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# Molecular Phylogeny of *Pseudocapillaroides xenopi* (Moravec et Cosgrov 1982) and Development of a Quantitative PCR Assay for its Detection in Aquarium Sediment

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**We used high-fidelity PCR to amplify a portion of the small ribosomal subunit (18S rRNA) of *Pseudocapillaroides xenopi*, a nematode that parasitizes the skin of *Xenopus laevis*. The 1113-bp amplicon was cloned, sequenced, and aligned with sequences from 22 other nematodes in the order Trichocephalida; *Caenorhabditis elegans* was used as the outgroup. Maximum-likelihood and Bayesian inference phylogenetic analyses clustered *P. xenopi* in a clade containing only members of the genus *Capillaria*. Our analyses support the following taxonomic relationships: 1) members of the family Trichuridae form a clade distinct from those in the family Trichocephalida; 2) members of the genera *Trichuris* and *Capillaria* form 2 distinct clades within the family Trichuridae; and 3) the genus *Trichuris* includes 2 distinct clades, one representing parasites that infect herbivores and the other representing parasites that infect omnivores and carnivores. Using 18S rRNA sequence unique to *P. xenopi*, we developed a *Taq*Man quantitative PCR assay to detect this *P. xenopi* sequence in total DNA isolated from aquarium sediment. The assay's lower limit of detection is 3 copies of target sequence in a reaction. The specificity of our assay was validated by using negative control DNA from 9 other pathogens of *Xenopus*. Our quantitative PCR assay detected *P. xenopi* DNA in the sediment of 2 of 12 aquaria from the source institution of the specimen used to develop the assay; these aquaria had been treated with ivermectin 6 mo previously.**

*Xenopus laevis* frogs are used extensively to study early vertebrate embryology due to the ease in maintaining these animals and the simplicity of observing and manipulating the fertilized eggs, which develop in an extrauterine environment. Infectious diseases of *X. laevis* have been described,[18] but there has been little effort to develop a definition of SPF status for this species. One parasite of *X. laevis*, first reported by Cosgrove and Jared,[6,7] is a capillarid nematode infecting the skin along the dorsum and was diagnosed in wild-caught frogs imported from South Africa. In the original description, adult worms and their eggs were located in tunnels within the skin of infected frogs; clinically the skin was grossly thickened and rough, skin fragments sloughed in copious amounts, frogs were anorexic and emaciated with muscle wasting, and the condition often lead to death.[6,7] The morphology of this nematode was consistent with a unique species of capillarid, which the authors named *Pseudocapillaroides xenopi* (Moravec et Cosgrove 1982).[13] In another report, the morphologic characteristics of this same parasite led to its naming as *Capillaria xenopodis*.[19] As additional outbreaks of this parasite were reported in colonies of *X. laevis*, the 2 scientific names were used interchangeably, leading to confusion in the literature regarding the nomenclature.[4,5,16] The more accepted of the scientific names is *P. xenopi*, which we use hereafter. When compared with other capillarid nematodes, *P. xenopi* has 2 unique morphologic features: infection of the skin and in utero embryonation of eggs.[13,14,19] Infection of *X. laevis* by *P. xenopi* occurs in the wild, but its prevalence is unknown.[18] Fulminate disease caused by *P. xenopi* appears to be kept in check by a thymus-dependent immune response;[5] nonspecific stressors have been reported to cause subclinical *P. xenopi* infection to escalate, increasing parasitic burden and often leading to death.[5]

Current methods for detecting *P. xenopi* infestation in laboratory *X. laevis* colonies require microscopic examination of either 1) unstained scrapings of the skin of suspect animals or large pieces of desquamated skin in aquaria sediment or 2) histologic sections of fixed tissue.[4,7,16] We obtained specimens of *P. xenopi* from a laboratory experiencing an outbreak in their *X. laevis* colony and determined the DNA sequence of a portion of the *P. xenopi* 18S rRNA gene. Using this sequence, we analyzed the nematode's phylogeny and found a short segment of unique DNA sequence, which we used to develop a *Taq*Man PCR assay for the detection of *P. xenopi* DNA in aquarium sediment.

## Materials and Methods

**Sources of *P. xenopi* and *X. laevis* aquarium samples.** During an outbreak of *P. xenopi* at the University of Rochester (NY), samples of sediment from 3 infected aquaria were provided (gifts of Fransisco De Jesús Andino and Dr. Jacques Robert, University of Rochester). Three decaying adult nematodes (one female and 2 male) were found in the debris when it was examined with a dissection microscope. These nematodes were washed in sterile PBS and photographed prior to isolating DNA from them. Six months after the colony had been treated with ivermectin and appeared to be free of infection by *P. xenopi*, additional sediment samples from 12 aquaria were submitted for analysis with the *P. xenopi Taq*Man PCR assay we developed.

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**DNA isolation.** DNA was isolated from the washed samples of *P. xenopi* and aquarium sediment samples, by using Q-biogene's FastDNA for Soil Spin Kit (MP Biomedical, Irvine, CA) and Q-biogene's FASTPrep orbital shaker (model FP120, MP Biomedical). Aquarium sediment samples were submitted as 1 to 1.5 mL of sediment and water obtained by siphon from the bottom of the tank during routine husbandry procedures and placed in microfuge tubes. Upon arrival at our laboratory, sediment samples were centrifuged at 13,000 × *g* for 5 min and the supernatant removed. The sediment pellets were suspended in 1 mL PBS prior to further processing for DNA isolation. DNA was isolated from the isolated nematodes and PBS-suspended sediment according to the manufacturer's recommendations and with the following modifications of the manufacturer's recommended washing methods. At step 9 in the protocol, the glassmilk binding matrix was pelleted in a 15-mL polypropylene conical tube at top speed in a clinical centrifuge for 1 min and the supernatant discarded. The pellets were resuspended by vortex in 1 mL 5.5 M guanidine thiocyanate, centrifuged for 1 min at top speed in a clinical centrifuge, and the supernatant discarded. Each pellet was resuspended in 1 mL humic acid wash solution by vortex, centrifuged for 1 min at top speed in a clinical centrifuge, and the supernatant discarded. The pellets were resuspended in the kit wash solution and transferred to spin filters. The manufacturer's protocol was followed hereafter, with the final DNA elution using 70 °C ultrapure water (MilliQ System, Millipore, Billerica, MA).

The humic acid wash solution is prepared by combining 9.78 mL sodium phosphate buffer, 1.22 mL MT buffer, and 250 µL PPS (kit components) in a 15-mL conical polypropylene centrifuge tube and mixing by vortex. The conical tube is centrifuged at top speed in a clinical centrifuge for 1 min. The supernatant is the humic acid wash solution, and the pellet is discarded.

DNA samples were quantified by using UV spectrophotometry, and purity was determined according to the A260:A280 ratio, with acceptable purity being defined as a value of 1.4 or greater. Between 100 ng and 200 ng of DNA were used in each reaction. For the *Taq*Man quantitative PCR (qPCR) assay, a duplicate sample of DNA isolated from each specimen was spiked with 100 copies of target sequence to test for the presence of PCR inhibitors.

**DNA amplicon cloning, sequencing and linearizing cloned positive controls.** A portion of the 18S rRNA ribosomal gene was PCR-amplified from the capillarid DNA in a 25-µL reaction containing 0.5 U Hotstart Ex*Taq* (Takara Mirus Bio, Madison, WI), 2 mM $MgCl_2$, 1× Ex*Taq* buffer, and 0.5 µM of each universal nematode 18S primer.[9] The thermal cycling conditions were 98 °C for 1 min 50 s (initial denaturation); 32 iterations of 98 °C for 10 s, 55 °C for 30 s, and 72 °C for 2 min; and a final extension at 72 °C for 5 min. The amplicon was ligated into TOPO TA pCR4 (Invitrogen, Carlsbad, CA) and transfected into One Shot TOP-10 *E. coli* (Invitrogen). Transformed bacteria were selected on LB–kanamycin agar (kanamycin, 50 µg/mL). Six clones from each amplicon were grown in LB–kanamycin broth overnight, and plasmids were isolated by using a DNA plasmid minikit (Qiagen, Valencia, CA). Approximately 250 ng of plasmid DNA was digested with 20 U *Eco* R1 (New England Biolabs, Ipswich, MA) at 37 °C for 2 h and analyzed by agarose gel electrophoresis to verify integration of the amplicon by comparison with the original amplicon after gel migration. Three validated clones were submitted to Davis Sequencing (Davis, CA) for DNA sequence determination. Each amplicon was sequenced from both ends by using the T3 and T7 sites in pCR4 plasmid.

One plasmid clone containing the amplicon was linearized for use as a positive control by digestion with *Bgl*II (New England Biolabs) at 37 °C for 2 h and followed by heat inactivation at 65 °C for 20 min. An aliquot was quantified by UV spectrophotometry at 260 mn. Serial dilution of the linear plasmid was performed, and the copy number of the target sequence in each dilution was calculated according to the amplicon length and the size of the plasmid by using an average molecular weight of 660 g per basepair and Avogadro's number.

**Computational molecular phylogenetic analysis.** Forward and reverse sequences from all 3 18S rRNA *P. xenopi* clones were aligned by using Clustal X.[12] Ambiguities at a given locus in the consensus sequence were resolved by simple majority rule. A search of NIH GenBank by using the order name Trichocephalida returned accessions containing partial or complete 18S rRNA sequences from 22 related nematodes. Table 1 lists the nematodes used in our phylogenetic analyses, their GenBank accession number, and some of their characteristics. Currently no accessions from capillarid parasites of aquatic species (for example, *P. tormentosa*) are available from GenBank to incorporate into the analysis. An alignment of the 18S rRNA sequence of *P. xenopi* with these Trichocephalida and the 18S rRNA region of *C. elegans* was performed. The sequences were truncated at the 5′ and 3′ ends to match the size of the *P. xenopi* sequence before further phylogenetic analysis was performed. For 3 accessions (*Capillaria plica*, *Capillaria hepatica*, and *Capillaria* [*Aonchotheca*] *putorii*), GenBank sequences were incorporated that provided only 559 to 588 bp of DNA sequence. The Clustal X alignment was manually optimized prior to output in the PHYLIP interleaved and NEXUS formats.

We used jModelTest 2.2.1 to compare 88 evolutionary models to find the model that best fit the data from the aligned sequences.[8] The parameters associated with the best evolutionary model determined by jModelTest (based on log likelihood calculations of Akaike informational criteria) and 100 data sets random in organism order were input into the phylogenetic bootstrap analysis program DNA PhyML.[11]

In addition, phylogenetic analysis was performed by Bayesian inference (Mr. Bayes 3.1.2) with parameters set as a standard nucleotide substitution model; a general time reversal model with γ-distributed rate variation and a proportion of invariable sites was used; 500,000 generations of posterior probability calculations were run with a sampling frequency of 10%; and burn-in value of 500 was incorporated.[1] Branch lengths were not constrained, and default values were used for all other parameters. The standard deviation of split frequencies was less than 0.01 at the end of the analysis.

Our final method of phylogenetic boot-strap analysis used 100 datasets generated from our alignment by SEQBOOT input into the PHYLIP DNAML maximum-likelihood algorithm.[10] The transition, transversion, and γ-shape parameters determined by jModelTest were input, the random number seed and jumble were set to 3, global rearrangement was set to off, and results were compiled by using PHYLIP CONSENSE.

In all 3 methods of phylogenetic computational analysis, *C. elegans* was identified as the outgroup to root the resulting phylograms. Phylograms were displayed by using TreeView[15] and exported as Windows- enhanced metafiles for text editing by using Corel Draw (Corel, Ottawa, Ontario, Canada) with export in EPS format.

**Parameters, sensitivity, and specificity of *Taq*Man assay.** The PCR assay for the detection of *P. xenopi* targets a 97-bp portion of our 18S rRNA gene sequence. The sequence target was identified by visual examination of the Clustal X sequence alignment

**Table 1.** Members of class Adenophorea, subclass Enoplia, and orders Trichurida, Enoplida, and Trichocephalida included in the analyses

| Nematode | Host | Organ affected | Length (no. of basepairs) | GenBank accession no. |
|---|---|---|---|---|
| *Pseudocapillaroides xenopi* | *Xenopus laevis* | Skin | 1113 | |
| *Capillaria plica* | *Canis familiaris* | Urinary bladder | 586 | JX456618 |
| *Capillaria hepatica* | Rodents, mammals | Liver | 559 | JX456635 |
| *Capillaria (Aonchotheca) putorii* | *Felis domesticus* | Stomach | 588 | JX456624 |
| *Capillaria tenuissima* | Raptors | Stomach and intestine | 1115 | EU004822 |
| *Eucoleus dispar* | Raptors | Esophagus | 1115 | EU004821 |
| *Trichuris vulpis* strain Tv1 | *Canis familiaris* | Large intestine and cecum | 1165 | HF586909 |
| *Trichuris leporis* strain Tl1 | *Oryctalagus* and *Lepus* rabbits | Large intestine and cecum | 1190 | HF586913 |
| *Trichuris skrjabini* strain Tsk1 | *Capris hircus* | Large intestine and cecum | 1190 | HF586912 |
| *Trichuris ovis* strain To1 | *Aries ovis* | Large intestine and cecum | 1179 | HF586911 |
| *Trichuris discolor* strain Td1 | *Bos taurus* | Large intestine and cecum | 1179 | HF586910 |
| *Trichuris muris* strain Tm1 | Rodents | Cecum | 1179 | HF586907 |
| *Trichuris* sp. CC-2013 strain Tsp1 | *Colobus guereza kikuyensis* | Large intestine and cecum | 1179 | HF586906 |
| *Trichuris suis* strain TS1 | *Sus scrofa* and mammals | Large intestine and cecum | 1179 | HF586905 |
| *Trichuris trichiura* strain Tt1 | *Macaca fuscata* | Large intestine and cecum | 1192 | AB699092 |
| *Trichinella murrelli* | *Ursus americanus* and mammals | Small intestine and striated muscle | 1108 | AY851259 |
| *Trichinella pseudospiralis* | Birds and mammals | Small intestine and striated muscle | 1108 | AY851258 |
| *Trichinella britovi* | *Sus scrofa* and mammals | Small intestine and striated muscle | 1108 | AY851257 |
| *Trichinella spiralis* strain ISS10 | *Sus scrofa* and mammals | Small intestine and striated muscle | 1108 | AY497012 |
| *Trichinella nativa* | *Ursus marimimus* and mammals | Small intestine and striated muscle | 1108 | AY851256 |
| *Trichinella nelsoni* | African predators and scavengers | Small intestine and striated muscle | 1108 | AY851261 |
| *Trichinella zimbabwensis* | Reptiles and mammals | Small intestine and striated muscle | 1108 | AY851264 |
| *Trichinella papuae* | Reptiles and mammals | Small intestine and striated muscle | 1108 | AY851263 |
| *Caenorhabditis elegans* | Soil | Bacterivorous | 1080 | NR00005 |

followed by primer and probe designer by using Beacon Designer 6.0 (Premier Biosoft, Palo Alto, CA) software. The *P. xenopi* -specific *Taq*Man sequences are: sense, 5′ GCC ACG GTC CTC TAA GTC AAG 3′; antisense, 5′ CCA GGA CAC TCG GTA AAG AGC 3′; and probe, 5′ FAM-ATT GGC TCT GCC GCC GTT GGT CAT-TAMRA 3′. Optimal primer and probe concentrations for the TaqMan assay were determined using the methods outlined by Applied Biosystems (Foster City, CA)[2] and are 0.15 µM for the probe and 0.6 µM for each primer. We used Perfecta qPCR 2× Supermix with UNG (Quanta Biosciences, Gaithersburg, MD), which incorporates an initial decontamination reaction using uracyl-N-glycosylase by substituting dUTP for dTTP in the master mix. The thermocycling conditions for our *P. xenopi* qPCR were: decontamination at 40 °C for 5 min; initial denaturation at 95 °C for 2.5 min; and 32 cycles of 95 °C for 10 s and 62 °C for 30 s. We used an IQ5 Optical System thermocycler and Optical analysis software (Biorad, Hercules, CA).

To determine the limit of detection (equivalent to 'sensitivity' as used herein) of the *P. xenopi Taq*Man assay, we assayed 10-fold serial dilutions of the linearized pCR4 plasmid containing the 1113-bp 18S rRNA clone in ultrapure water diluted to a concentration less than 1 target copy per microliter. Threshold cycle ($C_t$) compared with copy number to 32 cycles was determined for a series of reactions and graphically displayed by the IQ5 thermocycler as a semilog plot. To ensure that the assay uniquely detected *P. xenopi*, we included DNA isolated from *Mycobacterium xenopi*, *Mycobacterium liflandii*, *Mycobacterium chelonae*, *Mycobacterium marinum*, *Chlamydia psittaci*, *Cryptosporidium parvum*, *Batrachochytrium dendrobatidis* (chytrid), and frog virus 3 (kindly provided by Dr Jacques Robert, University of Rochester, NY). Total DNA isolated from uninfected aquaria sediment was our negative control, and ultrapure water was our no-template control.

## Results

**Identification of *P. xenopi* in aquarium sediment.** A single female and 2 male *P. xenopi* nematodes were found in the aquarium sediment. The female nematode was identified according to the presence of bioperculate eggs within the uterus (Figure 1 A, arrow); the male nematodes were identified by the everted spicule characteristic of this species (Figure 1 B, arrow). These specimens were washed, and DNA was isolated from them for amplification of portions of the small ribosomal subunit gene.

**DNA isolation, amplification, cloning, and sequence determination of *P. xenopi*.** DNA of sufficient quantity and quality was isolated from the *P. xenopi* specimens to amplify the target gene of interest. The amplicon was 1113 bp in size, comprising approximately 61.8% of the 18S rRNA gene. The amplicon contained 25.6% adenosine, 22.2% cytosine, 27.9% guanine, and 24.3% cytosine residues, with a guanine+cytosine content of 50.1%. A BLAST search of GenBank by using the 1113-bp sequence as the query demonstrated that *P. xenopi* has between 96% and 98% identity with 19 accessions of the genus Capillarid (for example, *C. hepatica*, 98%; *C. plica*, 97%; and *C. putorii*, 96%). The *P. xenopi* sequence determined spans from nucleotide 500 to nucleotide 1600 in the small ribosomal subunit gene; the sequence was deposited in the GenBank under accession number KJ415283. The A260:A280 ratio of DNA isolated from sediment varied between 1.89 and 3.04, confirming minimal contamination with extraneous protein.

**Phylogenetic relationship of *P. xenopi* to other Trichocephalida using partial18S rRNA gene sequence.** According to the jModelTest 2.2.1 analysis, the evolutionary model that best fit the data was that described by Tamura–Nei,[17] with a percentage of invariant sites (I) and γ distribution (G) of rates (abbreviated as TrN + I + G), based on the lowest calculated Aikake information criteria. The jModelTest 2.2.1 log likelihood score

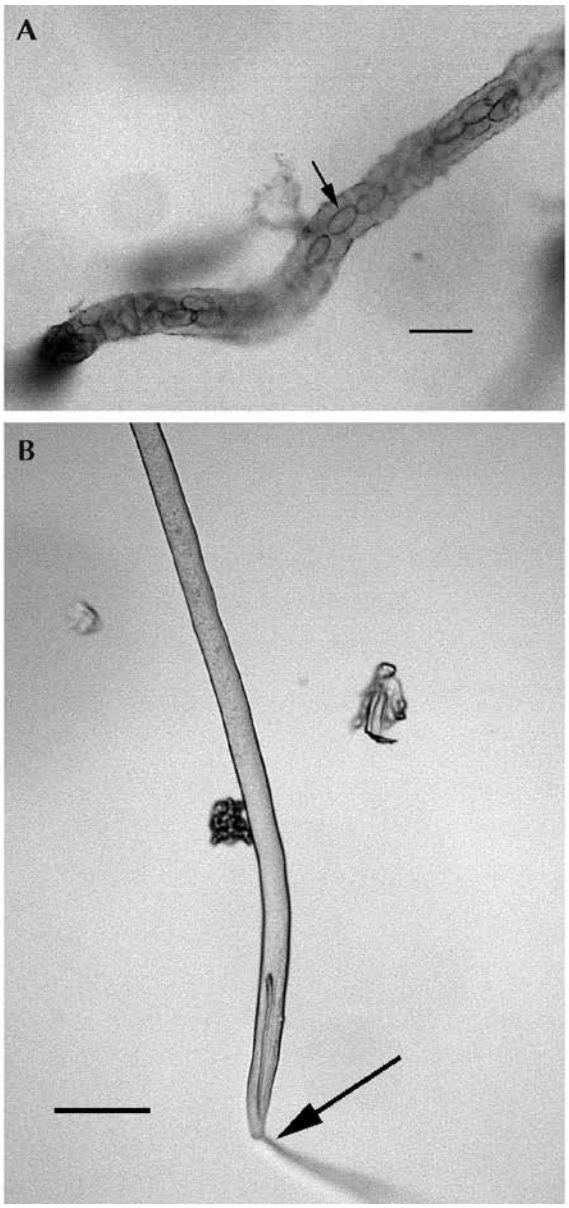


**Figure 1.** *Pseudocapillaroides xenopi*. (A) Female. (B) Male. The bioperculate ova (arrow) are seen in the reproductive tract of the decomposing female nematode, and the everted spicule (arrow) is shown at the posterior end of the male. Bar, 50 µm.

for TrN + I + G is 5220.4656 when comparing this model with 87 other models. The average frequency of base occurrences in the dataset were A = 0.2427, C = 0.2190, G = 0.2784, and T = 0.2599. The calculated transition rates were [AG] = 2.7578 and [CT] = 5.5439; transversion rates were set to 1.0 (by definition in the Tamura–Nei model).[17] The proportion of invariant sites among all sequences aligned was 0.1980, and the γ shape parameter was 0.5010.

Phylograms (rooted to *C. elegans*) generated by Bayesian inference by using the Mr. Bayes algorithm and DNA PhyML (maximum-likelihood using a bootstrap comparison of datasets) are shown in Figures 2 and 3, respectively. Results of phylogenetic analysis using PHYLIP DNAML maximum-likelihood bootstrap comparison of datasets was in agreement with the other 2 methods but was not statistically supported at some nodes (data not shown). All 3 analytical methods were in agreement regarding the phylogenetic relationships among these nematodes, placing *P. xenopi* in a clade containing the genus *Capillaria*. The *P. xenopi* DNA sequence is ancestral among the capillariids and positioned near a divergence into 2 groups representing those that infest birds (*E. dispar* and *C. tenuisssima*) and those that infest terrestrial mammals (*C. plica*, *C. hepatica*, and *C. putorii*). However, the separation of this clade into 2 parts may be an artifact caused by the shorter sequences for *C. purtorii*, *C. hepatica*, and *C. plica* used in the analyses. All 3 analyses depict the clade of trichinellids as giving rise to an ancestor that evolved into the parasites, forming a clade that contains both the genera *Trichuris* and *Capillaria*. Interestingly, all 3 phylograms distinguish between a clade of trichurids that infects carnivores and omnivores (*T. vulpis*, *T. trichuira*, *T. suis*, and *T. muris*) from the clade that infects herbivores (*T. discolor*, *T. ovis*, *T. leporis*, and *T. skrjabini*). Bayesian inference provided support for all nodes (branch points) in the phylogram generated. PhyML provided support for all nodes, with the exception of those distinguishing between the trichinelids *T. nelson*, *T. britovi*, *T. murelli*, *T. pseudospiralis*, *T. papuae*, and *T. zimbabwensis*. PHYLIP DNAML supported all nodes except 2: the node distinguishing *T. nelsoni* from the other trichinellids and that distinguishing *T. ovis* from *T. leporis*.

**Characterization of the *P. xenopi Taq*Man assay.** The *Taq*Man primers and probes were submitted as BLAST searches into the NIH GenBank. No significant similarities were found to the primers and probe. The sensitivity of the *P. xenopi Taq*Man assay determined by serial dilution of the linearized plasmid containing the 1113-bp cloned amplicon was 3 copies per reaction. The correlation coefficient ($R^2$) between threshold cycle and log copy number for the assay was 0.945; the linear regression had a slope of –2.472 and a *y* intercept of 32. The assay is specific in that relative fluorescence did not obtain a value sufficient to trigger a cycle threshold when tested with DNA from 9 other frog pathogens (data not shown). We cannot state definitively that our assay would not detect other capillariid parasites that infest aquatic species were sediment samples from *Xenopus* aquaria contaminated with these nematodes (that is, *P. tormentosa* of zebrafish).

DNA isolated from 12 aquaria sediment samples were tested for the presence of *P. xenopi* DNA by using 1 µL of resuspended DNA, which contained 100 to 200 ng. Two samples yielded 259 and 2420 copies of target sequence in the reactions (Figure 4). These 2 samples were interpreted as positive for the presence of *P. xenopi*, and this information was reported back to the source facility. When spiked with 100 copies of linear positive control DNA, all of the sediment DNA samples showed a cycle threshold corresponding to that value, meaning that the DNA samples isolated from the aquaria did not contain significant amounts of PCR inhibitors. Therefore our method of isolating DNA from aquarium sediment and washing it provides samples of reliable quality for use in nucleic acid amplification assays. This same DNA could be used to test for the presence of other pathogenic microorganisms that might be found in aquarium sediment.

## Discussion

The purpose of our current investigation was to develop a nucleic-acid–based assay for detection of the pathogen of *Xenopus* frogs. This report is the first to describe genomic DNA sequence from *P. xenopi*. We were able to use this sequence information to examine this parasites phylogeny and to develop a nucleic

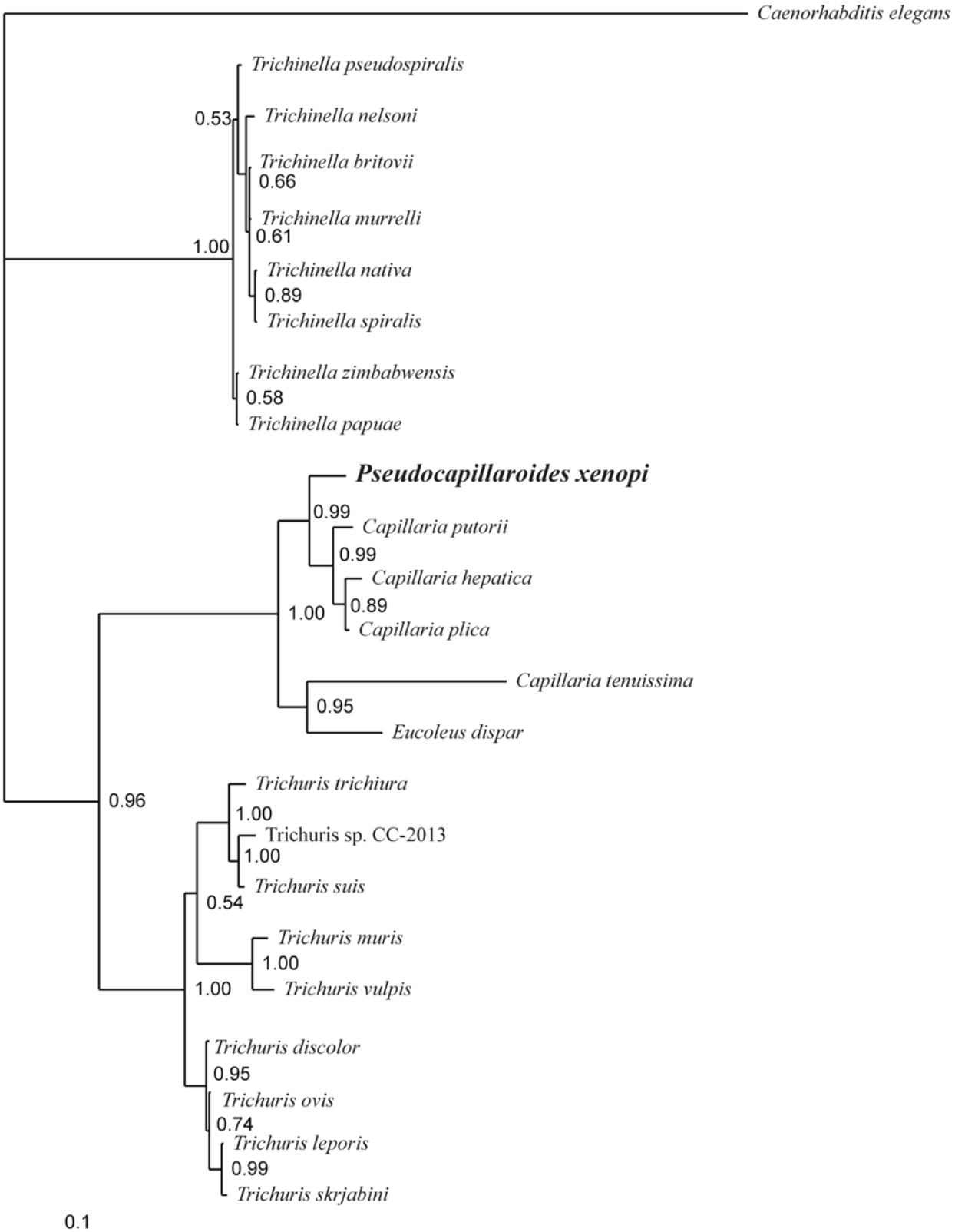


**Figure 2.** Phylogram determined by Bayesian inference analysis of an alignment of the *P. xenopi* 18S rRNA sequence with sequences from 22 other Trichocephalida and *C. elegans*. The analysis statistically supported all branch points. The size bar is the number of expected base changes per site and is related to the branch lengths given at each node. The percentage provided at each branch point indicates the statistical support for that node; values of 0.5 or greater are considered significant.

acid detection assay. Our phylogenetic analysis reveals several interesting aspects of the relationships among parasitic nematodes in the genera *Trichuris* and *Capillaria*. First we noted that the amphibian parasite *P. xenopi* has more 18S rRNA sequence similarity to the capillariids that parasitize mammals than do those that parasitize birds. In addition, the *P. xenopi* sequence appears to be ancestral to capillariids that parasitize mammals. Moreover, our analysis of some members of the genus *Trichuris*

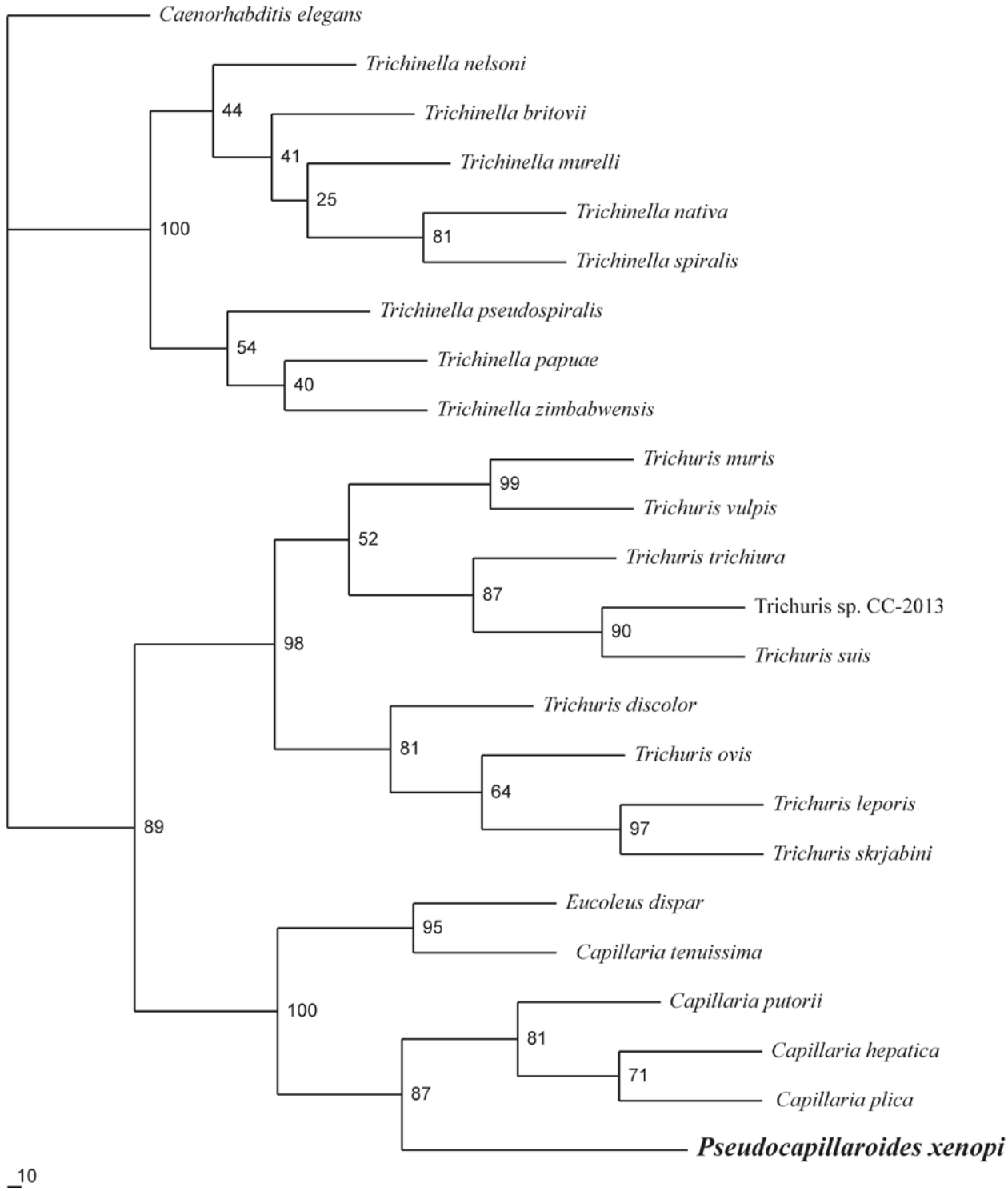


**Figure 3.** Phylogram was determined by PHyML analysis of an alignment of the *P. xenopi* 18S rRNA sequence with sequences from 22 other Trichocephalida and *C. elegans*. The size bar is the branch length equal to 10 base changes. The number provided at each branch point is the number of trees in agreement for that node out of 100 trees generated from the dataset. Values of 50 or greater are considered significant agreement in support of the clades defined at a branch point. The analysis statistically supported all branch points for the trichuriids and capillariids, but little support was given to branch points in the clade formed by the genus *Trichinella*, other than the node separating those that parasitize lizards from those that parasitize mammals.

divided this group into one clade that parasitizes carnivores and omnivores that is statistically distinct from another clade of trichurids that parasitizes herbivores. When this report was being prepared, no DNA sequence information on capillariids that infest aquatic species was available to include in our analysis.

The positive–negative cutoff value for the *P. xenopi* qPCR assay was established according to the amount of background fluorescence produced during thermal cycling and apparent in the results of negative control DNA samples. We assume that the negative background fluorescence seen in negative control samples arose from slight degradation of the *Taq*Man

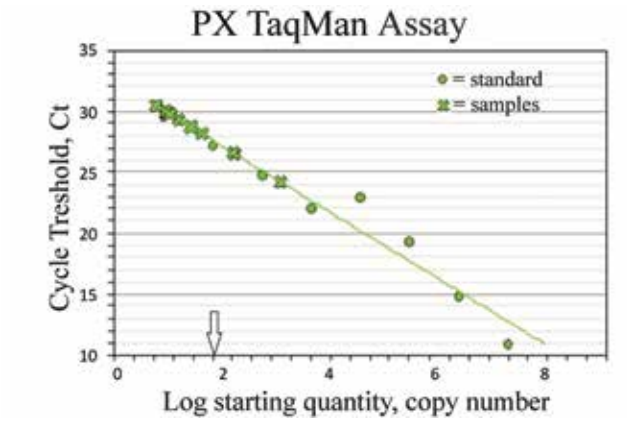


**Figure 4.** Sensitivity of the *P. xenopi Taq*Man assay. This semilog plot of a 10-fold dilution of linear target (green dots) demonstrates that as few as 3 copies of target sequence can be detected in this qPCR reaction. The assay correlation coefficient is 0.945. DNA samples from aquarium sediment were tested (X, some are overlapping), of which 2 samples were considered to be positive for the organism (that is, generated more than 100 copies per 1µL of sample).

probe during repetitive thermal cycling; this background fluorescence was always equivalent to less than 25 copies of target per microliter. Given that a typical nematode microorganism has 56 to 323 copies of the small ribosomal gene complex in tandem per cell in their genome[3] and the fact that sediment samples positive for *P. xenopi* were well beyond the 100 copies per microliter of sample DNA added to the quantitative PCR reaction, we surmised that a result of more than 25 copies per DNA sample would be a reasonable positive cutoff value for a definitive detection of *P. xenopi* DNA in our qPCR.

Laboratory *Xenopus* species have been used intensively for more than 70 y to facilitate studies in physiology, endocrinology, development biology, and, more recently, tissue regeneration. The important scientific advances that have emerged from this research in *Xenopus* in conjunction with the creation of transgenic and genetically defined *Xenopus* stocks have culminated in the creation of the National *Xenopus* Resource Center at Woods Hole Marine Biologic Laboratory (see URL http://www.mbl.edu/xenopus). These important advancements using *Xenopus* models emerged despite a lack of diagnostic testing for infectious diseases of these animals. Little is known about the prevalence of infectious diseases in laboratory-reared *Xenopus*, and little disease surveillance currently is performed during quarantine of *Xenopus* models at research institutions and commercial vendors. Therefore, most laboratory *Xenopus* colonies, including the National *Xenopus* Resource, are vulnerable to disease introduction. With the development of a diagnostic assay for the detection of *P. xenopi* DNA in *Xenopus* aquarium sediment, we have initiated progress toward the goal of defining the SPF status for *Xenopus* frogs and a method to validate that status.

## Acknowledgments

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