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FIRST RECORD OF THREE LARVALTREMATODES, *RHIPIDOCOTYLE CAMPANULA*, *PHYLLODISTOMUM* SP. AND *ECHINOSTOMA* SP. (DIGENEA: BUCEPHALIDAE, GORGODERIDAE AND ECHINOSTOMATIDAE) INFECTING FRESHWATER MUSSEL *NITIA TERETIUSCULA* IN EGYPT

By

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Abstract

Unionidae is the most diverse family of freshwater bivalves; however, infectious diseases of these molluscs have received little attention. A total of 200 freshwater mussels from five species were collected monthly during the period of February 2016 to August 2017 from Tura region in River Nile, Cairo, Egypt during the current investigation. The collected samples consisted of 50 *Corbicula fluminea*, 50 *Caelatura aegyptiaca*, 50 *Nitia teretiuscula*, 30 *Mutela rostrata* and 20 *Chambardia rubens*. Mussels were dissected and examined for detection of parasitic infection. Larval trematodes were recovered from mussel species *N. teretiuscula* with infection rate 2% (4/200). Light and scanning electron microscopic examinations showed that the pre-patent invasions of trematode parasites belong to three families, *Rhipidocotyle campanula* (Bucephalidae), *Phyllodistomum* sp. (Gorgoderidae) and *Echinostoma* sp. (Echinostomatidae). Also, the histopathological effects associated with larval trematode infection were studied in different tissues of *N. teretiuscula*. No infection was demonstrated in the mantle and visceral mass. The present study indicated that freshwater mussels can serve as first and second intermediate hosts for trematodes. In addition, this is the first study to report cercarial emergence from freshwater bivalves in this geographic region.

Key words: Freshwater mussels, *Nitia teretiuscula*, *Rhipidocotyle campanula*, *Phyllodistomum* sp., *Echinostoma* sp., Histopathological examination

Introduction

Invertebrate species represent a great percentage of animal diversity; however, they attract extremely minor research effort relative to vertebrates (Ricciardi and Rasmussen, 1999). Among them freshwater molluscs is one of the most diverse and endangered animals with limited research specialists (Carella *et al*, 2016). The use of freshwater molluscs in the biomedical research and environmental sentinels has dramatically grown in recent decades, as they have been known to play significant roles in public and veterinary health. Freshwater molluscs need to be scientifically explored (Supian and Ikhwanuddin, 2002) and extensively understand their comparative pathology (USEPA, 1992). Order Unionoida is a widespread group of bivalve molluscs commonly known as "freshwater mussels" including six families (Lydeard *et al*, 2004). Unionidae is the largest family with approximately 677 species (McElwain and Bullard, 2014), represented as worldwide distributed bivalves residing small ditches, ponds, lakes, canals and rivers (Strayer *et al*, 2004) and often used as a mall test in the eco-toxicological studies (Torres *et al*, 2004). Unionids provide a habitat for various free-living and parasitic symbionts as trematodes (Piechocki and Dyduch-Falniowska, 1993). Trematodes are responsible for a variety of parasitic diseases, some of which are transmitted through fish and shellfish (Aldana *et al*, 2009; Marcus *et al*, 2012). Different developmental stages of trematode parasites may use bivalves as the first intermediate host (sporocyst, redial and cercarial stages) or as the second intermediate host (metacercarial stage), or the bivalve may be the only host for all stages of the life cycle (Duobinis-Gray et al, 1991).

Bucephalidae is one of the largest digenean families with 25 genera containing hun-

dreds of described species characterized by having a muscular organ at the anterior end termed as "rhynchus" which is used to attach to their hosts (Muñoz and Bott, 2011). *Bucephalus* species are the most common parasites of commercial bivalves; the adult stage of the trematode life cycle is completed in different fish or bird species (Cribb *et al*, 2001). Also, genus *Phyllodistomum* is the only genus of family Gorgoderidae observed in freshwater unionids (Hoffman, 1999), but immature stages in this family are difficult to identify and have often been assigned only to the family Gorgoderidae (George-Nascimento *et al*, 1998). In addition, Echinostomatidae is a family of digenetic trematode worms (type genus *Echinostoma*) that are rare in man but common and widely distributed as parasites of invertebrate animals. Laruelle *et al*. (2002) only found an unidentified species of Echinostomatidae in zebra mussels from Europe. Few studies have specifically addressed the impact of larval trematodes on their hosts (Chai *et al*, 2008; Nguyen *et al*, 2009). Mussels parasitized by digenetic (host-castrating) trematodes exhibit decreased growth rates, physiological condition, larval production and tissue damage (Fisher *et al*, 2000; Gustafson *et al*, 2005a, b; Howard *et al*, 2004; Keiser and Utzinger 2009; Phan *et al*, 2010; Muñoz *et al*, 2013), so histopathological studies have been used as components for the examination of infected mussels (Chittick *et al*, 2001).

The aim of the present study was to perform a detailed morphological examination of the different larval stages infecting freshwater mussels using light and scanning electron microscopes. In addition, histopathological effects of the recorded parasites on their host tissues were studied.

Materials and Methods

Mussel samples collection: Adult mussels (n= 200) were monthly and randomly collected during the period of February 2016 to August 2017, from rocky muddy bottoms of Tura region (Helwan Governorate), and then transported immediately to Laboratory of Invertebrates at Zoology Department, Faculty of Science, Cairo University, Egypt. Mussels were held in small containers provided with well-aerated water and sediments, then sorted and maintained under the same conditions of food and temperature of its original environment. Identification of the freshwater mussels was performed according to Ibrahim et al. (1999) and Graf and Cummings (2007). The collected mussel specimens belonging to 3 families of 5 genera, 50 of each *C. fluminea* (Müller, 1774) (F: Cyrenidae), *C. aegyptiaca* (Cailliaud, 1827) (F: Unionidae), *N. teretiuscula* (Philippi, 1847) (F: Unionidae), 30 of *M. rostrata* (Rang, 1835) (F: Iridinidae) and 20 of *C. rubens* (Lamarck, 1819) (F: Iridinidae).

Parasitological investigation: Each mussel was dissected and examined for the presence of parasitic infections. Flushed parasites stages from the gills and reproductive tissue with saline solution were carefully isolated and preserved in 70% ethanol. Prevalence for parasite infections was statistically analyzed according to the guidelines stated by Bush *et al*. (1997). Morphology of the larval stages was studied on living and fixed specimens; neutral red and Nile blue stains were used for intra-vital staining. For morphological examination, fixed specimens were stained with acetocarmine, dehydrated in a graded ethanol series, cleared in clove oil, and then mounted permanently in Canada balsam. Photomicrographs were taken using Zeiss Axiovert 135 microscope supplied with a Canon Digital Camera. Measurements of larval stages are presented in micrometer; mean values are given in parentheses. Trematodes were identified based on the standard keys (Skrjabin *et al*, 1964; Yamaguti, 1971; Schell, 1985; Gibson and Bray, 2002; Campbell, 2008).

Scanning electron microscopic study: Recorded parasites were fixed in 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 critical point dryer "Bomer-900" with Freon 13, sputter-coated with gold–palladium in a Technics Hummer, and then examined and photographed under an Etec Autoscan at 20-kV Jeol scanning electron microscope in Electron Microscope Unit in Faculty of Science, Ain shams University, Egypt.

Histological examinations: Infected tissues (mantle, gills, viscera and reproductive tissues) of studied mussels were fixed in Bouin's solution for 48hrs. The fixed samples were washed in tap water overnight and exposed to ascending concentrations of ethyl alcohol (70%, 80%, 90% and 100%), then 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, then cut on a rotary microtome, stained with Harris' Hematoxylin and counter-stained with Eosin (H&E stain) and then examined and photographed by a Zeiss Research Photomicroscope. Histological terminology for Unionidae follows McElwain and Bullard (2014).

Ethical Standards: All procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals and have been approved and authorized by Institutional Animal Care and Use Committee (IACUC) in Faculty of Science, Cairo University, Egypt.

Results

Parasitological investigation indicated that out of 200 freshwater mussels 4 (2%) *N. teretiuscula* were infected (Figs. 1-5), while the other examined bivalves; *C. fluminea, C. aegyptiaca, M. rostrata* and *C. rubens* did not harbor any larval trematodes. Histological examination of soft parts of the infected mussels demonstrated larval trematodes in the gill lamellae, inter-lamellar connective tissues and spaces of the gills in addition to reproductive tissues for male and female bivalves. *N. teretsicula* showed equal infection for both sexes with ratio 1:1. The recovered larval trematodes were identified as sporocyst of *Rhipidocotyle campanula* (F: Bucephalidae), cercariae of *Phyllodistomum* sp. (F: Gorgoderidae), and metacercariae of Echinostomatids (F: Echinostomatidae).

Microscopic examinations of larval stages (Figs. 6-19):

1-*Rhipidocotyle campanula* (based on 20 live specimens): This larval stage appeared as ribbon of sporocysts with multi-branched constrictions or ramifications, dark in color and comprising a great number of cercariae at different stages of development. These sporocysts measured 0.520-1.620 (0.849μm) long and 0.300-0.870 (0.529μm) wide. This stage lacks suckers and having instead a muscular organ called a "rhynchus" at the front end which is used for attachment to host tissues.

2- Cercaria of *Phyllodistomum* sp. (based on 20 specimens from permanent mounts): The body was dorso-ventrally flattened with slightly narrower anterior end and broader posterior end. Body measured 0.395-0.860 (0.593μm) long with maximum width at the level of the anterior testis reached about 0.123-0.250 (0.173μm). Mouth sub-terminally. Oral sucker was sub-terminal and measured 0.063-0.143 (0.096μm) long and 0.063-0.108 (0.084μm) wide. Ventral sucker rounded, measured 0.060-0.145 (0.104μm) long and 0.070-0.138 (0.102μm) wide and located at 0.175-0.500 (0.325μm) from the anterior end. Tegument was thin with very fine papillae distributed irregularly from the anterior to posterior end of the body and condensed at the posterior part of the body. Spines were not observed.

3- Metacercaria of *Echinostoma* sp. (based on 20 specimens from permanent mounts): This stage was folded within a transparent cyst, spherical in shape with double walled, and enclosed in a thin sheet of connective tissue of the host. Also, excretory granules were detected. The mean diameter was within the range of 120-165 (157μm).

Histopathological examination (Figs. 20-37)

revealed different developmental stages in the gills and reproductive tissues of *N. teretiuscula* but was not perceived in the mantle and digestive gland. Also, inflammation of the connective tissue was observed and lesions appeared as either diffuse hemolytic infiltration of the vesicular connective tissue or as discrete focal accumulations that displaced connective tissues and organ systems associated with the infestation by larval trematodes. Moreover, in the gill tissues a large number of cysts were detected with highly accumulated numbers of hemocytes around the infection sites, indicating activation of the mussel defensive mechanisms. All examined mussels showed severe inflammation accompanied by an increased prevalence of granulocytomas (either as single or multiple lesions) within the interconnective tissues of the gill lamellae. Furthermore, larval stages may penetrate into the reproductive tissues of male and female mussels and producing disorders of the gametes.

Discussion

Studies on mollusc-transmitted diseases are very important for veterinary and public health (Krailas *et al*, 2008). Aquatic molluscs have been reported as the first and second intermediate hosts of trematodes and various studies have been conducted on molluscan fauna and their trematode infections (Grizzle and Brunner, 2009;Chantima *et al*, 2013). Unionids are important factors in the functioning of the freshwater ecosystems (Vaughn and Hakenkamp, 2001). They constitute a significant benthos biomass (Piechocki, 1999) and filter large volumes of water (Strayer *et al*, 2004), thus contributing to the purification of the aquatic systems (Pusch *et al*, 2001). They represented a good source of food for other organisms (Zahner-Meike and Hanson, 2001) and also provided a habitat for various free-living and parasitic symbionts (Piechocki and Dyduch-Falniowska, 1993).

In the present study, the only unionid species found infected by larval trematodes was *N. teretusicula* (2%), while other freshwater bivalves did not harbor any larval trematodes. This result demonstrates that male and female *N. teretusicula* showed equal infection with ratio 1:1 which agreed with the findings of Waffle (1967) who suggested that neither size nor sex of the host apparently have effect on infectivity by these parasites. These results contradicted with Taskinen and Valtonen (1995), followed by Heino and Kaitala (1996), they observed an increase in the prevalence of parasites in older mussels and higher infection rates in females. Also, Müller *et al*, (2015) recorded higher infection rates in older female mussels, *D. polymorpha* with *R. campanula* and *Phyllodistomum* sp. Previous studies by Stadnychenko (1974), Yanovich and Stadnychenko (1997), Chernomaz (2001), Laruelle *et al*. (2002) and Grizzle and Brunner (2009) suggested that *B. polymorphus* was specific to Dreissenidae not Unionidae, and the earlier data on its presence in unionids should be verified due to the high degree of similarity between the larval stages of *B. polymorphus* and *R. campanula* (Karatayev *et al*, 2000). Bucephalid trematodes in unionids belong to the genus *Rhipidocotyle*, now named *B. polymorphus* (Kelly, 1899; Yanovich and Stadnichenko, 1997). In the line of this expectation, *B. polymorphus* which is a synonym of *R. campanula* was also specific to Unionidae, this agreed with Baturo (1977) who found sporocysts of *R. campanula* in the painter's mussel *Unio pictorum* and provided detail description of the developmental stages of this parasite. This also agreed with our findings that record sporocysts of *R. campanula* in the unionid, *N. teretusicula.* Laruelle *et al*. (2002) reported sporocysts of *B. polymorphus* in the connective-tissue spaces, i.e. gonadal tissues, and gills. Also, Pina *et al*. (2009) observed cercaria of *B. minimus* infecting the digestive gland and gonads of its first intermediate host, the edible cockle *Cerasto dermaedule*. Also, Marchiori *et al*. (2010) demonstrated sporocysts and cercariae of *B. marg-*

aritae in the mantle, digestive gland and gonad of the brown mussel *Pernaperna.* Additionally, the findings stated by Müller *et al*, (2015) recorded trematode larvae in the gonads more than in hepato-pancreas of the duck mussels. These results agreed with our observations of the larval stages of bucephalid but with some respect to the mantle epithelium, the digestive gland and other visceral organs.

Also, in the present study, larval stages of *Phyllodistomum* sp. were detected in the gills and reproductive tissues of *N. teretusicula* belonging to family Unionidae. This result agreed with Thomas (1956) who reported that European gorgoderian *Phyllodistomum simile* is found in the urinary bladder of brown trout as an adult, and sporocysts are found in the epi-branchial cavity between the gill lamellae of *Sphaerium corneum*. Also, Waffle (1967) found cercariae of *Phyllodistomum staffordi* infecting gills of the fingernail clam *Sphaerium lacustre*, followed by the finding of Taskinen *et al*. (1997) who reported that the gonads of the duck mussels in two Finnish lakes were more heavily parasitized by *R. campanula* and *R. fennica* than any other organs. Additionally, Rantanen *et al*, (1998) recorded that the sphaeriid clam *Pisidium amnicum* was infested by larval *Phyllodistomum elongatum* which caused castration of the host and Shiver (2002) recorded that *Rhipidocotyle* sp. were found infecting the gonadal tissues of the freshwater clam *Lampsilis Rafines queana*. Moreover, Kudlai and Yanovich (2013) detected larval stages of *Phyllodistomum* sp. in bivalves of the families Unionidae and Sphaeriidae. Finally, Taskinen *et al*. (1997; 2016) reported that the ribbons of sporocyst of *R. campanula* and *R. fennica* were present in the gonadal tissues of the freshwater clam *Anodonta piscinalis*.

Various species of freshwater snails have been reported to play a dual role of the first and second intermediate hosts of echinostomes in Thailand, namely, *Indoplanorbis* spp., *Gyraulus* spp., *Lymnaea* spp., *Pila* spp., *Viviparus* spp., *Filopaludina* spp. and *Bithynia* spp. as recorded by Chantima *et al*, (2013). Also, Chantima *et al*, (2013) first reported metacercariae of *E. revolutum* in the snail host, *C. helena* and Zimmermann *et al*, (2015) observed larval stages of *Echinostoma* spp. parasitize gastropods as both first and second intermediate hosts. These data reversed our findings that record metacercariae of echinostome infecting the gills of unionid freshwater mussel, *N. teretusicula* which was considered as second intermediate host. However, it agreed with results of Molloy *et al*, (1997) who reported that zebra mussels are often second intermediate hosts to trematodes in the family Echinostomatidae. Also, Kanev *et al*. (1998) reported metacercariae of *Echinostoma* sp. infected pulmonate and Semenas *et al*, (1999) found metacercariae of Echinostomatidae infecting viscera and gonads of *Diplodon chilensis*. Also, Han *et al*, (2009) discovered metacercariae of *Himasthla alincia* (Echinostomatidae) in brackish water bivalves in the Republic of Korea. Lastly, Bakhmet *et al*. (2017) showed that metacercariae of echinostomatidae disrupt neuronal control of cardiac function and lower growth rate in the blue mussels *Mytilus edulis* in situ. Besides, Han *et al*. (2009) and Chantima *et al*. (2013) described metacercariae of echinostomatidae folded within a transparent cyst with a bilayered wall, spherical in shape and reached 136.0-195.0µm in diameter. Cyst wall consisted of outer transparent layer and inner opaque layer, collar spines (37 in total number), were presented in both fresh and fixed specimens. The excretory granules and suckers were visible, these results agreed with the present description of metacercariae of echinostomes but with the respect to the diameter the present results ranged from 120-165 (157µm).

The present investigation recorded localized (focal) tissue damage associated with larval trematodes in the histological sections of gills and reproductive tissues. Previous studies regarding parasitic infections in mus-

sels have provided little specificity regarding how these infections affect tissue (Vidrine, 1996; Wu *et al*, 2008). In addition, histopathological studies showed that gills were the most infected organ which contradicts with the study of Lajtner *et al*. (2008) that confirmed gonads of *D. polymorpha* were predominantly attacked by *B. polymorphus* infection more than any other organ. Also, Kudlai and Yanovich (2013) found larval stages of *Phyllodistomum* sp. in the gonads of the duck mussel *A. anatina*. Conversely, the present results agreed with their findings where digestive gland of the majority of infected mussels was not affected by trematode infection. Similar results were reported by Molloy *et al*. (1996) and Laruelle *et al*. (2002) as well as present results.

The most serious effect of bucephalidis is host sterility with gonadal tissues replaced by sporocysts also accompanied to follicle fibrosis. Additional lesions were also recorded by other studies (Kniskern, 1952; Yanovich and Stadnichenko, 1997). The present histopathological study showed follicle fibrosis and lesions in the connective tissues of the different gill parts and reproductive tissues in *N. teretusicula*. Also, an outer eosinophilic membrane was sometimes observed around larval stages in the gill, and around the integument of the reproductive tissue. However, this membrane was not always apparent, especially when larval stages were located in fibrous tissues such as interbranchial septum. A single layer of flattened hemocytes, eosinophilic cytoplasm and a small protruding nucleus were most evident in subcutaneous tissues of the reproductive tissue. An outer layer of hemocytes may either represent a limited host response or hemocytes that have become flattened. Previous studies by Humes and Jamnback (1950), Humes and Russell (1951), Humes and Harris (1952) indicated that *N. ingens* might limit mussel populations by obstructing water tubes of marsupia. Besides, Mitchell (1955) reported hyperplasia, followed by Flook and Ubelaker (1972) who reported hypertrophy of tissues infected with larval stages.

Conclusion

The current investigation may represent the first comprehensive series of bucephalid, gorgoderid and echinostomatid larval stages in addition to its pathological effects in the tissues of freshwater mussel *N. teretusicula*. Also, future studies should focus on the search for new trematode species, using: (i) various regions along the River Nile, (ii) many native and alien bivalve species, and (iii) different research methods, including morphological and molecular diagnosis of trematode species. The research should also contribute to answer the question of the environmental determinants of differences between the infection of Unionidae and Dreissenidae residing on their shells.

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Explanation of figures

Figs. 1-5: Micrographs of freshwater mussel *N. teretiuscula*; 1 External view (EV) of shell with anterior (AS) and posterior (PS) ends and oldest part of shell (umbo) (U). 2-5: Inner view (IV) of shell; 2 Soft parts of uninfected shell with gills (G), viscera (V), foot (F), and mantle (M). 3: Swollen brood gills of uninfected shell with its eggs. 4, 5: Infected mussel with white knot on gills.

Figs. 6-12 Photomicrographs of three larval stages infecting *N. teretiuscula*; 6-8 Metacercariae of Echinostomatids with outer membrane (OM), inner membrane (IM), excretory granules (EG), and cercariae (C); 100×, 400×. 9, 10 Cercariae of *Phyllostomium* sp. with oral sucker (OS) and ventral sucker (VS); 100×. 11, 12: Ribbon sporocysts of *Rhipidocotyle campanula* inside cyst (CW) (11) and outside cyst (12) with ramifications (RA); 100×.Figs. 13-19: Scanning electron micrographs of larval trematodes stages infecting *N. teretiuscula*; 13-15: Metacercariae (MC) of Echinostomatids. 16, 17: Cercariae of *Phyllodistomum* sp. with oral sucker (OS) and ventral sucker (VS). 18, 19: Ribbon sporocyst of *R. campanula* with ramifications (RA) and rynchus (R).

Figs. 20-21: Photomicrographs of gills of *N. teretiuscula*; 20: Uninfected gills with gill filaments (GF) and gill lamellae (L); 10×. 21: Infected gills with entanglement knot (EK) of ribbon sporocysts of *R. campanula*; 10×.

Figs. 22-28: Photomicrographs of histological sections; 22: Uninfected gills of *N. teretiuscula* with water tubes (WT), and gill lamellae (L) separated from each other by interlamaller spaces (ILS); 100×. 23 Non-infected gills showing larvae (La) of *N. teretiuscula*; 100×. 24-28 Infected gills of *N. teretiuscula* showing: 24, 25 Ribbon sporocyst (SP) of *R. campanula*. 26 Metacercariae (MC) of Echinostomatids and cercaria (C) of *Phyllodistomum* sp.; 400×. 27, 28 Cercaria(C) of *Phyllodistomum* sp. with oral sucker (OS) and ventral sucker (VS); 400×.
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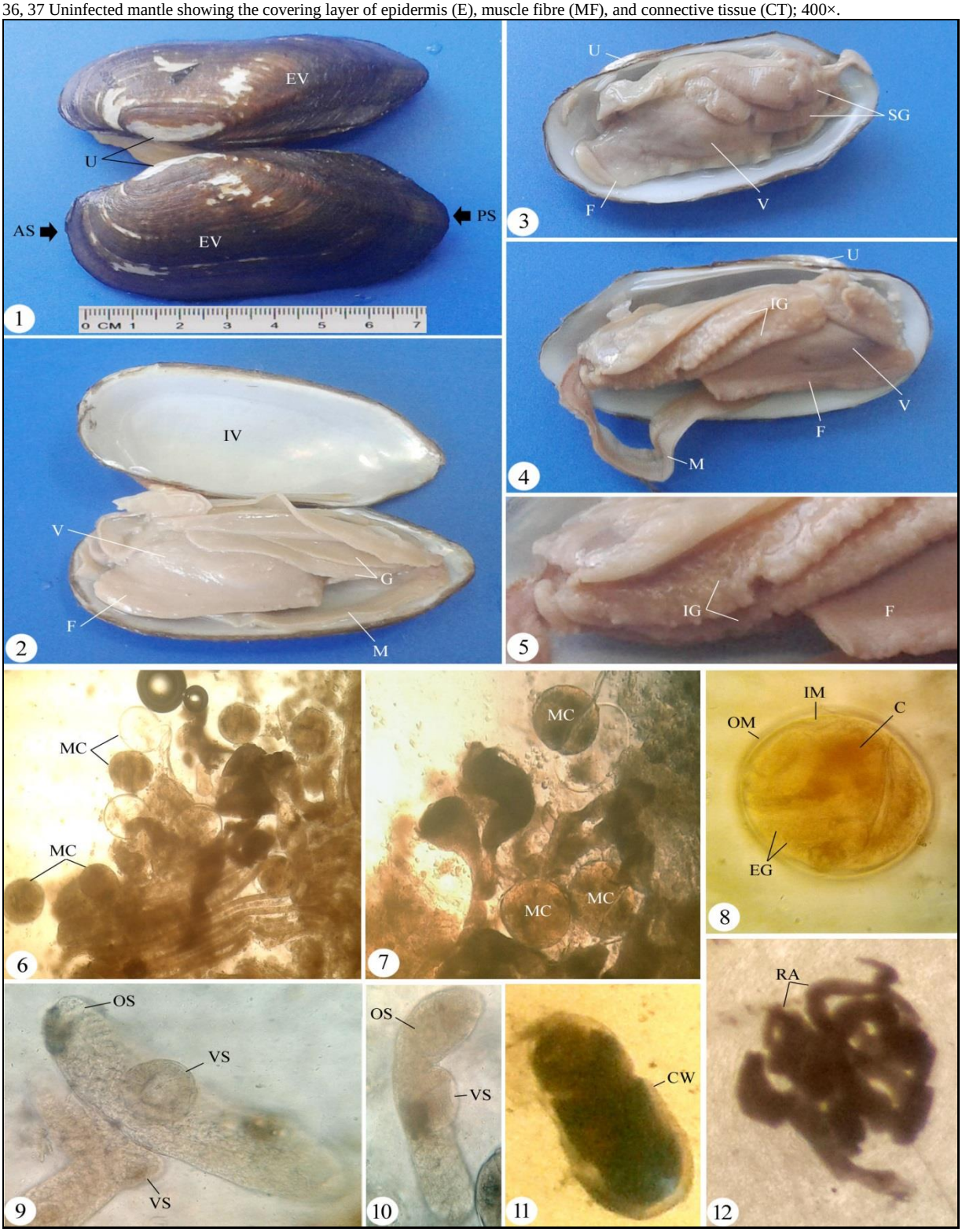

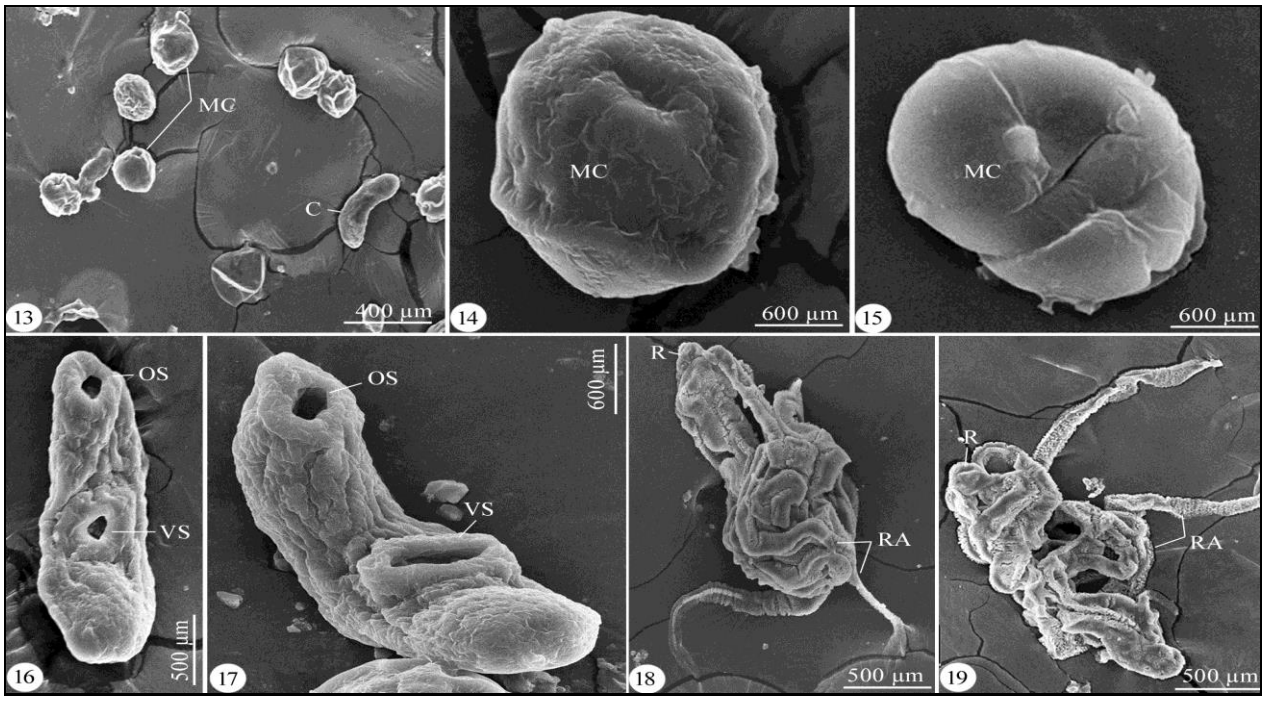
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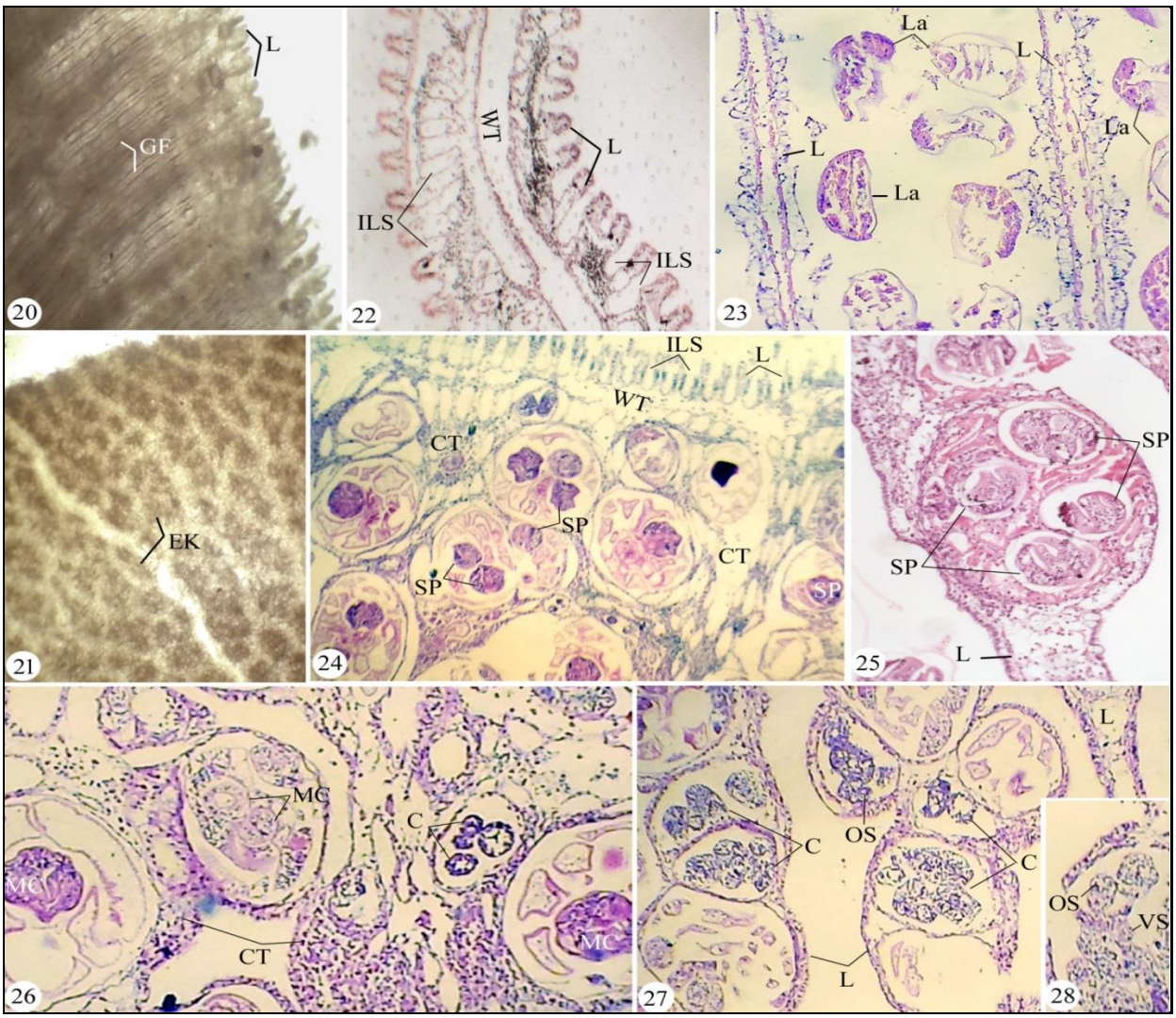
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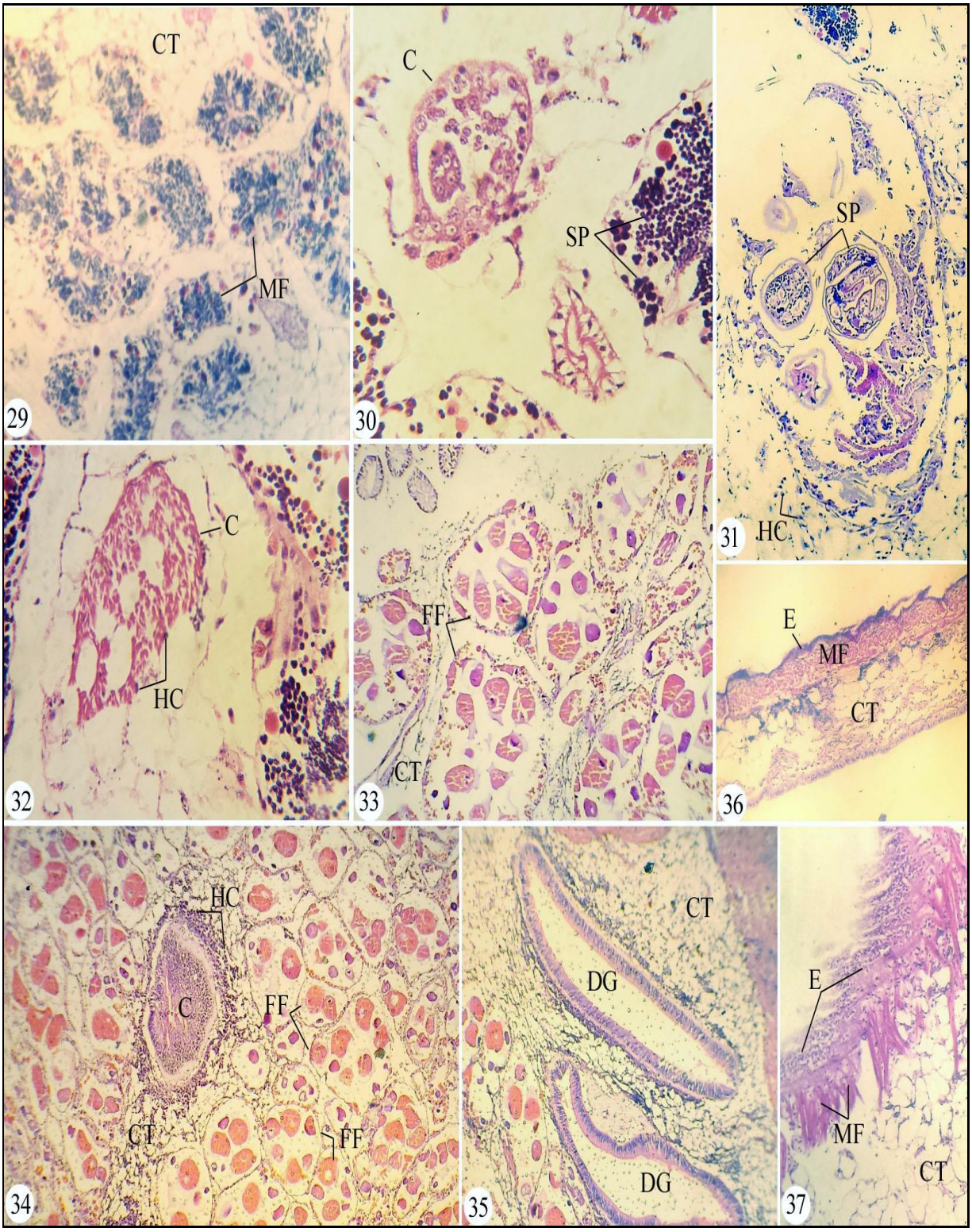
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EXPERIMENTAL EVALUATION OF THE ROLE OF SYMBIOTIC AND APOSYMBIOTIC, *CULEX PIPIENS* MOSQUITOES IN THE TRANSMISSION OF HEPATITIS C VIRUS (HCV)

By

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Abstract

The present study evaluated the potential role of *Culex pipiens* mosquitoes in the transmission of HCV, post being fed on an infected blood with a viral load of $1.3x10^6$IU/ml.in symbiotic mosquitoes and $1.2x10^6$IU/ml.in aposymbiotic one. Mouth parts, mid-gut and salivary glands in both symbiotic and aposymbiotic mosquitoes at different time intervals were tested for evaluation of viral load using real-time PCR for up to 60min and up to 5, 13 day in case of mouth parts, mid-gut and salivary glands respectively. It was observed that the viral load was decreased by increasing the time post feeding in mid-gut and mouth parts in both symbiotic and aposymbiotic mosquitoes so, there is a negative relationship between the HCV load and feeding time. On the other hand, HCV was not detected in salivary glands in both symbiotic and aposymbiotic mosquitoes during the period of detection. The results showed that the mechanical transmission through mouth parts in both symbiotic and aposymbiotic mosquitoes are plausible, while biological transmission through symbiotic and aposymbiotic mosquitoes did not occur.

Key words: *Culex pipiens*, Symbiotic, Aposymbiotic, HCV, Transmission.

Introduction

The mosquitoes are vectors of many vertebrate blood parasites. In Egypt *Culex pipens* has a wide distribution and is the main vector of Rift Valley Fever (RVF) virus (Megan *et al*, 1980; Darwish and Hoogastraal, 1981; El Bahnasawy *et al*, 2013a), *Wuchereria bancrofti* (Khalil *et al*, 1930; Gad *et al*, 1996; Abdel-Hamid *et al*, 2013) and Western Nile Virus (Pelah *et al*, 2002; El Bahnasawy *et al*, 2013b). Hassan *et al.* (2003); Pybus *et al*. (2007); Tarish *et al*. (2014) and El Kholy *et al*. (2017) studied the possibility of the HCV experimental transmission by different mosquitoes.

Hepatitis C Virus infection is one of the major public health problems in both developed and developing countries since discovering at 1989 (Choo *et al*, 1989; Alter *et al*, 1989). It was estimated that HCV infect 200 million peoples (3%) of the world's population and there are at least 21.3 million HCV carriers in the Eastern Mediterranean countries (Sy and Jamal, 2006). The infection acquired mainly via parenteral route (Karaca *et al*, 2006), and also perinatally (Indolfi and Resti, 2009), but there is (30-40%) of infected cases were without identifiable route (Hayashi and Furusyo, 2010).

Many investigators suggested that the mechanical transmission of HCV by mosquitoes was plausible (Germi *et al*, 2001; Hassan *et al*, 2003; Pybus *et al*, 2007). This was in accordance to Kamal (2008) who reported that one viral particle was perhaps sufficient to induce infection via parenteral route. El Kholy *et al.* (2017) reported that the mosquito *Cx. pipiens* may be a potential vector of HCV. However, the biological and mechanical of HCV transmission by mosquitoes showed negative results. Low HCV RNA titers in patient sera and different species tropisms for HCV/RNA replication are probably the reasons why the mechanical and biological HCV transmission did not occur in mosquitoes (Chang *et al*, 2001).