J. Parasitol., 104(4), 2018, pp. 359–371

LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDIES OF *UNIONICOLA TETRAFURCATUS* (ACARI: UNIONICOLIDAE) INFECTING FOUR FRESHWATER BIVALVE SPECIES AND HISTOPATHOLOGICAL EFFECT ON ITS HOSTS

Rewaida Abdel-Gaber[1,2], Mona Fol[2], and Saleh Al Quraishy[1]

[1] Zoology Department, College of Science, King Saud University, Riyadh-11451, Saudi Arabia.
[2] Zoology Department, Faculty of Science, Cairo University, 12631 Cairo, Egypt.
Correspondence should be sent to Rewaida Abdel-Gaber at: *rewaida@sci.cu.edu.eg*, *rabdelgaber.c@ksu.edu.sa*

ABSTRACT: Water mites of the genus *Unionicola* are the most common symbionts of freshwater bivalves. During the current investigation, a total of 120 live freshwater mussels representing 5 species, *Corbicula fluminea* (Veneroida), *Coelatura aegyptiaca* (Unionoidea), *Mutela rostrata*, and *Chambardia rubens* (Mutelidae), were collected from 2 localities in Tura (Helwan Governorate) and El Kanater (Qaluobiya Governorate), Egypt. Only 3 of the 4 bivalve species listed are considered freshwater bivalves (members of Unionoidea). *Corbicula fluminea* belong to the family Cyrenidae within Veneroida. Collected mussels were dissected and examined for the presence of unionicolid mites. It was found that 30.83% (37/120) were infected with a single mite species, *Unionicola tetrafurcatus* (Unionicolidae). The highest prevalence was observed during the summer with 83.33% (25/30) whereas the least was observed in autumn, i.e., 33.33% (10/30). Mites were recovered from the gills, gonads, and visceral mass of mussel hosts. Gills of host mussels were the primary site of oviposition for *Unionicola* mites. Smaller bivalves in size had significantly greater numbers of mites than did larger ones in size. Numbers of mites per host species was variable and the highest prevalence level of 83.33% (25/30) was recorded in *Cor. fluminea* while the lowest one of 16.66% (5/30) was found in *Ch. rubens*. Morphological and morphometric characterizations of mites revealed some differences between the present species and other related *Unionicola*. Histopathological responses of host mussels to the eggs, larvae, and cuticular remnants of *U. tetrafurcatus* were also studied. Therefore, the present study demonstrated that freshwater bivalves have a new host and locality records for infection with *U. tetrafurcatus*. Future studies are recommended to include advanced molecular characteristics for these mites.

Freshwater bivalves occurring in Egypt represent a neglected animal group, and little is known about them or their diversity (Ramadan, 2004a, 2004b; Sleem and Ali, 2008). They are considered as a supplemental protein source for humans (El-Assal et al., 2014). Ectoparasites have often been shown to have profound effects on host life-history parameters and fitness (Smith, 1988; Lehmann, 1993; Smith et al., 2010). In general, parasites may have a negative effect on host reproductive success (Luxton, 1990; Mùller, 1990a; Polak and Markow, 1995; Polak, 1996), reduce body mass or condition (Mùller, 1990b; Vidrine, 1996b; Polak and Starmer, 1998), or reduce life span (Forbes and Baker, 1991; Polak and Starmer, 1998). The role of parasites in the evolution, ecology, and conservation of freshwater bivalves (Bivalvia: Unionoida) remains poorly understood (Mitchell, 1955; Fuller, 1974; Smith and Oliver, 1986; U.S. Environmental Protection Agency, 1992).

Water mites of the genus *Unionicola* (Haldeman, 1842) (Acari: Unionicolidae) are cosmopolitan and have parasitic association with bivalve and gastropod molluscs as well as with sponges (Mitchell, 1955; Davids, 1973; Ramadan, 2003a; Edwards and Vidrine, 2013). Many species of the genus *Unionicola* were described from different regions in the world such as Europe, South and North America, Asia, Africa, and Australia (Vidrine, 1996a, 1996b; Aboul-Dahab, 1998). Water mites infecting freshwater mollusks comprise at least 247 nominal species assigned to 3 genera and 3 families (Hygrobatidae, *Dockovdia* with 2 species; Pionidae, *Najadicola* with 1 species; Unionicolidae, *Unionicola* with 244 species) (Conroy, 1974; Gledhill, 1985; Baker, 1991). In Egypt, only 5 species of unionicolid mites, namely *Unionicola anodontae*, *Unionicola niloticus*, *Unionicola palpatus*, *Unionicola tetrafurcatus*, and *Unionicola difurcatus*, were described from freshwater mussels by Aboul-Dahab (1998), Aboul-Dahab et al. (1998), and Ramadan and Aboul-Dahab (2002).

The life cycles of unionicolids are complex, and species complete their entire life cycle exclusively in bivalves (Jones, 1965; Smith and Oliver, 1986; Pennak, 1989; Ramadan and Aboul-Dahab, 1999a, 1999b, 2000), aquatic insects, and other arachnids (Smith et al., 2010). The vast majority of *Unionicola* spp. has been reported in chironomid (Diptera, Chironomidae) larvae or other aquatic invertebrates (Edwards and Dimock, 1995; Smith et al., 2010). Adult mites move about within the mantle cavity and some species consume host mucus and gill tissue (Fisher et al., 2000). Mite eggs are deposited in mussel gill tissues, mantle, labial palps, and integument of the visceral mass (Gordon et al., 1979). Larval mites also can encyst in mussel gill or mantle tissues prior to nymphal transformation (Fisher et al., 2000). Gills of freshwater bivalves serve both as respiratory/excretory organs and marsupia for the developing bivalve larvae (Forbes and Baker, 1991; Aboul-Dahab et al., 1996a, 1996b, 1997a, 1997b). Thus, it is possible that parasitic mites interfere not only with mussel gas exchange and nitrogenous waste removal but also glochidial development or retention, even if glochidia are not directly consumed (Edwards and Vidrine, 2013).

Regarding histopathology, Faust (1918), Mitchell (1955), Wu et al. (2008), and McElwain et al. (2016) characterized early life-history stages of *Unionicola* spp. embedded in mantle cavity tissues whereas Baker (1976) described tissue damage associated with mite appendages. Faust (1918) described *Unionicola aculeata* embedded in the connective tissue of mantle and foot of *Lampsilis*

Received 18 February 2018; revised 2 March 2018; accepted 16 March 2018.
DOI: 10.1645/18-31

siliquoidea Barnes (1823). Mitchell (1955) characterized the life cycle of *Najadicola ingens*, *U. aculeata*, *Unionicola abnormipes*, *Unionicola fossulata*, and *Unionicola serrata* and illustrated eggs in mantle, nymphochrysalis in gill, and teleochrysalis in foot and siphon of *L. siliquoidea*. Mitchell (1955) reported surface projections associated with embedded larvae and hyperplasia associated with eggs of *Unionicola* spp. Tissue damage and hemocytic infiltration associated with tarsal claw penetration and feeding was reported from gills of *Anodonta anatina* infected with *Unionicola intermedia* (Baker, 1976).

Our investigation was intended to (1) report on the geographic distribution of unionicolid parasites in freshwater bivalves from 2 different localities at the Nile River in Egypt, with reference to the first host record of *Cor. fluminea* for this unionicolid parasite; (2) determine the total and seasonal prevalence of infection in different tissues of naturally infested bivalves; (3) obtain more information on the morphological features of *U. tetrafurcatus* using light and scanning electron microscopy; and (4) describe any pathological changes observed to the gill, reproductive tissue, and visceral mass of infected bivalves.

MATERIALS AND METHODS

A total of 120 adult freshwater bivalves were randomly collected monthly for 1 yr during the period of September 2015 to August 2016 from rocky, muddy areas along the Nile River, the first area being Tura (Helwan Governerate) and the second El-Kanater (Qaluobiya Governerate). After collection, the mussels were transported immediately to the Laboratory of Invertebrates at the Zoology Department, Faculty of Science, Cairo University, Egypt. Bivalves were kept in small containers provided with well-aerated water and sediments, then sorted and maintained in the laboratory under the same conditions regarding food and temperature of the original environment. Identification of the collected freshwater bivalves was performed according to Graf and Cummings (2007). The bivalves belong to the family Unionoidae and were identified as *Coelatura aegyptiaca* Cailliaud (1827) (Unionnidae), *Mutela rostrate* Rang (1835) (Iridinidae), and *Chambardia rubens* Lamarck (1819) (Iridinidae), in addition to *Cor. fluminea* Müller (1774) (Veneroida).

Samples were dissected and parasites were flushed from the gills, reproductive tissue, and visceral mass with deionized water. The prevalence (%) and seasonal changes for selected parasites were statistically analyzed according to the guidelines as stated by Bush et al. (1997). Mites removed from infected tissues were placed into a small glass dish filled with deionized water prior to fixation because any attached mucus or debris would be liberated from the setiferous appendages as the mites swim/crawl. Clean mites were then fixed in 2-ml cryogenic storage vials containing modified Koenike's fluid according to Edwards and Vidrine (2013) and labeled. Photomicrographs were taken using a LEICA DM-2500 microscope incorporated with a digital camera (NIS ELEMENTS software, ver. 3.8; Leica Microsystems, Wetzlar, Germany). Identification of parasitic mites was performed according to the keys of Edwards and Vidrine (2013). Measurements of the recovered mites were made with an Olympus ocular micrometer (Olympus Corporation, Tokyo, Japan) and expressed in micrometers (μm) as a range followed by mean ± SD in parentheses, unless otherwise stated. Voucher specimens were deposited in the museum at the Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt.

Scanning electron microscopy

Recovered parasites were fixed with 3% glutaraldehyde, then washed in sodium cacodylate buffer, dehydrated in a graded series of ethanol, and infiltrated with amyl acetate. After passing through an ascending series of Genesolv D, they were processed in a LEICA EM CPD300 critical point dryer (Leica Microsystems) with Freon 13, sputter-coated with gold–palladium in an auto fine coater (JEOL, JEC-3000FC, Tokyo, Japan), and were finally examined and photographed at 10-kV under an Etec Autoscan JEOL scanning electron microscope (JSM-6060LV) in the Electron Microscope Unit at Micro-analytical Center, Faculty of Science, Beni Suef University, Egypt.

Histological examinations

Infected portions of the soft parts of bivalves were fixed in Bouin's solution for 48 hr. The fixed samples were washed in tap water overnight and exposed to ascending concentrations of ethyl alcohol (70, 80, 90, and 100%), cleared in xylene, infiltrated with liquid paraffin at 58 C, and finally embedded in paraffin blocks. The prepared blocks were trimmed and sectioned at 5–8 μm thick, cut on a rotary microtome, stained with Harris' hematoxylin and counter-stained with eosin (H&E stain), and examined and photographed by a Zeiss research photomicroscope (Carl Zeiss Microscopy GmbH, Jena, Germany). Histological terminology for Unionidae follows McElwain and Bullard (2014).

RESULTS

Prevalence of mites in bivalves

A total of 37 out of 120 (30.83%) specimens from 4 freshwater bivalves, *Cor. fluminea*, *Coe. aegyptiaca*, *M. rostrate*, and *Ch. rubens*, were examined (Fig. 1A–D) and found to be naturally infected with a single mite species. Freshwater bivalves collected from the Tura region showed a higher infection of mite prevalence than did the El-Kanater region. Mites parasitized in the suprabranchial cavity, integument of the visceral mass, and reproductive follicles of the infected mussel species. Additionally, the highest seasonal prevalence of 83.33% (25/30) was in summer whereas the lowest was found in autumn with 33.33% (10/30). Number of mites per host was variable and the highest prevalence of 63.33% (19/30) was recorded in *Cor. fluminea* followed by 33.335 (10/30) in *Coe. aegyptiaca* and 20% (6/30) in *M. rostrate* while the lowest of 6.66% (2/30) was found in *Ch. rubens*. All infected bivalves were parasitized by mites identified as *U. tetrafurcatus* (Unionicolidae).

Morphological description (Figs. 2, 3)

Mites dark-colored, dorsoventrally flattened with symmetrical body, very small-sized acarines. Bodies formed of a combined head and thorax section, with an abdomen. On the dorsal plate, idiosoma often covered by single sclerotized plate, i.e., carapace (Fig. 2B, C, E, G, K). Gnathosoma or capitulum usually set off sharply from idiosome and carrying feeding appendages, i.e., chelicerae and pedipalps. Posteriorly, genital pore opened through highly sclerotized area known as genital plate covered

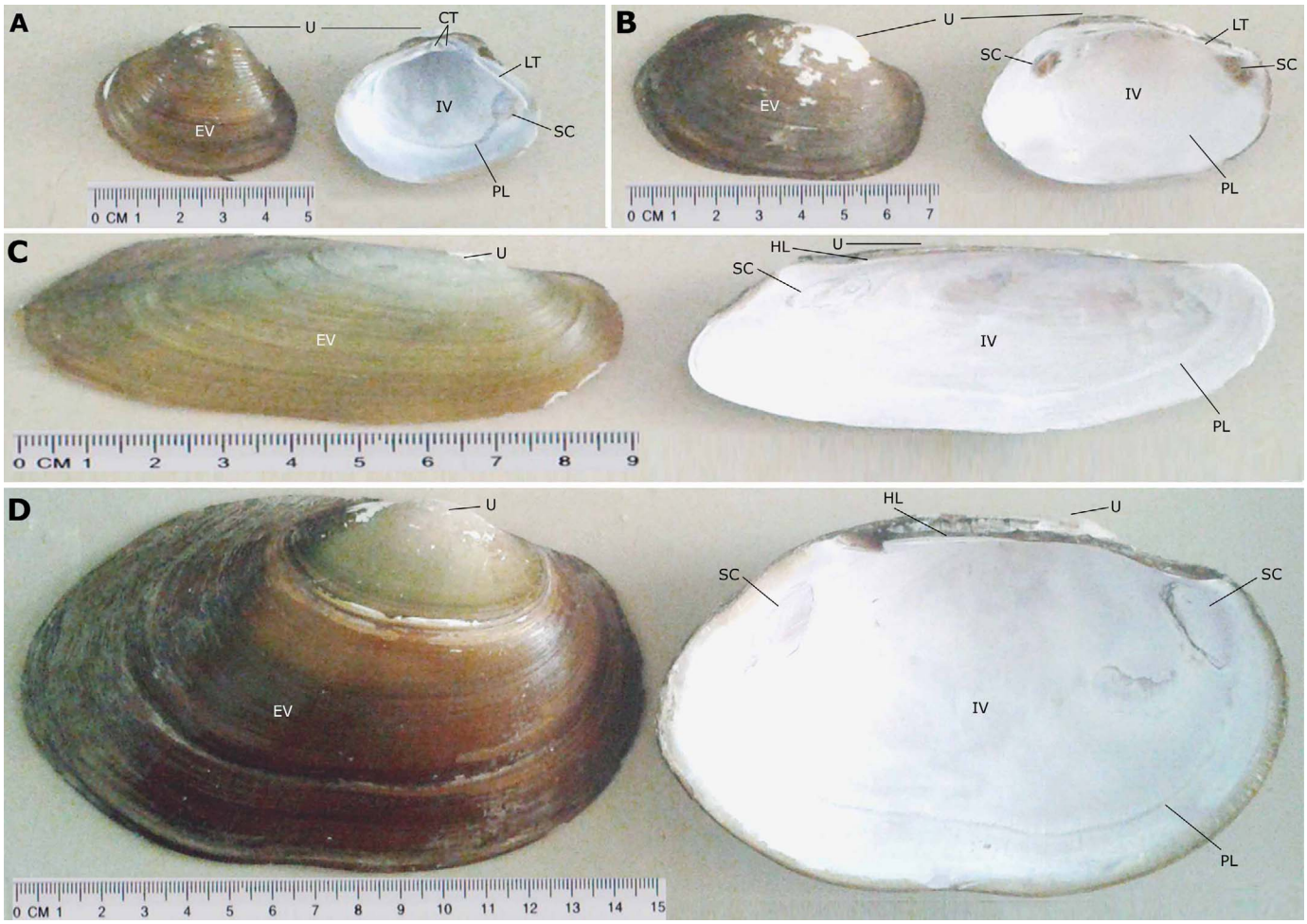

FIGURE 1. Photographs of the dorsal and ventral views of the 4 collected freshwater bivalves. (**A**) *Corbicula fluminea*. (**B**) *Coelatura aegyptiaca*. (**C**) *Mutela rostrata*. (**D**) *Chambardia rubens*. Abbreviations: CT, cardinal teeth; EV, external view; HL, hinge ligament; IV, inner view; LT, lateral teeth; PL, pallial line; SC, scar of adductor muscle; U, umbo. Color version available online.

with setae. The life cycle of mites includes 4 main stages; egg, larva, nymph, and adult.

Mite eggs were spherical in shape, varied from light cream when freshly laid through orange to nearly black when about to hatch, and reached about 0.1 ± 0.001 mm in length (Figs. 2L, K, 3H). Eggs formed a substantial, dome-like structure under cap including rim and ribs. The larval mite escaped by pushing aside the detached dome with its small adherent circle of shell membrane. The ribbed and frilled portion of the cap envelope was often left almost intact.

Eggs hatched into larvae (Fig. 2A). No apparent sexual dimorphism in the larval stage was observed. Larvae were pear shaped, more or less whitish, and very small (~190 μm in length and ~98 μm in width) with 3 pairs of legs. The nymph was lighter in color compared to any other mite life stages, with 4 pairs of legs that adhered tightly to the nymphal body as long as it remained enclosed within the skin of the larva until the adult stage was formed, and measured 390 × 130 μm in size (Fig. 2B). There was also apparent sexual dimorphism in the nymphal stage.

The adult was the reproductive stage with 4 pairs of legs (Fig. 2C). Males with oval body shape measured 479 ± 0.11 × 265 ± 0.09 μm in size, capitulum slender (Fig. 2D), genital field located at post-venter end and measured 40 ± 0.01 × 55 ± 0.02 μm in size, 1 pair of plates connected at posterior end, acetabula with latero-cleft on lateral margin of acetabular plates, pair of ventero-glandularia near genital field, ejaculatory complex reached about 20 ± 0.01 × 29 ± 0.01 μm in size, anal pore located at post-dorsum end of body (Figs. 2C–F, 3I–N). Palpal segments P-I short; P-II slightly stout bearing 4 spiculate spines; P-III with spiculate spine; P-IV with 3 papillous protrusions bearing seta, respectively, and dorsal seta; P-V curved and with 2 clawlets. Dorsal lengths of palpal segments: P-I 13, P-II 150 ± 0.11, P-III 49 ± 0.01, P-IV 134 ± 0.12, P-V 116 ± 0.10 μm; dorsal lengths of I leg segments: I-L-3 225 ± 0.12, I-L-4 397 ± 0.13, I-L-5 387 ± 0.13, I-L-6 278 ± 0.12 μm; dorsal lengths of IV leg segments: IV-L-3 374 ± 0.13, IV-L-4 443 ± 0.12, IV-L-5 534 ± 0.13, IV-L-6 498 ± 0.12 μm. Swimming setae on leg segments: I-L-2-5 2-5-10-14, II-L-2-5 1-3-5-6, III-L-2-5 1-21-10-12, IV-L-2-5 3-0-7-3; III-L-3-5 with 24-61-56 dorsal spines, IV leg sexually dimorphic, IV-L-4 bearing 20 spines and 5 elongate setae on dorsum, IV-L-6 with 5 long distal setae; IV-L-5 more slender than IV-L-4; bifid claws of legs with dorsal prong shorter than ventral prong (Figs. 2H–J, 3K, L).

FIGURE 2. Photomicrographs of the dorsal view of different stages of *Unionicola tetrafurcatus* showing: (**A**) Three-legged larva with IS provided with DP, CA bearing PP, and 3 pairs of swimming legs (I-L, II-L, III-L). (**B**) Four-legged nymph with IS provided with DP, CA bearing PP, the body covered with C, and 4 pairs of legs (I-L, II-L, III-L, IV-L). (**C**) Adult mite with well-developed IS provided anteriorly with CA bearing PP and posteriorly with GF and presence of 4 pairs of legs (I-L, II-L, III- L, IV-L); in addition, the body is covered with C. (D-L) High magnifications of: (**D**) CA bearing PP with palpal segments (P-I, P-II, P-III, P-IV, P-V) with observing the first and second pairs of legs (I-L, II-L); (**E**) Lateral portion of mite body showing C covering DP of the body and connecting with it 4 pairs of legs (I-L, II-L, III-L, IV-L); (**F**) Posterior portion of male mite with EC near GF and provided with S; (**G**) Posterior portion of female mite with well-developed GF surrounding GP; (**H**) Fourth swimming leg of adult stage with 6 segments (IV-L-1, IV-L-2, IV-L-3, IV-L-4, IV-L-5, IV-L-6) and surrounded with SP; (**I, J**) Posterior part of IV-L-6 of swimming leg ended with CL; (**K**) Posterior portion of the DP of female mite with EG. (**L**) EG. Abbreviations: C, carapace; CA, capitulum; CL, claws; DP, dorsal plate; EC, ejaculatory complex; EG, eggs; GF, genital field; GP, genital pore; IS, idiosoma; PP, pedipalp; S, setae; SP, spines. Color version available online.

Females with body color, palp, and claws of legs similar to those of males, body nearly ellipsoidal and flattened dorsum in shape, measured 565 ± 0.11 × 334 ± 0.10 μm in size, capitulum slender, genital field located at post-ventral end measured 56 ± 0.01 × 64 ± 0.01 μm in size, with 2 pairs of plates, anterior acetabular plates well sclerotized, with elongate anterior plates, 2 acetabula each and inner flap with 2 short spines on each side; posterior plates with 3 acetabula each and single, inner seta, anal pore located at post-dorsum end of body (Figs. 2G, 3A–E). Dorsal lengths of palpal segments: P-I 19 ± 0.01, P-II 129 ± 0.11, P-III 49 ± 0.02, P-IV 127 ± 0.11, P-V 92 ± 0.03 μm; dorsal lengths of I leg segments: I-L-3 256 ± 0.2, I-L-4 389 ± 0.19, I-L-5 365 ± 0.18, I-L-6 297 ± 0.10 μm; dorsal lengths of IV leg segments: IV-L-3 320 ± 0.12, IV-L-4 454 ± 0.13, IV-L-5 599 ± 0.12, IV-L-6 401 ± 0.11 μm. Swimming setae on leg segments: I-L-2-5 2-5-10-12, II-L-2-5 1-4-11-12, III-L-2-5 2-4-11-15, IV-L-2-5 2-3-8-7; III-L-3-5 with 13-22-20 spines on dorsum, IV-L-6 with 3 elongate setae on dorsum (Fig. 3F, G).

Histopathology

Histopathological findings revealed that uninfected gill tissue has a well-defined series of ciliated filaments, occasionally interrupted by ostia. Water tube walls consist of a thin layer of flattened cells, sometimes with an elliptical hemolymph vessel. Inner and outer lamellae are joined by septa, consisting of a simple columnar epithelium and underlying fibrous connective tissue (Fig. 4A, E). Female mites deposited their eggs longitudinally along the length of the interlamellar septa of the inner and outer demibranchs (Fig. 4B). Eggs and developing larvae were embedded subcutaneously in interlamellar septa throughout the ctenidia and typically oriented parallel along the long axis of a

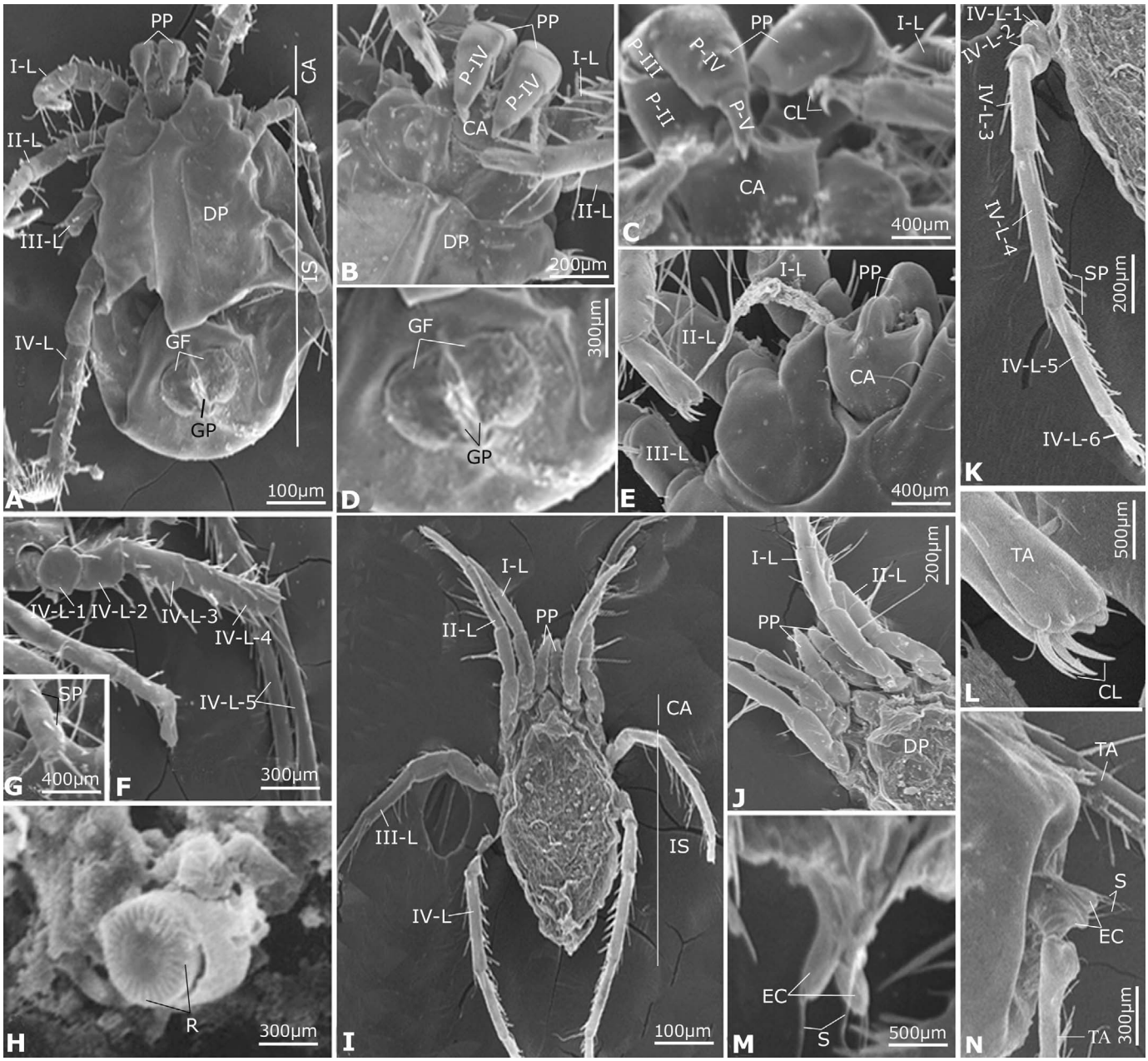

FIGURE 3. Scanning electron micrographs of the dorsal view of different stages of *Unionicola tetrafurcatus* showing: (**A**) Female mite with ventral view of IS and CA bearing PP, 4 legs (I-L, II-L, III-L, IV-L), and well-developed GF opened by GP. (**B–G**) High magnifications for different female body showing: (B, C, E) Anterior portion with CA bearing PP with palpal segments (P-II, P-III, P-IV) and with 3 pairs of legs (I-L, II-L, III-L); (D) GF opened by GP; (F, G) Fourth legs of female mite covered with numerous SP and segmented into IV-L-1, IV-L-2, IV-L-3, IV-L-4, IV-L-5; (**H**) Egg of the present species with R; (**I**) Adult male mite with IS provided with DP and CA bearing PP, and 4 legs (I-L, II-L, III-L, IV-L). (J–O) High magnifications for different male body showing: (**J**) Anterior portion with PP, DP, and with 2 pairs of legs (I-L, II-L); (**K**) Fourth legs of male mite with 6 segments (IV-L-1, IV-L-2, IV-L-3, IV-L-4, IV-L-5, IV-L-6) surrounded with SP; (**L**) Posterior part of swimming leg ended with CL at the TA. (**M, N**) Posterior portion of male mite with EC near genital field showing: (M) Ventral view with EC and S; (N) Lateral view with TA for legs, EC and S. Abbreviations: CA, capitulum; CL, claws; EC, ejaculatory complex; GF, genital field; GP, genital pore; IS, idiosoma; PP, pedipalp; R, ribs; S, setae; SP, spines; TA, tarsal segment.

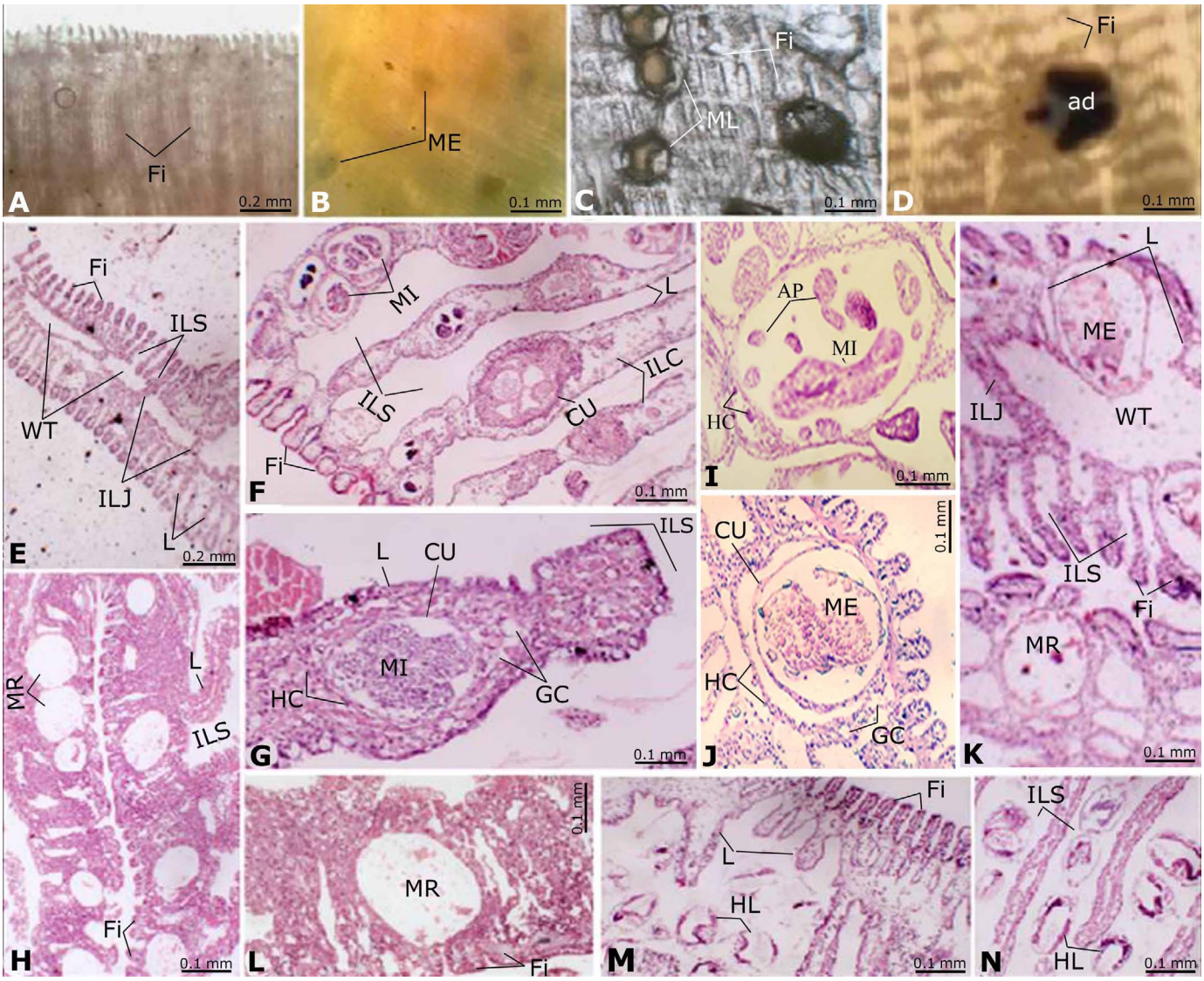

FIGURE 4. Gross anatomy images of gills of non-infected and infected freshwater bivalves stained with hematoxylin and eosin (H&E). (**A**) Non-infected gills. (**B–D**) Infected gills showing: (B) ME; (C) ML; (D) *Unionicola tetrafurcatus* at the stage of ad attached to the Fi. (**E–N**) Transverse histopathological findings stained with H&E stain of the gills of the freshwater bivalves showing: (E) Non-infected gill with normal architecture with Fi, inner and outer L joined by ILJ and separated by ILS underlying fibrous inter-lamellar connective tissue, and WT; (F–I) Infected gill of the bivalves contained its larva with embedded MI with its AP inside its cyst covered by CU surrounded by ILC, numerous HC and GC causing destruction for the main elements of the gills within L filled with inter-lamellar connective tissue and separated from each other by ILS; (J) ME within the gill lamellae surrounded by HC and GC; (K, L) Infected gills with degradation mite and appearing the MR in Fi, inner and outer L joined by ILJ and separated by ILS underlying fibrous inter-lamellar connective tissue, and WT; (M, N) Damaged L, ILS with observed HL. Abbreviations: ad, adult; AP, appendages; CU, cuticle; Fi, gill filaments; GC, goblet cells; HC, hemocytes; HL, host mussel larvae; ILC, interlamellar cuticle; ILJ, inter-lamellar junctions; ILS, inter-lamellar spaces; L, lamellae; ME, mite eggs; MI, mite; ML, mite larvae; MR, mite remnants; WT, water tubes. Color version available online.

water tube (Fig. 4C). A slight localized distension surrounded the eggs, and embedded mites in gill tissue were observed. Mite eggs were deposited in the demibranchs of infected bivalves while adults were found to be attached with their chelicerae to the gills, encountered in the tissue at the division of the inner and outer gill plates and observed crawling or swimming in the mantle cavity near the labial palps of live bivalves (Fig. 4D). Mites pierced the gills with their pedipalps, causing damage and inducing leukocytic infiltration and edema of the gill filaments (Fig. 4F–N). These mites typically had a surrounding hyaline membrane. Additionally, concentric layers of hemocytes and connective tissue fibers typically surrounded these embedded mites, and suprabranchial connective tissues were the only sites where such host responses were observed, resulting in mite degradation and the appearing of mite remnants (Fig. 4K–L). The integument surrounding visceral mass was a simple cuboidal epithelium interspersed with goblet cells (Fig. 5A). Thin layers of fibrous connective tissue supported the epithelium (Fig. 5B); below these layers were transverse and longitudinally oriented somatic musculature. Larvae of *Unionicola* sp. occupied the subcutaneous hemolymph sinuses (Fig. 5B–D). Furthermore, a thin layer of emarginated hemocytes and goblet cells near these embedded mites was observed (Fig. 5B, C).

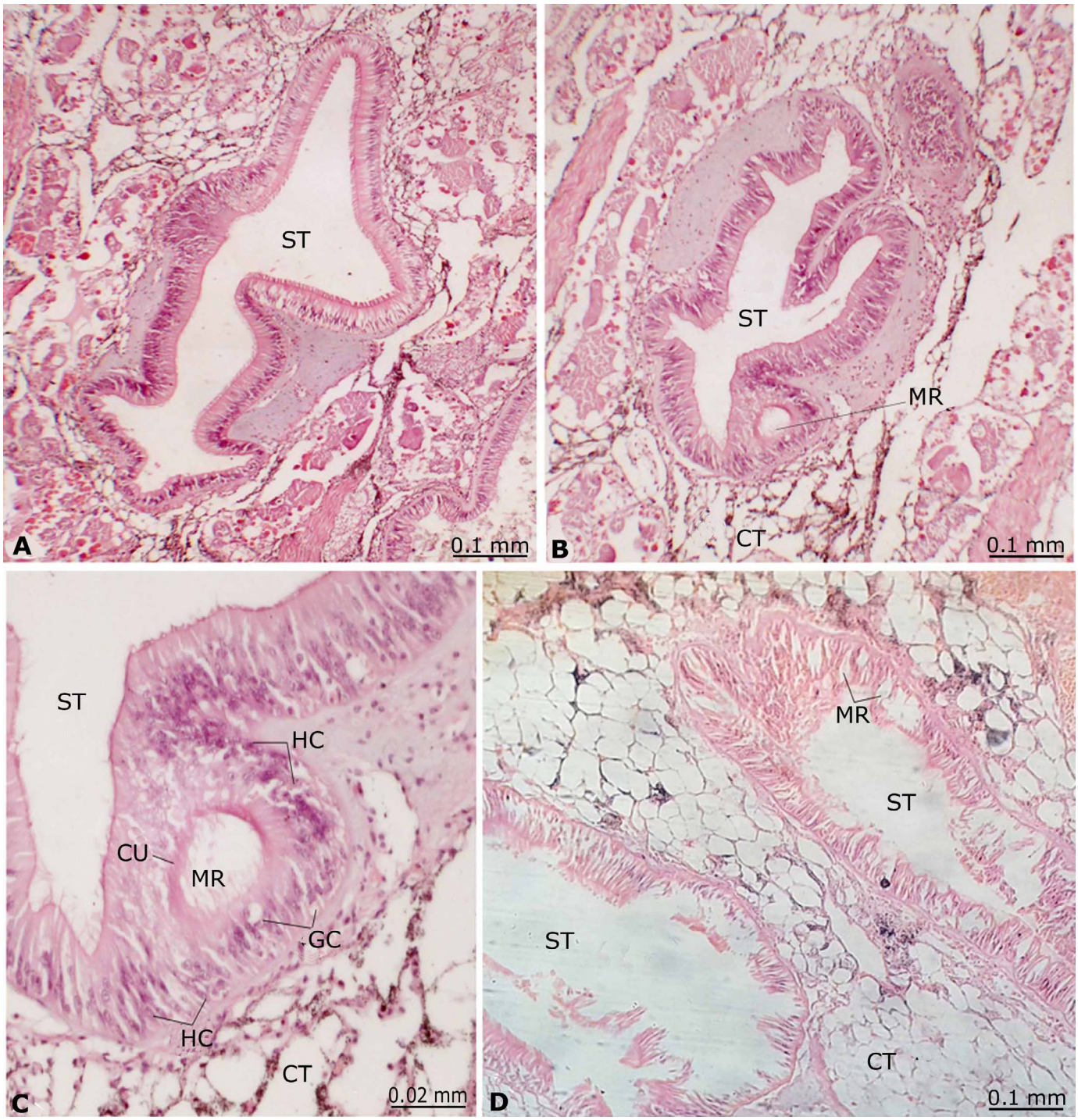

FIGURE 5. Gross anatomy images for visceral mass and reproductive follicles of the freshwater bivalves stained with H&E stain showing: (**A**) Non-infected ST covered with cuboidal epithelium; (**B–D**) Infected ST with the occurrence of MR due to degradation of the mites inside mite cyst covered with CU, surrounded with numerous HC and GC with the presence of CT. Abbreviations: CT, connective tissue; CU, cuticle; GC, goblet cells; HC, hemocytes; MR, mite remnants; ST, stomach. Color version available online.

Mites were also found inside the digestive diverticulum where the mite was partially or entirely encapsulated by hemocytes, and the membrane of the diverticulli appeared damaged (Fig. 5D). Moreover, mites may penetrate the reproductive tissues of male (Fig. 6A–D) and female bivalves (Fig. 6E–H) where they may produce disorder of the gametes, and a hemocyte reaction is observed around the invader.

DISCUSSION

The use of aquatic invertebrates in biomedical research and as environmental sentinels has dramatically grown in recent decades, with an increased need for understanding of the comparative pathology (Nachev et al., 2010). Bivalves are a group of worldwide-distributed molluscs residing in small ditches, ponds, lakes, canals, and rivers and are often used in animal tests of ecotoxicological studies (Naimo, 1995; Lafferty and Kuris, 1999; Jones et al., 2005; Vidrine et al., 2007; Wang et al., 2010; El-Assal and Fol, 2011). Little attention has been focused so far on the population of unionicolid mites parasitizing molluscan hosts mainly in Egypt, and few studies have been made on field populations of parasitic water mites (Ramadan, 2003a). There was a relation between host size and number of mites, which probably depends on both mite and host species (Ramadan, 2002, 2003b, 2003c; Cáceres-Martìnez et al., 2008; Carella et al., 2016). In the current investigation, there were a higher number of mites infecting smaller-size bivalves than larger ones, which agreed with previous studies by Humes and Jamnback (1950) who reported an inverse relation between prevalence of *N. ingens* and size of *Elliptio complanata* and *Pyganodon cataracta*. A negative relationship between mite density and the size of *Pyganodon grandis* was also reported by Gangloff et al. (2008), but the species of mites were not identified. In contrast, Gordon et al. (1979) followed by Dimock (1985) found positive correlations between the host size and the presence of *Unionicola formosa*. Additionally, Joy and Hively (1990) found that the number of mites per host was positively correlated with host shell length of the mussels in the first pond, but there was no correlation in a second pond. Moreover, Wen et al. (2006) reported that the abundance of *Unionicola arcuata* was positively correlated with host size but was not related to the gender of the host. Also, Downes (1986) stated that, firstly, larger bivalves may be easier to locate and have a stronger excurrent stream that is more easily detected by mites. Secondly, mites may reside in bivalves only up to a maximum density, thus larger bivalves can accommodate more mites.

The present study extends the previous preliminary knowledge of morphological details of eggs, larvae, nymphs, and adults of *U. tetrafurcatus*. The morphological details dealt with shape, measurements, armature of the legs, and surface sculpturing of the whole body regions in all developmental stages, using light and scanning electron microscopy examination, which revealed that the parasitic mite has all the generic characters of genus *Unionicola*, and we compared this with other known species of the same genus. The closest species to our specimen was *U. tetrafurcatus* according to studies made by Ramadan and Aboul-Dahab (2002) and *Unionicola agilex*, Wen, Hu, and Zhu (2008), in having 1 pair of plates connected with a latero-cleft on the lateral margin of the acetabular plates, the presence of a pair of ventero-glandularia near the genital field, distribution of setae and spines on the palpal segments, and armature of legs, but it differs from it in having different measurements for various body parts. In addition, it resembles *Unionicola lumbaria* described by Wen and Zhu (1998), but it can be distinguished from the latter by the elongated setae on the dorsum of male IV-L-4 (the latter male IV-L-4 bearing 8 elongate setae on dorsum), elongated setae on distal dorsum of male IV-L-6 (the latter male IV-L-6 with 2 long distal setae), and elongate setae on dorsum of female IV-L-6 (the latter only bearing 2 long setae on the venter of female IV-L-6). Also, it can be distinguished from *Unionicola parasitica* identified by Uchida and Imamura (1951), which have peg-like seta on the distal protrusion of pedipalp (the latter 3 papilla have each a minute hair), in the morphology of male dorsal genital plates (the latter morphology of male dorsal genital plates being narrow), and setae of male IV-L-4 (the latter with 2 long distal bristles); moreover, from *Unionicola palpatus* recognized by Aboul-Dahab (1998) in the absence of setation in the first and third palpal segments, the presence of triangular genital plates around the genital opening, the posterior end of each genital plate has along dorsal flap with a long seta, and a short ventral flap with 2 short setae; *Unionicola niloticus* demonstrated by Aboul-Dahab (1998) in having a smaller body size, presence of forked claws at the end of each leg, the genital opening is guarded by a pair of semi-oval genital plates, and each plate carried acetabulae and 2 pairs of short simple setae; also, *U. anodontae* recorded by Aboul-Dahab et al. (1998) having a larger body size, one pair of palpal process; genital opening is guarded by a pair of genital plates each one carried 10 acetabulae, and the posterior end of each genital plate has 6 short setae; furthermore, from *Unionicola difurcatus* described by Ramadan and Aboul-Dahab (2002) having a larger body size, the palpal tibia carries 2 simple setae, 1 solenidion and 1 lateral process, the palpal tarsus terminates with 1 solenidion and 2 clawlets, and each genital plate of both sexes carries 18 acetabulae (10 dorso-lateral and 8 ventral).

In the present study, adult *U. tetrafurcatus* were recorded crawling in the mantle cavity, as demonstrated by McElwain et al. (2016), as well as between the inner and outer gill layers and at the labial palp, which agreed with Kelly (1899) and Mitchell (1955) who reported on the presence of mites on the body surface between the gills or between the gills and abdomen, between the labial palps, or among the papillae fringing the mantle edges at the incurrent siphon; followed by Humes and Jamnback (1950) who observed that *N. ingens* inhabited the suprabranchial chambers, gills, and pericardial region of unionids; consequently, Wen et al. (2006) reported that the most common locations for *U. arcuata* in a Chinese unionid were the inner and outer gills. However, these results disagreed with previous studies by Humes and Jamnback (1950), Humes and Russell (1951), Humes and Harris (1952), Mitchell (1955, 1965), Flook and Ubelaker (1972), Davids (1973), Gordon et al. (1979), Dimock (1985), Edwards and Dimock (1988), Vidrine (1996a, 1996b), Wu et al. (2008), and Edwards and Vidrine (2013), who reported that many of the species in the subgenera of *Unionicola* use freshwater mussels as the preferred hosts tissues for egg-laying and metamorphosis (protonymphs and tritonymphs) while larvae, deutonymphs, and adults are found outside of the mussel host. In the present study, mite eggs were deposited only in the outer and inner gills whereas several authors described the distribution of mite eggs either in the body wall, the gills, or the mantle. This result agreed with data obtained by others who noted that oviposition sites within bivalves are limited and that various mites have distinctive genital

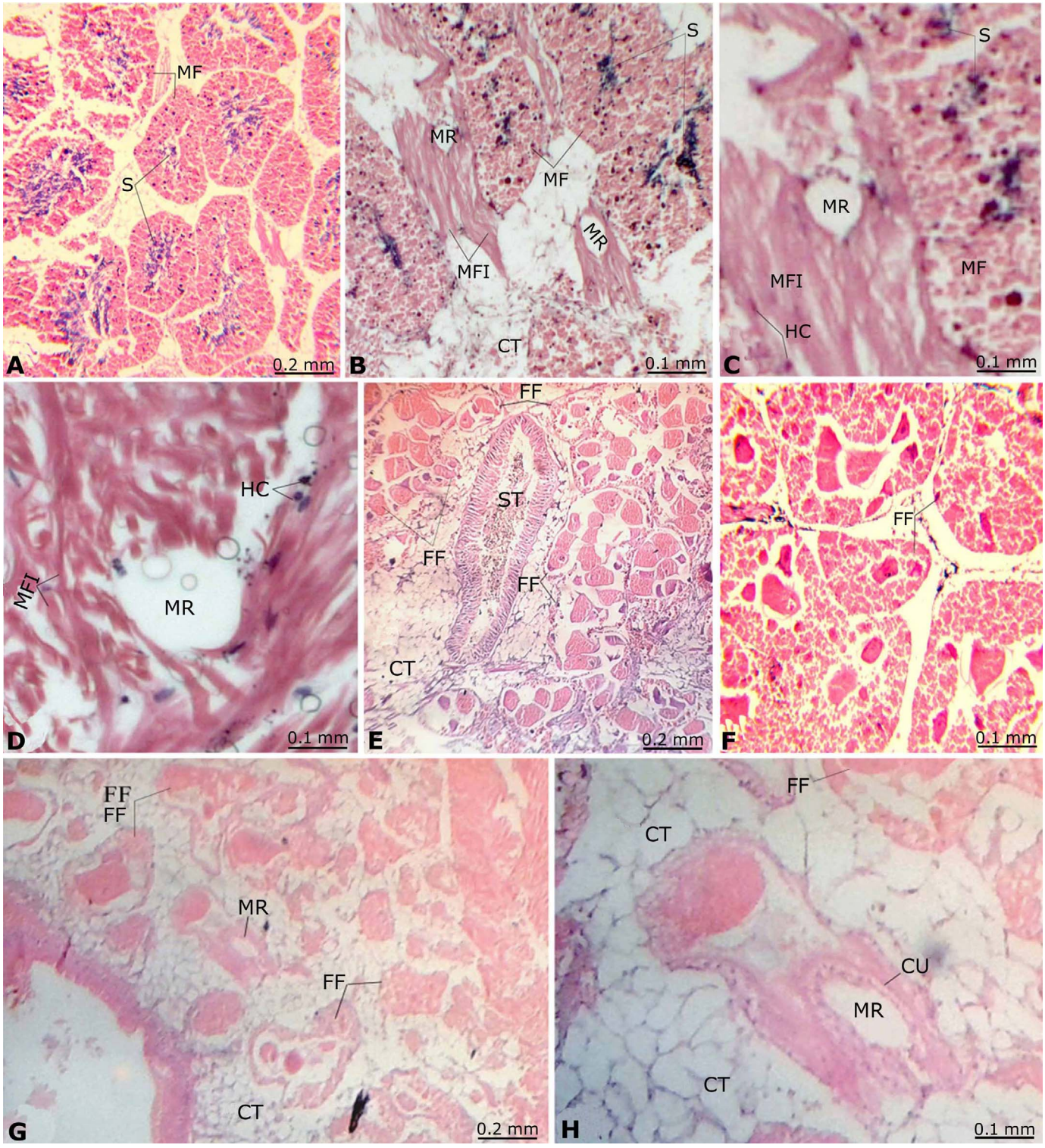

FIGURE 6. Gross anatomy images for reproductive tissues showing: (**A**) Non-infected follicles of MF filled with numerous S; (**B–D**) Infected follicles of MF filled with numerous S with the presence of MR, MFI with HC and CT; (**E, F**) Non-infected follicles of FF with appearing of ST and surrounded by CT; (**G, H**) Infected follicles of FF with MR covered with CU and CT. Abbreviations: CT, connective tissue; CU, cuticle; FF, female mussel; HC, hemocytes; MF, male mussel; MFI, myofibrils; MR, mite remnants; S, spermatids; ST: stomach. Color version available online.

fields that are adapted for oviposition in different tissue areas (Mitchell, 1965; Williams et al., 1993; Aboul-Dahab et al., 1996b, 1998; Ramadan and Aboul-Dahab, 2002; Vidrine et al., 2006; Starliper, 2011; Edwards and Vidrine, 2013).

Regarding histopathology, the present study observed that an inflammatory infiltration and epithelial proliferation are in different areas of the gills, reproductive tissue, and around the visceral mass of the freshwater mussels under investigation as a cellular reaction from the host toward the parasite. Similar findings were described by Baker (1976) who indicated tissue damage and leukocytic infiltration in the gills of *A. anatina* infected by *U. intermedia*. Also, Ramadan and Aboul-Dahab (2000) recorded a rupture, erosion, and cellular reactions in gill tissue of *Anodonta rubens* caused by infection with *U. niloticus* and its pedipalp displacement. Previous studies regarding mite eggs in bivalves have provided little specificity about how these infections affect tissue (Humes and Jamnback, 1950; Humes and Russell, 1951; Humes and Harris, 1952; Mitchell, 1955; Flook and Ubelaker, 1972; Ramadan, 1995, 2003a, 2003b, 2003c, 2004a, 2004b, 2005; Ramadan and Aboul-Dahab, 1999a, 2000; Wu et al., 2008; Song et al., 2010). Nevertheless, some of our observations of mite eggs in tissue corroborate the findings of Mitchell (1955) and Wu et al. (2008). Specifically, mite eggs were noticed to infect the fibrous tissue immediately beneath the simple epithelium of the mantle cavity tissues. Furthermore, an ovular space surrounding the eggs was demonstrated, which was also observed in *Cristaria plicata* by Wu et al. (2008). The seemingly vacuous space possibly represents the location of the fluid surrounding the eggs that was removed during tissue processing, but we are uncertain if this fluid represents a mite-derived secretion surrounding the eggs, an exudate, or if the space is an artifact of sectioning; we suspect this is unlikely given the lack of artifactitious separation of tissue layers adjacent to the aforementioned site/tissue. Wu et al. (2008) reported a seemingly morphologically comparable layer surrounding the embedded mites. This layer may represent a protective covering (i.e., a parasite-exuded cyst) surrounding the embedded mites, and it may be present in pre-hatch larvae and larvae that have invaded tissue. Whereas the inner membrane represents a substance derived from mites (i.e., the cyst), the outer membrane is suggestive of hemocytes, which would comprise a component of the host encapsulation. An outer eosinophilic membrane was sometimes observed around embedded mites in the gills and around the integument of the visceral mass and reproductive tissue. A single layer of emarginated hemocytes, having flattened, eosinophilic cytoplasm and a small, protruding nucleus, was most evident in subcutaneous tissues of the visceral mass and reproductive tissue. However, this membrane was not always apparent, especially when mite eggs were located in fibrous tissues such as inter-branchial septum as myofibers, connective tissue fibers, and emarginated hemocytes which have similar histological characteristics. An outer layer of emarginated hemocytes may either represent a limited host response or hemocytes that have become flattened in a confined space onto the surface of the parasitoid. Previous studies by Humes and Jamnback (1950), Humes and Russell (1951), and Humes and Harris (1952) indicated that *N. ingens* may limit mussel populations by obstructing the water tubes of the marsupia. Additionally, Mitchell (1955) reported hyperplasia, followed by Flook and Ubelaker (1972), who reported hypertrophy of tissues infected with mite eggs. Furthermore, localized tissue damage of these mites could interfere with host feeding because eggs were observed in the ctenidial hemolymph sinuses and inter-branchial septa but not in the ventral food groove of the inner demibranch or in the labial palps. This result contradicts other reports by Vidrine (1996a, 1996b) and Wu et al. (2008), who stated that bivalves infected with multitudes of mite eggs may lose the effectiveness of their tissues, but it was unclear what processes may be impaired or whether such changes were associated with the eggs of *Najadicola* spp. and *Unionicola* spp. Hypertrophic foci occurred where eggs were embedded, typically when intensities were high. In at least 1 individual of *Potamilus purpurata*, there were scattered clusters of brown, degenerative eggs surrounded by yellow or brown-pigmented host tissue (Flook and Ubelaker, 1972). In addition, mites were present in the connective tissue around the digestive diverticula of mussels and surrounded by mucus due to the initial degradation of the mite; however, the rest of the mites were not found in the stomach of the host, which would suggest a true degradation. On the other hand, if mites may reach the diverticula and the reproductive follicles, it would have shown that they have enough time to crawl and penetrate the host tissue, as stated by Song et al. (2010).

Therefore, it could be concluded that the present study provides valuable information about freshwater bivalves and represents a new hosts and new locality record for parasitic infection with *Unionicola* spp. In addition, more research is needed to determine whether other types of pathogens infect bivalves. Finally, molecular technique is important to differentiate between *Unionicola* spp. infecting different freshwater bivalves.

ACKNOWLEDGMENTS

The authors are thankful to the Faculty of Science, Cairo University, Cairo, Egypt. In addition, authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group No. RG-002.

LITERATURE CITED

Aboul-Dahab, H. M. 1998. Two new species of water mites parasitizing the freshwater mussel *Anodonta rubens*, in the River Nile, Egypt. Journal of the Egyptian-German Society of Zoology, Ser. D: Invertebrate Zoology and Parasitology **25:** 127–141.

Aboul-Dahab, H. M., M. A. Hussein, and S. A. Ramadan. 1996a. Parental care and brood protection in the parasitic water mite *Unionicola anodontae* n. sp. Bulletin of Faculty of Science, Assiut University, Egypt **25:** 11–21.

Aboul-Dahab, H. M., M. A. Hussein, and S. A. Ramadan. 1996b. The incidence of the parasitic mite *Unionicola anodontae* inside the molluscan host *Anodonta rubens*. Assiut Veterinary Medical Journal **35:** 188–207.

Aboul-Dahab, H. M., M. A. Hussein, and S. A. Ramadan. 1997a. Reproductive system and embryogenesis of the water mite *Unionicola anodontae* (Acari: Prostigmata). Bulletin of Faculty of Science, Assiut University, Egypt **26:** 1–31.

Aboul-Dahab, H. M., M. A. Hussein, and S. A. Ramadan. 1997b. Population dynamics of *Unionicola anodontae* n. sp. associated with the molluscan host, *Anodonta rubens*, in the River Nile, Egypt. Bulletin of Faculty of Science, Assiut University, Egypt **26:** 33–60.

Aboul-Dahab, H. M., M. A. Hussein, and S. A. Ramadan. 1998. *Unionicola anodontae* n. sp. (Acari: Unionicolidae), a parasitic mite collected from River Nile, Sohag, Egypt. Bulletin of Faculty of Science, Assiut University (E) Zoology, Egypt **27:** 1–23.

Baker, R. A. 1976. Tissue damage and leukocytic infiltration following attachment of the mite *Unionicola intermedia* to the gills of the bivalve mollusc *Anodonta anatina*. Journal of Invertebrate Pathology **27:** 371–376.

Baker, R. A. 1991. Development and life-history strategies in mussel mites (Hydrachnellae: Unionicolidae). *In* The Acari: Reproduction, development, and life-history strategies, R. Schuster and P. W. Murphy (eds.). Chapman and Hall, London, U.K. p. 65–73.

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology **83:** 575–583.

Cáceres-Martìnez, J., R. Vásquez-Yeomans, Y. G. Renterìa, S. Curiel-Ramìrez, J. A. Olivas Valdèz, and G. Rivas. 2008. The marine mites *Hyadesia* sp. and *Copidognathus* sp. associated with the mussel *Mytilus galloprovincialis*. Journal of Invertebrate Pathology **76:** 216–221.

Carella, F., G. Villari, N. Maio, and G. DeVico. 2016. Disease and disorders of freshwater unionid bivalves: A brief overview of recent studies. Frontiers in Physiology **7:** 489. doi:10.3389/fphys. 2016.00489.

Conroy, J. C. 1974. The taxonomy and ecology of *Unionicola crassipes crassipes* (Müller), a water mite parasitic on the freshwater sponge, *Spongilla lacustris* (Linnè). *In* The 4th International Congress of Acarology, Marion Lake, British Columbia, Canada, p. 959–964.

Davids, C. 1973. The relations between mites of the genus *Unionicola* and the bivalves *Anodonta* and *Unio*. Hydrobiologia **41:** 37–44.

Dimock, J. R. V. 1985. Population dynamics of *Unionicola formosa* (Acari: Unionicolidae), a water mite with a harem. American Midland Naturalist **114:** 168–178.

Downes, B. J. 1986. Guild structure in water mites (*Unionicola* spp.) inhabiting freshwater bivalves: Choice, competitive exclusion, and sex. Oecologia (Berlin) **70:** 457–465.

Edwards, D. D., and R. V. Dimock. 1988. A comparison of the population dynamics of *Unionicola formosa* from two anodontine bivalves in a North Carolina farm pond. Journal of the Elisha Mitchell Scientific Society **104:** 90–98.

Edwards, D. D., and R. V. Dimock. 1995. Life history characteristics of larval *Unionicola* (Acari: Unionicolidae) parasitic on *Chironomus tentans* (Diptera: Chironomidae). Journal of Natural History **29:** 1197–1208.

Edwards, D. D., and M. F. Vidrine. 2013. Mites of freshwater mollusks. Malcolm F. Vidrine, Eunice, Louisiana, 336 p.

El-Assal, F., and M. Fol. 2011. *Corbicula* species (Bivalvia: Veneroida), from the River Nile in Egypt as bioindicators of pollution. Environmental Pollution Ecological Impacts Health Issues and Management, p. 82–91.

El-Assal, M. F., S. F. Sabet, K. G. Varjabedian, and M. F. Fol. 2014. Pollution of freshwater *Coelatura* species (Mollusca: Bivalvia: Unionidae) with heavy metals and its impact on the ecosystem of the River Nile in Egypt. International Journal of Waste Resources **4:** 1–11.

Faust, E. C. 1918. Additions to our knowledge of *Unionicola aculeata* (Koenike). Transactions of the American Microscopical Society **37:** 125–128.

Fisher, G. R., R. V. Dimock, and R. E. Kuhn. 2000. The symbiotic water mite *Unionicola formosa* (Acari: Unionicolidae) ingests mucus and tissue of its molluscan host. Journal of Parasitology **86:** 1254–1258.

Flook, J. M., and J. E. Ubelaker. 1972. A survey of metazoan parasites in unionid bivalves of Garza-Little Elm Reservoir, Denton County, Texas. Texas Journal of Science **23:** 381–392.

Forbes, M. R. L., and R. L. Baker. 1991. Condition and fecundity of the damselfly, *Enallagma ebrium* (Hagen): The importance of ectoparasites. Oecologia **86:** 335–341.

Fuller, S. L. H. 1974. Clams and bivalves (Mollusca: Bivalvia). *In* Pollution ecology of freshwater invertebrates, C. W. Hart and S. L. H. Fuller (eds.). Academic Press, New York, New York, p. 215–273.

Gangloff, M. M., K. K. Lenertz, and J. W. Feminella. 2008. Parasitic mite and trematode abundance are associated with reduced reproductive output and physiological condition of freshwater bivalves. Hydrobiologia **610:** 25–31.

Gledhill, T. 1985. A new species of water-mite, *Unionicola* (*Pentatax*) *macani* (Unionicolidae, Hydrachnellae, Acari), from the mantle cavity of the prosobranch mollusc *Lanistes ovum* Peters in Nigeria, with remarks on some aspects of host/parasite relationships between unionicolids and molluscs. Archiv für Hydrobiologie **104:** 77–92.

Gordon, M. J., B. K. Swan, and C. G. Paterson. 1979. The biology of *Unionicola formosa* (Dana and Whelpley): A water mite parasitic in the unionid bivalve, *Anodonta cataracta* in a New Brunswick Lake. Canadian Journal of Zoology **57:** 1748–1756.

Graf, D. L., and K. S. Cummings. 2007. Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). Journal of Molluscan Studies **73:** 291–314.

Humes, A. G., and S. K. Harris. 1952. The clam hosts of *Najadicola ingens* (K.) Acarina in a Quebec lake. Canadian Field-Naturalist **66:** 83–84.

Humes, A. G., and H. A. Jamnback. 1950. *Najadicola ingens* (Koenike), a water-mite parasitic in freshwater clams. Psyche **57:** 77–87.

Humes, A. G., and H. D. Russell. 1951. Seasonal distribution of *Najadicola ingens* (Koenike) (Acarina) in a New Hampshire pond. Psyche **58:** 111–119.

Jones, R. K. H. 1965. Parasitism of larvae of *Unionicola intermedia* Koenike and another *Unionicola* sp. (Acarina, Pionae) on chironomids. Nature **207:** 317–318.

Jones, J. W., R. A. Mair, and R. J. Neves. 2005. Factors affecting survival and growth of juvenile freshwater bivalves cultured in recirculating aquaculture systems. North American Journal of Aquaculture **67:** 210–220.

Joy, J. E., and J. W. Hively. 1990. Population dynamics of adult *Unionicola formosa* (Acari: Hydracarina), a parasite of *Anodonta imbecillis* (Mollusca: Bivalvia), in West Virginia. Brimleyana **16:** 33–42.

Kelly, H. M. 1899. A statistical study of the parasites of the Unionidae. Bulletin of the Illinois State Laboratory of Natural History **5:** 399–418.

Lafferty, K. D., and A. M. Kuris. 1999. How environmental stress affects the impacts of parasites. Limnology and Oceanography **44:** 925–931.

Lehmann, T. 1993. Ectoparasites: Direct impact on host fitness. Parasitology Today **9:** 8–13.

Luxton, M. 1990. The marine littoral mites of the New Zealand region. Journal of the Royal Society of New Zealand **20:** 367–418.

McElwain, A., and S. A. Bullard. 2014. Histological atlas of freshwater bivalves (Bivalvia, Unionidae) *Villosa nebulosa* (Ambleminae: Lampsilini), *Fusconaia cerina* (Ambleminae: Pleurobemini) and *Strophitus connasaugaensis* (Unioninae: Anodontini). Malacologia **57:** 99–239.

McElwain, A., R. Fleming, M. Lajoie, C. Maney, B. Springall, and S. A. Bullard. 2016. Pathological changes associated with eggs and larvae of *Unionicola* sp. (Acari: Unionicolidae) infecting *Strophitus connasaugaensis* (Bivalvia: Unionidae) from Alabama creeks. Journal of Parasitology **102:** 75–86.

Mitchell, R. 1965. Population regulation of a water mite parasitic on unionid bivalves. Journal of Parasitology **51:** 990–996.

Mitchell, R. D. 1955. Anatomy, life history, and evolution of the mites parasitizing freshwater bivalves. Miscellaneous Publications Museum of Zoology, University of Michigan **89:** 1–28.

Mùller, A. P. 1990a. Effects of a haemtophagous mite on the barn swallow (*Hirundo rustica*): A test of the Hamilton and Zuk hypothesis. Evolution **44:** 771–784.

Mùller, A. P. 1990b. Effects of parasitism by a haematophagous mite on reproduction in the barn swallow. Ecology **71:** 2345–2357.

Nachev, M., S. Zimmermann, T. Rigaud, and B. Sures. 2010. Is metal accumulation in *Pomphorhynchus laevis* dependent on parasite sex or infra population size? Parasitology **137:** 1239–1248.

Naimo, T. J. 1995. A review of the effects of heavy metals on freshwater bivalves. Ecotoxicology **4:** 341–362.

Pennak, R. W. 1989. Fresh-water invertebrates of the United States: Protozoa to Mollusca, 3rd edition. John Wiley and Sons, Inc., New York, New York, 822 p.

Polak, M. 1996. Ectoparasitic effects on host survival and reproduction: The *Drosophila-Macrocheles* association. Ecology **77:** 1379–1389.

Polak, M., and T. A. Markow. 1995. Effect of ectoparasitic mites on sexual selection in a Sonoran desert. Evolution **49:** 660–669.

Polak, M., and W. T. Starmer. 1998. Parasite-induced risk of mortality elevates reproductive effort in male Drosophila. Proceedings of the Royal Society of London. Series B, Biological Sciences **265:** 2197–2201.

Ramadan, S. A. 1995. Biological studies on some aquatic parasitic mites. Ph.D. Dissertation. Sohag University, Sohag, Egypt, 168 p.

Ramadan, S. A. 2002. On the distribution and population density of the water mite *Unionicola tetrafurcatus* inside two molluscan hosts from the River Nile, Sohag, Egypt. Egyptian Journal of Zoology **39:** 541–559.

Ramadan, S. A. 2003a. On the occurrence of a protozoan parasite inside the water mite *Unionicola anodontae* with emphasis on histopathological changes of the host. Journal of the Egyptian-German Society of Zoology **40:** 101–113.

Ramadan, S. A. 2003b. A comparison of the population structure of the water mite *Unionicola tetrafurcatus* inside two molluscan hosts from the River Nile, Egypt. Egyptian Journal of Zoology **41:** 229–247.

Ramadan, S. A. 2003c. Spermatogenesis and sperm structure in the water mite *Unionicola tetrafurcatus* (Unionicolidae) with remarks on the male reproductive system. Journal of Egyptian German Society of Zoology **42:** 11–25.

Ramadan, S. A. 2004a. Description of epibiont ciliates on the water mite *Unionicola tetrafurcatus* and their distribution on the host legs. Egyptian Journal of Zoology **42:** 97–112.

Ramadan, S. A. 2004b. Numerical taxonomic studies of some aquatic mites collected from the River Nile and Red Sea, Egypt. Egyptian Journal of Zoology **43:** 257–275.

Ramadan, S. A. 2005. Ultrastructure of haemocytes in the water mite *Unionicola tetrafucatus* (Acari: Unionicolidae) with reference to their role in phagocytosis. Assiut University (G) Zoology **34:** 1–9.

Ramadan, S. A., and H. M. Aboul-Dahab. 1999a. Sexual dimorphism in three freshwater mite species (Acari: Unionicolidae). Bulletin of Faculty of Science, Assiut University, Egypt **28:** 101–118.

Ramadan, S. A., and H. M. Aboul-Dahab. 1999b. Life history of freshwater mite *Unionicola niloticus* (Unionicolidae) parasitizing on the molluscan host, *Anodonta rubens*. Bulletin of Faculty of Science, Assiut University, Egypt **28:** 141–158.

Ramadan, S. A., and H. M. Aboul-Dahab. 2000. Tissue damage and internal defence reactions to the molluscan host *Anodonta rubens* infected by the water mite *Unionicola niloticus*. Journal of the Egyptian German Society of Zoology **33:** 19–30.

Ramadan, S. A., and H. M. Aboul-Dahab. 2002. Scanning electron microscopic study of two new species of water mites collected from unionid mussels in Egypt. Journal of the Egyptian German Society of Zoology **37:** 9–22.

Sleem, S. H., and T. G. Ali. 2008. Application of RAPD-PCR in taxonomy of certain freshwater bivalves of genus *Coelatura*. Global Journal of Molecular Sciences **3:** 27–31.

Smith, B. P. 1988. Host–parasite interaction and impact of larval water mites on insects. Annual Review of Entomology **33:** 487–507.

Smith, I. M., D. R. Cook, and B. P. Smith. 2010. Water mites (Hydrachnidiae) and other arachnids. *In* Ecology and classification of North American freshwater invertebrates, 3rd ed., T. H. Thorp and A. P. Covich (eds.). Academic Press, London, U.K., p. 485–586.

Smith, I. M., and D. R. Oliver. 1986. Review of parasitic associations of larval water mites (Acari: Parasitengona: Hydrachnida) with insect hosts. Canadian Entomologist **118:** 407–472.

Song, L., L. Wang, L. Qiu, and H. Zhang. 2010. Bivalve Immunity. *In* Invertebrate immunity, K. Soderhall (ed.). Springer, New York, New York, 316 p.

Starliper, C. E. 2011. Pathogens and diseases of freshwater bivalves in the United States: Studies on bacterial transmission and depuration in bridging America and Russia with shared perspectives on aquatic animal health. Proceedings of the Third Bilateral Conference between Russia and the

United States, 12–20 July, 2009, held in Shepherdstown, West Virginia, R. C. Cipriano, A. W. Bruckner, and I. S. Shchelkunov (eds.). Khaled bin Sultan Living Oceans Foundations, Landover, Maryland, p. 47–55.

Uchida, T., and T. Imamura. 1951. Some water-mites from China. Journal of the Faculty of Science, Hokkaido University. Series 6, Zoology **10:** 324–358.

U.S. Environmental Protection Agency. 1992. Framework for ecological risk assessment. EPA/630/R-92-001, Risk assessment forum. U.S. Environmental Protection Agency, Washington, DC.

Vidrine, M. F. 1996a. North American *Najadicola* and *Unionicola*: Systematics and coevolution. Gail Q. Vidrine Collectibles, Eunice, Louisiana, 146 p.

Vidrine, M. F. 1996b. North American *Najadicola* and *Unionicola*: Diagnoses and distributions. Gail Q. Vidrine Collectibles, Eunice, Louisiana, 356 p.

Vidrine, M. F., Z. Lötter, J. G. Van As, M. Bastian-Stanford, and S. R. Hazelton-Robichaux. 2006. *Unionicola* (Iridinicola) *botswaniana* n. subgen., n. sp. (Acari: Unionicolidae) from freshwater mussels in Botswana. International Journal of Acarology **32:** 1–4.

Vidrine, M. F., Z. Lötter, J. G. Van As, A. E. Bogn, and M. Bastian-Stanford. 2007. *Unionicola* (Coelaturicola) *gredhilli* n. subgen., n. sp. (Acari: Unionicolidae) from freshwater mussels in Botswana and East Africa. International Journal of Acarology **33:** 167–171.

Wang, N., C. G. Ingersoll, C. D. Ivey, D. K. Hardesty, and T. W. May. 2010. Sensitivity of early life stages of freshwater bivalves (Unionidae) to acute and chronic toxicity of lead, cadmium, and zinc in water. Environmental Toxicology and Chemistry **29:** 2053–2063.

Wen, C. G., and Z. M. Zhu. 1998. A new species of the genus *Unionicola* from China. Systematic and Applied Acarology **3:** 171–174.

Wen, C., B. Hu, and Z. Zhu. 2008. A new subgenus *Vidrinatax* (Acari: Unionicolidae: Unionicola) with description of two new species of water mites in the genus *Unionicola* from China. Acarologia **3:** 191–196.

Wen, C., P. Nie, and Z. Zhu. 2006. Population dynamics of the water mite *Unionicola arcuata* (Unionicolidae) in the freshwater bivalve *Cristaria plicata* (Unionidae) in Poyang Lake, eastern China. Diseases of Aquatic Organisms **70:** 123–127.

Williams, J. D., M. L. Warren, K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater bivalves of the United States and Canada. Fisheries **18:** 6–22.

Wu, H., Y. Xie, C. Wen, and T. Feng. 2008. Histopathological effect of *Unionicola arcuata* eggs on different tissues in *Cristaria plicata*. Jiangxi Science **26:** 544–547.