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Host Penetration and Emergence Patterns of the Mosquito-Parasitic Mermithids *Romanomermis iyengari* and *Strelkovimermis spiculatus* (Nematoda: Mermithidae)

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Abstract: Romanomermis iyengari and *Strelkovimermis spiculatus* are mermithid nematodes that parasitize mosquito larvae. We describe host penetration and emergence patterns of *Romanomermis iyengari* and *Strelkovimermis spiculatus* in laboratory exposures against *Culex pipiens pipiens* larvae. The mermithid species differed in host penetration behavior, with *R. iyengari* juveniles attaching to the host integument before assuming a rigid penetration posture at the lateral thorax (66.7%) or abdominal segments V to VIII (33.3%). *Strelkovimermis spiculatus* attached first to a host hair in a coiled posture that provided a stable base for penetration, usually through the lateral thorax (83.3%). Superparasitism was reduced by discriminating against previously infected hosts, but *R. iyengari's* ability to avoid superparasitism declined at a higher inoculum rate. Host emergence was signaled by robust nematode movements that induced aberrant host swimming. Postparasites of *R. iyengari* usually emerged from the lateral prothorax (93.2%), whereas *S. spiculatus* emergence was peri-anal. In superparasitized hosts, emergence was initiated by males in *R. iyengari* and females in *S. spiculatus;* emergence was otherwise nearly synchronous. Protandry was observed in *R. iyengari*. The ability of *S. spiculatus* to sustain an optimal sex ratio suggested superior self-regulation. Mermithid penetration and emergence behaviors and sites may be supplementary clues for identification. Species differences could be useful in developing production and release strategies.

Key words: Culex pipiens pipiens, insect-parasitic, host emergence, host penetration, mermithid, Mermithidae, mosquito, nematode behavior, *Romanomermis iyengari*, *Strelkovimermis spiculatus*.

Mermithids nematodes may be terrestrial, semiterrestrial, or aquatic, but all are obligate endoparasites of members of the phylum Arthropoda, particularly insects. Mermithids tend to be host specific, usually to a single host species or family (Poinar, 1979). Most aquatic mermithids have seen limited study with the exception of species parasitizing mosquitoes.

The infective unit of mosquito mermithids is the preparasite, which is a second-stage juvenile (J2). The infective juveniles swim in search of larval hosts immediately after hatching. Once in contact with a suitable mosquito larva, they use their odontostylet to pierce the host cuticle and enter. Shamseldean and Platzer (1989) described aspects of the penetration process for *Romanomermis culicivorax* Ross & Smith using light and scanning electron microscopes. Camino and Reboredo (2000) reported that infective juveniles prefer to infect early-stage hosts, with 80% successful infection of 1st and 2nd instars, compared with 52% and 38% of 3rd and 4th instars. The host immune system rapidly recognizes the invaders, but the parasites secrete an extracellular surface coat that aids immune evasion (Shamseldean et al., 2006, 2007). The coat is a disposable, renewable barrier between parasite and host that is intermittently shed to cleanse the nematode of adhering host immune products. The parasitic stage takes nourishment from the host's hemolymph by transcuticular uptake (Poinar and Hess, 1977; Platzer and Platzer, 1985), growing slowly for the first 3 to 4 d before rapidly increasing in size. There are four molts in mosquito mermithids but only a single molt occurs within the host. When development is complete, the postparasite stage (J3) exits the host, with most mosquito mermithids emerging from larval hosts although a few species emerge from adult mosquitoes (Gaugler et al., 1984; Blackmore, 1994). The emergence wound is invariably fatal. After emergence, postparasites burrow into the soil at the bottom of the mosquito pool, form large mating clusters, make a double molt to the adult stage (Poinar and Otieno, 1974), mate, and lay eggs to complete the life cycle.

Because they attack medically important disease vectors, mosquito mermithids have received attention as biological alternatives to chemical insecticides (Petersen, 1973; Platzer et al., 2005; Abagli et al., 2012). *Romanomermis culicivorax* is the most extensively studied of all mermithid nematodes, and this species has demonstrated an ability to suppress mosquito populations (Platzer, 2007). Most notable was a large-scale field release in El Salvador that reduced an anopheline larval population 17-fold (Petersen et al., 1978). Political unrest unfortunately disrupted plans to determine the long-term impact of the release. Other mosquito mermithids have begun to receive attention in recent years, most notably *Romanomermis iyengari* Welch and *Strelkovimermis spiculatus* Poinar & Camino. Platzer (2007) recognized these two species to present the best biological control opportunities with mermithids other than *R. culicivorax*. The tolerance of these two species to saline and polluted environments distinguishes them from their better-known and studied rival. *Romanomermis iyengari* Welch was first reported in India from *Anopheles* and *Culex* larvae (Gajanana et al., 1978), whereas *S. spiculatus* was originally described from *Aedes albifasciatus* (Macquart) in Argentina (Poinar and Camino,

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1986) and subsequently isolated from *Culex pipiens pipiens* Linnaeus. Platzer (2007) reviews the special biological control attributes of these two mermithids. Although eclipsed by the commercial development of *Bacillus thuringiensis* var. *israelensis* as a storage-stable, inexpensively produced biological insecticide, mosquito mermithids have a role to play where inoculative rather than short-term, repeated, inundative biological control is the objective (Platzer et al., 2005). But their development as either inoculative or inundative agents will require an improved understanding of their biology and host-parasite interactions. We describe host penetration and emergence patterns of *Romanomermis iyengari* and *Strelkovimermis spiculatus*, with special attention to differences that may be useful as simple, supplementary tools for identification.

Materials and Methods

Host larvae of *C. pipiens pipiens* were obtained from a colony established from eggs collected in Mercer County, New Jersey, were used as the host. The colony was maintained at 26°C and a relative humidity of 75% with a 16L:8D photoperiod. Adults were held in 0.51-m^3 aluminum screen cages and supplied with 10% sucrose solution on cotton wicks. Restrained adult quail were used to blood-feed female mosquitoes (Rutgers Animal Use Protocol #86-129). Egg rafts were collected from a black, 400-ml plastic container. Resulting larvae were held in enamel trays with 1 liter of dechlorinated water and 0.15 g of Brewer's yeast: lactalbumin (50:50). The water was replaced with fresh water alternate days, whereas food was added daily. Second instars were used in initiating all nematode infections.

Mermithid cultures were initially obtained from the Applied Center for Entomonematodes, Cairo University, Egypt. Nematode cultures were kept in 21- × 14- × 6-cm plastic containers containing sand with 1.4- to 2.0-mm particle sizes. Eggs were stored in moist sand for at least 6 wk at 26 ± 2°C. As needed for experiments, 5 g of the sand cultures was flooded to stimulate egg hatching and the emergence of infective juveniles.

Host penetration behaviors of *R. iyengari* and *S. spiculatus* infective juveniles were observed in 1:1 host-parasite exposures. A host larva and juvenile nematode were separately transferred via pipet to a droplet of water on a 35- × 10-mm petri dish. Nematode attachment and penetration behaviors observed continually until a host had been infected. This was replicated 10 times for each mermithid species, and the experiment was repeated three times, yielding 30 penetration events per species as all hosts become infected. Infective juvenile host penetration behaviors were video-recorded under light microscopy for further analysis and select recordings posted at http://www.youtube.com/watch?v=bV_wwBBhNwI (*R. iyengari*) and http://www.youtube.com/watch?v=gJLACI-X–U (*S. spiculatus*).

Infections were conducted in 100-ml glass beakers with 20 ml of water and 64 *Culex* larvae. Nematode concentrations for exposures were determined using the method described by Petersen and Willis (1972). The larvae were exposed to *R. iyengari* and *S. spiculatus* infective-stage juveniles at host-parasite ratios of 1:3 or 1:5. A 1:3 ratio is optimal for *R iyengari* infections (Paily and Balaraman, 1990) whereas 1:5 is optimal for *S. spiculatus* (Becnel and Johnson, 1998), but both ratios were tested here to facilitate comparisons. The experiments were replicated three times for each inoculation and species treatment. All tested larvae were parasitized.

Each treatment (2 inoculation ratios × 2 mermithid species) was transferred to enamel trays with 1-liter of water 16-hr postexposure, and maintained as described above. Six days postexposure, 4th instars were transferred to individual wells of a 12-well cell culture plate with 4 ml of water. On day 7, larvae were observed at hourly intervals to identify the initiation of postparasite emergence. Once emergence commenced, larvae were observed continually. Mosquitoes displaying the characteristic aberrant movements associated with nematodes preparing to emerge were transferred to a small petri dish and observed by microscope. Postparasite emergence site, number and gender of emerging nematodes were recorded for each host. The study was terminated when all postparasites had emerged. Postparasite host emergence behaviors were video-recorded and select recordings posted at http://www.youtube.com/watch?v=m8HaZPV5wIs (*R. iyengari*) and http://www.youtube.com/watch?v=M5N_yPqUH0I (*S. spiculatus*).

Statistical analysis: All data were analyzed by one-way analysis of variance (ANOVA) using Fisher's least significant difference (LSD) in multiple range tests among the means ($P \leq 0.05$ or $P \leq 0.01$). Data are presented as mean ± SE.

Results and Discussion

Penetration: Striking differences in mermithid penetration sites were observed ($P \leq 0.05$), with *R. iyengari* preferring to pierce the host abdomen (Fig. 1A) and *S. spiculatus* the thorax (Fig. 1B). Of the 30 *R. iyengari* infective juveniles observed during penetration, 20 (66.7%) pierced the posterior abdomen (exclusively the most posterior segments V to VIII), 10 (33.3%) pierced the thorax, and none (0%) pierced the head. Juveniles of *S. spiculatus* were more restrictive in choice of penetration site (Fig. 1B), with nonthorax sites appearing to be outliers. Of 30 *S. spiculatus* juveniles, 25 (83.3%) pierced the thorax, 3 (10%) pierced the abdomen, and 2 (6.67%) pierced the head. All portals of entry were located either laterally or dorso-laterally.

There were also distinct differences in penetration behavior between the species. As *R. iyengari* infective juveniles swim to within one juvenile body length, they invariably pause briefly (1 to 2 sec), before pushing

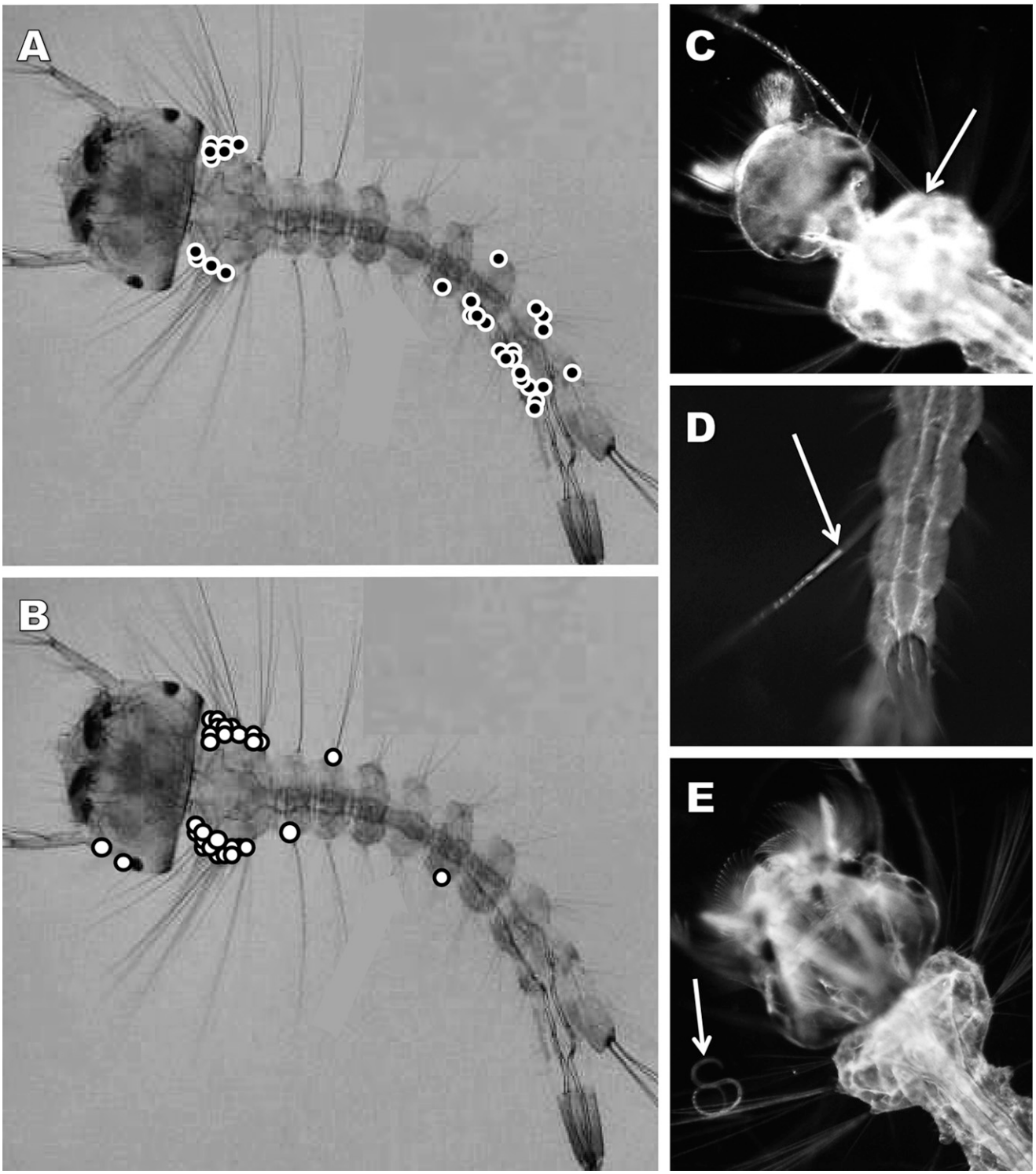

FIG. 1. Penetration behaviors of infective juveniles (J2) (arrows) of *Romanomermis iyengari* and *Strelkovimermis spiculatus* attacking 2nd-instar *Culex pipiens pipiens*. (A) Route-of-entry sites for *R. iyengari*. (B) Route-of-entry sites for *S. spiculatus*. (C) *R. iyengari* attached to host prothorax and displaying the stiff body poster associated with penetration. (D) *R. iyengari* attached to host posterior abdomen and displaying the stiff body poster associated with penetration. (E) *S. spiculatus* attached to host thorax hair by coiling before migrating proximate to the host thorax.

forward and attaching by their stoma to the host integument. The juvenile body gently flexes during this phase, during which the host becomes immobile. Within 1 min, the nematode becomes stiff and immobile (Fig. 1C,D). The duration of this arrow-like posture is brief, approximately 25 to 35 sec, and terminates when the nematode abruptly begins entry into the host body cavity. Passage through the integument is swift, being completed in 4 to 5 sec. The posterior portion of the juvenile exterior to the host during penetration maintains its inelastic pose, whereas the anterior portion begins coiling immediately as the host is entered. The host

recovers and assumes normal behaviors 1 to 2 min postpenetration.

Infective juveniles of *S. spiculatus* do not pause briefly before initiating their attack. Upon locating the host, they immediately attach by coiling around a mosquito hair showing a strong preference for the thoracic hairs (Fig. 1E). Soon thereafter they migrate down the hair to reach the host body wall. The anterior portion of the juvenile begins to uncoil from the hair within 1 to 2 min. The juvenile head begins to sweep briefly over the host cuticle without making contact initially, before the stoma locks onto the host integument. The posterior portion of the nematode remains tightly coiled around the host body hair so the parasite is firmly attached anteriorally and posteriorally. The host becomes inactive 1 to 2 min later, triggering a sudden penetration of 2 to 3 sec duration, during which the posterior portion uncoils from the hair. Unlike *R. iyengari*, no portion of *S. spiculatus* becomes stiff and rigid during the penetration process. Immediate coiling within the body cavity and host recovery is similar in all respects to *R. iyengari*.

The penetration behavior and sites for *R. iyengari* agrees closely with that described for *R. culicivorax* by Shamseldean and Platzer (1989). These authors reported attachment of the juvenile stoma by a "secreted adhesive material," following by host paralysis, and stylet thrusting to create an opening for juvenile entry. Infective juveniles of *S. spiculatus* completed a similar infection pathway, with the exception of attachment. These juveniles initially attach by coiling about hairs, particularly those on the thorax that are the longest, most dense, and therefore more easily contacted larval hairs, before attaching by their stoma. Coiling about a hair provides a stable base to push from in initiating the penetration process. We did not detect an adhesive. The small size of S. *spiculatus* infectives, one-third the size of *R. iyengari*, may account for its requirement for a support base to increase.

Parasite load: Both mermithid species reduced superparasitism by discriminating against previously infected mosquito larvae ($P \leq 0.05$) (Fig. 2). At the lower inoculum rate, there was no difference between species in parasite load (Fig. 2A), with most (66.49 ± 4.38%) mosquito larvae harboring one or two parasites. A parasite load up to eight was found in rare instances (1.06 ± 0.53%) ($P \leq 0.05$). Overall, the greater the load, the greater the discrimination against those hosts. At the higher rate, dissimilarities between the species became apparent (Fig. 2B). *Strelkovimermis spiculatus* maintained its strong preference for uninfected hosts as approximately 43.92 ± 6% of hosts were found infected with a single parasite regardless of rate ($P \leq 0.05$). But *R. iyengari's* ability to discriminate failed at the higher inoculum rate, with no differences detected in parasite load. Infection of unparasitized hosts declined by nearly two-thirds from 26.98 ± 5.87% to 9.52 ± 5.1% at the 1:3 and 1:5 concentrations ($P \leq 0.05$). An upper parasite load of 12 was recorded.

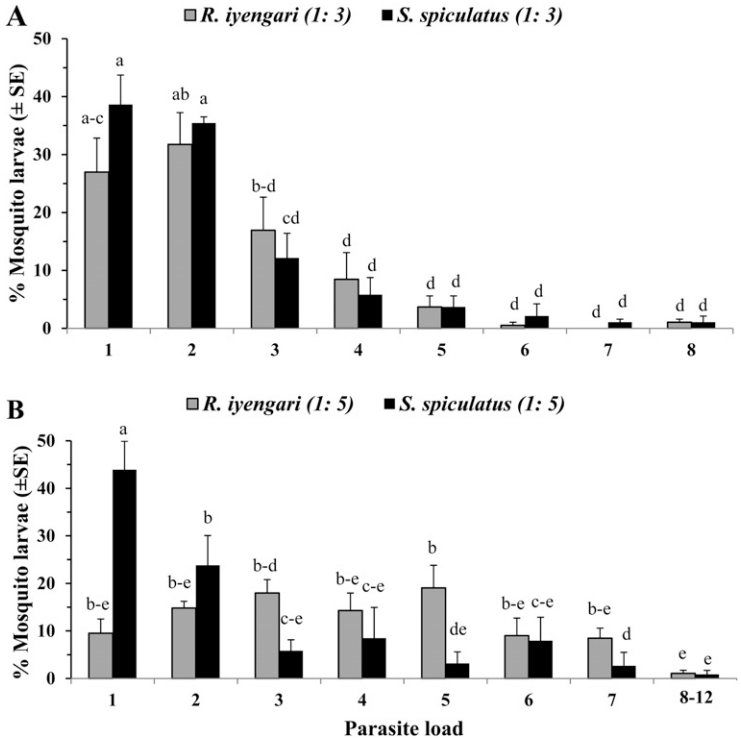

FIG. 2. Parasite load (number of postparasitic nematodes emerging from single hosts) of mosquito larvae infected with *Romanomermis iyengari* and *Strelkovimermis spiculatus* at host-parasite inoculation ratios of (A) 1:3, and (B) 1:5. Bars with same letters are not significantly different ($P \leq 0.05$).

Nematode load is related to sex ratio, with strong male bias as parasite density per host and therefore competition for nutrients increases (Petersen, 1972). The superior capability of *S. spiculatus* to sustain optimal sex ratio regardless of inoculum rate, suggests this species is similarly superior at reducing intraspecific competition and regulating its population. This could offer an advantage over *R. iyengari* in inoculative biological control efforts where establishment and recycling for long-term control are goals. Unfortunately the potential of *S. spiculatus* to meet these goals has not been field tested (Platzer, 2007).

Emergence behavior and site: Postparasite emergence from larvae is first signaled by robust nematode movement within the host as they seek a suitable emergence site. Searching activity, once initiated by a single parasite, is then observed concurrently in all parasites in superparasitized larvae. This, in turn, induces aberrant host swimming movements that are easily recognized. Emergence of postparasites commences 3 to 5 min later. Emergence is a product of vigorous pushing and mechanical pressure (unlike infective juveniles, postparasites lack a stylet) that generates an exit wound. Just as with searching, emergence occurs nearly synchronously in superparasitized hosts. That is, once initiated emergence is completed in 9 to 10 sec regardless of parasite load. During emergence, hosts infected by *S. spiculatus* continue to show aberrant movements indicative of irritation, whereas hosts infected by *R. iyengari* sharply reduce movement.

The emergence site is sharply differentiated and localized in the two parasite species, with no differences based on inoculum ratio. Parasitic development of *R. iyengari* occurs within the abdominal cavity, but nearly all postparasites emerge from narrowly delineated lateral locations of the prothorax (95.0 ± 2.34%) ($P \leq 0.05$) (Fig. 3A). Only a few exited from the abdomen (5.0 ± 1.68%) and none from the head ($P \leq 0.05$). This contrasts with *R. culicivorax*, which both develops and emerges from the thorax (Petersen, 1972). Exit wounds were easily detected from the residues of extruded body fluids (Fig. 3B). Parasitic development and emergence of *S. spiculatus* occurs from the abdomen. Regardless of inoculum level, 100% of *S. spiculatus* postparasites emerged peri-anally (Fig. 3C), exiting from the anus or base of the anal gills. The torn rectum is ejected from the host body as the postparasite escapes (Fig. 3D).

In hosts co-infected by male and female nematodes, female postparasites of *S. spiculatus* tended to generate the peri-anal exit portal used by all subsequent emergents (92.99 ± 1.29%). However, postparasite emergence through the thorax was triggered by males in *R. iyengari* (93.44 ± 4%) ($P \leq 0.05$). All *R. iyengari* males that failed to emerge before females died. These males were invariably small, weak, and often deformed. Female *S. spiculatus* are more than twice as large as male postparasites (19- vs. 9-mm length) and easily generate the mechanical force needed to breach the host. The size differential in *R. iyengari* is less extreme (17-mm-female vs. 12.5-mm-male length). Generating an exit wound via the thorax would seem far more challenging than a peri-anal exit, suggesting that the smaller diameter *R. iyengari* males (184 vs. 133 μm) may exit first to generate a pilot hole—a smaller hole bored into a surface to facilitate the subsequent insertion of a wider object.

Kobylinski et al. (2012) found unidentified mermithids that emerged from the anus of field-collected *Anopheles* spp. adults in a malarial region of Senegal. The meager pool of mermithid sequences available in GenBank indicated the nematodes were most closely related to *Strelkovimermis spiculatus*, although this species does not parasitize adults. We suggest that the emergence wounds left by postparasites may provide clues to mermithid species identification, supplementing a thin morphological and molecular taxonomic base, even in cases where the nematodes have exited and are lost.

Postparasites of *S. spiculatus* always exited the host through a single wound regardless of the parasite load, whereas *R. iyengari* exited from one (36.98 ± 8.38% and 63.02 ± 8.38%) at 1:3 and 1:5 ratios) ($P \leq 0.05$) or two (40.1 ± 9.08% and 74.90 ± 9.42% at 1:3 and 1:5) ($P \leq 0.05$) exit wounds. Emergence of the first postparasite signals near synchronous exit of all nematodes, because the wound renders the host quickly unsuitable for the remaining parasites. Even when two exit wounds were observed, the second wound is created less than 1 sec after the first wound. Emergence of *R. iyengari* kills mosquito larvae within 1 to 2 hr, approximately twice as rapidly as *S. spiculatus*. Hominick and Welch (1980) reported that the emergence of mermithids from mayflies signals quick host death from mechanical injury and lost hemolymph. The more protracted death in *S. spiculatus* hosts is likely because the peri-anal region is less sensitive to mechanical damage than the thorax; this parasite causes fewer emergence wounds, and visibly less hemorrhaging and fluid loss results than from thoracic wounds.

Daily emergence: Parasite development was complete and host emergence initiated seven days postinfection for both mermithids (Fig. 4). Most postparasitic juveniles emerged over the next 24 hr and emergence was essentially complete within 48 hr, with inconsequential emergence thereafter. At the lower inoculation ratio (Fig. 4A), emergence patterns were identical between species with 62.76 ± 13.87% ($P \leq 0.05$) of emergence occurring in the first 24 hr. At the higher inoculation ratio (Fig. 4B); however, *S. spiculatus* emergence on day 7 increased to 80.16 ± 9.94% and *R. iyengari* had decreased to 42.29 ± 9.68% ($P \leq 0.01$).

Postparasite emergence occurs when the host is depleted and parasite development is complete (Petersen, 1975). We observed different responses to the increased

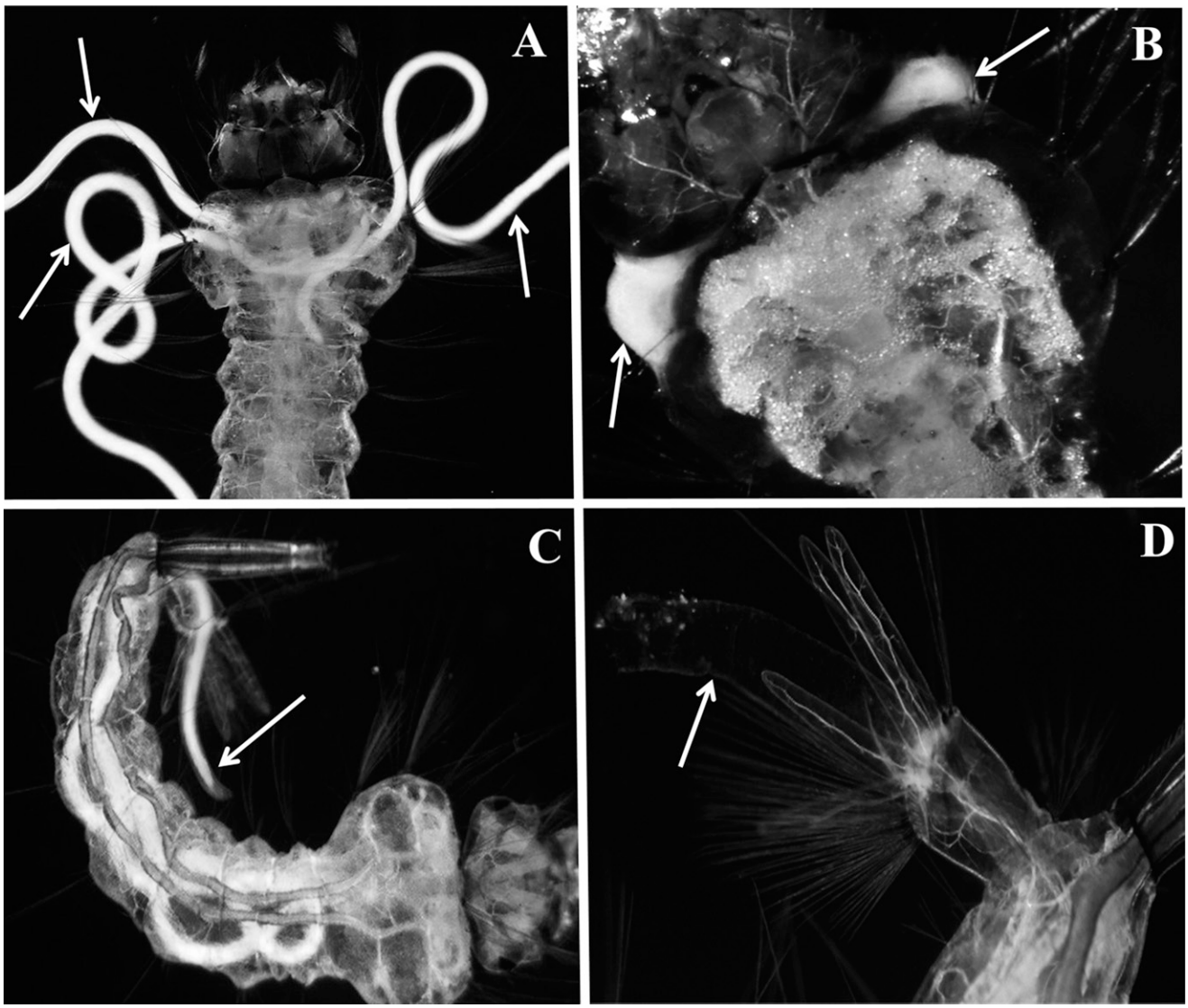

Fig. 3. Emergence of *Romanomermis iyengari* and *Strelkovimermis spiculatus* postparasites from *Culex pipiens pipiens* larvae. (A) Three *R. iyengari* postparasites (J3) exiting from the host anterior prothorax. (B) Host fluids extruded from exit wounds (arrows) at host prothorax following *R. iyengari* emergence. (C) Emergence of *S. spiculatus* (arrow) between the anal gills and anus. (D) Ejected rectum (arrow) of *Culex pipiens pipiens* larva indicating the peri-anal exit portal of *S. spiculatus* postparasites.

parasite load associated with higher inoculum rate and consequently more rapid host depletion: *R. iyengari* delayed and *S. spiculatus* accelerated development. This observation may have practical implications for optimizing mermithid mass production (Achinelly and Micieli, 2011). That is, would accelerating development to increase production come at the expense of reduced parasite fitness?

Sex ratio: Mermithid gender is determined post-penetration (Charnov and Bull, 1977) and is a function of parasite burden, with superparasitism being strongly associated with male production. That is, the proportion of males produced increases as parasite load increases which serves an essential role as a population self-damping mechanism (Nickle, 1973; Petersen, 1977; Paily and Balaraman, 1990). Our study with *R. iyengari* and *S. spiculatus* lends further support to this fundamental principle of mermithid biology. A low parasite burden of one yielded 7.65 ± 3.95 and 11.87 ± 7.53% male *R. iyengari* and *S. spiculatus*; a median burden of two yielded a balanced sex ratio with 42.92 ± 11.05 and 52.89 ± 1.69% males; a high burden of four yielded 80.78 ± 9.61 and 58.32 ± 10.43% males; and severe superparasitism of six or more resulted in 97.5 ± 2.26 and 98.43 ± 6.61% *R. iyengari* and *S. spiculatus* males.

Protandry: Males of *R. iyengari* emerged earlier than females (Fig. 5A,B), a phenomenon known as protandry. Regardless of the host-parasite inoculation ratio, only male emergence was recorded the first day (7 days postinfection) with no females. By day 2, emerging nematodes were predominately female at the lower rate (63.86 ± 10.72%) although this difference was not significant ($P \leq 0.05$). At the higher rate, females comprised a significantly smaller portion relative to males

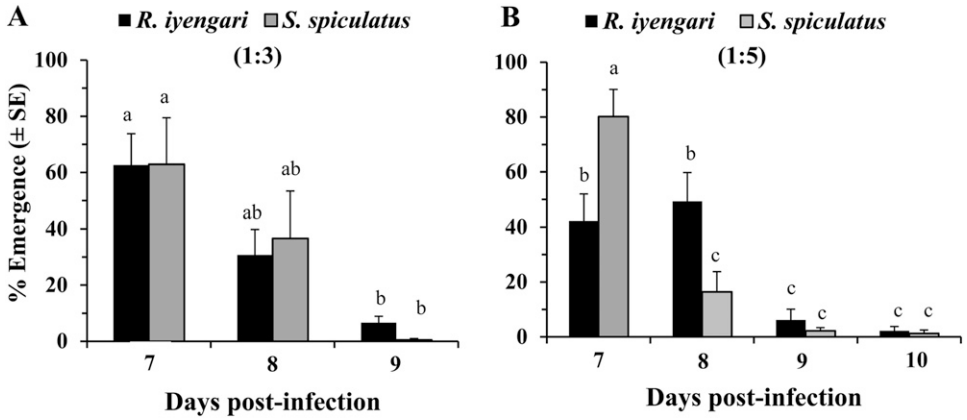

FIG. 4. Daily emergence of total postparasites of *Romanomermis iyengari* and *Strelkovimermis spiculatus* from *Culex pipiens pipiens* larvae at two host-parasite inoculation ratios (1:3 and 1:5). Bars with the same letters are not significantly different (A) ($P \leq 0.05$), and (B) ($P \leq 0.01$).

at 1:5 (43.37 ± 2.64%) ($P > 0.05$). By day 3, all emerging postparasites were female at 1:3 compared with 68.41 ± 15.13% at 1:5. Emergence was protracted to day 4 at this later rate, presumably reflecting a need for extended developmental time as parasite load increases, and all emergents were females. However, protandry was not observed in *S. spiculatus* even at superparasitism levels of five or more nematodes per mosquito. There was no difference between *S. spiculatus* male and female emergence from the initial emergence day in *S. spiculatus* at a 1:3 host-parasite ratio (48.87 ± 1.13% males and 51.13 ± 1.13% females) ($P > 0.05$) (Fig. 5C). Females dominated by day 2, comprising 85.05 ± 10.09% ($P \leq 0.05$) of that day's emergence. Similar results were obtained at the 1:5 concentration ($P \leq 0.05$) (Fig. 5D).

The reasons for protandry in *R. iyengari* and its absence in *S. spiculatus* are unclear. Protandry is common and exists in several phyla. It is most frequently observed in species where females mate once, generating intense evolutionary pressure for males to reach sexual maturity faster or reach breeding sites earlier than competitors (Torbjorn and Wiklund, 1982). Females of *R. iyengari* and *S. spiculatus*, however, mate multiple times (Petersen, 1978; Torbjorn and Wiklund, 1982; Undeen et al., 1996). Petersen (1972) previously noted that males of *R. culicivorax* tended to emerge before females and attributed this to the earlier death of multiple-infected mosquitoes. We also made this observation for *R. iyengari*, but only at extreme parasite loads of seven or greater which was a rare (2.29%) occurrence.

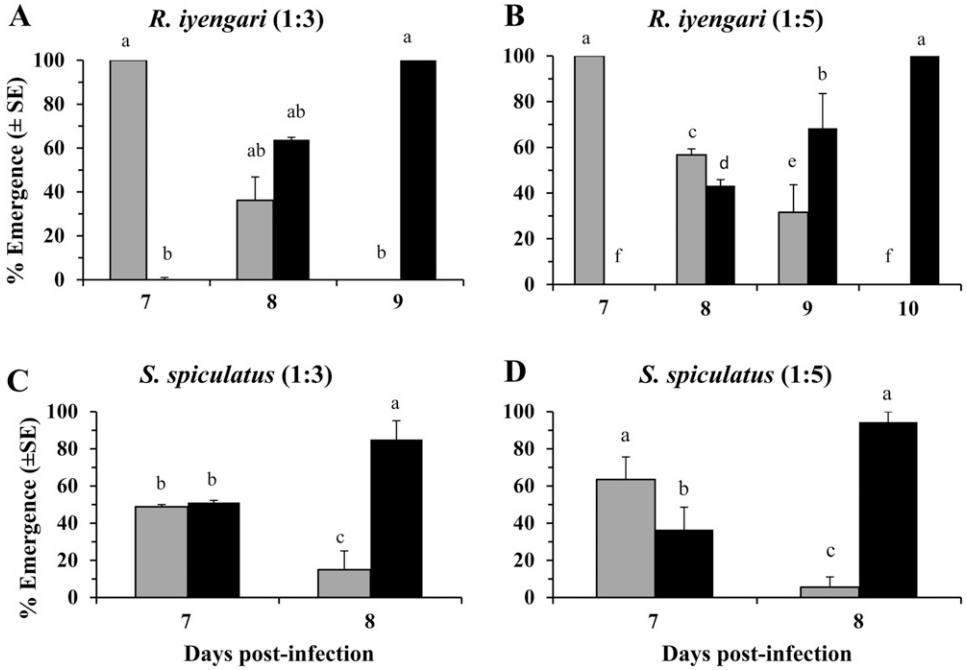

FIG. 5. Daily emergence of male (gray bars) and female (black bars) postparasites (J3) of *Romanomermis iyengari* and *Strelkovimermis spiculatus* from *Culex pipiens pipiens* larvae at two different host-parasite inoculation ratios (1:3 and 1:5). Bars with same letters are not significantly different ($P \leq 0.05$).

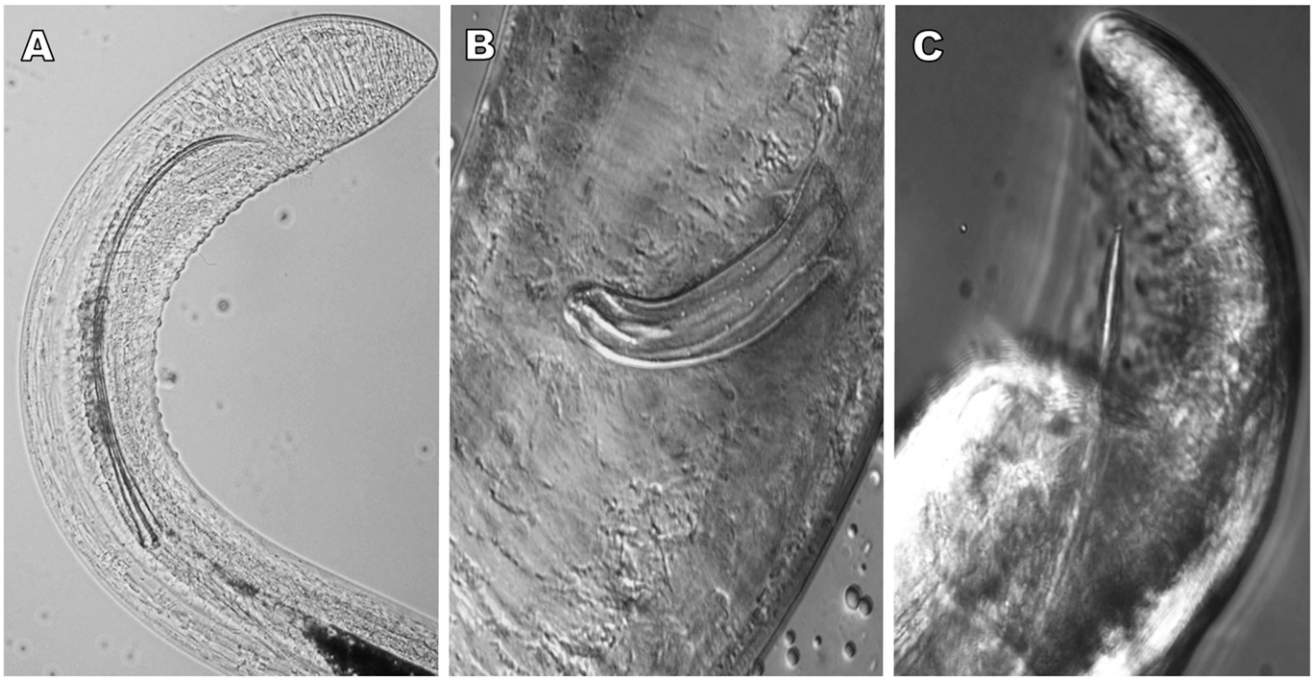

FIG. 6. Spicules of adult male (A) *Romanomermis iyengari* and (B) *Strelkovimermis spiculatus*. (C) Distal portion of spicule of *R. iyengari* male immediately after forced mating separation, showing extent of spicule protrusion during mating.

Moreover, even at extreme parasite loads we did not note this in *S. spiculatus* ($P \leq 0.05$).

If there is one striking difference between the two species it is spicule morphology (Fig. 6): *R. iyengari* possesses unusually long (478 μm), thin, needle-like spicules (Fig. 6A), whereas *S. spiculatus* has short (94 μm), thick, blunt spicules (Fig. 6B). Yet *R. iyengari* females have a short vagina, so that less than 15% of spicule length is inserted during mating (Fig. 6C and unpublished observations). Therefore spicule morphology does not appear to be designed exclusively for sperm transfer. We hypothesize that these lengthy spicules may be deployed in male-male aggressive behaviors within mating clusters. Although behaviors are difficult to observe in clusters, we have multiple times detected *R. iyengari* using their spicules to snag competitors and expel them from the cluster in a swift whip-like movement. Male expulsion behavior was not observed against females. Further, we frequently detected dead males in *R. iyengari* during early stages of mating cluster formation, whereas dead females were rare, hinting that the sharply pointed spicule tips could be wielded in sexual conflict. There is precedent for this behavior, as penis fighting has been reported, for example, from flatworms (Michiels and Newman, 1998). In short, we offer the working hypotheses that protandry in *R. iyengari* accelerates male maturation to the adult stage to equip males earlier to combat competing males. Mermithids may offer a window into a topic virtually unstudied in nematodes: male-male competition for mates.

The host-parasite interactions between mermithids and their mosquito hosts are highly sophisticated and deserving of examination. But their study has been driven by their long-held but unrealized promise for biological control. Despite studies demonstrating efficacy for mosquito control, mermithid advantages over chemical insecticides including safety, specificity, registration, and lethality, are vastly offset by the unfavorable economics of mass production (Petersen, 1985). There are no prospects currently envisioned for mermithid development as commercial products. The single viable strategy for mermithid deployment in the future is a nonprofit model where public health is the prime goal; that is, a government not a business model. New Jersey provides a template. Here the state assumes responsibility for mass production of mosquito fish and copepods, which are provided cost-free to county mosquito control agencies for release into mosquito habitats. Exploiting this model for mosquito mermithids will require an expanded portfolio of biocontrol-ready species coupled with an enhanced understanding of their life cycles. Our study is intended to contribute to this future pathway, as well as to generate new interest in a field that has been nearly moribund since the arrival of *B.t. israelensis* and the departure of mermithid icon James J. Petersen.

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