

Morphological and Functional Effects of Cytoskeletal and Ion-Channel Agents on the Protoscolex of *Echinococcus granulosus sensu lato*

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Keywords

Echinococcus granulosus; Protoscoleces; Motility; Viability; Cytoskeleton; Ion Channels, Scanning Electron Microscopy.

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Abstract

Helminthiasis remains a major global health burden, and limitations of current anthelmintic therapies highlight the need for new pharmacological targets. In this study, we examined the effects of ion-channel and cytoskeletal modulators on bovine lung protoscoleces (PSCs) of *Echinococcus granulosus sensu lato*. Compounds acting on ion channels (praziquantel, amiloride, and amlodipine) and cytoskeletal components (albendazole and cytochalasin D) were evaluated using a semi-automated motility assay, methylene blue exclusion to assess viability, and scanning electron microscopy (SEM) to characterize structural damage. All compounds produced concentration-dependent reductions in PSCs' motility. Amlodipine was the most potent inhibitor of motility, whereas praziquantel and cytochalasin D produced pronounced tegumental alterations and strong correlations between motility impairment and parasite death. In contrast, amiloride markedly reduced motility with comparatively minor effects on viability, indicating a primarily paralytic effect. Cytoskeletal disruption induced severe structural damage and parallel declines in motility and viability. SEM analysis revealed extensive tegumental collapse, loss of glycocalyx, and microtrichial damage in PSCs exposed to cytoskeletal and calcium-modulating agents. These findings highlight cytoskeletal organization and calcium-dependent ion fluxes as key physiological vulnerabilities in *E. granulosus*. Comparative analysis of these pharmacological targets provides mechanistic insight into how disruptions in cytoskeletal dynamics and cation homeostasis compromise parasite motility and survival.

Introduction

Cystic echinococcosis (CE) is a neglected zoonotic disease of worldwide concern. It is caused by larval stages of species of the *Echinococcus granulosus sensu lato* complex, affecting humans and livestock on every continent except Antarctica (Alvarez Rojas et al., 2014; Deplazes et al., 2017; WHO, 2023). Infection occurs following ingestion of parasite eggs from contaminated environments, leading to the development of hydatid cysts, primarily in the liver and lungs (Romig et al., 2017).

Within the hydatid cyst, the protoscolex (PSC) emerges as the key infectious stage. They are enclosed by a laminated and germinal layer that provides structural support and facilitates immune evasion, thereby promoting parasite survival (Morseth, 1967). Cysts expand slowly and may remain asymptomatic for years, with clinical manifestations emerging as they enlarge and compress surrounding tissues (Kern et al., 2017). Surgical removal of the entire cyst is considered curative. However, accidental spillage of viable PSC during surgery frequently results in secondary infection and disease recurrence (Prousalidis et al., 2012; Sielaff et al., 2001).

Pharmacological treatment relies mainly on benzimidazoles and, in some cases, praziquantel. Yet, both present important limitations, including incomplete efficacy, variable clinical outcomes, adverse side effects, and the risk of emerging resistance (Doenhoff et al., 2008; Krämer et al., 2025). These shortcomings underscore the need to explore new therapeutic targets that exploit parasite-specific vulnerabilities. Among the most promising candidates are the cytoskeleton and ion channels, two systems essential for parasite physiology yet sufficiently divergent from their host counterparts to serve as selective drug targets (Perez-Serrano et al., 1995; Sasaki et al., 2002; Wolstenholme, 2011).

The parasite cytoskeleton plays a fundamental role in maintaining PSC morphology, motility, nutrient uptake, and tegumental integrity (Chen et al., 2008). Its divergence from mammalian cytoskeletal proteins has already been successfully exploited by benzimidazole carbamates, which selectively bind parasite β -tubulin (Guarnaschelli & Koziol, 2025). Beyond microtubules, actin filaments also support attachment and movement through specialized structures such as suckers and the rostellum, suggesting that disruption of actin dynamics could profoundly compromise parasite viability (La-Rocca et al., 2019).

Ion channels also govern essential physiological processes, including neuromuscular coordination, osmoregulation, and signal transduction (Choudhary et al., 2022; Wolstenholme, 2011). Targeting ion fluxes has already proven highly effective in flatworm control. For instance, praziquantel's mode of action is closely linked to calcium entry, causing rapid muscle contraction, tegumental disruption, and paralysis, thereby implicating Ca^{2+} -permeable channels as pivotal drug targets (Nogueira et al., 2022; Park & Marchant, 2020). Moreover, genomic analyses of *E. granulosus* have identified genes encoding Na^{+} -like channel subunits, suggesting that cation channels may play important functional roles and represent underexplored pharmacological opportunities (Zheng et al., 2013).

In this study, we evaluated the *in vitro* effects of cytoskeletal and ion-channel modulators on bovine lung PSCs of *E. granulosus*. Using motility assays, methylene blue viability staining, and Scanning Electron Microscopy (SEM), we generated dose–response curves, calculated IC_{50} values, and assessed structural damage at pharmacologically relevant doses. By integrating functional and morphological readouts, our work provides comparative insights into how disruptions in cytoskeletal dynamics and cation-channel activity compromise PSC motility and viability, highlighting both systems as promising therapeutic targets for cestode infections.

Material and Methods

Chemicals

Dimethyl sulfoxide (DMSO), penicillin-streptomycin solution, cytochalasin D (CD), praziquantel (PZQ), albendazole (ABZ), amiloride (AMI), and amlodipine (AML) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Dulbecco's Modified Eagle's medium (DMEM) was supplied by Thermo Fisher Scientific (Foster, CA, USA).

Parasites and Incubation Media

Protoscoleces (PSCs) of *Echinococcus granulosus s.l.* were extracted under sterile conditions from intact cysts of naturally infected cattle lungs. Briefly, as previously reported, aseptically collected PSCs were washed three times with sterile PBS (pH 7.2) (Carabajal et al., 2022). After the last sedimentation, PSCs were resuspended in prewarmed culture medium (DMEM containing 0.4% (w/v) glucose, 100 IU/ml of penicillin, and 100 µg/ml of streptomycin). They were incubated at 37°C in a humidified incubator with 5% CO₂. Parasites were cultured for at least 24 h until the motility assay was performed.

Motility Assessment

Motility assays were conducted in sterile 96-well flat-bottom microplates. Cultured PSCs were washed with sterile phosphate-buffered saline (PBS; pH 7.4) and resuspended in pre-warmed Dulbecco's Modified Eagle Medium (DMEM) supplemented with 0.4% (w/v) glucose. Aliquots were dispensed into each well to obtain 50–60 PSCs per well. Test compounds were dissolved in DMSO, and 10 µL of each stock solution was added to reach final concentrations ranging from 10⁻¹⁰ M to 10⁻² M. Solvent and untreated controls were included in all experiments. Microplates were incubated for 24 h at 37°C in a humidified atmosphere containing 5% CO₂.

PSC motility was evaluated using a live-cell imaging system (Zeiss AxioScope A1 microscope equipped with an AxioCam 105 5.0 MP camera) and ZEISS ZEN Microscopy Software. For each well, two sequential images were captured at 10-second intervals under 50× magnification (Fig. 1). Motility was quantified by calculating pixel-based displacement between the two frames in ImageJ v1.46, following the macro described by Ritler et al. (2017), with minor modifications. The motility index for each well was expressed as the percentage of movement relative to the DMSO control. Mean ± standard error (SE) values were calculated from three independent experiments per condition. To evaluate the reversibility of drug-induced motility impairment, PSCs were exposed to each compound for 24 h and thoroughly washed with pre-warmed sterile PBS to remove residual drugs. Then, PSCs were resuspended in fresh drug-free DMEM and incubated for an additional 24 h under identical culture conditions. Motility was quantified immediately

after the initial 24 h exposure and again following the 24 h recovery period in the absence of drug. Motility values were expressed as a percentage of untreated controls.

Viability Assessment

Parasite viability was determined by the Methylene Blue (MB) exclusion assay, which remains one of the most commonly used methods for evaluating protoscolex viability *in vitro* because it reflects tegumental membrane integrity and provides a rapid, reproducible estimate of parasite survival (Casado et al., 1986; Ritler et al., 2017). Briefly, after 24 h of incubation with each tested compound, 0.1% MB solution was added to each well and mixed gently. After 5 min at room temperature, samples were examined under an inverted microscope (40X). Viable PSCs were identified by exclusion of the dye and preservation of normal morphology, whereas non-viable PSCs exhibited uniform blue staining due to loss of membrane integrity. Each treatment was tested in triplicate across two independent experiments. Dose–response curves were generated by plotting the percentage of viable PSCs against drug concentration.

Scanning Electron Microscopy (SEM)

To evaluate tegumental morphological damage induced by the treatments, Scanning Electron Microscopy (SEM) was performed as described in Rasuk et al. (2017), with slight modifications for parasite specimens. Briefly, PSCs were washed in PBS (pH 7.4) and fixed with Karnovsky’s fixative, containing 2.66% w/v paraformaldehyde and 1.66% w/v glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). After fixation, PSCs were washed with 0.1 M phosphate buffer twice and dehydrated in a graded ethanol series (50%, 70%, 90%, and 100%), followed by 100% acetone. Critical-point drying was performed using a Denton Vacuum DCP-1. The samples were then mounted on aluminum stubs and subjected to gold sputtering (JEOL model JFC-1100). Control and treated PSC were observed under Zeiss Supra 55VP scanning electron microscope (Carl Zeiss NTS GmbH, Germany), at the Electron Microscopy Core Facility (CIME-CONICET-UNT).

Statistical Analysis

IC₅₀ and LC₅₀ values were obtained by nonlinear regression to a four-parameter logistic model in GraphPad Prism v6.0 (GraphPad Software, San Diego, USA). Differences between dose–response curves were assessed by an extra-sum-of-squares F-test.

Dose-response curves were fitted by nonlinear regression using a four-parameter logistic model (4PL):

$$Y = Bottom + \frac{Top - Bottom}{1 + 10^{(\log IC_{50} - X) \cdot HillSlope}}$$

For motility data, the upper and lower limits were constrained to 100% and 0%, respectively, because values were normalized to the DMSO control. Correlation between motility and viability was analyzed using Spearman's rank (r_s) correlation coefficient,

$$r_s = 1 - \frac{6\sum d_i^2}{n(n^2 - 1)}$$

with the significance assessed by

$$t = r_s \sqrt{\frac{n-2}{1-r_s^2}}$$

using $n-2$ degrees of freedom. To test whether the relationship between motility inhibition and viability followed a biphasic pattern, segmented regression was also applied:

$$V = \begin{cases} \beta_0 + \beta_1 M + \varepsilon, & M \leq M_b \\ \beta_0 + \beta_1 M_b + \beta_2 (M - M_b) + \varepsilon, & M > M_b \end{cases}$$

Model fits were compared using an F-test based on the residual sums of squares. Statistical significance was set at $p < 0.05$.

To assess whether drug-induced motility inhibition persisted after washout, motility data were analyzed using two-way ANOVA followed by Sidak's multiple comparisons test to compare motility between treatment and post-treatment conditions at each concentration. Differences were considered significant at $p < 0.05$. Data are presented as mean $\pm$ SEM from two independent experiments.

Results

Functional Impact of Ion-Channel Modulators on Motility and Viability

Within the set of ion channel-acting compounds, all compounds reduced the motility of *E. granulosus* PSCs in a concentration-dependent manner after 24 h of incubation (Fig. 2A–C). The experimental points for both motility and viability were satisfactorily fitted by a four-parameter logistic model (motility: $R^2 = 0.65$ – 0.90 ; viability: $R^2 = 0.90$ – 0.98 ; Table 1), enabling quantitative comparison of potency and maximal efficacy.

Amlodipine (AML) was clearly the most effective treatment, producing complete motility loss at nanomolar concentrations ($IC_{50} = 5.5$ nM; 95% CI = 0.0033–0.0093 μ M, $n = 6$; Table 1). Praziquantel (PZQ) displayed intermediate potency ($IC_{50} = 0.23$ μ M; 95% CI = 0.07–0.75 μ M, $n = 6$; Table 1), whereas the amiloride (AMI) treatment was only effective at much higher concentrations ($IC_{50} = 35$ μ M; 95% CI = 20–62 μ M, $n = 6$; Table 1).

When parasite viability was evaluated, a similar potency ranking was observed; however, the LC_{50} values were substantially higher than those calculated for motility (Table 1). AML showed the highest response, with the lowest LC_{50} (0.4 ± 0.1 mM), whereas PZQ and AMI required millimolar concentrations to reduce viability by 50%.

Effect of Cytoskeletal Drugs on Motility and Viability

Both cytoskeletal modulators—albendazole (ABZ) and cytochalasin D (CD)—also produced clear, concentration-dependent reduction in motility (Fig. 2D–E). Their potencies did not differ significantly ($p > 0.05$), with IC_{50} values of 0.022 ± 0.005 μ M for ABZ and 0.047 ± 0.029 μ M for CD. Although both compounds impaired viability, the maximal lethality was lower than that observed with ion-channel modulators (Table 1). CD was approximately 6-fold more potent than ABZ in reducing viability ($LC_{50} = 0.22 \pm 0.05$ mM); however, as mentioned above, neither compound substantially reduced viability, consistent with a predominantly paralytic rather than fully cytotoxic effect.

Divergent Relationships Between Functional Inhibition and Parasite Death

To determine whether drug-induced impairment of motility reflects loss of parasite viability, the association between both parameters was analyzed across the full concentration range tested for each compound. Correlations were assessed using Spearman’s rank coefficient (Table 2; Fig. 3).

Among ion-channel modulators, praziquantel exhibited a strong and statistically significant inverse association between motility and viability ($r_s = -0.98$, $p = 0.0004$; Fig. 3A), indicating that reductions in movement closely paralleled loss of viability. Amlodipine also showed a significant monotonic association between the two variables ($r_s = 0.81$, $p = 0.035$), although the data points were more concentrated at higher levels of motility inhibition. In contrast, Amiloride displayed no significant correlation ($r_s = 0.12$, $p = 0.793$), suggesting a clear dissociation between motility impairment and viability loss.

For cytoskeletal modulators, both Cytochalasin D and Albendazole exhibited strong and statistically significant associations ($r_s = 0.93$, $p = 0.0022$; $r_s = 0.96$, $p = 0.0028$, respectively), indicating more consistent coupling between the parameters.

Inspection of the motility inhibition–viability plots (Fig. 3A–E) revealed that a single linear trend did not adequately describe these relationships. Instead, most compounds exhibited a nonlinear, two-phase pattern: an initial region in which motility decreased substantially while viability remained relatively preserved, followed by a second phase marked by a sharp decline in viability.

Segmented regression analysis supported this interpretation. For praziquantel, the broken-stick model provided a significantly better fit than a linear model ($p = 0.004$), with a breakpoint at approximately 60.5% motility inhibition. Below this threshold, viability changed minimally despite reductions in motility, whereas beyond this point, viability declined steeply. A similar threshold-like behavior was observed for amlodipine, with a breakpoint near 92% motility inhibition. However, the limited number of data points at low inhibition levels reduced statistical support for the segmented model ($p = 0.119$).

Amiloride displayed the most pronounced decoupling between both parameters. Viability remained largely unchanged across a wide range of motility inhibition and decreased abruptly only at the highest concentrations. This behavior was well captured by a segmented model with a breakpoint around 78.3% motility inhibition ($p < 0.001$), consistent with a delayed transition from functional impairment to lethal damage.

Cytoskeletal agents showed comparable biphasic behavior. For cytochalasin D, the segmented model significantly improved the fit ($p = 0.005$), with a breakpoint at approximately 51.6% motility inhibition, followed by a steeper decline in viability. Albendazole also favored a segmented relationship ($p = 0.019$), with a breakpoint near 87.6%, although, as with amlodipine, most observations clustered at high levels of motility inhibition (Fig. 3E).

Thus, segmented regression analysis indicated that a two-phase model better described the relationship between motility inhibition and viability than a single linear fit. When all drugs were analyzed together, the pooled breakpoint occurred at approximately 90% motility inhibition, after which viability declined much more steeply. Class-specific analysis further showed that ion-channel agents and cytoskeletal modulators followed significantly different segmented profiles (Fig. 3F), with ion-channel agents displaying a sharper post-threshold decline in viability.

Taken together, these findings indicate that inhibition of motility does not uniformly predict parasite death. Instead, most compounds induce an early phase of functional impairment—consistent with paralysis with preserved membrane integrity—followed by a second phase associated with irreversible structural damage and loss of viability. This pattern is particularly evident for amiloride and praziquantel, and remains discernible, although less pronounced, for cytoskeletal modulators.

Finally, to assess whether motility inhibition represented transient paralysis or sustained functional impairment, PSCs were subjected to a washout protocol after 24 h of treatment exposure (Fig. 4). After an additional 24 h in drug-free medium, motility remained markedly suppressed at all concentrations tested. No statistically significant recovery was detected, indicating that the inhibitory effects were not reversible under the experimental conditions.

Ultrastructural Damage of PSCs induced by Cytoskeletal and Ion Channel-Targeting Drugs

Scanning electron microscopy (SEM) revealed that all tested compounds induced marked alterations in the tegument and overall morphology of the PSCs. However, the severity and patterns of damage differed according to their mechanism of action (Fig. 5). Under control conditions, PSCs exhibited a well-preserved architecture, with clearly defined rostellum, suckers, and body (Fig. 5A). The tegument was continuous and densely covered with uniformly distributed microtrichia, consistent with intact structural integrity and viability. ABZ treatment (Fig. 5B) resulted in moderate tegumental damage, partial distortion of the body and suckers, and surface irregularities. At higher magnification, the tegument showed disorganization and partial loss of microtrichia, indicating impairment of cytoskeletal stability and early tegumental disruption. In contrast, the actin filament disruptor CD induced severe morphological alterations (Fig. 5C). PSCs exhibited pronounced deformation and collapse, particularly affecting the muscular suckers and body. The tegument was extensively disrupted, with marked loss, shortening, and disorganization of microtrichia. The surface frequently appeared corrugated and eroded, reflecting profound destabilization of actin-dependent structural integrity.

Among ion channel modulators, PZQ caused the most dramatic damage. PSCs displayed a severely collapsed tegument, often exposing the underlying syncytial layer (Fig. 5D). The rostellar pad appeared distorted, and hooks were displaced or embedded within folded tegument, suggesting muscular collapse likely associated with altered calcium homeostasis. Microtrichia were extensively damaged, either truncated or completely absent. Treatment with AML also produced substantial alterations, characterized by strong body contraction and the formation of thick tegumental folds and vesicle-like structures, particularly around the suckers. The tegument appeared highly disorganized, with abundant detached debris (Fig. 5E). Microtrichia were sparse, shortened, and irregularly arranged, indicating significant surface remodeling. In contrast, AMI induced comparatively milder lesions. PSCs exhibited an irregular surface with shallow erosions and slight collapse in the rostellar region (Fig. 5F). Although the tegument remained partially preserved, microtrichia were less dense and unevenly oriented. Notably, rostellar hooks retained their structural integrity, suggesting a less disruptive effect on parasite morphology.

274 **Table 1.** Summary of dose–response parameters for viability and motility inhibition of *Echinococcus granulosus* protoscoleces after 24
 275 h exposure to cytoskeletal disruptors and ion channel agents.

		<i>Motility</i>			<i>Viability</i>		
		IC ₅₀ (M ×10 ⁻⁶)	E _{max} (%)	R ²	LC ₅₀ (M ×10 ⁻³)	E _{max} (%)	R ²
Cytoskeletal Modulators	Albendazole	0.022 ± 0.005	93.5 ± 0.8	0.91	1.3 ± 0.4	49.1 ± 1.5	0.96
	Cytochalasin D	0.047 ± 0.029	94.4 ± 4.3	0.65	0.22 ± 0.05	62.3 ± 5.9	0.91
Ion Channel Agents	Praziquantel	0.23 ± 0.14	99.6 ± 0.1	0.70	1.8 ± 0.4	85.2 ± 4.0	0.94
	Amlodipine	0.0055 ± 0.001	94.4 ± 0.8	0.85	0.4 ± 0.1	100.0 ± 0.0	0.90
	Amiloride	35.4 ± 9.9	98.3 ± 0.8	0.90	3.7 ± 1.1	92.8 ± 4.6	0.98

276
 277 **Table 2. Correlation analysis between motility (%) and viability (%) under different drug treatments.** The correlation coefficient
 278 (Spearman’s rho, r_s) usually presents a weak (r < 0.5), moderate (0.5 < r < 0.8) and strong (0.8 < r) relationship.

Spearman’s rank correlation coefficient	
Praziquantel	-0.98 (<i>p</i> = 0.0004)
Amlodipine	-0.81 (<i>p</i> = 0.0302)
Amiloride	0.12 (<i>p</i> = 0.793)
Cytochalasin D	-0.93 (<i>p</i> = 0.0022)
Albendazole	-0.96 (<i>p</i> = 0.0028)

Discussion

Cystic echinococcosis remains a neglected tropical disease for which chemotherapy is limited and often ineffective, leaving surgery as the only curative option despite the risks of recurrence and postoperative complications (Kern, 2010; Kern et al., 2017; Prousalidis et al., 2012). This therapeutic gap underscores the importance of phenotypic screening platforms capable of identifying compounds that rapidly impair *E. granulosus* survival. Movement-based assays have gained increasing relevance in anthelmintic discovery because they provide an objective and quantifiable readout of neuromuscular activity and overall parasite fitness (Buckingham et al., 2014; Ritler et al., 2017). In this study, we evaluated compounds targeting two essential biological systems in PSCs: cytoskeletal organization and ion-transport mechanisms, both of which are central to tegumental architecture, intracellular transport, signaling, and host–parasite interaction (Martinac, 2014; Ríos-Valencia et al., 2023; Robertson & Martin, 2007).

PZQ and ABZ are well-known anthelmintics with well-described molecular targets in flatworms (Cioli et al., 2014; Falagas & Bliziotis, 2007; Moreno et al., 2001). ABZ targets β -tubulin and inhibits microtubule polymerization, thereby disrupting vesicular transport, glucose uptake, and tegumental turnover (Guarnaschelli & Koziol, 2025). Of interest was the observation of the preferential deformation of the rostellar region after PZQ and ABZ treatments, likely reflecting its high dependence on cytoskeletal integrity and calcium-regulated muscular activity. Disruption of microtubule dynamics by ABZ and Ca^{2+} -mediated hypercontraction induced by PZQ converges in this structurally complex region, leading to early deformation, hook displacement, and tegumental collapse.

In this work, we also tested the effect of CD, which targets the cytoskeleton's complementary arm: this compound inhibits actin filament polymerization—an essential process for tegumental stability and parasite morphology (Ferreira de Lima et al., 2021; La-Rocca et al., 2019; Lacey, 1990). Both ABZ and CD impaired motility at micromolar concentrations, but CD was markedly more lethal and caused more extensive structural damage (Figs. 2 and 5).

Among ion-channel agents, PZQ is widely used against trematodes and many cestodes (Urrea-Paris et al., 1999); in schistosomes, it produces a characteristic triad of rapid depolarization, sustained muscle contraction, and extensive tegumental damage (Chulkov et al., 2023). These effects are now linked to activation of a TRP channel which mediates a non-selective Ca^{2+} influx in excitable cells and underpins the Ca^{2+} -dependent phenotypes observed *in vitro* and *in vivo* (Chulkov et al., 2023; Chulkov et al., 2021; Park & Marchant, 2020). Dihydropyridine calcium channel blockers, such as AML, are not classical anthelmintics but flatworm neuromuscular systems are highly sensitive to Ca^{2+} -channel modulation; neuropeptides and other modulators enhance Ca^{2+} currents in schistosome muscle, and a range of Ca^{2+} channel antagonists can blunt these responses, underscoring voltage-gated Ca^{2+} entry as a key control point

for motility and tegumental function (Jeziorski & Greenberg, 2006; McVeigh et al., 2006). Here, AML emerged as the most potent compound overall, abolishing motility at nanomolar concentrations and causing pronounced tegumental disruption at higher doses.

Amiloride represents a mechanistically distinct ion-transport modulator in this panel. In vertebrate epithelia, it is a prototypical blocker of epithelial Na^+ channels (ENaC) and Na^+/H^+ exchangers, reducing Na^+ entry and altering pH homeostasis (Sariban-Sohraby & Benos, 1986). Comparable sensitivities have been reported in other platyhelminths: in *Schistosoma mansoni*, microelectrode recordings showed that amiloride impairs recovery from acid loads across the tegument, indicating the presence of an amiloride-sensitive Na^+/H^+ exchange mechanism that regulates surface pH and cation handling (Pax & Bennett, 2009). Our previous work in *E. granulosus* demonstrated that amiloride alters tegumental electrical potentials under high- K^+ , low- Ca^{2+} conditions, consistent with the involvement of a Ca^{2+} -dependent, cation-selective pathway at the parasite surface (Carabajal et al., 2023). These results suggest that amiloride primarily perturbs electrodiffusional cation movement across the tegument—modulating membrane potential and neuromuscular excitability—without strongly compromising structural integrity or energy metabolism.

The correlation analyses performed in this study further highlight the mechanistic diversity underlying drug-induced phenotypes in PSCs. Inspection of the correlation plots suggests that motility impairment precedes loss of parasite viability, producing a non-linear relationship between both parameters. This pattern likely reflects an initial phase of neuromuscular paralysis followed by irreversible structural damage at higher drug concentrations. For compounds targeting Ca^{2+} signaling or actin cytoskeleton, motility inhibition was strongly associated with parasite death, indicating that progressive functional impairment closely parallels loss of viability. In contrast, amiloride produced substantial reductions in motility without a corresponding decline in parasite viability, suggesting that paralysis can occur independently of lethal damage. This dissociation indicates that motility-based readouts primarily reflect disturbances in neuromuscular excitability rather than irreversible cellular injury.

To determine whether the paralysis induced by these compounds was reversible, we evaluated the recovery of PSC motility after removal of the drug from the culture medium. Following a 24 h exposure period, parasites were washed and maintained under drug-free conditions to assess whether motility could be restored. The absence of recovery suggests that the effects induced by these compounds are not transient but rather reflect sustained functional impairment across the entire dose range, possibly associated with structural or physiological damage to critical cellular systems. However, it is worth mentioning that while the methylene blue exclusion method primarily reflects the integrity of the parasite's tegument, it does not detect early or subtle physiological damage. Consequently, the apparent discrepancy between motility impairment and viability measurements for some treatments, such as amiloride, may reflect limitations in

the sensitivity of this assay. Future studies should therefore incorporate complementary methodologies capable of detecting metabolic or physiological alterations with greater sensitivity.

Lastly, SEM analysis revealed that cytoskeletal- and ion channel-targeting drugs induce distinct yet partially overlapping patterns of tegumental disruption, accompanied by pronounced alterations in overall parasite morphology, as well as in specific anatomical regions. The most severe damage was observed following treatment with PZQ and CD, both of which resulted in extensive tegumental collapse and a marked loss of microtrichia. ABZ and AML induced intermediate phenotypes, characterized by structural disorganization and partial tegumental damage, whereas amiloride produced comparatively mild effects. Notably, a consistent feature across all treatments was the alteration or loss of microtrichia, underscoring their high sensitivity to both cytoskeletal disruption and ion imbalance. Given their essential role in nutrient absorption and host-parasite interactions, the impairment of these structures likely represents a key mechanism underlying drug-induced parasite dysfunction.

Conclusion

This study demonstrates that compounds targeting ion channels and cytoskeletal components exert distinct, quantifiable effects on both the morphological and physiological parameters of *E. granulosus* PSCs, supporting the interpretation that motility inhibition and loss of viability reflect partially distinct biological processes. In particular, ion-channel modulators appear capable of inducing a marked paralytic state before overt loss of membrane integrity. In contrast, cytoskeletal disruptors show a closer and more progressive coupling between functional impairment and parasite death. Agents that disrupt Ca^{2+} homeostasis or cytoskeletal organization produced pronounced and correlated reductions in motility and viability, accompanied by severe tegumental damage. These findings highlight the vulnerability of calcium-dependent signaling pathways and the actin cytoskeleton, both of which play central roles in neuromuscular coordination and tegumental integrity in cestodes. Taken together, this work contributes to a more refined framework for understanding drug action in *E. granulosus* and supports future efforts to identify novel scolicidal compounds. In particular, therapeutic strategies that simultaneously target Ca^{2+} -mediated signaling and actin cytoskeletal organization may represent a promising avenue to enhance antiparasitic efficacy through combined or synergistic drug actions.

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484

Figure Legends

Fig. 1: Representative images of motility analysis in *Echinococcus granulosus* PSCs. Photomicrographs illustrate the motility assessment under different experimental conditions. For each treatment, two consecutive frames separated by a 10-second interval are shown (first and second rows), allowing visualization of parasite displacement over time. Columns correspond to the experimental groups: untreated control (left), praziquantel-treated PSCs (middle), and cytochalasin D-treated PSCs (right). The bottom row shows the processed difference images obtained by pixel subtraction between the two frames, which highlight areas of movement and were used for quantitative motility analysis. Scale bar = 200 μ m.

Fig. 2: Concentration-dependent effects on motility and viability of *E. granulosus* PSCs. Dose-response curves of parasite motility and viability under different concentrations for (A) Praziquantel, (B) Amlodipine, (C) Amiloride, (D) Albendazole, and (E) Cytochalasin D. Data represent mean $\pm$ SEM from three independent experiments performed in duplicate.

Fig. 3: Correlation analysis between motility inhibition (%) and viability (%). Scatter plots show the relationship between both parameters for (A) Praziquantel, (B) Amlodipine, (C) Amiloride, (D) Cytochalasin D, and (E) Albendazole. Each point represents the mean values of motility and viability at a given concentration. Solid lines indicate segmented regression fits. (F) Combined analysis of all compounds, showing distinct profiles for ion-channel agents (orange) and cytoskeletal modulators (blue).

Fig. 4: Assessment of reversibility of drug-induced motility inhibition in *E. granulosus* PSCs. Dose-response curves for motility measured after 24 h of treatment and after an additional 24 h in drug-free medium (24 h post-treatment). (A) Ion-channel agents (B) Cytoskeletal modulators. Motility values correspond to mean $\pm$ SEM (n=6). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 (two-way ANOVA).

Fig. 5: Scanning electron microscopy of *E. granulosus* protoscoleces (PSCs) treated with cytoskeletal and ion channel drugs. Parasites were incubated with: (A) 0.1 % DMSO (solvent control), (B) 0.1 mM Albendazole (ABZ), (C) 0.1 mM Cytochalasin D (CD), (D) 1 mM Praziquantel (PZQ), (E) 1 mM Amlodipine (AML), and (F) 1 mM Amiloride (AMI). For each condition, panels show the whole parasite (left), tegument surface detail at high magnification (second column), the scolex region (third column), and microtrichia detail (right). r: rostellum; s: sucker; b: body; ms: muscular sucker; mt: microtrichia; h: hooks.

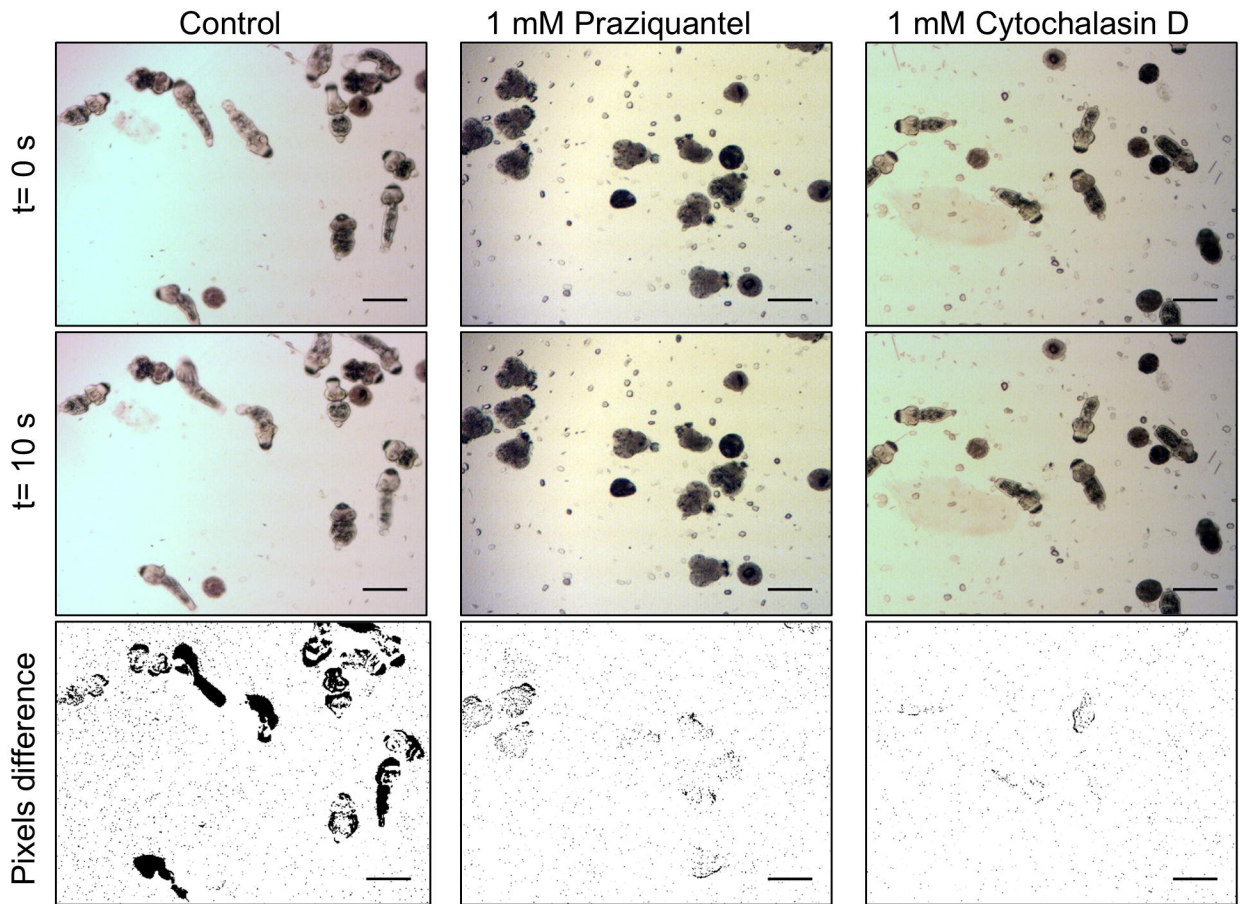


Figure 1

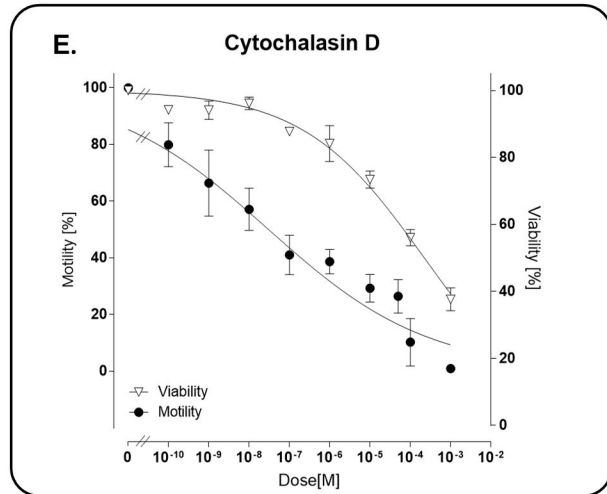
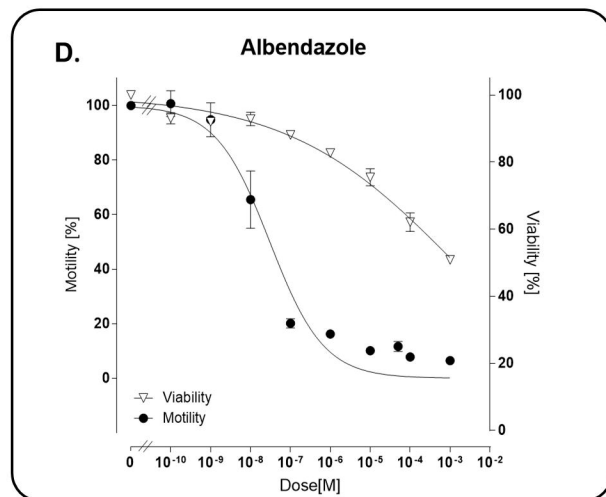
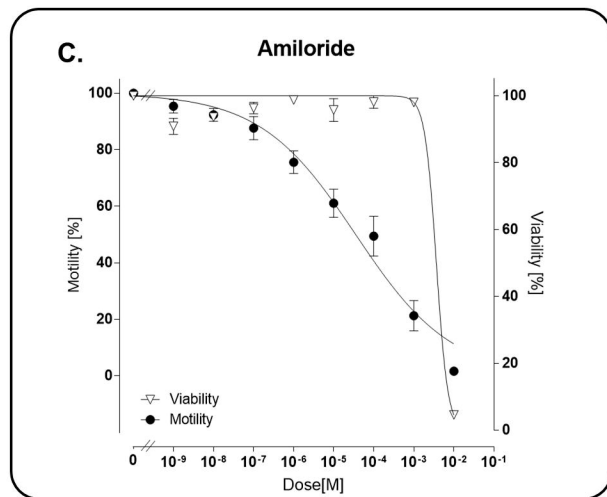
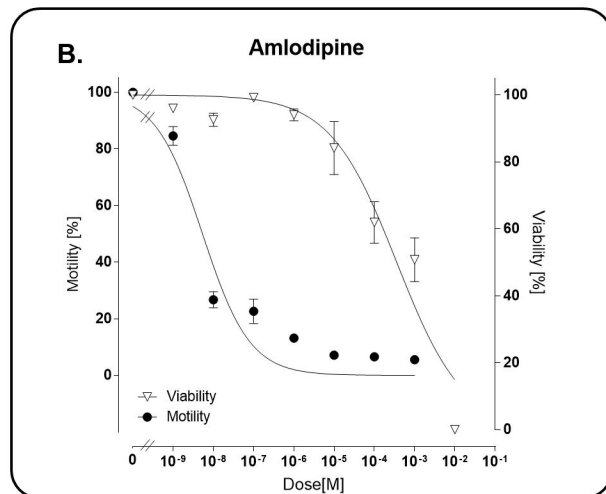
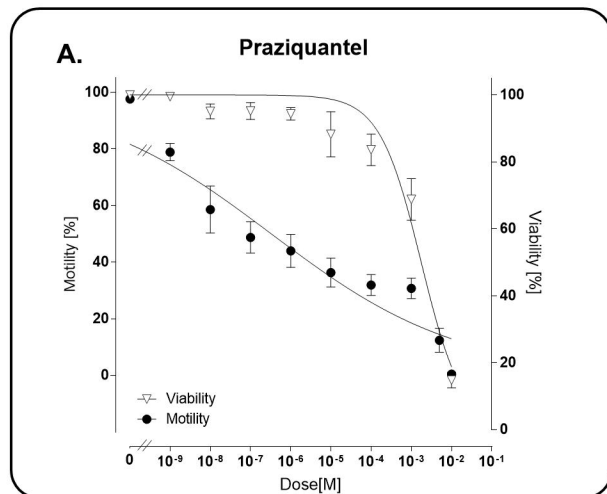


Figure 2

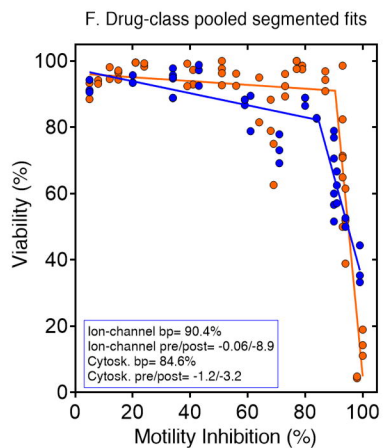
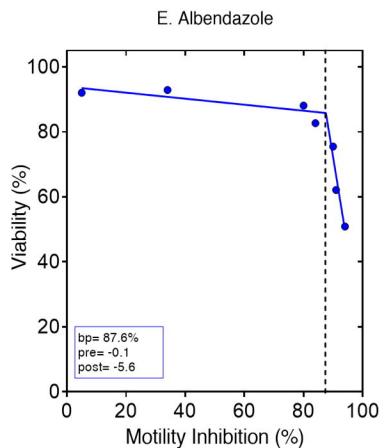
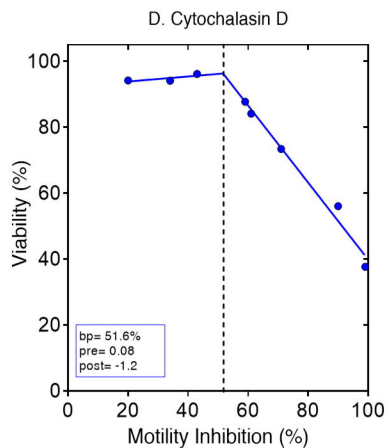
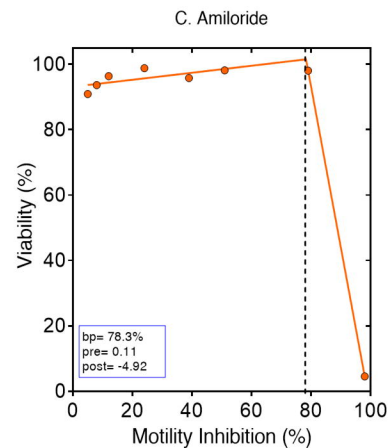
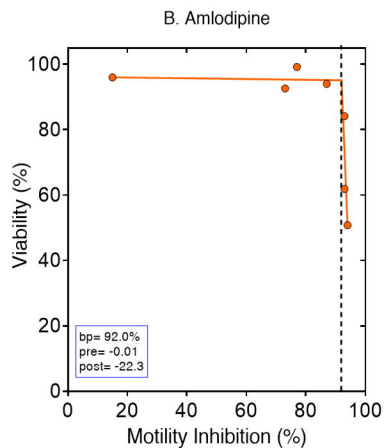
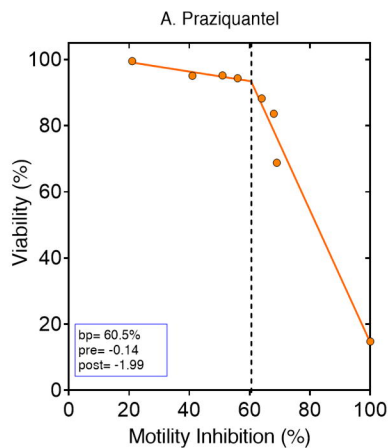
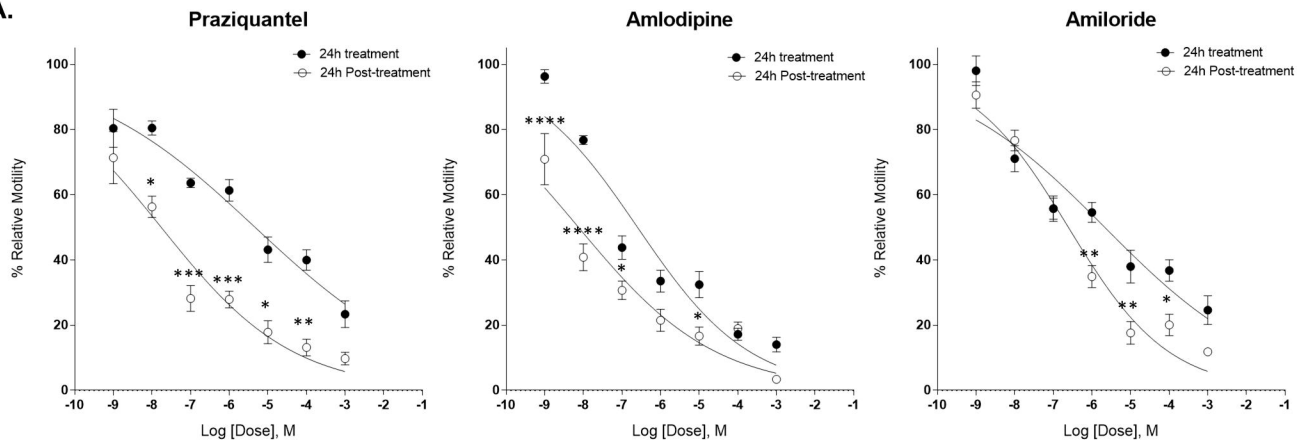
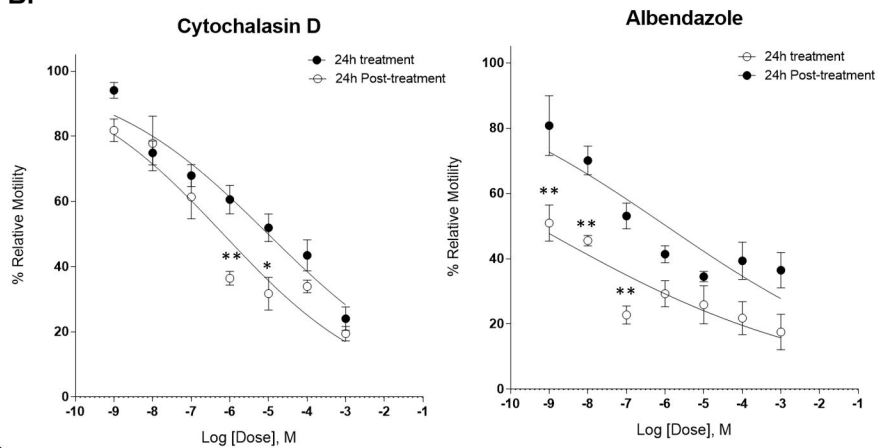


Figure 3

A.**B.****Figure 4**

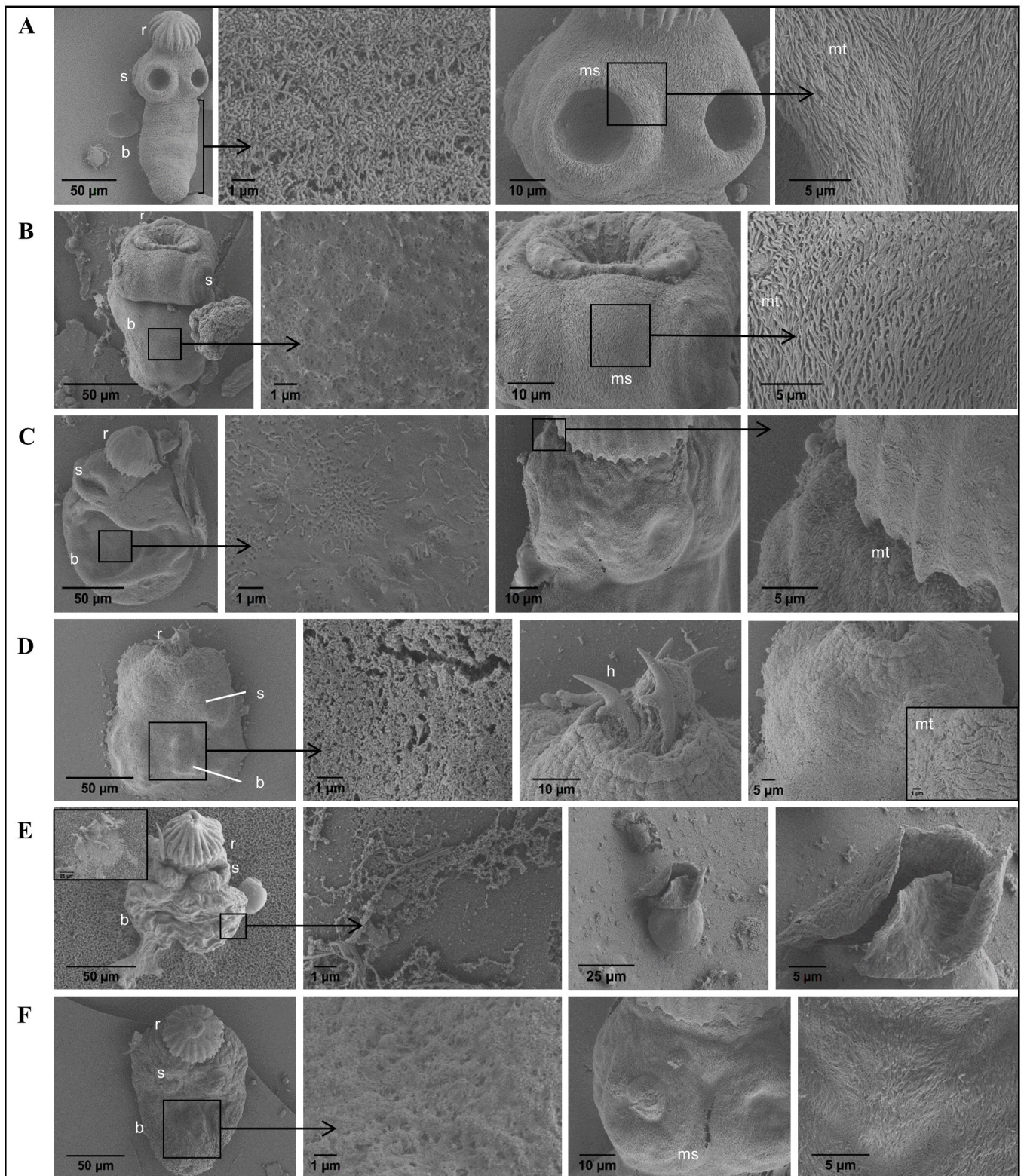


Figure 5