	<i>Pipa</i>	<i>carvalhoi</i>	Alexander Haas	(36)
DQ283156	Anura	Pelobatoidea	Pelobatidae	*	<i>Scaphiopus</i>	<i>holbrookii</i>	AMNH A168434	(36)
EF397243	Anura	Pelobatoidea	Megophryidae	*	<i>Xenophrys</i>	<i>nankiangensis</i>	CIB-XM835	(51)
DQ283432	Anura	Bombinatoridae	*	*	<i>Bombina</i>	<i>orientalis</i>	Rafael de Sa	(36)
EF566971	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Acris</i>	<i>gryllus</i>	ECM0052	Lemmon, E M unpublished
DQ283257	Anura	Neobatrachia	Ranoidea	Ranidae	<i>Lithobates</i> (<i>Rana</i>)	<i>catesbeianus</i> (<i>catesbeiana</i>)	Bruce L. Christman	(36)
AY779228	Anura	Neobatrachia	Ranoidea	Ranidae	<i>Lithobates</i> (<i>Rana</i>)	<i>palustris</i>	KU 204425	(52)
JX996027	Anura	Neobatrachia	Ranoidea	Arthroleptidae	<i>Leptopelis</i>	<i>barbouri</i>	CAS:168472	Portillo, F. and Greenbaum, E. unpublished

DQ283361	Anura	Neobatrachia	Ranoidea	Arthroleptidae	<i>Nyctibates</i>	<i>corrugatus</i>	UTA A44461	(36)
DQ283223	Anura	Neobatrachia	Ranoidea	Phrynobatrachiidae	<i>Phrynobatrachus</i>	<i>dispar</i>	CAS 218995	(36)
AB640932	Anura	Neobatrachia	Ranoidea	Ranidae	<i>Pelophylax</i> (<i>Rana</i>)	<i>ridibundus</i>	KTUFS-1	(53)
AY549345	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Dendropsophus</i> (<i>Hyla</i>)	<i>minutus</i> (<i>minuta</i>)	MACN 33799	(49)
JF790084	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Dendropsophus</i>	<i>minutus</i>	AS0474	(54)
DQ022351	Anura	Neobatrachia	Ranoidea	Ranidae	<i>Hylarana</i> (<i>Amnirana</i>)	<i>albolabris</i>	TMSA84177	(55)
DQ283369	Anura	Neobatrachia	Ranoidea	Ranidae	<i>Hylarana</i> (<i>Amnirana</i>)	<i>albilabris</i>	UTA A44424	(36)
AY014377	Anura	Neobatrachia	Ranoidea	Ranidae	<i>Hylarana</i>	<i>lepus</i>	*	(56)

DQ283433	Anura	Neobatrachia	Hyloidea	Leptodactylidae	<i>Leptodactylus</i>	<i>discodactylus</i>	Rafael de Sa	(36)
DQ283404	Anura	Neobatrachia	Hyloidea	Leptodactylidae	<i>Leptodactylus</i>	<i>fuscus</i>	AMNH A139088	(36)
DQ283163	Anura	Neobatrachia	Hyloidea	Buonidae	<i>Amietophrynum</i> (<i>Bufo</i>)	<i>regularis</i>	FMNH 251386	(36)
AY843740	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Pseudis</i>	<i>paradoxa</i>	MACN 38642	(34)
DQ830810	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Hyla</i>	<i>cinerea</i>	LSUMZ 48181	Smith, S. A. unpublished
AY843563	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Agalychnis</i>	<i>callidryas</i>	RdS 537	(34)
AY843692	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Litoria</i>	<i>caerulea</i>	AMNH A-168409	(34)
GQ204694	Anura	Neobatrachia	Ranoidea	Rhacophoridae	<i>Polypedates</i>	<i>maculatus</i>	WHTKANT	(57)

AY779252	Anura	Neobatrachia	Ranoidea	Ranidae	<i>Lithobates</i> (<i>Rana</i>)	<i>sphenocephalus</i> (<i>sphenocephala</i>)	USC 7448	(52)
AF215441	Anura	Neobatrachia	Ranoidea	Hyperoliidae	<i>Hyperolius</i>	<i>viridiflavus</i>	*	(42)
AY581644	Anura	*	Pipidae	Xenopodinae	<i>Xenopus</i>	<i>laevis</i>	MHNG 2644.53	(58)
DQ283045	Anura	Neobatrachia	Hyloidea	Dendrobatidae	<i>Allobates</i>	<i>femoralis</i>	LSUMZ 17552	(36)
GQ366286	Anura	Neobatrachia	Hyloidea	Hylidae	<i>Phyllomedusa</i>	<i>tomopterna</i>	CFBH 2451	(44)
DQ092266	Caudata	*	Salamandroidea	Salamandridae	<i>Pleurodeles</i>	<i>nebulosus</i>	Anc12	(59)
DQ283445	Caudata	*	Salamandroidea	Salamandridae	<i>Pleurodeles</i>	<i>waltl</i>	AMNH A168418	(36)
DQ283407	Caudata	*	Salamandroidea	Ambystomatidae	<i>Ambystoma</i>	<i>tigrinum</i>	AMNH A164658	(36)

EF107170 Caudata * Salamandridae Ambystomatidae *Ambystoma mexicanum* 0907AmbMex (47)

Supplementary Table S10: Detail of environmental sequences sampled from GenBank and reduced to triangles on Figure 1.

Clade number	Accession no.	Environmental clone	Environment	Location	Environment type	Reference
1	EU162624	PAA9SP2005	Lake Pavin	Massif Central, France	Oligo-mesotrophic lake	(8)
1	EF527175	SA2_2G9	Framvaren Fjord	South-western Norway	Axonic/sulfidic waters	(9)
1	EU162630	PAC8SP2005	Lake Pavin	Massif Central, France	Oligo-mesotrophic lake	(8)
1	JN090897	KRL01E37	Lake Karla	Greece	Lake	(17)
1	AY642744	A31	Lake Aydat	Massif Central, France	Eutrophic lake	(60)
1	JN090873	KRL01E13	Lake Karla	Greece	Lake	(17)

1	DQ455739	BB01-172.24w	Estuary	USA: Barnegat Bay, NJ	Marine waters	Lim <i>et al.</i> unpublished
1	EU162628	PAD1SP2005	Lake Pavin	Massif Central, France	Oligo-mesotrophic lake	(8)
1	HQ191345	PA2009C9	Lake Pavin	Massif Central, France	Oligo-mesotrophic lake	(10)
1	DQ103802	M2_18C03	Mariager Fjord	Danish east coast	Anoxic marine waters	(13)
2	EU162625	PAG5SP2005	Lake Pavin	Massif Central, France	Oligo-mesotrophic lake	(8)
2	EU162627	PAD12SP2005	Lake Pavin	Massif Central, France	Oligo-mesotrophic lake	(8)
3	AY919720	LG10-12	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(61)
3	AY919821	LG53-06	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(61)

3	AY919809	LG36-11	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(61)
4	DQ244020	PAB11AU2004	Lake Pavin	Massif Central, France	Oligo-mesotrophic lake	(62)
4	DQ244021	PAD10AU2004	Lake Pavin	Massif Central, France	Oligo-mesotrophic lake	(62)
4	DQ244035	PAD7AU2004	Lake Pavin	Massif Central, France	Oligo-mesotrophic lake	(62)
4	DQ244037	PAF7AU2004	Lake Pavin	Massif Central, France	Oligo-mesotrophic lake	(62)
4	DQ244034	PAG2AU2004	Lake Pavin	Massif Central, France	Oligo-mesotrophic lake	(62)
4	AY642737	A20	Lake Aydat	Massif Central, France	Eutrophic lake	(60)
4	AY919736	LG15-10	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(61)

5	AY919682	LG02-01	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(61)
5	AY919712	LG09-02	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(61)
5	AY919769	LG25-01	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(61)
5	DQ244031	PAF8AU2004	Lake Pavin	Massif Central, France	Oligo-mesotrophic lake	(62)
5	DQ244027	PAA22AU2004	Lake Pavin	Massif Central, France	Oligo-mesotrophic lake	(62)
5	GU290070	TKR07E.24	Lake Tanganyika	East Africa	Oligotrophic lake	Tarbe et al. unpublished
5	AY919820	LG50-10	Lake George	Adirondack Park North-Eastern New York, USA	Oligotrophic lake	(61)
6	EF526795	NA2_4B10	Framvaren Fjord	Norway	Anoxic marine waters	(9)

6	EF526831	NA1_3C6	Framvaren Fjord	Norway	Anoxic marine waters	(9)
6	EF526760	NA2_2D10	Framvaren Fjord	Norway	Anoxic marine waters	(9)

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