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TRPtracker: a community database for monitoring praziquantel sensitivity at $TRPM_{PZQ}$ variants

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ABSTRACT

The anthelmintic praziquantel (PZQ) has been used for decades as the clinical therapy for schistosomiasis, and remains the only available drug. As a cheap and effective drug therapy for all human disease-causing *Schistosoma* species, usage of PZQ underpins mass drug administration strategies aimed at eliminating schistosomiasis as a public health problem by 2030. Concern over the potential emergence of resistance to PZQ is therefore warranted, as it would constitute a major threat to this approach. In terms of molecular adaptations conferring PZQ resistance, variation in the sequence and/or expression of the drug target is an obvious mechanism and should be a priority for surveillance efforts. The target of PZQ is a transient receptor potential ion channel, $TRPM_{PZQ}$, which is established as a locus that regulates schistosome sensitivity to PZQ. Here, we describe the establishment of a community resource, 'TRPtracker', which coalesces data on $TRPM_{PZQ}$ natural variants together with measurements of individual $TRPM_{PZQ}$ variant sensitivity to PZQ assessed by profiling $TRPM_{PZQ}$ in a heterologous expression system. A compendium of laboratory-generated mutants in $TRPM_{PZQ}$ is also compiled in the TRPtracker database to delimit regions within $TRPM_{PZQ}$ that are critical for PZQ sensitivity. Aggregation of data from multiple research groups into TRPtracker catalogues which $TRPM_{PZQ}$ variants have been functionally profiled, where geographically these variants have been found, their frequency within populations, and their potential impact on PZQ sensitivity. The overall goal is to facilitate rapid community-wide exchange of data to monitor predicted variants of concern that are likely to be associated with decreased PZQ efficacy.

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1. Introduction

Schistosomiasis is a disease caused by infection with parasitic blood flukes of the genus *Schistosoma* (McManus et al., 2018). Over 240 million people are estimated to be infected worldwide, and the current World Health Organization roadmap advances the goal of elimination of schistosomiasis as a public health problem, with interruption of transmission in selected regions, by 2030 (Diaz et al., 2023; Buonfrate et al., 2025).

A key part of this strategy centers on mass drug administration (MDA) campaigns utilizing praziquantel (PZQ), an anti-schistosomal drug originally discovered in the 1970s. While an 'old' drug, PZQ has proven an effective therapy, both in terms of treatment costs and outcomes (Fukushige et al., 2021; Aboagye and Addison, 2023). However clinical dependence on a single therapy has nurtured lingering concern over the emergence of drug resistance. This concern has heightened following increased rollout of MDA centered on PZQ, with reduced drug efficacies already reported within human populations under the longest and strongest PZQ pressures (Crellen et al., 2016). This is further exacerbated through scenarios of poor compliance with dosing and/or inappropriate dosing (Bustinduy et al., 2025), as well as non-ideal PZQ usage (for example treatment of livestock (Diaz et al., 2023)). Given that decreased sensitivity to PZQ is a selectable trait in laboratory schistosome populations and schistosomiasis treatment failures have been reported in the literature, caution over whether reliance on PZQ will be sufficient to reach elimination goals appears prudent (Fallon, 1998; Wang et al., 2012). Recent reports of PZQ resistance in cestodes from studies in companion animals and horses heightens concern over the potential for emergence of resistance (Jesudoss Chelladurai et al., 2018; Loftus et al., 2022; Nielsen, 2023; Oehm et al., 2024).

One challenge in assessing and monitoring PZQ efficacy, has been a paucity of genetic markers associated with decreased PZQ sensitivity (Summers et al., 2022). One reason for this is that the basis of PZQ action has long remained mysterious as no parasite target for PZQ was known. This picture has thankfully changed, following the recent discovery of an ion channel from the transient receptor potential melastatin subfamily ($TRPM_{PZQ}$) that is activated by PZQ (Park et al., 2019). Various lines of evidence support $TRPM_{PZQ}$ as the clinically relevant target of PZQ in schistosomes and other parasitic flatworms sensitive to PZQ (Marchant, 2024). Identification of $TRPM_{PZQ}$ as the parasite PZQ binding target defines an obvious locus for profiling PZQ sensitivity of natural $TRPM_{PZQ}$ variants, consistent with a plethora of examples of 'on-target' mutations that confer decreased sensitivity to a variety of drugs (Freedy and Liau, 2021; Liu et al., 2024). While the PZQ binding pocket in $TRPM_{PZQ}$ is highly conserved across parasitic flatworms (Rohr et al., 2023), it has been demonstrated that the identity of a single amino acid within the PZQ binding pocket of *Fasciola spp*. $TRPM_{PZQ}$ renders *Fasciola spp*. $TRPM_{PZQ}$ refractory to PZQ. This is consistent with the insensitivity of these liver fluke to PZQ and the inability to cure fascioliasis with PZQ. Similarly, another instance of sequence variation within the $TRPM_{PZQ}$ binding pocket of pseudophyllidean cestodes decreases sensitivity to PZQ into the micromolar range (Sprague et al., 2025), an observation consistent with the poor resolution of clinical infections caused by these tapeworms with standard PZQ dosing. Therefore, sequence variation within $TRPM_{PZQ}$ dictates clinical treatment strategies for different infections.

These examples underscore the importance of sampling variation at the $TRPM_{PZQ}$ locus within natural schistosome populations, as variation could impact the efficacy of PZQ in treating schistosomiasis. Indeed, *Smp_246790*, the *Schistosoma mansoni* gene encoding $TRPM_{PZQ}$ has been linked to decreased PZQ sensitivity in a laboratory population of *S. mansoni* selected for PZQ resistance (Le Clec'h et al., 2021; Chevalier et al., 2024). Clinically, we need to know whether poor schistosomiasis cure rates after multiple rounds of PZQ chemotherapy, often referred to as localized 'hot spots' of disease persistence, relate to the presence of specific $TRPM_{PZQ}$ variants, and/or lowered expression of $TRPM_{PZQ}$ within different schistosome populations. How extensive is standing genetic variation in $TRPM_{PZQ}$? Do specific single nucleotide polymorphisms (SNPs), indels, copy number variants or more complex adaptations contribute to lower $TRPM_{PZQ}$ responsivity to PZQ? Do comparable resistant mechanisms exist more broadly across the *Schistosoma* genus, encompassing other major disease-causing species such as *S. japonicum* and *S. haematobium*? Alternatively, is natural variation in $TRPM_{PZQ}$ completely benign without any impact on PZQ efficacy? The identification of $TRPM_{PZQ}$ now facilitates such analyses. This knowledge is critically important for design of effective schistosomiasis control and elimination strategies, enabling surveillance of the geographic distribution of any $TRPM_{PZQ}$ variants of concern and informing PZQ treatment approaches in areas where such low sensitivity variants persist. Given the availability, low cost, and general effectiveness of PZQ as the sole treatment for schistosomiasis, any decline in the clinical effectiveness of this drug would prove a serious global health challenge.

Following the identification of $TRPM_{PZQ}$, it is now timely to profile the PZQ sensitivity of natural $TRPM_{PZQ}$ genetic variants and ensure these data are broadly available to help surveillance efforts of PZQ effectiveness worldwide. In this manuscript, we report the establishment of a community resource (www.TRPtracker.live) which compiles $TRPM_{PZQ}$ variant data contributed from multiple research groups in parallel with measurements of PZQ sensitivity and $TRPM_{PZQ}$ expression from a cell-based Ca^{2+} reporter assay. Combination of these data into a single resource permits easy reference of which $TRPM_{PZQ}$ variants have been functionally profiled to date, where these variants have been found geographically, and whether any specific variants are associated with decreases in PZQ sensitivity.

2. Methods

Materials & Reagents. (±)-PZQ was purchased from Sigma (St. Louis, MO). All cell culture reagents were from Invitrogen (Waltham, MA). HEK293 cells (CRL-1573) were sourced from ATCC (Manassas, VA) and were tested monthly for mycoplasma contamination using the LookOut® Mycoplasma PCR Detection Kit (Sigma).

Functional assays of $TRPM_{PZQ}$ variants. For profiling PZQ sensitivity of different $TRPM_{PZQ}$ variants, specific mutants were made within the canonical reference $TRPM_{PZQ}$ sequence (*Smp_246790.1*, WormBase Parasite, SM_V10 (Howe et al., 2017),) by Genscript (Piscataway, NJ) with nucleotide changes confirmed by sequencing. $TRPM_{PZQ}$ variant sensitivity to racemic PZQ ((±)-PZQ) was assessed using a cytoplasmic Ca^{2+} reporter assay using a high affinity Ca^{2+} dye (Fluo-4 NW, Thermo Fisher Scientific). This Ca^{2+} reporter assay was performed in black-walled, clear-bottomed 384-well plates previously coated with poly-L-lysine (Greiner Bio-One, Germany). Non-transfected, or single $TRPM_{PZQ}$ variant transfected, cells were seeded (20,000 cells/well) in individual wells and incubated in DMEM growth media containing 10% FBS. After 24 h, this medium was replaced with 20 μl of Fluo-4 NW dye loading solution (Molecular Devices) previously reconstituted in assay buffer (Hanks' balanced salt solution containing 0.126 mM Ca^{2+}, 0.49 mM Mg^{2+}, 20 mM HEPES and 2.5 mM probenecid). Cells were incubated for 30 min at 37 °C (5% CO_2) followed by an additional 30 min incubation at room temperature. Changes in fluorescence were then resolved in real-time using a Fluorescence Imaging Plate Reader ($FLIPR^{TETRA}$, Molecular Devices). Basal fluorescence (filter settings λ_{ex} = 470-495 nm, λ_{em} = 515-575 nm) from each well was captured for 20s, then (±)-PZQ (5 μl) or vehicle solution (5 μl), was added (25 μl total volume) and the signal recorded over a subsequent 250s. Changes in fluorescence were represented as relative fluorescence units after subtracting the average basal fluorescence (averaged over 20s) from the recorded values. Concentration-response analysis was performed using four parameter sigmoidal curve fitting functions in Prism (v10.4.2, GraphPad Software, Boston, Massachusetts USA, www.graphpad.com)) from n ≥ 3 technical replicates per mutant/variant. For constructs where data diverged from wild type *Sm*.$TRPM_{PZQ}$ sensitivity, data collection was repeated from n

≥ 3 independent transfections. Sensitivity of *Sm.*TRPM$_{PZQ}$ to PZQ was profiled in response to a PZQ concentration range spanning 0.1 nM to 100 μM. The relative activity (RA) value describing PZQ sensitivity at individual TRPM$_{PZQ}$ variants was calculated from the reference (wild type) vs variant half maximal effective concentration (EC_{50}) and peak response (B_{max}) using the following equation: RA = [$EC_{50,wild\ type}$ x $B_{max,\ variant}$]/[$EC_{50,variant}$ x $B_{max,\ wild\ type}$]. All functional assays were performed in the Marchant laboratory, under standardized conditions with data expressed relative to PZQ which was included as a positive control on every plate. We note that this functional profiling methodology is also accessible for testing combinations of variants, either by co-transfection or through use of a bicistronic expression vector. Such co-expression analyses would be important to profile the impact of heteromerization of 'wild type' and 'resistant' TRPM$_{PZQ}$ subunits to approximate the natural diploid background.

Western Blotting. Cell lysates for Western blotting were prepared from HEK293 cells transfected with different TRPM$_{PZQ}$ constructs. In brief, confluent cells were washed twice with ice-cold PBS, harvested into 1 ml of PBS, and pelleted at 1000×*g* for 5 min. The cell pellet was then resuspended in 300-500 μl of lysis buffer (1x PBS, 1% Triton) containing a complete protease inhibitor mixture (Roche Applied Science). Samples were then incubated for 30 min at 4 °C on a rotator. The sample was then centrifuged at 10,000×*g* for 10 min, and the supernatant used for Western blot analysis. Protein concentrations were determined using a BCA assay (Bio-Rad) and equal amounts of protein (20–30 μg) were loaded into individual lanes of a 4-15% Tris-glycine gel (Bio-Rad). After electrophoresis, samples were transferred to a PVDF membrane, blocked with blocking buffer in (5% BSA, 0.1% Tween/Tris buffered saline (TBST)) for 1 h at room temperature. The membrane was incubated overnight at 4 °C with either a custom TRPM$_{PZQ}$ antibody (1:1000)

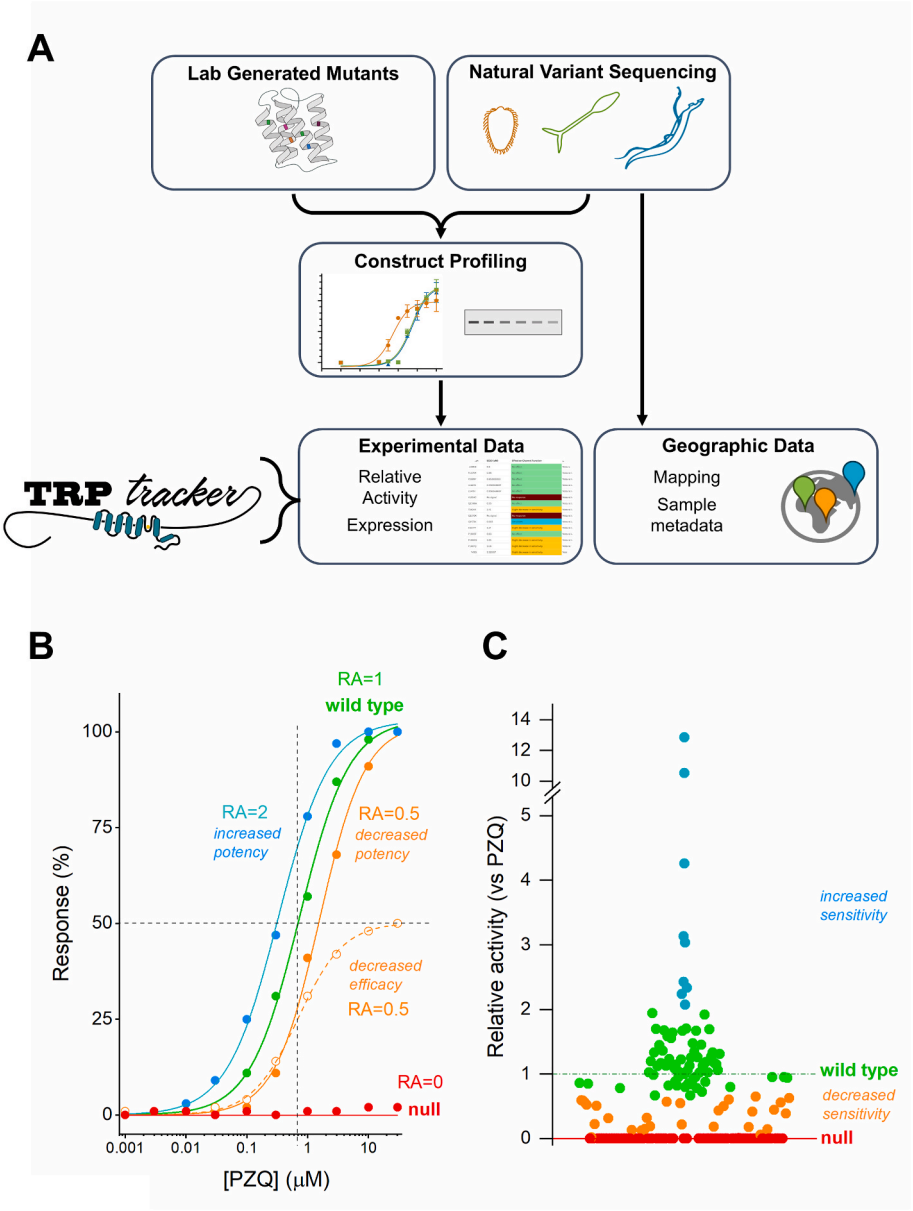

Fig. 1. Schematic overview of TRPtracker and functional profiling of TRPM$_{PZQ}$. (**A**) Schematic overview of profiling workflow for lab-generated mutants and natural variants, which can be profiled functionally and in terms of expression levels. These data and sample metadata are compiled in the TRPtracker resource. (**B**) Example concentration response curves to illustrate impact of different TRPM$_{PZQ}$ variants on the relative activity (RA) of PZQ. Increases in potency yield higher RA values (blue), decreases in potency or decreases in efficacy yield lower RA values (orange) relative to the wild type TRPM$_{PZQ}$ concentration response curve (green). (**C**) Relative activity plot of *Sm.*TRPM$_{PZQ}$ variants from the TRPtracker dataset, stratified into different function categories: null (RA = 0, red), decreased sensitivity (RA = 0 - 0.6, orange), wild type (RA = 0.6 - 2, green) and increased sensitivity (RA> 2 blue).

raised against the COOH-terminal region of $TRPM_{PZQ}$ (1709-1997 amino acids), or a β-actin antibody (1:2000). After washing with TBST, the membrane was incubated (1 h, room temperature) with a horseradish peroxidase-conjugated secondary antibody (Invitrogen). Following additional washes, protein bands were visualized using a chemiluminescent substrate (ECL, Invitrogen) and quantified using the Chem-iDoc™ MP Imaging System (BioRad).

3. Results

The TRPtracker database collates data reporting PZQ sensitivity of individual $TRPM_{PZQ}$ variants determined in a Ca^{2+} reporter assay after heterologous expression of individual constructs in a human cell line. These data were compiled from two sources (Fig. 1A). The first dataset represents a collection of 'laboratory' $TRPM_{PZQ}$ mutants made to probe the structure-function relationship of $TRPM_{PZQ}$ (Park et al., 2019, 2021). The second dataset represents natural $TRPM_{PZQ}$ variants, informed by sequencing data from natural schistosome populations that have been prioritized for functional evaluation by various research groups. Both datasets provide utility to this resource. While lab generated mutants impart no clinical relevance, they do inform about regions of $TRPM_{PZQ}$ relevant for function which may in turn guide selection of natural variants for functional profiling from large sequencing datasets. At the time of writing, both datasets establish a 'library' of ~170 TRPtracker database entries. Each entry is clearly labelled as to their derivation (i.e. lab mutant ('lab') or natural variant ('field')). Database entries are limited to those $TRPM_{PZQ}$ variants that have been subjected to functional profiling.

Functional profiling was performed using an *in vitro* Ca^{2+} reporter assay based upon heterologous expression of individual $TRPM_{PZQ}$ variants in HEK293 cells. $TRPM_{PZQ}$ is a non-selective cation channel, and changes in Ca^{2+} concentration resulting from $TRPM_{PZQ}$ activity can be resolved using a fluorescent Ca^{2+} indicator dye loaded into cells. $TRPM_{PZQ}$ activation by PZQ displays an EC_{50} in the sub-micromolar range for the reference *Schistosoma mansoni* $TRPM_{PZQ}$ sequence (EC_{50} of 739 ± 171 nM for (±)-PZQ at *Sm*.$TRPM_{PZQ}$). This approach provides insight into $TRPM_{PZQ}$ responsiveness, albeit in the background of the human HEK293 cell line used for construct expression. There is no available parasitic flatworm cell line, or currently accessible transgenic approach to evaluate these variants in their natural context. A Western blotting assay to resolve $TRPM_{PZQ}$ protein expression was also used for a subset of variants to probe the basis of changed PZQ sensitivity. These experimental data have been compiled together with natural variant sample metadata, which typically includes information about sample collection, including geographical sites and any recent PZQ treatment history. The complete profiling workflow is summarized schematically in Fig. 1A.

The functional impact of individual mutants is quantified as a 'relative activity' measure (RA, (Ehlert et al., 1999)) relative to PZQ action at the *Sm*.$TRPM_{PZQ}$ reference sequence. The RA parameter was chosen as it incorporates how each mutant impacts PZQ potency (left/right shift in curve EC_{50}) as well as PZQ efficacy (up/down shift in curve maximum, B_{max}) relative to values measured at the reference 'wild type' $TRPM_{PZQ}$. This is important as changes in either parameter – ligand potency or efficacy – will impact overall worm 'sensitivity' toward PZQ. Mutants that worsen potency or efficacy result in RA values < 1, whereas mutants that increase PZQ potency at $TRPM_{PZQ}$ result in RA values > 1. For example, a mutant that increases PZQ potency by 2-fold equates to a RA value of 2. In contrast, a mutant that decreases the measured EC_{50} of PZQ at $TRPM_{PZQ}$ by 2-fold or a mutant that decreases the efficacy of PZQ by 50% equate to a RA value of 0.5 (Fig. 1B). The distribution of RA values for the current compendium of mutants is shown in Fig. 1C. The measured RA values span a broad range, with a mean of ~1. Data were stratified into functional categories with the distinction between 'wild type' and 'decreased PZQ sensitivity' groups based on the standard deviation for the population of natural variants. These categories comprised: 'null' (RA = 0, red), 'decreased sensitivity' to PZQ (RA >0 but <0.6, orange), 'wild type' (RA, 0.6 – 2, green) and 'increased sensitivity' to PZQ (RA, >2, blue). All data, including sample metadata, are available for reference at the portal (www.TRPtracker.live). Functional profiling data are summarized in Supplementary Table 1 and can also be downloaded at the TRPtracker website. Insight from the 'lab' mutants and 'field' variants that were functionally profiled are discussed in the following two sections.

$TRPM_{PZQ}$ laboratory mutants. Ongoing functional analyses of $TRPM_{PZQ}$ has generated a collection of ~140 point mutants and truncations within the reference sequence of *Sm*.$TRPM_{PZQ}$. The distribution of these point mutants is currently biased to the transmembrane spanning regions of $TPRM_{PZQ}$ (~60% of mutants in the voltage sensor-like domains (VSLD), pore and TRP domains) because of prior work to define the binding sites of the $TRPM_{PZQ}$ ligands PZQ and BZQ (Park et al., 2021; Sprague et al., 2023). Multiple 'null' mutants that completely ablated PZQ sensitivity were identified (Fig. 2A), many of which were localized within the transmembrane spanning domains of $TRPM_{PZQ}$. While this weighting reflects the aforementioned bias in studying this region of $TRPM_{PZQ}$, the data do underscore the criticality of the VSLD architecture (S1-S4, containing the PZQ binding pocket), the pore domain (S5-S6), TRP helix and their intervening connections for normal channel function.

However, it is important to recognize that mutations in other regions of $TRPM_{PZQ}$ may also compromise PZQ action. Considering examples of human TRPM channels, the distribution of *in silico* predicted deleterious mutants spans multiple protein domains throughout the entire coding sequence. Predictions using the 'pathogenicity' function of AlphaMissense (Cheng et al., 2023) are shown in Fig. 2B for human TRPM2 (*Hs*.TRPM2) and human TRPM8 (*Hs*.TRPM8), both of which display structural conservation with $TRPM_{PZQ}$ (Park and Marchant, 2020; Park et al., 2021). In the human TRPM models, benign variation is colored blue and deleterious mutants are scored with increasing warm coloration. As evident through this structural comparison, predicted deleterious mutants span multiple domains within the human TRPM channels beyond the transmembrane spanning region, throughout domains that show conservation with $TRPM_{PZQ}$ (Fig. 2B).

Consistent with this prediction, a cluster of functionally 'null' mutants was evident at the NH_2-terminus of *Sm*.$TRPM_{PZQ}$ within the experimental dataset. For example, the dual mutants *Sm*.$TRPM_{PZQ}$[F107A] and *Sm*.$TRPM_{PZQ}$[G108A] exhibited no responsiveness to PZQ (Fig. 2C). These residues are found in a region of the MHR1/2 domain that is well conserved between $TRPM_{PZQ}$ orthologs (Supplementary Fig. 1). Structural comparison of this region in *Sm*.$TRPM_{PZQ}$ compared with *Hs*.TRPM2 shows these dual residues are located at the end of a β-strand (β5 in the *Hs*.TRPM2 MHR1/2 domain) which displays a similar predicted folding structure to the similar region of *Sm*.$TRPM_{PZQ}$ (Fig. 2D). Interestingly, the β5 strand in *Hs*.TRPM2 is known to form important interactions with an endogenous ligand, ADP ribose, which serves as an obligatory *Hs*.TRPM2 co-agonist while also conferring redox sensitivity to the ion channel (Huang et al., 2019; Szollosi, 2021). These data support further investigation of the role of endogenous nucleotides in regulating $TRPM_{PZQ}$ expression and/or function (Chulkov et al., 2023b). This could be of interest in light of the customized nucleotide metabolic pathways present in schistosomes (Senft et al., 1972; Skelly et al., 2022).

The highest sensitizing mutants to PZQ in *Sm*.$TRPM_{PZQ}$ (RA values of >10) Fig. 1C) merit comment. These two mutants were *Sm*.$TRPM_{PZQ}$[F1521A] (RA ~10.5) and *Sm*.$TRPM_{PZQ}$[W1667A] (RA ~13, Fig. 1C). Both mutants increased PZQ potency into the <100 nM potency range. While each residue occurs in a different region of *Sm*.$TRPM_{PZQ}$ (F1521 lies within the S4-S5 linker, W1667 is located in the TRP helix), they appear as potentially interacting residues (Fig. 2E). This interaction likely serves as a 'brake' on ligand-evoked conformational change between the VSLD and pore domain necessary for channel opening. Consequently, ablation of either residue by alanine mutation serves to

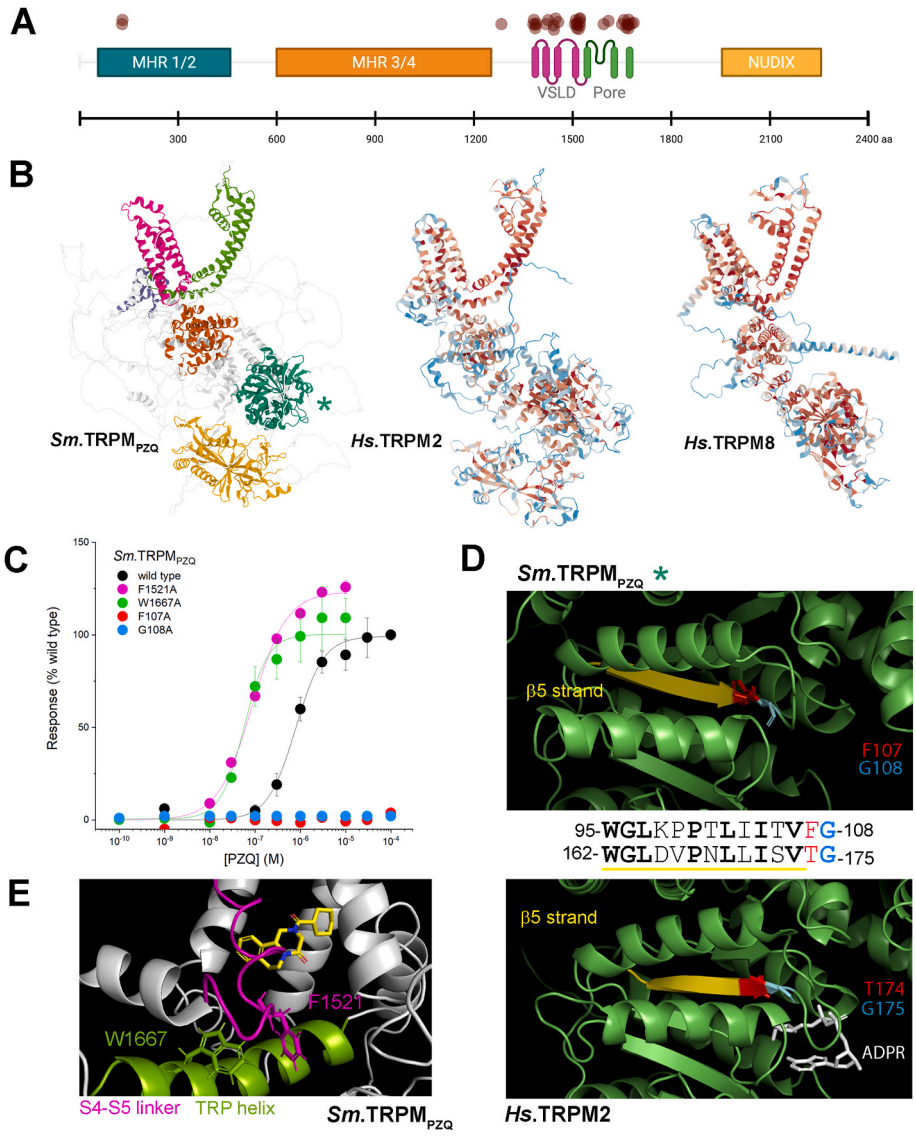

Fig. 2. Functional effects of various TRPM$_{PZQ}$ mutants. (**A**) Distribution of functionally 'null' laboratory mutants (red circles) along the length of the *Sm*.TRPM$_{PZQ}$ coding sequence. (**B**) Representations from the AlphaFold protein structure database (Jumper et al., 2021; Varadi et al., 2024) for a monomer of *Sm*.TRPM$_{PZQ}$ (left), *Hs*.TRPM2 (O94759, middle) and *Hs*.TRPM8 (Q7Z2W7,right). The *Sm*.TRPM$_{PZQ}$ prediction shows several predicted structural domains (Lau et al., 2024) corresponding to the voltage-sensing like domain (VSLD, S1-S4, magenta), pore domains (S5-S6, green), MHR3/4 (orange), MHR1/2 (turquoise), nudix hydrolase domain (yellow). Overall, *Sm*.TRPM$_{PZQ}$ presents with homology to the vertebrate TRPM8-like menthol binding pocket found in the VSLD (S1-S4) within a broader protein structure with cytoplasmic domain homology to *Hs*.TRPM2 (Park and Marchant, 2020). The monomers of *Hs*.TRPM2 and *Hs*.TRPM8 are colored in terms of predicted effect on protein 'pathogenicity' function using AlphaMissense (Cheng et al., 2023), with benign variation colored blue and deleterious mutants scored with increasing warm coloration toward red. (**C**) Functional profiling of *Sm*.TRPM$_{PZQ}$ mutants in response to PZQ. These include null mutants (*Sm*.TRPM$_{PZQ}$ [F107A] and *Sm*.TRPM$_{PZQ}$[G108A]) as well sensitizing mutants (*Sm*.TRPM$_{PZQ}$ [F1521A] and *Sm*.TRPM$_{PZQ}$[W1667A]). (**D**) Interrogation of a null mutant 'hot-spot' in the NH_2-terminal MHR1/2 domain of TRPM$_{PZQ}$. The null mutants found in *Sm*.TRPM$_{PZQ}$ profiled in (C) localize to the end of a β-strand (F107 in red, G108 in cyan, top) which shows structural and sequence conservation with the β5-strand in *Hs*.TRPM2 (T174 in red, G175 in cyan; bottom (Huang et al., 2018),). This region of TRPM2 is implicated in the binding of ADP-ribose (Huang et al., 2018), shown in white (PDB, 8E6V). (**E**) Projection of location of the sensitizing mutants *Sm*.TRPM$_{PZQ}$ [F1521A] (magenta, S4-S5 linker) and *Sm*.TRPM$_{PZQ}$[W1667A] (green, TRP domain) relative to the PZQ (yellow) in the VSLD binding pocket.

release this brake, more efficiently transducing ligand binding energy into the open state transition. The double mutant *Sm*.TRPM$_{PZQ}$ [F1521A][W1667A] did not show enhanced potency to PZQ (EC_{50} of 68 ± 11 nM) consistent with the idea that these residues interact with each other. Both these residues are well conserved in TRPM$_{PZQ}$ orthologs (Supplementary Fig. 2), suggesting this interplay may be a generalized feature of TRPM$_{PZQ}$ activation.

In summary, these examples of 'null' and 'sensitizing' TRPM$_{PZQ}$ mutants, highlight the principle that single residue variation found across the entire TRPM$_{PZQ}$ coding sequence impacts TRPM$_{PZQ}$ sensitivity to PZQ.

TRPM$_{PZQ}$ field variants. Studies of laboratory mutants, while informative for understanding how *Sm*.TRPM$_{PZQ}$ works, have no relevance to clinical infections unless similar variation is present and prevalent in the field. Profiling PZQ sensitivity of worms associated with specific clinical infections is challenging, requiring collection of viable egg samples, with hatched miracidia needing to pass through snail and vertebrate experimental host stages, before any adult worms could be obtained for phenotypic profiling and parallel genotyping. Profiling TRPM$_{PZQ}$ variation within natural schistosome populations by sequencing eggs/miracidia and then heterologously expressing these variants in a mammalian system is therefore a logical and accessible first step to

assess the scope of variation in this drug target that can help inform about the risks of decreased PZQ efficacy at this target. Readers are referred to ongoing studies for information regarding samples collected and specific variants prioritized for functional profiling from sequencing analyses (Berger et al., 2024; Summers, 2025). Most data derive from *S. mansoni* miracidia, preserved after hatching from eggs isolated from fecal samples.

From all of this ongoing work, ~30 natural coding variants have now been profiled and entered into the TRPtracker database. Many other variants have been reported but have not yet been functionally profiled. Data from the profiled variants, color coded in terms of sensitivity to PZQ is shown in Fig. 3A, together with their geographical distribution in Fig. 3B. Several of these variants have been found in samples collected and/or analysed by different research groups. The vast majority of these variants (~75%) exhibit, by our stratification of functional classes, no change in sensitivity toward PZQ (Fig. 3A), with the average RA of the entire natural variation collection being 1.2 ± 0.55 (mean ± sd). These are reassuring observations in the context of surveillance for 'resistant' variants within the natural *S. mansoni* populations that have been sampled to date. It is also worth noting that the functionality of these variants have been assessed as a homomeric $TRPM_{PZQ}$ channel, reflecting a 'homozygous' state. Rare variants will present in a heterozygous state, associated with a mixed $TRPM_{PZQ}$ heterotetramer, which may have less of an impact on overall PZQ sensitivity.

However, the dataset shown in Fig. 3A does provide examples of natural variants which are functionally 'null' (Y1554C, Q1670K) or exhibit low RA values (<0.6, outside the standard deviation of the entire group, R1375H, T1624K, R1843Q). These lower sensitivity variants are mostly proximal (e.g. the pre-S1 helix) or within the transmembrane spanning region of $TRPM_{PZQ}$ (Fig. 3C). It is important to emphasize that many of these variants stratified into the 'null' or 'impaired function' categories are found at very low frequency in miracidial samples

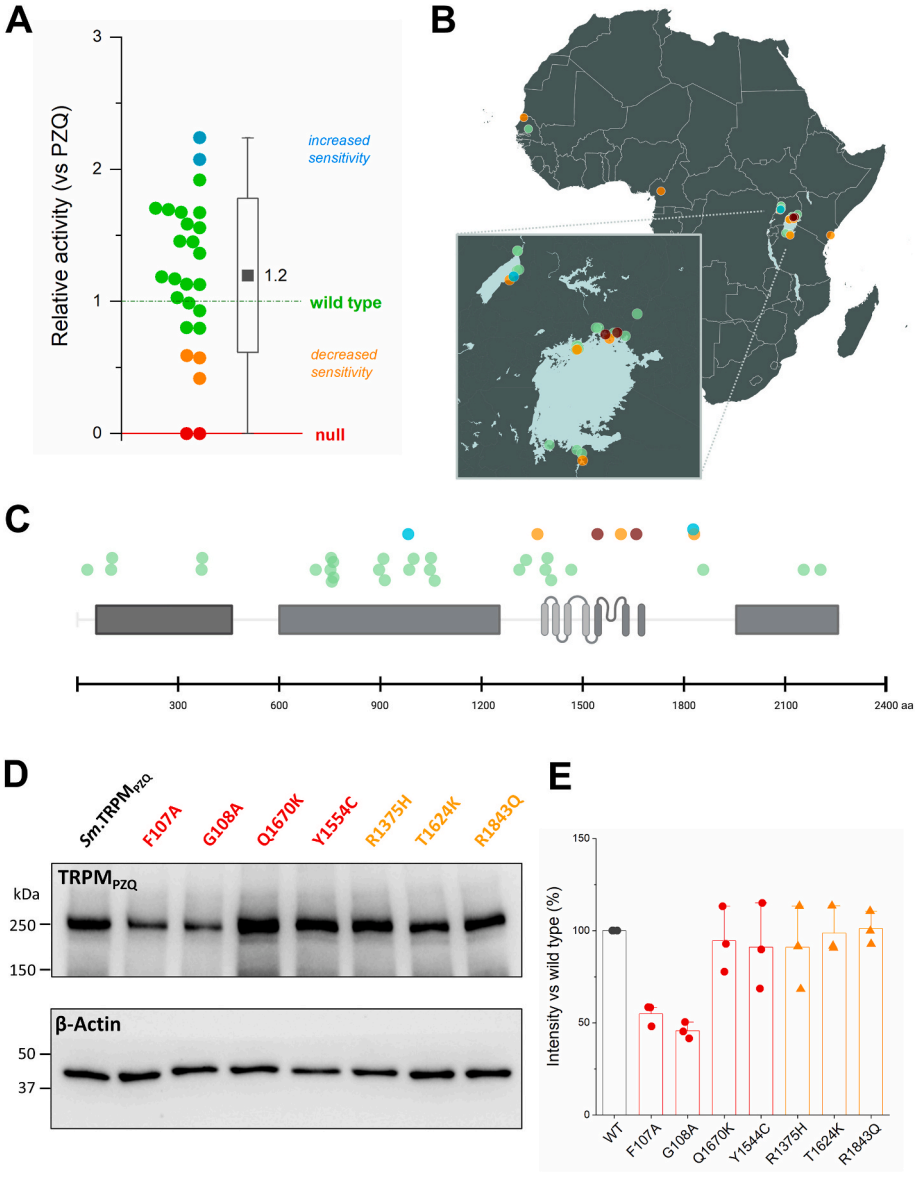

Fig. 3. Profiling function and expression of various natural $TRPM_{PZQ}$ variants. (**A**) Distribution of RA values for natural $TRPM_{PZQ}$ variants that have been functionally profiled in in the database. Data is presented as a half-box plot, with data on the left and the standard deviation (box), range (vertical line) and mean (black square) of this population shown on the right. (**B**) Collection sites where the natural $TRPM_{PZQ}$ variants that have been functionally profiled in this study were sampled. (**C**) Distribution of normal sensitivity (green), decreased sensitivity (orange) and functionally 'null' natural variants of *Sm*.$TRPM_{PZQ}$ (red circles) identified by various groups along the length of the *Sm*.$TRPM_{PZQ}$. (**D**) representative Western blot for expression of indicated $TRPM_{PZQ}$ variants. (**E**) densitometric measurements from independent blots (n = 3) quantified for these same $TRPM_{PZQ}$ variants.

collected to date (Berger et al., 2024; Summers, 2025). Where data is available in the original reports, information about variant frequency is being incorporated into the TRPtracker resource. Unless a variant is identified in several samples at a specific site, or found by different groups across multiple sampling locales, caution is warranted as to whether these are *bona fide* natural variants or simply false calls from sequencing errors or amplification biases. Further sequencing and surveillance efforts will be of paramount importance to verify the occurrence and frequency of any predicted 'variants of concern' within natural worm populations.

Finally, another route toward manifestation of a lower worm sensitivity to PZQ is decreased expression of $TRPM_{PZQ}$. Expression of several natural $TRPM_{PZQ}$ variants, as well as lab mutants *Sm*.$TRPM_{PZQ}$[F107A] and *Sm*.$TRPM_{PZQ}$[G108A], was therefore examined by Western blotting following transient transfection in HEK293 cells (Fig. 3D). Expression levels were assessed by densitometry relative to the intensity of the ~250 kDa band in the *Sm*.$TRPM_{PZQ}$ reference sequence (Fig. 3E). Each natural variant that exhibited reduced PZQ sensitivity (R1375H, T1624K, R1843Q) or no PZQ sensitivity (Y1554C, Q1670K) displayed no difference in total $TRPM_{PZQ}$ expression. These data suggest these variants impair *Sm*.$TRPM_{PZQ}$ sensitivity to PZQ rather than *Sm*.$TRPM_{PZQ}$ expression. This contrasts with the lack of function of the 'null' NH_2-terminal lab mutants (*Sm*.$TRPM_{PZQ}$[F107A] and *Sm*.$TRPM_{PZQ}$[G108A], Fig. 2C) where decreased *Sm*.$TRPM_{PZQ}$ expression was evident by Western blotting (Fig. 3D and E). This suggests a role for these NH_2-terminal residues in regulating folding, stability or multimerization of $TRPM_{PZQ}$, at least within the mammalian expression background. These *in vitro* data suggest that decreases in both $TRPM_{PZQ}$ expression and/or 'on target' PZQ potency underpin the lack of PZQ activity at the 'null function' $TRPM_{PZQ}$ variants.

4. Discussion

As a consortium of schistosomiasis researchers, we advance TRPtracker as an online resource for collating data on the distribution and PZQ sensitivity of natural $TRPM_{PZQ}$ sequence variants. This is timely as sequencing efforts to catalogue $TRPM_{PZQ}$ variants are currently being performed by several groups (for example (Berger et al., 2024; Summers, 2025)). Our vision is that this will be a living database regularly updated with data from ongoing collection and sequencing efforts, such that data can be easily shared and accessed by the community. Data entries will include pertinent metadata, for example variant frequency at collection sites, sample descriptors, collection dates and PZQ treatment histories. Contributors are encouraged to submit such details through the website portal. Community input for additional suggestions to integrate into this resource are also welcome. The intent is that aggregating this information on a single platform should minimize redundancy by not reprofiling variants already characterized, while maximizing comparability across datasets by using standardized assays performed under identical conditions for all variants to allow side-by-side comparison of data. Currently, this database contains data for the PZQ sensitivity of ~170 different *Schistosoma mansoni* $TRPM_{PZQ}$ variants. However, it is envisaged that data entries will in time expand to include functional data on other *Schistosoma* species that infect humans and livestock, allelic variants of $TRPM_{PZQ}$, and data on $TRPM_{PZQ}$ expression. These data could eventually be folded into an existing resource (such as the variant function of the Ensembl platform) or remain as a standalone catalogue like other initiatives (e.g. WWARN) that track drug resistance markers.

Some speculative discussion is nevertheless possible as to what ongoing collection and sequencing efforts will likely reveal, by drawing upon existing knowledge of TRP channel evolution as well as prior studies of polymorphism frequency within human TRP channels. We advance three predictions, justified in the following sections.

: first, considerable variation in $TRPM_{PZQ}$ will be resolved and this variation will not be evenly spread across the gene,

: second, established precedent that TRP channel polymorphisms alter ligand sensitivity without impairing channel function portend a similar scenario for $TRPM_{PZQ}$ unless fitness costs preclude selection of such variants,

: third, current analyses are yet to anticipate the impact of non-coding variation, where PZQ 'resistant' phenotypes may occur through regulatory changes in $TRPM_{PZQ}$ expression.

The landscape of $TRPM_{PZQ}$ coding variants. $TRPM_{PZQ}$ is a long TRP (2268 amino acids) encoded by a gene with 36 predicted exons. A human TRPM that is of similar length is hTRPM6 (2022 amino acids, 39 exons) and over 72,000 variant alleles of hTRPM6 have been catalogued to date. $TRPM_{PZQ}$ shares homology with both hTRPM8 (transmembrane binding pocket (Park et al., 2021)) and hTRPM2 (COOH terminal NUDT9H homology (Park and Marchant, 2020), Fig. 2B). Over 45,000 variant alleles have been described within both these human genes. In terms of coding sequence variation within hTRPM8, over 1000 missense variations, at least 50 premature stop codons (the majority removing the entire transmembrane domain) as well as 70 frameshift mutations have been identified. This variation is distributed unevenly across the hTRPM8 coding sequence, with less variation evident within the transmembrane spanning region compared to the NH_2 terminus (Majhi et al., 2015; Hilton et al., 2019). This constraint likely reflects the criticality of the voltage sensor-like domains (VSLD, transmembrane helices S1-S4), the pore domain (S5-S6) and the TRP helix/TRP box for channel function which exhibits higher constraint and lower tolerance for variation. SNPs found in this region will have a higher probability of being deleterious to channel function. Similarly, from an evolutionary perspective, the transmembrane regions of TRPM8 are highly conserved through vertebrate evolution with very high levels of sequence conservation in TM4, TM5 and TM6 and the TRP box (Majhi et al., 2015). Such evolutionary scale analyses also implicate co-evolution of residues that form networks of allosteric regulation between the VSLD and pore domain of hTRPM8, again underscoring the importance of these regions to channel function (Hilton et al., 2019).

More broadly across the coding sequence, conservation levels vary reflective of different selection pressures during vertebrate TRPM8 evolution (Majhi et al., 2015; Hilton et al., 2019). This does not imply that regions with higher variability beyond the transmembrane spanning domain are benign (Fig. 2B). Various diseases (TRP 'channelopathies' (Nilius and Owsianik, 2010)) resulting from 'gain-of' and 'loss-of' -function mutants that perturb channel expression and/or regulation are found throughout the entire coding sequence of TRP channels. For example, we have identified a cluster of NH_2-terminal mutants that ablate the PZQ sensitivity of $TRPM_{PZQ}$ (Fig. 2). In short, considerable variation will likely be evident and functional profiling across lab mutants, natural variants, and $TRPM_{PZQ}$ splice variants (Bidaux et al., 2012) which will help advance our understanding of how this ion channel works.

Sequence variation and ligand sensitivity. In studies of vertebrate TRP channels, many examples have emerged of amino acid variation impacting sensitivity to environmental cues. For example, the temperature sensitivity of thermosensitive TRP channels has been correlated with sequence variation between different species (Chen et al., 2013; Matos-Cruz et al., 2017; Yang et al., 2020; Luu et al., 2024). The sensitivity of TRP orthologs in different species to natural products also varies between species. For example, the loss of capsaicin sensitivity in birds and in a specific mammal (hTRPA1 (Jordt and Julius, 2002; Han et al., 2018)). Similarly changes in menthol sensitivity during vertebrate evolution (hTRPM8 (Luu et al., 2024)). All such changes likely reflect selection of adaptive advantage as the sensory role of TRP channels attunes to the environmental milieu. That specific residues mediate sensitivity to discrete agonists is supported by a wealth of mutagenesis data, often guided by observation of species-specific insensitivities – for example icilin insensitivity in birds (Chuang et al., 2004), and, of particular relevance to this topic, the PZQ insensitivity of *Fasciola* spp.

$TRPM_{PZQ}$ (Park et al., 2021).

These studies demonstrate that TRP channels can lose sensitivity to specific chemotypes, without impairment of responsiveness to other activators or overall channel function. This underscores the polymodality of TRP channel activation where multiple modes of channel activation co-exist. This is true for $TRPM_{PZQ}$, as the channel can be activated by different ligands as well as by membrane stretch (Chulkov et al., 2023a). Therefore, while it seems likely that PZQ-insensitive $TRPM_{PZQ}$ variants will emerge in schistosome populations, the critical consideration is whether selection of such variants will confer a deleterious fitness cost preventing their expansion in natural populations (King et al., 2000). $TRPM_{PZQ}$ is known to depolarize neurons (Chulkov et al., 2023a) and channel function may simply be essential for sensory and/or locomotory functions critical for life cycle progression.

Finally, thinking simply in terms of 'gain' or 'loss' of PZQ sensitivity is likely insufficient. Even small decreases in the PZQ sensitivity of schistosome $TRPM_{PZQ}$ could reduce cure rates and be clinically significant. Quantitatively, it is the sensitivity of $TRPM_{PZQ}$ to PZQ relative to PZQ exposure (the pharmacokinetic profile of PZQ concentrations in host mesenteric vessels after dosing) that is critical for the action of PZQ against adult worms. Even a slight decrease in $TRPM_{PZQ}$ sensitivity to PZQ will shrink the effective kill zone within the pharmacokinetic profile of PZQ that mediates worm elimination.

Non-coding variation. The current focus on variation within the coding sequence of $TRPM_{PZQ}$ will reveal only a small fraction of the variation associated with the *$TRPM_{PZQ}$* gene. Non-coding variants, spanning the introns and UTRs of $TRPM_{PZQ}$, as well as neighboring regulatory and structural variants will contribute the majority of variation and many of these variants may prove relevant in terms of PZQ sensitivity. Of note is the study of Le Clec'h et al. (Le Clec'h et al., 2021), who mapped markers associated with decreased PZQ sensitivity to regions close to, but not within *$TRPM_{PZQ}$*, which were speculated to impact full length $TRPM_{PZQ}$ expression. An example relevant for human TRP channel function is a non-coding SNP (rs10166942, C/T) found ~1 kb upstream of the human TRPM8 coding sequence. This SNP displays one of the strongest genome-wide associations with migraine in humans (Freilinger et al., 2012; Anttila et al., 2013), with the ancestral 'C' allele being associated with reduced migraine risk and lower hTRPM8 expression (Key et al., 2018; Gavva et al., 2019). The derived 'T' allele is found at higher frequency in human populations in a latitude-dependent manner with higher TRPM8 expression conferring stronger, and presumably protective, cold temperature responsivity in cold-climates despite association with an increased migraine risk observed in European populations. This example underscores how selection of a non-coding variant has impacted TRP channel expression and activity across distinct populations. In a similar vein, studies of non-coding *$TRPM_{PZQ}$* variants will also be important for understanding schistosome sensitivity to PZQ.

In conclusion, the TRPtracker resource establishes a community-driven portal to progressively compile data on the genetic variation within $TRPM_{PZQ}$ across natural *Schistosoma* populations together with the predicted functional impact of these variants assessed by heterologous expression analyses. Cross-referencing these data will allow predictions of whether particular schistosome populations are likely to exhibit lower sensitivity to PZQ, and permit surveillance of the geographic spread of any specific variants of concern. This resource may then allow for more tailored control interventions in specific populations.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpddr.2026.100639.

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