Received: 24 April 2023 | Accepted: 2 October 2023

DOI: 10.1111/2041-210X.14236

Methods in Ecology and Evolution

RESEARCH ARTICLE

FINDeM: A CRISPR-based, molecular method for rapid, inexpensive and field-deployable organism detection

Brandon D. Hoenig[1,2] | Jakub Zegar[3] | Michel E. B. Ohmer[3] | Macie M. Chess[4] | Brady A. Porter[4] | Myah Madril[1,2] | Corinne L. Richards-Zawacki[1,2]

[1]Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

[2]Pymatuning Laboratory of Ecology, Linesville, Pennsylvania, USA

[3]Department of Biology, The University of Mississippi, University Park, Mississippi, USA

[4]Department of Biological Sciences, Duquesne University, Pittsburgh, Pennsylvania, USA

Correspondence
Brandon D. Hoenig
Email: brandonhoenig@gmail.com

Funding information
American Museum of Natural History; National Science Foundation, Grant/Award Number: DBI-2120084; Pymatuning Lab of Ecology

Handling Editor: Ai-bing Zhang

Abstract

1. The field of ecology has undergone a molecular revolution, with researchers increasingly relying on DNA-based methods for organism detection. Unfortunately, these techniques often require expensive equipment, dedicated laboratory spaces and specialized training in molecular and computational techniques; limitations that may exclude field researchers, underfunded programmes and citizen scientists from contributing to cutting-edge science.
2. It is for these reasons that we have designed a simplified, inexpensive method for field-based molecular organism detection—FINDeM (**F**ield-deployable **I**sothermal **N**ucleotide-based **De**tection **M**ethod). In this approach, DNA is extracted using chemical cell lysis and a cellulose filter disc, followed by two body-heat inducible reactions—recombinase polymerase amplification and a CRISPR-Cas12a fluorescent reporter assay—to amplify and detect target DNA, respectively.
3. Here, we introduce and validate FINDeM in detecting *Batrachochytrium dendrobatidis*, the causative agent of amphibian chytridiomycosis, and show that this approach can identify single-digit DNA copies from epidermal swabs in under 1 h using low-cost supplies and field-friendly equipment.
4. This research signifies a breakthrough in ecology, as we demonstrate a field-deployable platform that requires only basic supplies (i.e. micropipettes, plastic consumables and a UV flashlight), inexpensive reagents (~$1.29 USD/sample) and emanated body heat for highly sensitive, DNA-based organism detection. By presenting FINDeM in an ecological system with pressing, global biodiversity implications, we aim to not only highlight how CRISPR-based applications promise to revolutionize organism detection but also how the continued development of such techniques will allow for additional, more diversely trained researchers to answer the most pressing questions in ecology.

KEYWORDS
Batrachochytrium dendrobatidis, CRISPR-Cas12a, DETECTR, field-based, guide RNA, isothermal, rapid DNA extraction, recombinase polymerase amplification

1 | INTRODUCTION

Until recently, answering the fundamental question, 'Which species are present in which environments?', required that researchers observe individuals directly, with methods such as the identification of collected specimens (Sherry, 1984) or documentation of individuals during point counts (Ralph et al., 1995). However, the introduction of molecular methods to the field of ecology has revolutionized how scientists characterize complex communities, particularly in the use of 'DNA barcoding' and its extensions (e.g. DNA metabarcoding; Taberlet et al., 2012). DNA barcodes, or taxon-specific genetic sequences used for organism detection and classification (Hebert et al., 2003), are increasingly applied in ecological studies (Valentini et al., 2009) and have enabled the detection of invasive species (Armstrong & Ball, 2005), rediscovery of presumed-extinct species (Campbell et al., 2008) and characterizations of ecological communities from water (Rees et al., 2014), soil (Kirse et al., 2021) and even air samples (Clare et al., 2021).

However, while molecular methods yield unrivalled sensitivity when used for organism detection (Darling & Mahon, 2011), these approaches bear limitations that may restrict their use by researchers desiring to apply DNA-based techniques in their work. For example, quantitative PCR (qPCR), a common approach for molecular detection and quantification of organisms, may require multi-day sample preparation and is typically performed on instruments costing greater than $10,000 USD. Furthermore, individuals performing these experiments must have familiarity with techniques ranging from operating micropipettes to more complex tasks such as interpreting the resulting qPCR data. Finally, even if one acquires the funding, instrumentation and training to employ qPCR, this approach is nearly always performed in a dedicated laboratory space—though see Franklin by Biomeme portable thermal cycler ($19,000/unit) in Thomas et al. (2020)—which effectively precludes many field researchers from harnessing the sensitivity of molecular techniques within their studies.

Fortunately, recent technological advancements have increased the accessibility and cost-effectiveness of molecular biological techniques, promising to not only revolutionize *how* organism detection is performed but also *who* is performing it and *where* it is performed. For example, although standard DNA-based protocols often require samples to be incubated at extreme temperatures for extended time periods, rapid methods have been developed that can extract (Wang et al., 1993) and amplify DNA (Hill-Cawthorne et al., 2014) in under 15 min, without requiring toxic chemicals, thermal cycling, or high-speed centrifugation. However, likely the most important breakthrough in molecular organism identification can be attributed to the utilization of CRISPR-Cas (Clustered Regularly Interspersed Short Palindromic Repeats—CRISPR-associated) systems for DNA detection. Though most notably recognized for their gene editing applications (e.g. Cas9; Doudna & Charpentier, 2014), other CRISPR-Cas systems—such as DETECTR (i.e. Cas12a; Chen et al., 2018) SHERLOCK (i.e. Cas13a; Gootenberg et al., 2017) or CDetection (i.e. Cas12b; Teng et al., 2019)—have been designed for nucleic acid detection, owing to the off-target effects that these Cas proteins exhibit upon recognition of target nucleic acid molecules. Yet, although variations of each of these approaches have been applied in ecological research, we are not aware of any study that has empirically demonstrated their ability to detect organisms in the limiting, non-laboratory settings that field researchers, underfunded programmes and citizen scientists tend to operate.

It is for this reason that we have developed FINDeM (Field-deployable Isothermal Nucleotide-based Detection Method), a field-friendly approach for DNA-based organism detection, requiring only standard laboratory plastics, inexpensive reagents (~$1.29/sample) and supplies, and emanated heat from the human body (Figure 1). This approach begins with chemical cell lysis to release DNA from the sample (Figure 1a), followed by the addition of cellulose filter discs to concentrate DNA, and incubation within Tris buffer to elute DNA from the filter in under 2 min without centrifugation or heat treatment (Zou et al., 2017). This extract is then used as the template in a recombinase polymerase amplification (RPA) reaction (Figure 1b; Piepenburg et al., 2006), which, when incubated at 37°C, can amplify a single copy of target DNA (Kalsi et al., 2015) and yield detectable levels of target DNA in as little as 5 min (Hill-Cawthorne et al., 2014). The product of this amplification reaction is then used as template for another body-heat inducible reaction, which uses a CRISPR-associated, RNA-guided DNA nuclease (i.e. DNase), Cas12a (Zetsche et al., 2015), to recognize the target DNA sequence and, upon binding to the target molecule, trigger indiscriminate single-stranded DNase activity (Figure 1c; Chen et al., 2018). To detect this single-stranded DNase activity, and by extension the presence of the DNA sequence of interest, we use single-stranded reporter DNA (ssDNA), which emits fluorescence only upon digestion. When in the presence of amplified target DNA, this reaction yields visible fluorescence under ultraviolet (UV) or blue light in as little as 5 min (Figure 1d; Xie et al., 2021). Finally, when optimized, FINDeM may even yield coarse, quantitative information about a sample's target DNA concentration.

Here, we demonstrate the utility of FINDeM with the field-based detection of an amphibian pathogen, *Batrachochytrium dendrobatidis* (*Bd*), the causative agent of chytridiomycosis (Berger et al., 1998)—a disease that has resulted in 'the most spectacular loss of vertebrate biodiversity due to disease in recorded history' (Skerratt et al., 2007). By providing a field-deployable approach for the detection of *Bd*—a species that is nearly impossible to observe directly without laboratory equipment—we believe that we demonstrate an important application of this technique, as it will: (1) allow field researchers to design experiments and apply treatments that depend on knowledge of an individual's health status; (2) provide disease monitoring teams, such as those at zoos or ports of entry, with a rapid and cost-effective method to identify infected individuals; and (3) empower citizen scientists and even classrooms to not only engage with, but directly benefit, an ongoing conservation effort to preserve biodiversity across the globe.

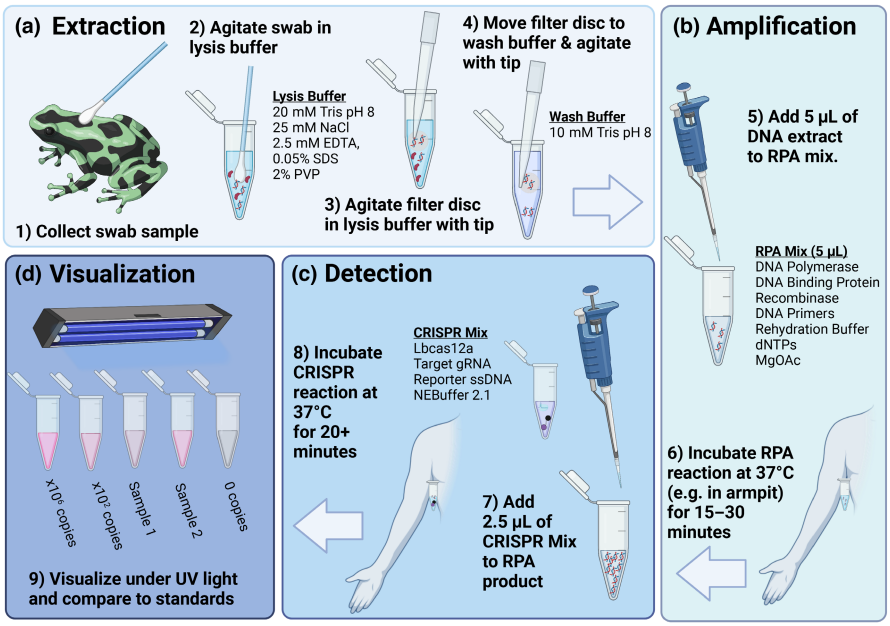

FIGURE 1 FINDeM protocol for rapid, inexpensive, field-deployable species detection. DNA extraction (a) begins with a sample (e.g. amphibian skin swab) being agitated within a lysis buffer, followed by the addition of a 3 mm cellulose filter disc. This disc is then moved to a 10 mM Tris buffer for DNA elution and is agitated before 5 µL of the eluted DNA is used as the template for the recombinase polymerase amplification (RPA) reaction (b). After a 30-min incubation at 37°C, 2.5 µL of a CRISPR-Cas12a/gRNA/reporter ssDNA mix is added to the RPA product (c) and the mixture is incubated at 37°C for an additional 15 min. Sample reaction tubes are viewed under ultraviolet light to visualize emitted fluorescence and are compared with DNA standards for detection and coarse quantification of target DNA (d).

2 | MATERIALS AND METHODS

2.1 | Study system

B. dendrobatidis (Family: Batrachochytriaceae) is an aquatic fungal pathogen and causative agent of amphibian chytridiomycosis, a panzootic that has contributed to the decline of over 400 species and the presumed extinction of at least 90 others (Scheele et al., 2019). While clinical indicators of chytridiomycosis are sometimes apparent in affected individuals (e.g. skin sloughing or discoloration; Mutschmann, 2015), these symptoms are not unique to chytridiomycosis, leaving histological analysis of amphibian epidermis as the most reliable approach for definitive diagnosis. However, due to the invasive and time-intensive nature of histology, many researchers instead rely on molecular approaches for the quantification of *Bd* DNA copies on amphibian skin, which can serve as a proxy for the pathogen load carried by an individual. The most frequently used DNA-based method in such studies is qPCR (Boyle et al., 2004), which monitors the amplification of target DNA molecules over time with fluorescent probes and compares these sample-derived fluorescence profiles to those of known target DNA quantities to estimate the number of target DNA molecules within a sample. Although molecular approaches cannot definitively diagnose chytridiomycosis without histological confirmation (Smith, 2007), the importance of qPCR in *Bd*-related studies to date cannot be understated. Because of its widespread application, qPCR has even allowed researchers to identify relationships between an individual's *Bd* DNA load and the pathogenic outcomes of chytridiomycosis (e.g. Vredenberg's 10,000 zoospore rule; Kinney et al., 2011). Here, we provide a side-by-side comparison between standard qPCR-based assessments and FINDeM for the detection of *Bd*.

2.2 | Identifying a molecular marker for FINDeM

To yield the greatest comparability between FINDeM and qPCR-based approaches for *Bd* detection and quantification, we targeted a ~146-bp region of the internal transcribed spacer (ITS) of fungal ribosomal DNA that serves as the standard marker for *Bd* detection and quantification (Boyle et al., 2004). Typical qPCR-based approaches amplify this region using the 'ITS1-3' (5′-CCTTGATATAA TACAGTGTGCCATATGTC-3′) and '5.8S' (5′-AGCCAAGAGATCCG TTGTCAAA-3′) primers, and amplification of this region is indicated by the fluorescence resulting from the cleavage of a single-stranded minor groove binding DNA probe modified with a 5′ fluorophore and 3′ quencher molecule ('MGB2'; 5′-6FAM CGAGTCGAACAAAAT MGBNFQ-3′). While our FINDeM system uses these primers to amplify the same DNA region as in qPCR studies, instead of using a modified single-stranded DNA probe for detection, FINDeM targets a 20–24 bp-long DNA region with a single-stranded guide RNA (hereafter, gRNA) that matches target DNA located adjacent to a 5′-TTTN-3′ (i.e. a protospacer adjacent motif or PAM) and does not contain a 5′-AAA-3′ sequence within it (Zetsche et al., 2015). Though our initial trials attempted to increase comparability to qPCR by targeting a region with 13-bp overlap with the 'MGB2' probe region (5′-**TTTG**TTCGACTCGTGACATATGGCACAC-3′; overlap underlined,

PAM bolded), we found target overlap complementing 14 bp of the 3′ region of the 'ITS1-3′ primer (5′-**TTTG**TTCGACTCGTGACATAT GGCACAC-3′; overlap underlined, PAM bolded) caused background fluorescence leading to ambiguous results. Therefore, we instead targeted a region of the gene that did not overlap with either primer site while still satisfying the requirements for Cas12a activation (5′-**TTTT**TAATATTTATTTTCAATTTTT-3′; PAM bolded).

2.3 | Limit of detection and quantification of Bd plasmid standards and zoospore genome equivalents

When quantifying *Bd* load, researchers often analyse known quantities of *Bd* DNA to serve as reference values for the sample DNA concentrations. Typically, these are serial dilutions of DNA extracted from known quantities of *Bd* zoospores or solutions with known concentrations of synthetic plasmids containing the *Bd* ITS target gene. Thus, we performed qPCR and FINDeM in triplicate using *Bd* plasmid standards ranging in order of magnitude from 2.6 to 2,600,000 copies per reaction (Pisces Molecular; Boulder, Colorado, USA) as well as *Bd* zoospore-based standards ranging in order of magnitude from 0.01 to 10,000 zoospores per reaction; negative controls for each were Buffer AE (Qiagen; Hilden, Germany) and nuclease-free water, respectively. We estimated the zoospore density of a liquid *Bd* culture using a haemocytometer and created six 10,000 zoospore stock dilutions; each of the less concentrated zoospore standards was created via 1:10 serial dilution from these stock dilutions. Each set of triplicate zoospore dilutions underwent two DNA extraction protocols: a modified Qiagen Blood and Tissue Kit extraction (hereafter, 'Qiagen'; Brannelly et al., 2020) or a rapid, chemical cell lysis (hereafter, 'Quick'). The Quick extraction approach entailed adding 50 μL of each dilution to 100 μL of lysis buffer (20 mM Tris HCl pH 8.0, 25 mM NaCl, 2.5 mM EDTA, 0.05% SDS and 2% PVP-40; Zou et al., 2017) followed by a single 3 mm Whatman #1 filter disc. The disc was agitated within the extraction buffer using a sterile pipette tip for 30s, and, using the pipette tip and fresh gloves, the filter disc was moved to 200 μL of 10 mM Tris HCl pH 8.0 where it was agitated with a pipette tip for an additional 30s to elute DNA (Zou et al., 2017).

We prepared qPCR reactions in 25 μL volumes with 5 μL of each *Bd* plasmid standard, Qiagen-extracted zoospore dilution or Quick-extracted zoospore dilution as template and analysed them using a QuantStudio 3 instrument and default software (Applied Biosystems; Waltham, MA, USA) following Brannelly et al. (2020). An additional series of *Bd* plasmid standards was analysed as a reference to determine whether *Bd* DNA in each sample's replicate was amplified (i.e. a sample's delta normalized reporter value, ΔRn, was higher than the standard-calculated threshold denoting target amplification with ≥90% confidence), inconclusive (i.e. a sample's ΔRn was higher than the standard-calculated threshold denoting target amplification, though with <90% confidence) or unamplified (i.e. a sample's ΔRn was lower than the standard-calculated threshold denoting target amplification) as well as to estimate the number of *Bd* ITS copies in each sample. Finally, as the lowest concentration of *Bd* plasmid standard—which was verified to contain, on average, 2.6 copies in each reaction—was, at best, only able to achieve inconclusive evidence of amplification and because each negative control only ever returned unamplified results, we regard conclusive and inconclusive amplification as detections of target DNA within this study.

For each FINDeM reaction, 1 μL of 10x NEBuffer 2.1 (New England Biolabs/NEB, Ipswich, MA, USA), 0.5 μL of 25 μM 5′-ROX-NNNNNNNNNNNN-BHQ2-3′ reporter ssDNA (Integrated DNA Technologies/IDT, Coralville, Iowa, USA), 0.5 μL of 1.25 μM *Bd* guide RNA (5′-UAAUUUCUACUAAGUGUAGAUUAAUAUUUAU UUUCAAUUUUU-3′; IDT) and 0.5 μL of 1 μM LbCas12a (NEB) were mixed and incubated for 30 min at ambient temperature (hereafter, 'CRISPR mix'). During this incubation period, 17.7 μL of Primer Free Rehydration buffer and 4.8 μL of a 20 mM each mixture of ITS1-3 and 5.8S primers were added to a single lyophilized RPA master mix (Basic RPA Kit; TwistDx, Cambridge, England). A 5 μL volume of *Bd* plasmid standard, Qiagen-extracted zoospore DNA or Quick-extracted zoospore DNA was mixed with 4.5 μL of the RPA mix, and 0.5 μL of 280 mM MgOAc was pipetted to the side wall of each reaction well. The plate containing these samples was then pulse spun to ensure that all reactions were initiated simultaneously, and the plate was incubated in a thermal cycler at 37°C for 30 min. Following this incubation step, the plate was placed on ice to halt DNA amplification and 2.5 μL of the CRISPR mix was added to the side wall of each reaction well. The plate was then pulse spun to ensure all reactions were initiated simultaneously, and the plate was incubated at 37°C on a QuantStudio 3 with ROX fluorescence monitored every 30 s for 20 min. Upon completion of the reaction, 8 μL from each well was immediately moved to a new 0.2 mL plastic tube and photographed under UV light. Finally, to determine whether this protocol could return information about starting DNA concentrations, we performed FINDeM on *Bd* plasmid standards ranging from 2.6 copies to 26,000 copies in triplicate, using the identical protocol though lowering the RPA incubation period from 30 to 15 min.

2.4 | Detecting Bd from amphibian swab samples

To determine whether FINDeM would be applicable in real-world scenarios, we applied this approach in two settings: a standard molecular laboratory with a formally trained molecular biologist (BD Hoenig) and a field research site with a formally trained field researcher with limited molecular biology training (J Zegar). Using fresh gloves, amphibians were collected from ponds surrounding the Pymatuning Lab of Ecology (lab-based detection; Linesville, Pennsylvania, USA; Collection Permit #2022-01-1222; IACUC Protocol #IS00021074) in the Summer 2022 or the University of Mississippi Field Station (field-based detection; University, Mississippi, USA; Collection Permit #1220211; IACUC Protocol #22-001) in the Fall 2022 and swabbed 40–50 times across their body with two rayon swabs (Medical Wire and Equipment Co., Corsham, England). For the lab-based approach,

a subset of these swabs ($n = 8$ individuals) was stored at −80°C for up to 3 months until DNA was extracted using either the Qiagen or Quick methods. Swabs collected for the field-based approach ($n = 26$ individuals) were either processed with the Quick DNA extraction method on-site (Figure 2a) or stored frozen at −20°C until they were processed using the Qiagen DNA extraction and analysed with qPCR up to 3 weeks later; *Bd* plasmid standards for field-based samples ranged from 4.2 to 4,200,000 copies.

The DNA extracts processed in the laboratory were used as templates for the qPCR and FINDeM protocols described above, though we present images of the tubes used in the assay to show that resulting end-point fluorescence is apparent without a qPCR instrument. We also performed FINDeM in a laboratory—though achieving incubation temperatures by instead taping the tubes within a researcher's armpit and lowering the arm (i.e. 'human body incubation')—on two samples (PA-9 and PA-24) and three zoospore standards (10,000, 10 and 0 zoospores) to assess whether field-based, DNA quantification was feasible; initial attempts with handheld incubation did not return rapid results and were therefore abandoned. Samples extracted in the field were immediately used as templates in the FINDeM assay alongside positive (DNA extract of *Bd* liquid culture) and negative (molecular grade water) controls. Field-based FINDeM reactions were performed as described above with the following alterations: Reaction temperatures were achieved using the human body incubation approach described above or by sandwiching the tubes between two air-activated heat packs; centrifugation was performed with a salad spinner modified with a 0.2 mL microcentrifuge rotor or a 0.2 mL microcentrifuge plugged into an automobile auxiliary power outlet; and fluorescence was induced by a handheld UV flashlight, visualized in a black tape covered box to limit outside light and documented with a cell phone camera. The full, step-by-step field-based detection and quantification protocol can be found at https://osf.io/3mbdf/.

2.5 | Data analysis and statistics

All statistics and data visualizations were performed in R (version 4.1.2), and the data and code to reproduce our analysis can be found at https://osf.io/3mbdf/. All qPCR *Bd* DNA amplification statuses and

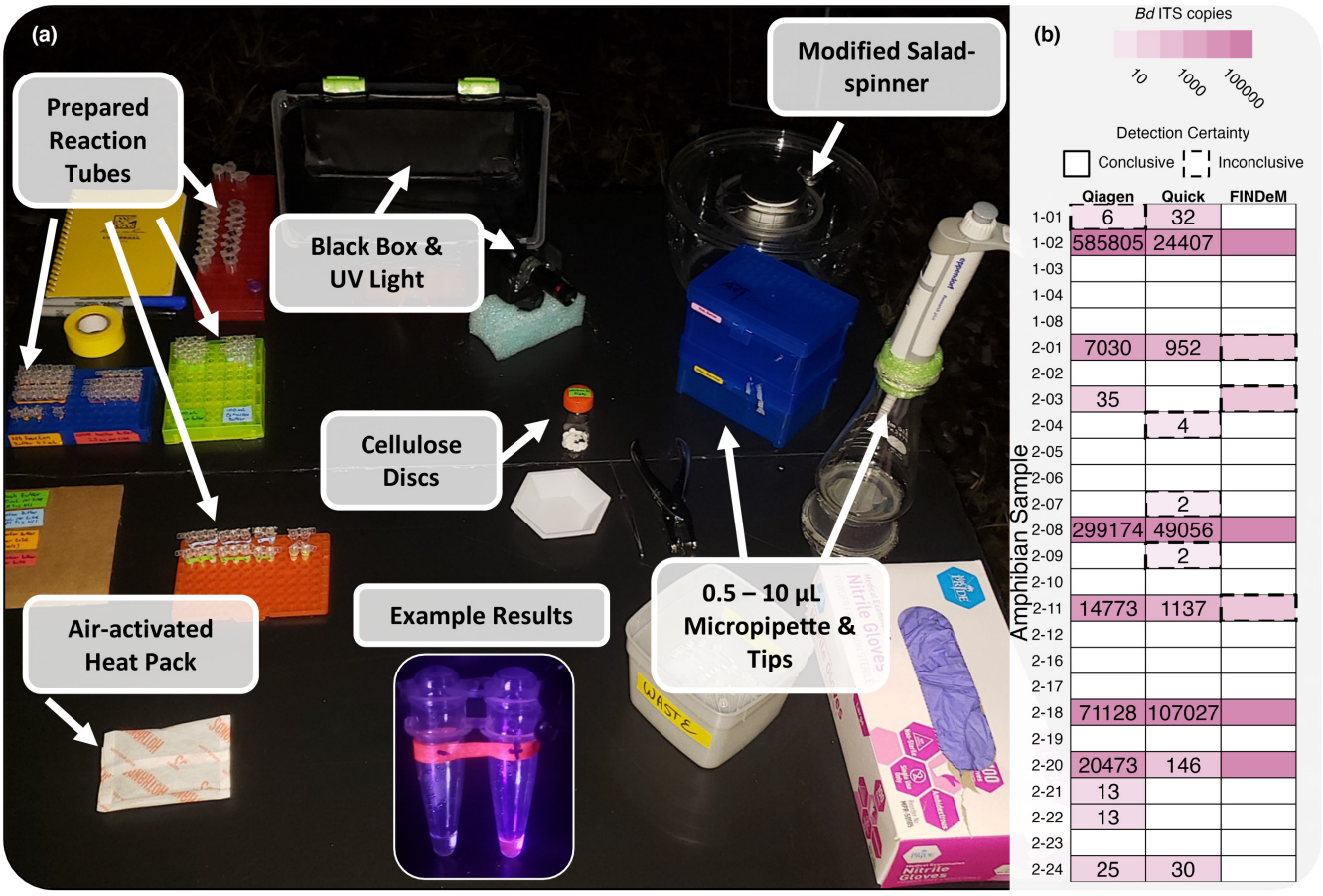

FIGURE 2 FINDeM offers a field-deployable approach for the detection of the amphibian fungal pathogen, *Batrachochytrium dendrobatidis*. A field sampling set-up used for FINDeM-based *Bd* detection in University, Mississippi (a) alongside a heatmap comparing Qiagen-extracted qPCR-based *Bd* DNA copy number estimates, Quick-extracted qPCR-based *Bd* DNA copy estimates and results from field-based FINDeM assays (b). *Bd* ITS copy number estimates are found within each qPCR-positive cell while FINDeM assessments of amplified (dark pink), inconclusive (light pink) and unamplified (white) are found within all FINDeM cells. FINDeM reactions were considered 'inconclusive' if supporting photographic evidence of field-based detection did not demonstrate unambiguous fluorescence.

copy number estimates were derived from the analyses performed by the QuantStudio 3 software. To replicate what would be possible in a field setting, all FINDeM-based *Bd* detection statuses were determined on-site, based on apparent fluorescence differences when compared to controls; we present images of each reaction either in text or the supplemental materials for external verification. FINDeM reactions were considered 'inconclusive' if supporting photographic evidence of field-called '*Bd*-positive' samples did not demonstrate unambiguous fluorescence. The coefficient of variation (CV) is defined as the ratio of the standard deviation to the mean for each of the following experiment's results: qPCR-based detection (Cycle threshold or 'Ct' values), qPCR-based quantification (number of *Bd* copies) and FINDeM-based detection (relative fluorescence in arbitrary units).

3 | RESULTS

3.1 | Comparing qPCR and FINDeM on Bd DNA standards

To understand how FINDeM compared with qPCR in terms of detection limits, we applied each approach to *Bd* plasmid standards and found that the FINDeM protocol was as sensitive and consistent as the traditional qPCR-based approach—enabling the detection of as many replicates of each dilution as qPCR, including the lowest of 2.6 *Bd* ITS copies (Figure 3). With that said, final relative fluorescence values from FINDeM did not exhibit concentration-dependent fluorescence and displayed a higher coefficient of variation among positive triplicates (CV range: 19.3%–61.42%) than the Ct values returned by qPCR (Figure 3; CV range: 0.1%–0.76%). We did find that by shortening the amplification reaction to 15 min, differences in fluorescent profile across varying DNA concentrations were apparent (Figure 4a), and 30-min, human body incubation was even able to yield coarse, but visible differences in fluorescence (Figure 4b). We then aimed to determine how the Quick DNA extraction protocol, which would be used alongside FINDeM in a field setting, compared with standard Qiagen DNA extractions. We found that, in general, Qiagen DNA extractions returned DNA yields that were more consistent (CV range: 2.7%–45.8% for Qiagen vs. 37.1%–79.8% for Quick) and roughly an order of magnitude greater than those offered by Quick extractions when extracting from >1 zoospores per reaction (Figure 5). However, at lower template concentrations, the Quick method appeared as effective at detecting *Bd* DNA with qPCR, even detecting 0.1 zoospores in a single replicate where Qiagen detected none of the three replicates (Figure 5).

When comparing qPCR to FINDeM on Qiagen-extracted zoospores, we found that FINDeM was able to offer greater sensitivity, detecting the DNA of a single replicate of 0.1 zoospores per reaction, while qPCR was only able to detect the DNA of a single replicate of 1 zoospore per reaction (Figure 5). However, we did find that qPCR offered more consistent *Bd* DNA detection in the presence of 1 or 10 Qiagen-extracted zoospores per reaction though each method was able to reliably detect 100 to 10,000 zoospores per reaction (Figure 5). When coupled with the Quick DNA extraction method, we found that FINDeM was able to detect the DNA of a single replicate of 100 zoospores, as well as each replicate of the 1000 and 10,000 zoospore dilutions. Finally, we found that *Bd*-negative, Qiagen-extracted samples exhibited higher baseline ROX fluorescence values (mean = 185,081, SD = 29,053) than *Bd*-negative, Quick-extracted samples (mean = 76,824, SD = 32,258), though this baseline fluorescence was not apparent under UV light.

3.2 | Comparing qPCR and FINDeM on amphibian swab samples

In the lab-based setting, we found that Pennsylvania-collected amphibian swab samples extracted with the Quick method generally yielded DNA concentrations that were as consistent (CV range: 11.70%–84.96% for Qiagen samples vs. 20.59%–108.14% for Quick samples) but roughly an order of magnitude lower than those produced by the Qiagen approach (Figure 6a). Additionally, we found that each of the Qiagen-extracted samples yielding definitive qPCR-based *Bd* amplification was also qPCR-amplified—albeit at times inconclusively—with Quick extractions (Figure 6a; PA9, PA24 and PA31). When using FINDeM on Qiagen-extracted swab samples, we were able to detect *Bd* DNA in each of the samples that returned qPCR-based amplifications (Figure 6b; PA9, PA24 and PA29) and even detect single-digit copies of *Bd* DNA in two samples (Figure 6b; PA29 and PA31). Once again, we found that Qiagen-extracted swab samples emitted higher baseline ROX fluorescence than Quick-extracted samples, though the highest background fluorescence values were only ~40% as high as those of the lowest Qiagen-extracted positive sample and were noticeably dimmer when compared to other positive samples under UV light. Finally, in our field-based demonstration in Mississippi (Figure 2b), we found that FINDeM agreed with the standard Qiagen-extraction and qPCR results in 19 of 26 samples (73.1%), though this percentage increased when considering inconclusive FINDeM results as positives (22/26; 84.6%). We did not observe any instances of false positives with FINDeM when comparing to qPCR results; however, three Quick-extracted swabs did exhibit inconclusively amplifiable levels (<5 copies) of *Bd* DNA with qPCR while their Qiagen-extracted counterparts did not (Figure 2b).

4 | DISCUSSION

In this study, we designed and tested a field-deployable, DNA-based organism detection protocol—FINDeM—which can identify single-digit copies of target DNA in under 1 h and return results that are largely comparable to qPCR, all while requiring little more than basic supplies, relatively inexpensive reagents and normal human body temperatures. We then validated this methodology to detect the amphibian fungal pathogen, *Bd*, from amphibian swabs processed

Methods in Ecology and Evolution

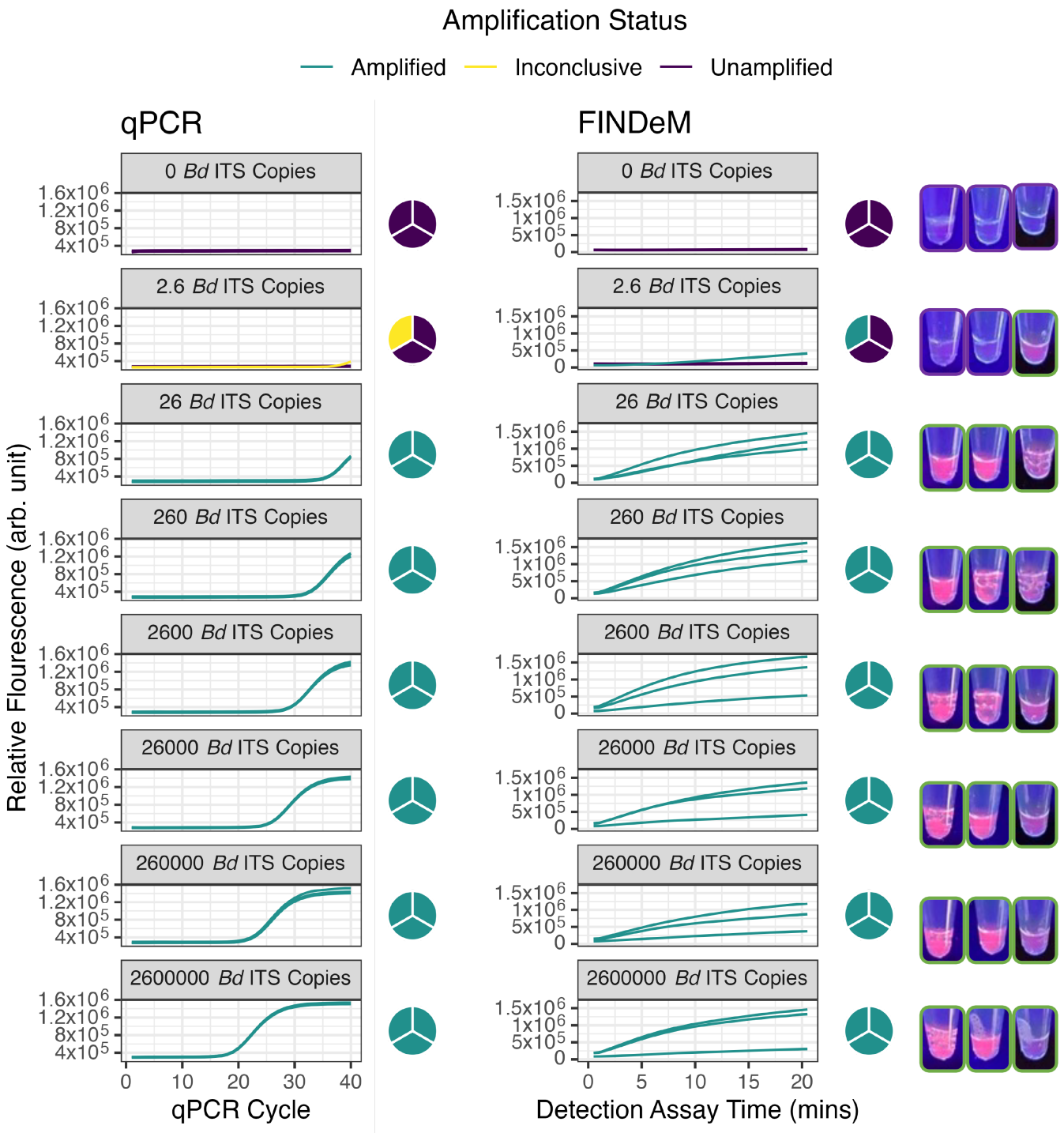

FIGURE 3 FINDeM offers a comparable limit of DNA detection to qPCR and may detect single-digit copies of *Bd* DNA from plasmid standards. Known *Bd* DNA copy quantities (0 to 2,600,000 copies per reaction) from plasmid standards were used as template in qPCR (left) and FINDeM (middle and right images) reactions. qPCR detection statuses were derived from default regression analysis using QuantStudio 3 software, while FINDeM detection statuses were assessed by visually comparing fluorescence of each template to the negative control sample in each set of replicates.

in two manners: (1) using supplies attainable by most scientific and educational laboratories and (2) using supplies that could reasonably be brought into a field setting and used by researchers with little to no training in molecular biology. In each scenario, we found that FINDeM could accurately detect the presence of *Bd* DNA, though at times with lower consistency and sensitivity than traditional assessments. With that said, we did find instances in which combining rapid approaches alongside more traditional ones—such as CRISPR-based detection on Qiagen-extracted samples—detected DNA with greater sensitivity, indicating that these methods are not

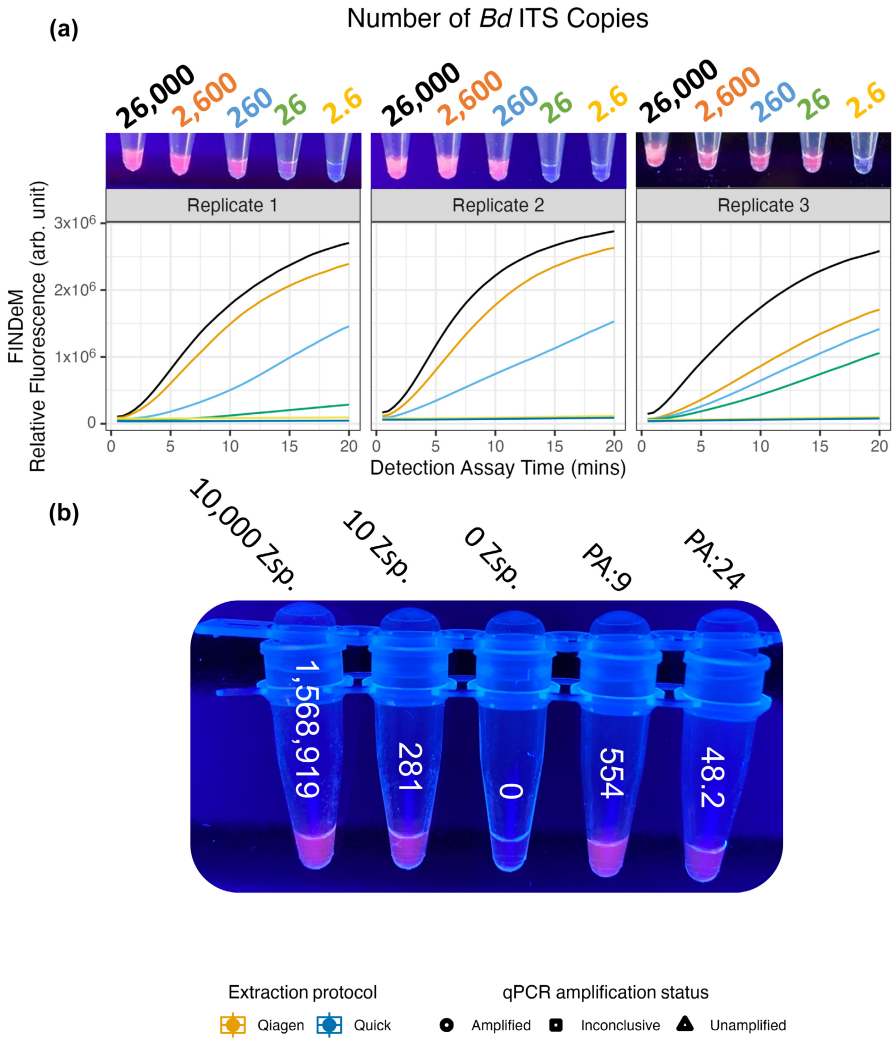

FIGURE 4 When optimized, FINDeM returns concentration-dependent fluorescence profiles that can be used to yield coarse quantitative insights about a sample's target DNA concentration. The FINDeM protocol was modified by reducing DNA amplification times from 30 to 15 min to aid in differentiating between samples with varying target DNA concentrations, though these differences were more pronounced with the aid of a qPCR instrument than with UV-induced visual assessment alone (a). Similar findings were obtained with a set of tubes that were armpit-incubated for 30 min as opposed to being incubated by air-activated heat packs or a thermal cycler (b). Zoospore count (abbreviated Zsp.) per reaction is indicated in black above each tube, while qPCR-estimated *Bd* ITS DNA copy number from each zoospore count is in white within the tube.

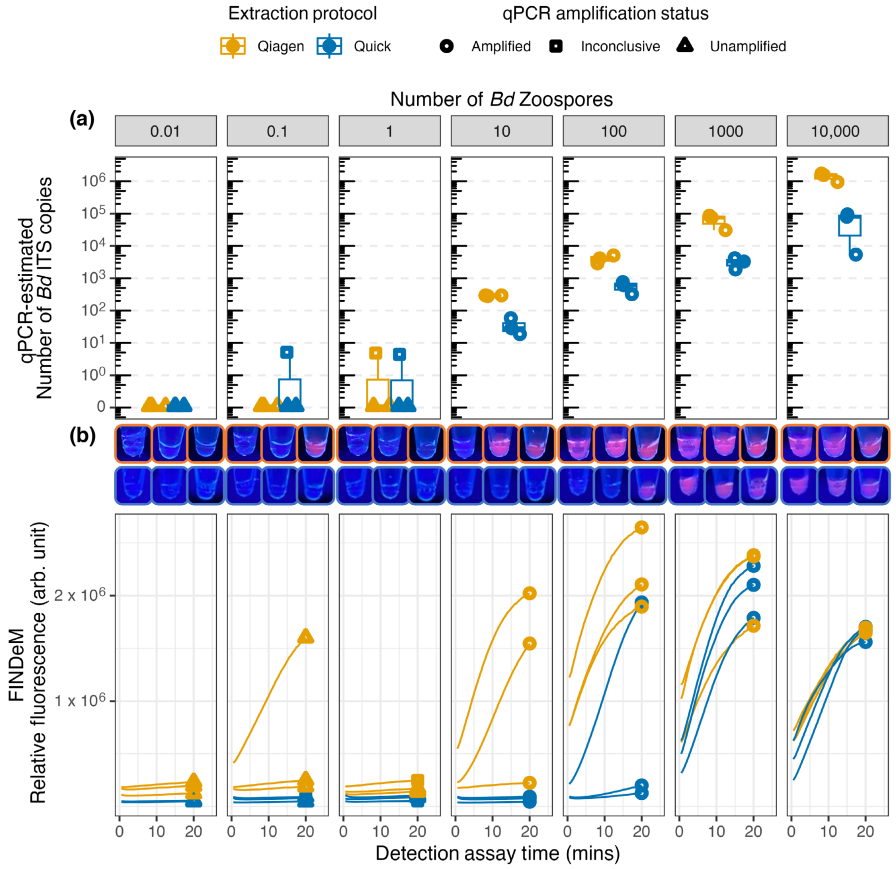

FIGURE 5 Two-min, field-ready DNA extraction method yields qPCR detection sensitivities which are comparable to those using kit-based extraction and can be used with Cas12a-based detection methods to identify subclinical concentrations of *Bd*. Dilutions of *Bd* zoospore concentrations were used as template in triplicate qPCR (a; top) and FINDeM assays (b; middle images and bottom) and were analysed for detection status and estimated copy number (qPCR) or fluorescence over time (FINDeM). Images of tubes show the final fluorescence of Qiagen-extracted (top row, orange outline) and Quick-extracted (bottom row, blue outline) FINDeM assays. Shapes for each dilution in the FINDeM results indicate if that biological replicate was amplified, inconclusive or unamplified by qPCR. Estimations for *Bd* ITS copy number are mapped onto a log-10 scale.

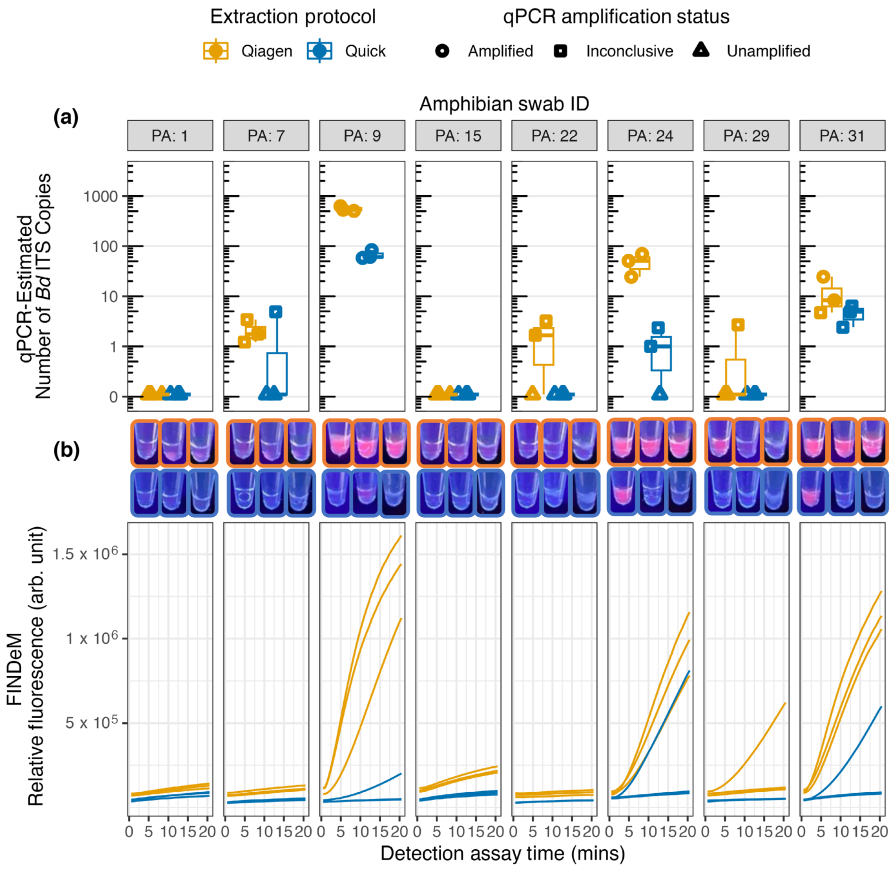

FIGURE 6 Using FINDeM with limited electronic instrumentation allows for the detection of single-digit copies of *Bd* target DNA from amphibian skin swab samples. Amphibian swab samples collected from the Pymatuning Lab of Ecology in Northwest Pennsylvania were analysed using qPCR (a; top) and FINDeM (b; middle images and bottom) assays. Images of tubes show the final fluorescence of Qiagen-extracted (top row, orange outline) and Quick-extracted (bottom row, blue outline) FINDeM assays. FINDeM results are presented without shapes as they represent technical replicates of a single sample as opposed to the biological replicates found in Figure 5. Estimations for *Bd* ITS copy number are mapped onto a log-10 scale.

'all-or-nothing' and can be interchangeable based on the researchers' needs.

For example, biologists in charge of captive breeding programmes for species threatened with extinction due to *Bd*—such as the Panamanian golden frog, *Atelopus zeteki*—may desire the increased yields offered by kit-based DNA extraction for greater sensitivity but worry less about the ability to precisely quantify *Bd* loads, instead opting for the faster, less expensive and less equipment-dependent FINDeM approach (<1 h, ~$1.29/sample) over qPCR (>2 h, ~$2.19/sample). Other applications may also opt to replace RPA with high-fidelity PCR—particularly if the assay is to be performed on low-quality or low-template samples, such as environmental DNA—before using the CRISPR-based reaction for organism detection; therefore, attaining the sensitivity of a probe-based qPCR assay without the associated limitations noted earlier. Finally, researchers who wish to only analyse samples from *Bd*-positive individuals can use FINDeM as a rapid and cost-effective screening step; an application that may be particularly attractive to underfunded laboratories looking to maximize the number of positive samples that can be analysed or research operations that do not have the instrumentation (e.g. thermal cyclers or gel electrophoresis supplies) required for PCR-based screening. Additional refinements of this approach—such as using lateral flow strips for electricity-free detection (Baerwald et al., 2020), using wax-coated, cellulose paper strips (e.g. 'DNA dipsticks'; Zou et al., 2017) to replace filter discs for DNA extraction and pipettes for moving reaction products (Sullivan et al., 2019), or performing each reaction within a microfluidic chip (*Chytritect*; iGEM 2021, NYU-Abu Dhabi)—are likely to increase this approach's accessibility, and we look forward to the adaptations that allow additional users to apply molecular ecological techniques within their own work.

4.1 | Generalizing FINDeM for additional applications

One of the greatest benefits of CRISPR-based approaches is their highly programmable nature (Jinek et al., 2012), meaning that adapting FINDeM for other study systems can be as simple as changing RPA primers and gRNA to match the genetic sequence found within the organism of interest. The minimal requirements of FINDeM—a reliably amplified gene with a 20-bp to 24-bp-long sequence that does not contain a 5′-AAA-3′ and is adjacent to a 5′-TTTN-3′ PAM site (Chen et al., 2018; Zetsche et al., 2015)—are likely common within the genomes of most, if not all, taxa; a statement empirically supported by the presence of two such sites within the ~146-bp target gene in this study. However, even if such sites did not exist or were not specific to a single taxon, researchers have begun to identify variants of LbCas12a which recognize additional PAM sites (i.e. 'impLbCas12a'; Tóth et al., 2020) and have developed approaches with Cas12a (Zhou et al., 2022) and Cas13a (Gootenberg et al., 2017), which can even operate in a PAM-independent manner, potentially allowing for an even greater number of target sequences and, as a result, a greater number of target taxa. Finally, it is also

possible for FINDeM to detect RNA—thus allowing for the detection of certain viruses (e.g. Ebola) and transcripts (e.g. those associated with amphibian life stages; Parsley & Goldberg, 2023)—by using reverse transcriptase enzymes within the RPA reaction to convert target RNA to DNA prior to detection (Sun et al., 2021). Therefore, it appears the applications for which field-friendly, CRISPR-based detection of an organism's genetic material are limited only by the imaginations of its users.

In addition to the *Bd*-related approaches outlined above, this technique may be particularly useful for those interested in monitoring the arrival of invasive species to protected areas (e.g. eDNA of Asian Carp in the Great Lakes; Jerde et al., 2013); selectively collecting tissues only from infected individuals or removing infected individuals from population entirely (e.g. birds infected with Avian Influenza; Liu et al., 2020); classifying morphologically indistinguishable individuals prior to release (e.g. 'Traill's' flycatcher species complex at bird banding labs; Seutin, 1991); or even detecting the presence of low-density species during traditional assessments (e.g. understanding snow leopard density in real-time from faeces; Chetri et al., 2019). Similar CRISPR-based organism detection systems are already being used to manage concerns specific to humanity—such as in agriculture (e.g. genetically modified rice; Zhang et al., 2020), aquaculture (e.g. viruses infecting Pacific white shrimp; Sullivan et al., 2019) and human health (e.g. SARS-CoV-2; Ding et al., 2020). Therefore, we hope that our demonstration in an ecological system with global biodiversity implications encourages future conservation-driven applications of this approach.

4.2 | Methodological considerations

Although our presentation of a field-deployable, CRISPR-based system for organism detection stands as a novelty in ecology, many of the methodological decisions made in this study came because of the integration of prior attempts from other systems. For instance, in the first application of Cas12a-based detection, termed DETECTR (sensu Chen et al., 2018), on eDNA samples, Williams et al. (2019) used a 50 nM final concentration of FAM-labelled ssDNA, which required a 2-h incubation and assistance by a qPCR instrument to detect the fluorescence emitted by the reporter molecules (though see modifications in Williams et al., 2023). FINDeM, however, uses a ROX-labelled ssDNA at a 1 μM final concentration, as Xie et al. (2021) found that using ROX-labelled ssDNA exhibited lower ambiguity between positive and negative results, showed greater emission of fluorescence at lower ssDNA concentrations and even allowed for naked-eye detection when used at higher concentrations. Though we believe 1 μM of reporter ssDNA used in this study struck an adequate balance between the benefits of rapid detection alongside the monetary costs associated with higher concentrations, the flexibility of CRISPR-based systems allows for various modifications—such as ssDNA concentrations, reaction volumes and incubation procedures—to be implemented with relative ease.

In another study that relied on instrument-based incubation, Baerwald et al. (2020) applied a system similar to DETECTR, termed SHERLOCK (sensu Gootenberg et al., 2017), which amplifies and transcribes target DNA into RNA copies which are then degraded by Cas13a (Shmakov et al., 2017), an RNA-guided RNase that exhibits collateral single-stranded RNA (ssRNA) digestion analogous to that of ssDNA by Cas12a. However, instead of using concentrations of fluorescent reporter ssDNA that could be detected by the human eye, these researchers used Biotin-labelled ssRNA fragments which would then attach to lateral flow strips to serve as indicators of target DNA presence. Using this method, the researchers were able to differentiate between three species of smelt (Family: Osmeridae) in under 1 h, though they did not evaluate their lateral flow strip approach for copy number quantification (Baerwald et al., 2020). While we believe that methods targeting DNA and using fluorescent reporter ssDNA present a more dependable, cost-effective and informative application than purely qualitative approaches which rely on transcription after DNA amplification and the relative instability of ssRNA molecules, it is nevertheless exciting to see how alternative approaches for CRISPR-based DNA detection are already being applied in other ecological settings.

4.3 | Future directions

In addition to the modifications of previous protocols that we have incorporated into the present study, it is possible that the use of our study design in other systems would also be benefited by alterations. First, although the method of rapid DNA extraction used in this study was as sensitive as a kit-based DNA extraction in terms of qPCR-based *Bd* zoospore detection limits, DNA yields from the Quick method were considerably lower than those offered by the Qiagen protocol, which in turn yielded lower *Bd* detection rates when paired with both qPCR and FINDeM. Although we found that using passive chemical lysis and cellulose filter discs isolated DNA from fungal cells effectively, researchers attempting to extract DNA from other robust cell types (e.g. plant cells or bacterial endospores) may have greater success with alternative methods, such as alkaline cell lysis followed by sample dilution (Wang et al., 1993) or by coupling mechanical processes with extraction buffers to increase the efficiency of cell lysis (Sullivan et al., 2019). Another potential avenue that would increase the ease of use in ecological settings would be to couple DNA amplification and detection within the same reaction (Aman et al., 2020). Our initial trials attempted to do this, but we found that final fluorescence within each sample—regardless of starting DNA concentration—was undetectable without a qPCR instrument after 45 min of incubation, which possibly came as a result of decreased DNA amplification caused by simultaneous Cas12a-degradation of the ssDNA primers.

Likely due to the ssRNA degradation properties of Cas13a that would leave ssDNA primers intact, Baerwald et al. (2023) were able to design a 'one-pot' SHERLOCK assay that was as sensitive as qPCR and the two-step FINDeM assay, detecting single-digit copies of

synthetic target DNA. However, Baerwald et al. (2023) did not test the sensitivity of their 'one-pot' assay on biological samples, which may contain proteins and chemicals that hinder or even entirely inhibit molecular reactions. Therefore, it is difficult to say if the 'one-pot' SHERLOCK approach would exhibit the same sensitivity as the FINDeM protocol, which was able to definitively detect single-digit DNA copies from amphibian swabs in a lab setting (Figure 6b; PA31, PA24) and inconclusively detect double-digit copies from amphibian swabs in the field (Figure 2b; 2-03). Nevertheless, it appears that alterations that increase the ease of use, either in nucleic acid extraction or in downstream detection, will impact the sensitivity of the assay, leaving practitioners to decide which aspects of the method are most important for their needs.

The final, and likely most appealing, area of improvement for CRISPR-based DNA detection lies in its ability to precisely quantify the target DNA concentration found within a sample. Unlike qPCR, where the excitation of a single fluorescent probe is associated with the amplification of a single target molecule, the fluorescence emitted in FINDeM is not linked to amplification directly and is instead associated with the multiple-turnover, ssDNAase activity of Cas12a. While this uncoupling of amplification and fluorescent detection may increase the detectability of molecules from samples that traditionally suffer from inhibition—such as faeces or soil—(i.e. the detection of a single target molecule by Cas12a leads to the digestion of multiple reporter molecules, thus yielding greater 'per-target-molecule' fluorescence than qPCR), it also muddles the relationship between the starting concentration of target DNA and the final fluorescence of the reaction. Although the full-length FINDeM assays (i.e. 30-min RPA and 20-min Cas12a reactions) presented within this study did not yield DNA concentration-dependent fluorescence, we did find that by shortening DNA amplification times to 15 min or by incubating the reactions at presumably lower and less consistent temperatures via human body incubation, we were able to identify concentration-dependent fluorescence profiles throughout the reaction and at times even visible differences in final fluorescence among varying DNA concentrations. One hypothesis for this is that by impeding the amplification reaction with lower temperatures or shorter incubation times, the final DNA amplicon concentration better correlates with the starting DNA template concentrations that are otherwise overwhelmed by amplifications reaching their effective asymptotic maxima. However, while this approach may yield coarse quantitative estimates of a sample's target DNA concentration, it may also incur a higher risk of false negatives, particularly in samples with low target DNA concentrations. For this reason, we recommend that field researchers requiring coarse quantitative estimates first perform a 'detection reaction' optimized for detecting target DNA of any concentration followed by a shorter or lower temperature 'quantification reaction'. Those interested in more precise, CRISPR-based quantifications should do so with the aid of a handheld thermal cycling and fluorimetry instrument to differentiate emitted fluorescence more acutely.

5 | CONCLUSIONS

The introduction of molecular techniques to ecology has allowed researchers to uncover insights about the natural world with a level of precision that was previously impossible with more traditional approaches. However, until recently, the power of molecular methods has been relegated almost entirely to laboratory settings, only to be performed by those researchers fortunate enough to have obtained the training and funding to undertake such projects. Here, we demonstrate the utility of a rapid, inexpensive and field-deployable system—FINDeM—which requires little more than inexpensive reagents, non-technical instrumentation and emanated human body heat for the molecular detection of organisms. In our validation of this approach, we show that FINDeM may detect single-digit copies of target DNA and identify the presence of a fraction of a zoospore in under 1 h, even returning coarse quantification of target DNA found within a sample upon proper optimization. There is little doubt that CRISPR-based methods will attract more individuals to the field of ecology, and we look forward to the breakthroughs that stem from putting DNA-based techniques into the hands—and armpits—of every ecologist.

AUTHOR CONTRIBUTIONS

The methods were designed by Brandon D. Hoenig, Macie M. Chess, Brady A. Porter and Corinne L. Richards-Zawacki with field optimization performed by Michel E. B. Ohmer and Jakub Zegar. Brandon D. Hoenig and Myah Madril collected and analysed laboratory-based data, while Jakub Zegar and Michel E. B. Ohmer collected and analysed field-based data. Brandon D. Hoenig wrote the first draft of the manuscript, and all authors contributed significantly to revisions.

ACKNOWLEDGEMENTS

This research was funded by a Chapman Grant (American Museum of Natural History) and a Frank J. Schwartz Early Career Research Fellowship (Pymatuning Lab of Ecology) awarded to Brandon D. Hoenig and was supported by the RIBBiTR Biological Integration Institute led by Corinne L. Richards-Zawacki through funding from the National Science Foundation (DBI-2120084). We are grateful for the assistance provided by Hailey Hoffman (University of Mississippi) and Miranda Kosowsky (University of Pittsburgh) as well as the on-site assistance provided by Chris Davis and Jessica Barabas (Pymatuning Lab of Ecology). We are appreciative of Michael Vorisek as well as the residents of The Great Outdoors Family Campground who allowed collection of samples from their private residences and to the Pymatuning State Park for allowing sample collection on public lands. All amphibians were collected under appropriate permits (University of Mississippi, #1220211; University of Pittsburgh, #2022-01-0122), and sampling protocols followed all IACUC-approved methods (University of Mississippi, #22-001; University of Pittsburgh, #IS00021074).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/2041-210X.14236.

DATA AVAILABILITY STATEMENT

The data supporting this manuscript's results are archived on OSF at https://osf.io/3mbdf/ (Hoenig et al., 2023).

ORCID

Brandon D. Hoenig https://orcid.org/0000-0001-6344-3202
Michel E. B. Ohmer https://orcid.org/0000-0002-5937-6585

REFERENCES

Aman, R., Mahas, A., Marsic, T., Hassan, N., & Mahfouz, M. M. (2020). Efficient, rapid, and sensitive detection of plant RNA viruses with one-pot RT-RPA–CRISPR/Cas12a assay. *Frontiers in Microbiology*, *11*, 610872.

Armstrong, K., & Ball, S. (2005). DNA barcodes for biosecurity: Invasive species identification. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*, 1813–1823.

Baerwald, M. R., Funk, E. C., Goodbla, A. M., Campbell, M. A., Thompson, T., Meek, M. H., & Schreier, A. D. (2023). Rapid CRISPR-Cas13a genetic identification enables new opportunities for listed Chinook salmon management. *Molecular Ecology Resources*. https://doi.org/10.1111/1755-0998.13777

Baerwald, M. R., Goodbla, A. M., Nagarajan, R. P., Gootenberg, J. S., Abudayyeh, O. O., Zhang, F., & Schreier, A. D. (2020). Rapid and accurate species identification for ecological studies and monitoring using CRISPR-based SHERLOCK. *Molecular Ecology Resources*, *20*, 961–970.

Berger, L., Speare, R., Daszak, P., Green, D. E., Cunningham, A. A., Goggin, C. L., Slocombe, R., Ragan, M. A., Hyatt, A. D., McDonald, K. R., Hines, H. B., Lips, K. R., Marantelli, G., & Parkes, H. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Sciences of the United States of America*, *95*, 9031–9036.

Boyle, D. G., Boyle, D., Olsen, V., Morgan, J., & Hyatt, A. (2004). Rapid quantitative detection of chytridiomycosis (*Batrachochytrium dendrobatidis*) in amphibian samples using real-time Taqman PCR assay. *Diseases of Aquatic Organisms*, *60*, 141–148.

Brannelly, L. A., Wetzel, D. P., West, M., & Richards-Zawacki, C. L. (2020). Optimized Batrachochytrium dendrobatidis DNA extraction of swab samples results in imperfect detection particularly when infection intensities are low. *Diseases of Aquatic Organisms*, *139*, 233–243.

Campbell, D. C., Johnson, P. D., Williams, J. D., Rindsberg, A. K., Serb, J. M., Small, K. K., & Lydeard, C. (2008). Identification of 'extinct'freshwater mussel species using DNA barcoding. *Molecular Ecology Resources*, *8*, 711–724.

Chen, J. S., Ma, E., Harrington, L. B., Da Costa, M., Tian, X., Palefsky, J. M., & Doudna, J. A. (2018). CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, *360*, 436–439.

Chetri, M., Odden, M., Sharma, K., Flagstad, Ø., & Wegge, P. (2019). Estimating snow leopard density using fecal DNA in a large landscape in north-central Nepal. *Global Ecology and Conservation*, *17*, e00548.

Clare, E. L., Economou, C. K., Faulkes, C. G., Gilbert, J. D., Bennett, F., Drinkwater, R., & Littlefair, J. E. (2021). eDNAir: Proof of concept that animal DNA can be collected from air sampling. *PeerJ*, *9*, e11030.

Darling, J. A., & Mahon, A. R. (2011). From molecules to management: Adopting DNA-based methods for monitoring biological invasions in aquatic environments. *Environmental Research*, *111*, 978–988.

Ding, X., Yin, K., Li, Z., Lalla, R. V., Ballesteros, E., Sfeir, M. M., & Liu, C. (2020). Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay. *Nature Communications*, *11*, 1–10.

Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. *Science*, *346*, 1258096.

Gootenberg, J. S., Abudayyeh, O. O., Lee, J. W., Essletzbichler, P., Dy, A. J., Joung, J., Verdine, V., Donghia, N., Daringer, N. M., Freije, C. A., Myhrvold, C., Bhattacharyya, R. P., Livny, J., Regev, A., Koonin, E. V., Hung, D. T., Sabeti, P. C., Collins, J. J., & Zhang, F. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science*, *356*, 438–442.

Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*, 313–321.

Hill-Cawthorne, G. A., Hudson, L. O., El Ghany, M. F. A., Piepenburg, O., Nair, M., Dodgson, A., Forrest, M. S., Clark, T. G., & Pain, A. (2014). Recombinations in staphylococcal cassette chromosome mec elements compromise the molecular detection of methicillin resistance in *Staphylococcus aureus*. *PLoS One*, *9*, e101419.

Hoenig, B. D., Zegar, J., Ohmer, M. E., Chess, M. M., Porter, B. A., Madril, M., & Richards-Zawacki, C. L. (2023). Data for "FINDeM: A CRISPR-based, molecular method for rapid, inexpensive, and field-deployable organism detection". Open Science Framework. https://doi.org/10.17605/OSF.IO/3MBDF

IGEM (2021). Chytritect. https://2021.igem.org/Team:NYU_Abu_Dhabi

Jerde, C. L., Chadderton, W. L., Mahon, A. R., Renshaw, M. A., Corush, J., Budny, M. L., Mysorekar, S., & Lodge, D. M. (2013). Detection of Asian carp DNA as part of a Great Lakes basin-wide surveillance program. *Canadian Journal of Fisheries and Aquatic Sciences*, *70*, 522–526.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science*, *337*, 816–821.

Kalsi, S., Valiadi, M., Tsaloglou, M.-N., Parry-Jones, L., Jacobs, A., Watson, R., Turner, C., Amos, R., Hadwen, B., Buse, J., Brown, C., Sutton, M., & Morgan, H. (2015). Rapid and sensitive detection of antibiotic resistance on a programmable digital microfluidic platform. *Lab on a Chip*, *15*, 3065–3075.

Kinney, V. C., Heemeyer, J. L., Pessier, A. P., & Lannoo, M. J. (2011). Seasonal pattern of Batrachochytrium dendrobatidis infection and mortality in *Lithobates areolatus*: Affirmation of Vredenburg's "10,000 zoospore rule". *PLoS One*, *6*, e16708.

Kirse, A., Bourlat, S. J., Langen, K., & Fonseca, V. G. (2021). Metabarcoding malaise traps and soil eDNA reveals seasonal and local arthropod diversity shifts. *Scientific Reports*, *11*, 1–12.

Liu, S., Zhuang, Q., Wang, S., Jiang, W., Jin, J., Peng, C., Hou, G., Li, J., Yu, J., Yu, X., Liu, H., Sun, S., Yuan, L., & Chen, J. (2020). Control of avian influenza in China: Strategies and lessons. *Transboundary and Emerging Diseases*, *67*, 1463–1471.

Mutschmann, F. (2015). Chytridiomycosis in amphibians. *Journal of Exotic Pet Medicine*, *24*, 276–282.

Parsley, M. B., & Goldberg, C. S. (2023). Environmental RNA can distinguish life stages in amphibian populations. *Molecular Ecology Resources*. https://doi.org/10.1111/1755-0998.13857

Piepenburg, O., Williams, C. H., Stemple, D. L., & Armes, N. A. (2006). DNA detection using recombination proteins. *PLoS Biology*, *4*, e204.

Ralph, C. J., Droege, S., & Sauer, J. R. (1995). Managing and monitoring birds using point counts: Standards and applications. In C. J. Ralph, J. R. Sauer, & S. Droege (Eds.), *Monitoring bird populations by point counts*. Gen. Tech. Rep. PSW-GTR-149 (pp. 161–168). US Department of Agriculture, Forest Service, Pacific Southwest Research Station, 149.

Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R., & Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA—A review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*, *51*, 1450–1459.

Scheele, B. C., Pasmans, F., Skerratt, L. F., Berger, L., Martel, A., Beukema, W., Acevedo, A. A., Burrowes, P. A., Carvalho, T., Catenazzi, A., de la Riva, I., Fisher, M. C., Flechas, S. V., Foster, C. N., Frías-Álvarez, P., Garner, T. W. J., Gratwicke, B., Guayasamin, J. M., Hirschfeld, M., … Canessa, S. (2019). Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. *Science*, *363*, 1459–1463.

Seutin, G. (1991). Morphometric identification of Traill's flycatchers: An assessment of Stein's formula (Identificación Morfométrica del Papamoscas de Traill, Una evaluación de la Ecuación de Stein). *Journal of Field Ornithology*, *62*, 308–313.

Sherry, T. W. (1984). Comparative dietary ecology of sympatric, insectivorous Neotropical flycatchers (Tyrannidae). *Ecological Monographs*, *54*, 313–338.

Shmakov, S., Smargon, A., Scott, D., Cox, D., Pyzocha, N., Yan, W., Abudayyeh, O. O., Gootenberg, J. S., Makarova, K. S., Wolf, Y. I., Severinov, K., Zhang, F., & Koonin, E. V. (2017). Diversity and evolution of class 2 CRISPR–Cas systems. *Nature Reviews Microbiology*, *15*, 169–182.

Skerratt, L. F., Berger, L., Speare, R., Cashins, S., McDonald, K. R., Phillott, A. D., Hines, H. B., & Kenyon, N. (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth*, *4*, 125–134.

Smith, K. G. (2007). Use of quantitative PCR assay for amphibian chytrid detection: Comment on Kriger et al. (2006a,b). *Diseases of Aquatic Organisms*, *73*, 253.

Sullivan, T. J., Dhar, A. K., Cruz-Flores, R., & Bodnar, A. G. (2019). Rapid, CRISPR-based, field-deployable detection of white spot syndrome virus in shrimp. *Scientific Reports*, *9*, 1–7.

Sun, Y., Yu, L., Liu, C., Ye, S., Chen, W., Li, D., & Huang, W. (2021). One-tube SARS-CoV-2 detection platform based on RT-RPA and CRISPR/Cas12a. *Journal of Translational Medicine*, *19*, 1–10.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, *21*, 2045–2050.

Teng, F., Guo, L., Cui, T., Wang, X.-G., Xu, K., Gao, Q., Zhou, Q., & Li, W. (2019). CDetection: CRISPR-Cas12b-based DNA detection with sub-attomolar sensitivity and single-base specificity. *Genome Biology*, *20*, 1–7.

Thomas, A. C., Tank, S., Nguyen, P. L., Ponce, J., Sinnesael, M., & Goldberg, C. S. (2020). A system for rapid eDNA detection of aquatic invasive species. *Environmental DNA*, *2*, 261–270.

Tóth, E., Varga, E., Kulcsár, P. I., Kocsis-Jutka, V., Krausz, S. L., Nyeste, A., Welker, Z., Huszár, K., Ligeti, Z., Tálas, A., & Welker, E. (2020). Improved LbCas12a variants with altered PAM specificities further broaden the genome targeting range of Cas12a nucleases. *Nucleic Acids Research*, *48*, 3722–3733.

Valentini, A., Pompanon, F., & Taberlet, P. (2009). DNA barcoding for ecologists. *Trends in Ecology & Evolution*, *24*, 110–117.

Wang, H., Qi, M., & Cutler, A. J. (1993). A simple method of preparing plant samples for PCR. *Nucleic Acids Research*, *21*, 4153.

Williams, M. A., de Eyto, E., Caestecker, S., Regan, F., & Parle-McDermott, A. (2023). Development and field validation of RPA-CRISPR-Cas environmental DNA assays for the detection of brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*). *Environmental DNA*, *5*, 240–250.

Williams, M. A., O'Grady, J., Ball, B., Carlsson, J., de Eyto, E., McGinnity, P., Jennings, E., Regan, F., & Parle-McDermott, A. (2019). The application of CRISPR-Cas for single species identification from environmental DNA. *Molecular Ecology Resources*, *19*, 1106–1114.

Xie, S., Tao, D., Fu, Y., Xu, B., Tang, Y., Steinaa, L., Hemmink, J. D., Pan, W., Huang, X., Nie, X., Zhao, C., Ruan, J., Zhang, Y., Han, J., Fu, L., Ma, Y., Li, X., Liu, X., & Zhao, S. (2021). Rapid visual CRISPR assay: A naked-eye colorimetric detection method for nucleic acids based on CRISPR/Cas12a and a convolutional neural network. *ACS Synthetic Biology*, *11*, 383–396.

Zetsche, B., Gootenberg, J. S., Abudayyeh, O. O., Slaymaker, I. M., Makarova, K. S., Essletzbichler, P., Volz, S. E., Joung, J., van der Oost, J., Regev, A., Koonin, E. V., & Zhang, F. (2015). Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell*, *163*, 759–771.

Zhang, Y.-M., Zhang, Y., & Xie, K. (2020). Evaluation of CRISPR/Cas12a-based DNA detection for fast pathogen diagnosis and GMO test in rice. *Molecular Breeding*, *40*, 1–12.

Zhou, S., Dong, J., Deng, L., Wang, G., Yang, M., Wang, Y., Huo, D., & Hou, C. (2022). Endonuclease-assisted PAM-free recombinase polymerase amplification coupling with CRISPR/Cas12a (E-PfRPA/Cas) for sensitive detection of DNA methylation. *ACS Sensors*, *7*, 3032–3040.

Zou, Y., Mason, M. G., Wang, Y., Wee, E., Turni, C., Blackall, P. J., Trau, M., & Botella, J. R. (2017). Nucleic acid purification from plants, animals and microbes in under 30 seconds. *PLoS Biology*, *15*, e2003916.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Supporting Information S1. Example protocol for the field-based detection and quantification of *Batrachochytrium dendrobatidis* DNA from swab samples.

How to cite this article: Hoenig, B. D., Zegar, J., Ohmer, M. E. B., Chess, M. M., Porter, B. A., Madril, M., & Richards-Zawacki, C. L. (2023). FINDeM: A CRISPR-based, molecular method for rapid, inexpensive and field-deployable organism detection. *Methods in Ecology and Evolution*, *14*, 3055–3067. https://doi.org/10.1111/2041-210X.14236