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Source: Systematic and Applied Acarology, 27(11) : 2258-2268

Published By: Systematic and Applied Acarology Society

URL: https://doi.org/10.11158/saa.27.11.11

Systematic & Applied Acarology 27(11): 2258–2268 (2022)
https://doi.org/10.11158/saa.27.11.11

ISSN 1362-1971 (print)
ISSN 2056-6069 (online)

Article http://zoobank.org/urn:lsid:zoobank.org:pub:22550777-3C80-4FF5-9A8E-E1F1B1BDDD9F

Aspects of the biology, behavior and mating of *Uroactinia* sp. (Acari: Mesostigmata: Uropodina: Uroactiniidae)

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Abstract

The mating process in *Uroactinia* sp. (Mesostigmata: Uropodina: Uroactiniidae) is described. Mating is venter to venter with the male on top. Spermatophore production is relatively slow, and both partners cooperate in emptying the spermatophore. Observations on mating behavior are compared with those for other Uropodina. Spermatophore morphology and the process of spermatophore formation appear to be similar to those described in ticks (Ixodida).

Key words: Mating behavior, spermatophore

Introduction

A general outline of the mating behavior and sperm transfer in Mesostigmata is well established. Mesostigmata use direct sperm transfer, but in two different ways (Alberti 2002; Alberti & Coons 1999; Walter & Proctor 2013). In Dermanyssidae and Heterozerconidae the males have a spermatodactyl on, respectively, the movable and fixed digit of the chelicera, which they use to transfer sperm to the secondary genital opening(s) of the females—podospermy *sensu* Athias-Henriot (1968). The alternative, and presumably the more ancestral condition, involved males using unmodified chelicerae to transfer a spermatophore to the primary genital opening of the females—tocospermy. The latter condition is also found in Ixodida and suspected for Opilioacarida and Holothyrida (Alberti & Coons 1999 and references therein). Mating and sperm transfer have been studied in considerable detail in Ixodida (e.g. Coons & Alberti 1999), but detailed descriptions of sperm transfer in individual taxa of tocospermous Mesostigmata are still relatively rare (Marquardt & Kaczmarek 2013).

The focus of this study is on one tocospermous lineage of Mesostigmata, the Uropodina. Studies on mating behavior in Uropodina include Radinovsky (1965) for *Leiodinychus orbicularis* (Koch) (Trematuridae), Faasch (1967) for *Uropoda orbicularis* (Müller) (Uropodidae) and *Fuscuropoda marginata* (Koch) (Urodinychidae), Woodring and Galbraith (1976) for *Uroactinia agitans* Banks (Uroactiniidae), Compton and Krantz (1978) for *Caminella peraphora* Krantz & Ainscough (Trachytidae), Athias-Binche (1981a) for *Oodinychus alveola* Athias-Binche (Trematuridae) and Marquardt and Kaczmarek (2013) for *Oodinychus ovalis* (Koch). Comparisons among these studies are complicated because they each focus on slightly different aspects of mating behavior, but it is clear that in addition to broad similarities there are also some distinct differences (Marquardt &

Kaczmarek 2013). Second, the process of spermatophore formation in Parasitiformes has been described for a few ticks (Feldman-Muhsam 1967; Feldman-Muhsam & Borut 1978) but not (yet) for Uropodina, although elements of the structure of the spermatophore have been described for some Uropodina (Athias-Binche 1981b; Faasch 1967; Marquardt & Kaczmarek 2013; Radinovsky 1965; Woodring & Galbraith 1976).

The availability of an undescribed species of *Uroactinia* (Uroactiniidae) in culture allowed new observations on some aspects of mating behavior and spermatophore formation in this species, supplementing previous studies for Uropodina in these areas.

Materials and methods

Material

Uroactinia n.sp. (Uroactiniidae). A taxonomic description of this species is in progress. Voucher specimens have been deposited at OSAL (Ohio State University Acarology Collection, Columbus) (5 females, 5 males, 1 slide of spermatophore), UAEQROO (Universidad Autónoma del Estado de Quintana Roo, Chetumal) (10 females, 10 males, 1 slide of spermatophore), and UNAM (Acarology Collection of the Universidad Autónoma de Mexico, Mexico City) (5 females, 5 males, 1 slide of spermatophore).

Rearing methods. Mites for use in this study were obtained from rotting fruit like mango, banana and orange, that had been maintained in a tray on top of a litter layer (Fig. 1). The tray was maintained in an open but shaded place (Fig. 2, 3B). The mites were then extracted from a piece of decaying orange peel and transferred to a culture box. The unit was maintained at about 20 °C for 10 h / day, and at 18–20 °C room temperature for the remainder of the day. The mites were permanently in the shade, except during the time they were being examined. The lid of the culture box had a hole for ventilation, covered with fine screen to prevent mites from escaping (Fig. 3A). The substrate for the culture box included burnt pieces of trunks and leaves rather than organic charcoal (Fig. 3C). Drops of water were added about once a week to maintain humidity inside the box. The specimens were fed by adding one spoonful of fresh manure, collected in the field, to the culture box once a week, while the dry manure was removed. Notably, the culture box contained large numbers of mites (Fig. 3B).

Behavioral observations. Mites were observed under a dissecting microscope (Zeiss, Stemi-2000-c) twice a day, each time for a period of one hour. The observation periods started at 10 AM and 3 PM, respectively. Three layers of colored paper (green, blue and red) were used to cover the LED light source of 35 thousand lumens, to obtain a black-light effect, and diminish the amount of light and heat produced in order to reduce mite disturbance. The observations were recorded using a Sony Exwave HAD video camera connected to a computer.

Slide preparation and slide-based observations. After observations mites used were mounted on microscope slides in Hoyer´s medium for examination under a Zeiss Axioscope A1 phase contrast compound microscope. Three spermatophores were obtained and mounted separately on microscope slides. One of these was taken from the mouth parts (chelicerae) of a male, another was collected from the substrate. The drawings of the spermatophores were done with the aid of a drawing tube. Measurements of the spermatophores are in micrometers (μm) and taken as indicated in Fig. 4.

FIGURE 1. Rearing substrate for *Uroactinia* sp., rotting fruit and vegetables.

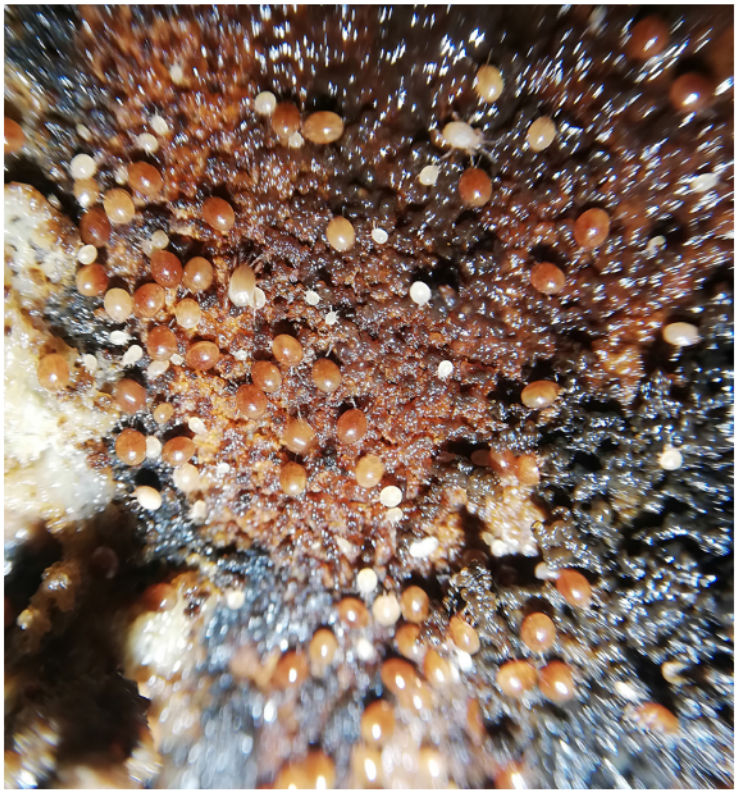

FIGURE 2. *Uroactinia* sp. on compost.

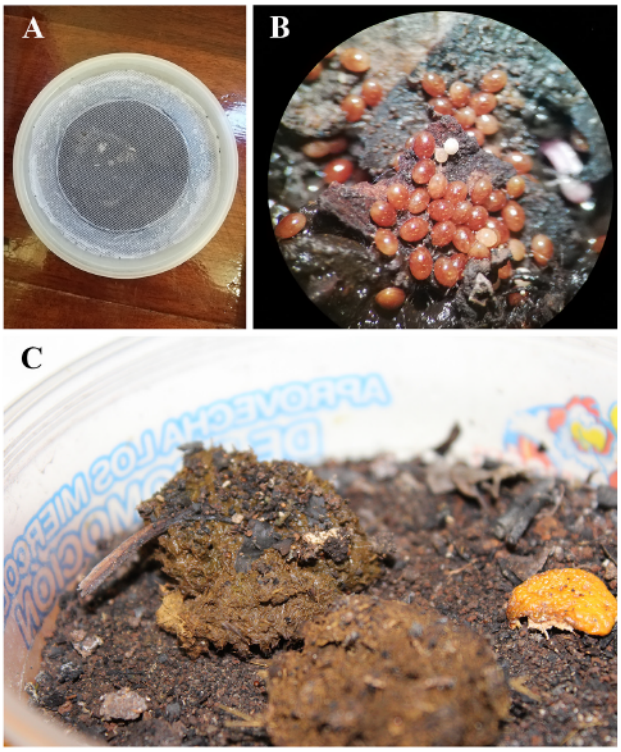

FIGURE 3. Culture box for *Uroactinia* sp. A, top view with mesh screen; B, mites in culture box; C. overview habitat.

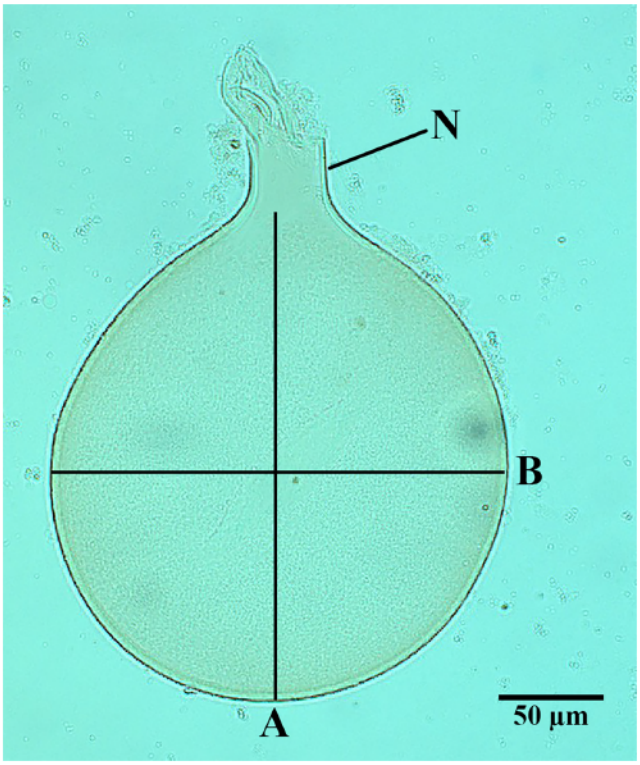

FIGURE 4. Measurements for ectospermatophore of *Uroactinia sp*, A, length; B, width. Labels: N, neck.

Results

Observations on the entire process from initial courtship to separation of partners was observed for at least 20 mating pairs. The process from start to finish for each couple lasted 40–60 min. Seven phases can be identified:

1. Courtship.
2. Positioning for sperm transfer.
3. Distancing between the venter of the partners and formation of the spermatophore.
4. Filling of the spermatophore.
5. Spermatophore transfer.
6. Spermatophore emptying.
7. Separation of the female and male.

Description of the individual phases

Phase 1) Courtship began with the male approaching the female and climbing on her dorsum; this process lasted 15–40 minutes. During this period, the male secreted a viscous liquid from its mouthparts (hypostome) on the dorsum of the female (Fig. 5A–B). The male changed body position repeatedly, possibly to stimulate the female (video clip 1; https://youtu.be/BhiefAcxMlo). Notably, there did not appear to be an optimal angle for males to take. Males could position themselves aligned with their female partner, at a 90° angle, or in reverse position (Fig. 5A–C). Females were relatively quiet in this phase. Phase 1 ended with the male in reverse position.

Phase 2) Positioning for sperm transfer. While in reverse position, the male touched the posterior margin of the female dorsal shield with his chelicera and palps. At this time both partners remained quiet for around 2–3 seconds (Fig. 5C). After this, the male positioned itself lateral to the female and attempted to turn her upside down. If successful, he rapidly climbed on top of her (Fig. 5D-E). This phase lasted 5–7 min.

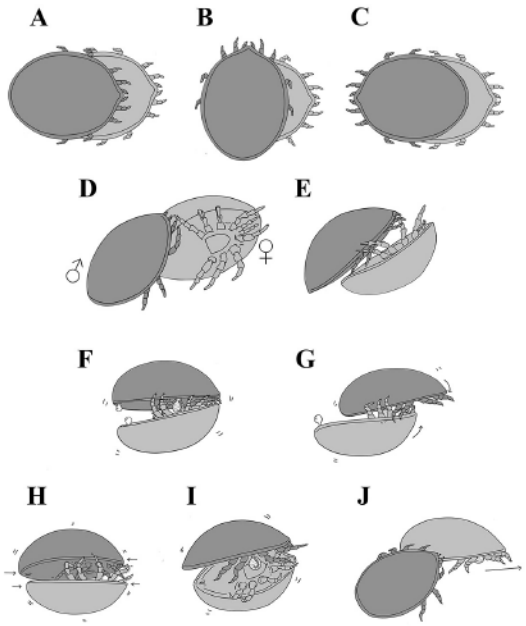

FIGURE 5. Courtship and mating behavior of *Uroactinia* sp. Female light gray, male dark grey.

During phases 1 and 2 the females could end the process by walking away, presumably rejecting the male. However, this was relatively uncommon.

Phase 3) Distancing and formation of the spermatophore. On at least 20 occasions we observed the males and females in venter-to-venter position with the male on top (Fig. 5F), holding the female using legs III–IV. Although close, the male and female maintained some distance between their bodies. More specifically, the anterior parts of their bodies were closely adjacent while the posterior regions were separate (Fig. 5F). This phase lasted 3–5 min.

During this phase the formation of the spermatophore started with the emergence of a small bubble which grew rapidly over 3–5 minutes (video clip 2; https://youtu.be/_97I_hMgn5Q). Both male and female manipulated the developing spermatophore with their chelicerae and palps. Around this time both partners also excreted a drop of unidentified liquid from their anus, first in the female, soon after in the male (Fig. 5F; video clip 3; https://youtu.be/NL5fjjrFkBk).

Phase 4) Filling of the spermatophore. In this phase the spermatophore was clearly visible. Examination of the three spermatophores of *Uroactinia* sp. from the culture box allowed us to add some details. The ectospermatophore in *Uroactinia* is bag-like and its contents appears hyaline or milky. The evaginating endospermatophore showed a well-developed neck with curved sperm ducts terminating into roundish capsules (Figs. 7, 8A). Woodring & Galbraith (1976) reported a spermatophore size of "at least 100 µm" for *Uroactinia agitans*, and Radinovsky (1965) reported a diameter of 210 µm for the spermatophore of *L. krameri*. Our measurements show a noticeably greater size, with maximum length 310 ± 0 and width 272 ± 2 (270–275) (N=3).

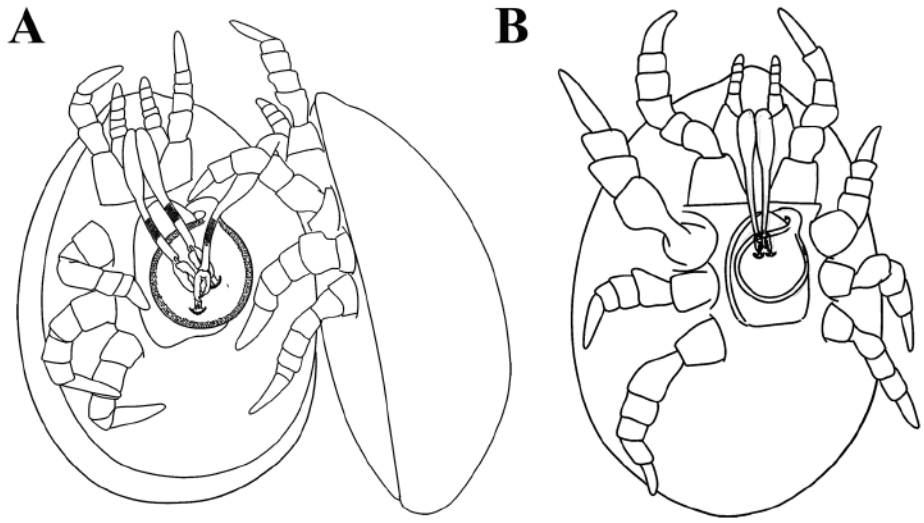

FIGURE. 6. Semi-schematic drawings of spermatophore manipulation in *Uroactinia* sp. A, male and female; B, female only.

Phase 5) Spermatophore transfer. The spermatophore was transferred to the outside of the female genital shield by the male using its chelicera (Figs. 6, 9), but apparently with assistance of the female. There were hints of involvement of the umbrella-like membranous extension of the fixed digit (Fig. 9A) but this could not be confirmed. In this phase, the female slid partially away from under the male seemingly to get space to use her mouthparts (chelicera and palps) (Figs. 5G, 6; video clip 4, 5; https://youtu.be/LapmQRVxj58; https://youtu.be/zMEhJ-mu3aY). The chelicera and palps of the female were now at the level of the genital plate of the male, actively palpitating the male.

Meanwhile the male was touching the venter of the female with its legs III and IV. This phase lasted 3–4 min (video clip 6; https://youtu.be/m87gq87BjYE).

Phase 6) Spermatophore emptying. In this phase the pair re-established the venter-to-venter position, holding each other in place using legs II and III. Next, both female and male pushed towards each other several times, although most effort seemed to come from the female. She used legs IV to pull the body of the male towards her (Fig. 5H). This phase lasted 5–7 min. The effect of this behavior is most likely the complete emptying of the spermatophore into the genital atrium of the female.

Phase 7) Separation of the female and male. Once they finish pushing, the male moved away, and the female straightened herself. She continued to carry the empty spermatophore for some time (Fig. 5J). This phase lasted 3–4 min. Interestingly, although the behavior in phase 6 is assumed to empty the spermatophore by pressure, the spermatophore did not appear to be crushed, perhaps indicating some elasticity in this structure.

Discussion

Our comments are focused on two topics, first the spermatophore and second the general mating behavior.

According to the literature, spermatophores in Parasitiformes are not formed internally and extruded, they are formed externally. For example, in Argasidae formation of the spermatophore starts with secretion of a "bubble" composed of the exterior of three layers forming the outer lining of the ectospermatophore (Feldman-Muhsam & Borut 1978). Next this balloon-like structure is filled with prospermia and seminal fluid, rapidly increasing its size (Feldman-Muhsam 1967; Feldman-Muhsam & Borut 1978). Formation of the spermatophore is completed with the formation of an endospermatophore that closes off the ectospermatophore. The endospermatophore in all species studied consists of a "plug" part and an external "neck" with some variability in the shape and size of the spermatophore parts. After attachment of the spermatophore to the female genital shield, the endospermatophore evaginates, entering the female genital atrium (Coons & Alberti 1999). In the process a pair of capsules is formed at the distal end of the endospermatophore which contain the prospermia (Feldman-Muhsam 1967).

Although spermatophore structure has not been studied in detail in Uropodina, available literature data and our observations suggest a structure and sequence of events similar to that described for Argasidae. For example, the formation of the initial bubble and the evagination of the endospermatophore in *Uroactinia* sp. terminating in two capsules (see Figs. 7, 8) match. Similarly, Faasch (1967) observed evagination and curving of the neck into the genital atrium. This suggest that many details of spermatophore formation and "action" may be shared among all tocospermous Parasitiformes. Notably, the mechanism of the evagination process and the means by which direction of the neck of the endospermatophore is determined are not fully resolved. Suggestions include creation of a partial vacuum in the genital atrium (Woodring & Galbraith 1976) or guidance by female and/or male chelicera.

While the general mating behavior is similar among all uropodine species studied, there are also some clear areas of variability. Mating position in most Mesostigmata features the male crawling under the female, but in *L. orbicularis* and *Uroactinia* the male is positioned on top of the female (Radinovsky 1965), and in *O. ovalis* orientation seems to be more gnathosoma down (Marquardt & Kaczmarek 2013). As a side note, the body morphology of many uropodids, heavily sclerotized with a strongly domed dorsum, almost requires a structured environment to achieve a stable mating position. In fact, without a way to "wedge" one partner may roll away from the other. Faasch (1967)

observed that both partners in *F. marginata* appear to require some level of physical contact with the environment. Absence of such contact often lead to termination of the mating sequence. Interestingly, the same was not true for *U. orbicularis* (Faasch 1967).

The distancing of the two bodies (phase 3), with one or both partners pushing away from each other, has also been reported by several authors (e.g. Athias-Binche 1981b; Radinovsky 1965). There is general agreement that this may be required to allow space for the formation of the spermatophore. Formation of the spermatophore can be very rapid, e.g. ~20 sec in *Oodinychus ovalis* (Marquardt & Kaczmarek 2013), and ~1 min for *L. orbicularis* (Radinovsky 1965), but this process took 3–5 minutes in the *Uroactinia* sp. specimens studied.

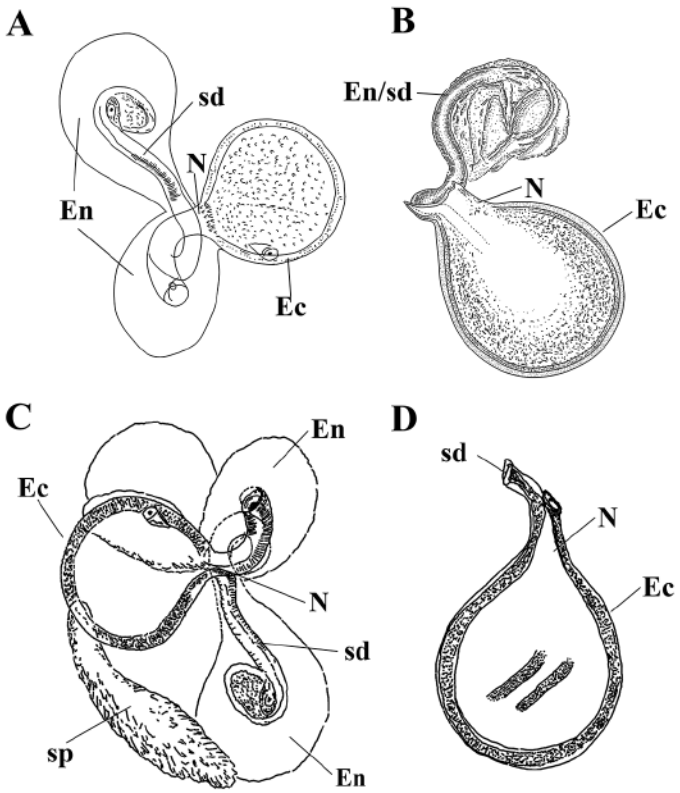

FIGURE 7. A–C. Semi-schematic drawings of the three spermatophores collected from males of *Uroactinia* sp. before sperm transfer; D, spent ectospermatophore with part of endospermatophore neck still attached. Labels: Ec, main section of ectospermatophore; En, endospermatophore capsules with sperm duct; N, neck of endospermatophore; sd, sperm duct; sp, sperm packet.

In phase 4–5, the spermatophore is transferred and attached externally to the female genital plate, usually near the anterior rim of the plate. All available data from tocospermous Parasitiformes suggest this is done by the male using his chelicera (at least in some cases holding the spermatophore by the neck; Fig. 9) with possible aid from the palps.

Based on the limited sample of studies available, one of the most variable aspects of the mating process in Uropodina may involve the emptying of the spermatophore (phase 6). In *Uropoda orbicularis* the male repeatedly inserts its chelicera into the female genital atrium, while in *Fuscuropoda marginata*, the female "pumps" using her genital shield (Faasch 1967). In *O. ovalis* and *Uroactinia* sp. the male and female press their bodies together, presumably to empty the

spermatophore into the female genital atrium (Marquardt & Kaczmarek 2013). The most bizarre option occurs in the semi-aquatic *C. peraphora*, where mating induces formation of a ring-shaped external spermatheca in the female (Compton & Krantz 1978). It is worth noting that the limited available data do not support an obvious phylogenetic correlation in distribution of these patterns (e.g. *Fuscuropoda* (Urodinychidae) and *Uroactinia* (in the closely related family Uroactiniidae) show quite distinct behaviors). Detailed observations on a larger diversity of species would be very helpful in that context.

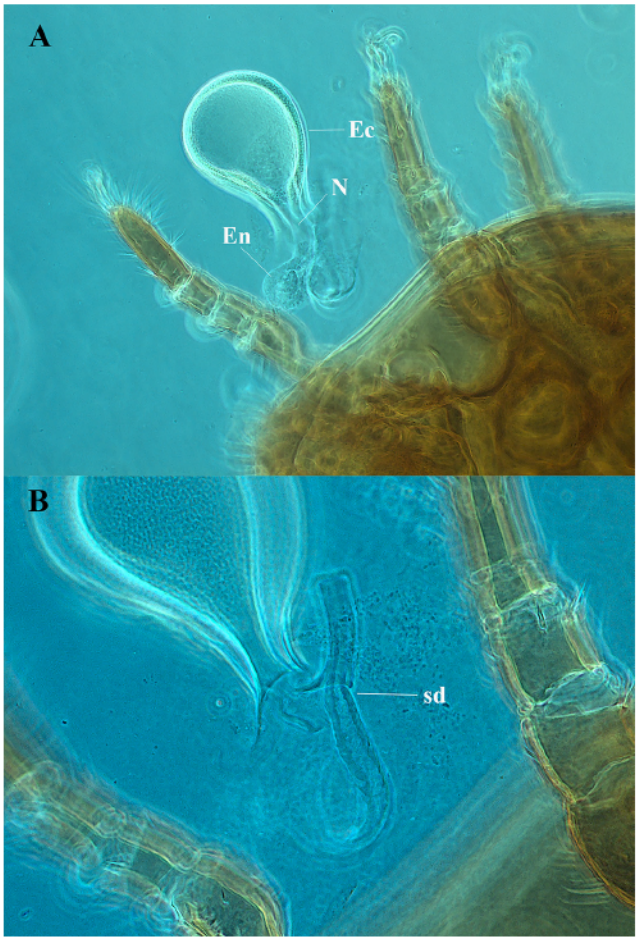

FIGURE 8. Spermatophore in *Uroactinia* sp. A. endospermatophore in process of evagination; B, detail of sperm duct. Labels: Ec, ectospermatophore; En, endospermatophore capsules; N, neck of the ectospermatophore; sd, sperm duct.

During separation of the mating pair (phase 7) *O. alveola* and *O. ovalis* are reported to consume the remnants of the ectospermatophore (Athias-Binche 1981a; Marquardt & Kaczmarek 2013). This has not been observed for other species including *Uroactinia* sp.

While the current observations answer some questions on uropodid mating behavior, they definitely raise new ones about specific details. Why do females palpitate (stimulate?) the males after the spermatophore has been transferred (phase 5)? One possible answer might be to keep the males engaged as their assistance seems necessary in the presumed emptying of the spermatophore (phase 6) but evidence for this is currently lacking. Second, questions concerning fluid production, based on a set of novel observations in this study. The first is the production of a liquid by the mouthparts

of the male in phase 1, the second involves near simultaneous liquid production from the anus by both male and female in phase 3. We hypothesize that the male secretions in phase 1 may function in courtship, but the exact function of either secretion is unknown. Neither has been reported in previous studies.

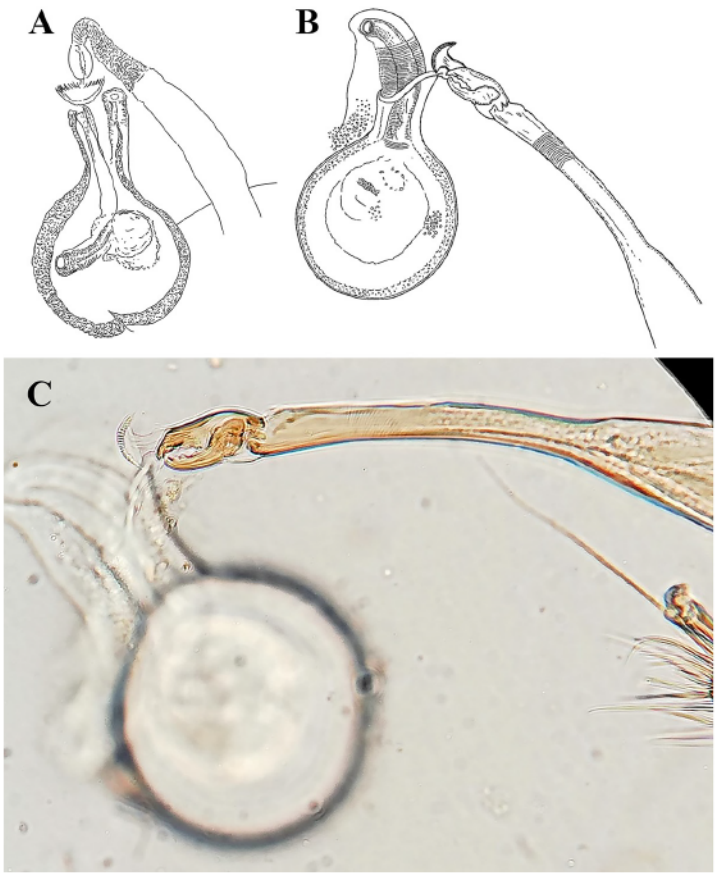

FIGURE 9. Different views of *Uroactinia* sp. Male manipulating a spermatophore with their chelicera.

Acknowledgements

We thank Dr. Antonella Di Palma and an anonymous reviewer for their detailed comments which significantly improved this study. The project was funded in part through U.S. National Science Foundation grant DEB 2017439 (H. K.).

Literature cited

Alberti, G. (2002) Ultrastructural investigations of sperm and genital systems in Gamasida (Acari: Anactinotrichida). Current state and perspectives for future research. *Acarologia*, 42, 107–126.

Alberti, G. & Coons, L.B. (1999) Volume C. Acari: Mites. *In:* Harrison, F.W. & Foelix, R.F. (Eds.) *Microscopic Anatomy of Invertebrates. Vol. 8. Chelicerate Arthropoda*. New York, NY, John Wiley & Sons, Inc., pp. 515–1215.

Athias-Binche, F. (1981a) Contribution a la connaissance des uropodides libres (Arachnides: Anactinotriches)

de quelque écosystèmes forestiers européens, 308 pp. Ph. D. thesis, Univ. P. M. Curie Paris VI, Paris.
Athias-Binche, F. (1981b) Écologie des Uropodides édaphiques (Arachnides: Parasitiformes) de trois écosystèmes forestiers. 1. Introduction, matériel, biologie. *Vie et Milieu*, 31, 137–147.
Athias-Henriot, C. (1968) L'appareil d'insémination laelapoïde (Acariens anactinotriches: Laelapoidea). Premières observations. Possibilité d'emploi à des fins taxonomiques. *Bulletin Scientifique de Bourgogne*, 25, 175–228.
Compton, G.L. & Krantz, G.W. (1978) Mating behavior and related morphological specialization in the uropodine mite, *Caminella peraphora*. *Science, Washington, D.C.*, 200, 1300–1301.
https://doi.org/10.1126/science.200.4347.1300
Coons, L.B. & Alberti, G. (1999) Volume B. Acari: ticks. *In:* Harrison, F.W. & Foelix, R.F. (Eds.), *Microscopic Anatomy of Invertebrates. Vol. 8. Chelicerate Arthropoda*. New York, NY, John Wiley & Sons, Inc., pp. 267–514.
Faasch, H. (1967) Beitrag zur biologie der einheimischen Uropodiden *Uroobovella marginata* (C.L. Koch 1839) und *Uropoda orbicularis* (O.F. Müller 1776) und experimentelle Analyse ihres Phoresieverhaltens. *Zoologische Jahrbücher für Systematik*, 94, 521–608.
Feldman-Muhsam, B. (1967) Spermatophore formation and sperm transfer in *Ornithodoros* ticks. *Science*, 156, 1252–1253.
https://doi.org/10.1126/science.156.3779.1252
Feldman-Muhsam, B. & Borut, S. (1978) Further observations on spermatophore formation in argasid ticks. *Journal of Insect Physiology*, 24, 693–697.
https://doi.org/10.1016/0022-1910(78)90066-5
Marquardt, T. & Kaczmarek, S. (2013) Mating behaviour of *Trichouropoda ovalis* (Acari: Mesostigmata: Uropodina: Trematuridae) with notes on phylogeny of reproductive biology in the Parasitiformes. *International Journal of Acarology*, 39, 369–376.
https://doi.org/10.1080/01647954.2013.800133
Radinovsky, S. (1965) The biology and ecology of granary mites of the Pacific Northwest. IV. Various aspects of the reproductive behavior of *Leiodinychus krameri* (Acarina: Uropodidae). *Annals of the Entomological Society of America*, 58, 267–272.
https://doi.org/10.1093/aesa/58.3.267
Walter, D.E. & Proctor, H.C. (2013) *Mites: Ecology, Evolution & Behaviour*. Dordrecht, Springer, 494 pp.
Woodring, J.P. & Galbraith, C.A. (1976) The anatomy of the adult uropodid *Fuscouropoda agitans* (Arachnida: Acari), with comparative observations on the other Acari. *Journal of Morphology*, 150, 19–58.
https://doi.org/10.1002/jmor.1051500103

Submitted: 12 May 2022; accepted by Zhi-Qiang Zhang: 23 Aug. 2022; published: 19 Oct. 2022