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Micro Fauna Associated with Aquatic Snails, their Control Roles, and the Phylogenetic of New Species *Euplotes elhusseini*

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ABSTRACT

microorganisms, including nematodes, ciliates. Manv rotifers. cheatogastriches, and ostracods, have been found to be associated with freshwater snails in their natural habitats; some of these act as snail biocontrol agents. The current study sought to identify the microfauna associated with aquatic snails and how they interact to know if they have a role of snail biological control. During the 2020-2023 seasons, a survey of freshwater snails and their associated microfauna was conducted in the governorates of Giza and Qualyobyia in Egypt. The 18S ribosomal (18S rRNA) gene sequences for the new ciliate species were determined. The survey identified two snail parasitic nematodes, nine inquiline rotifers, nine ciliates (one of which, Euplotes elhusseini n.sp., was described morphologically and phylogenetically), one actinophryd, two gastrotrichs, and one ostracod was associated with five snail species from each of the Prosobranchiate and Pulmonate snails. The interaction between some microorganisms may affect their roles as control bioagents. Morphological and molecular characteristics of the new species, Euplotes elhusseini, were described. In conclusion, rotifers and snail parasitic nematodes are the only species that might be called control bioagents of harmful gastropods.

INTRODUCTION

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The gastropods contain the most species of molluscs. Mollusca is the second most species-rich phylum after arthropods, with an estimated 80,000–100,000 identified species and a potential total diversity of 200,000 according to **Strong and Colleagues (2008)**. Rice plants suffered severe damage from pulmonates and prosobranchiates, some of which act as intermediate hosts for parasitic diseases (Godan, 1983; Azzam, 2006; Azzam & Belal, 2006; Zaki *et al.*, 2019).

Numerous microorganisms, such as rotifers (Ramakrishna, 1993; Azzam, 2011; De Smet *et al.*, 2015; Chatterjee *et al.*, 2021; Lin *et al.*, 2021; Gavrilko *et al.*, 2024), Heliozoa (Mikrjukov & Patterson, 2001; Weithoff, 2022), nematodes (Azzam, 2003; Azzam, 2006, 2023,2025; Azzam and El-Abd, 2021; Denver *et al.*, 2024), ciliates (Dias

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et al., 2006, 2008 and 2010; Utz, 2007; Azzam, 2011; Abrahama *et al.*, 2021; Chatterjee *et al.*, 2021), Cheatogastriches (Balsamo *et al.*, 2015; Todaro *et al.*, 2015, 2019), Ostracod (Martens *et al.*, 2008; Karanovic, 2012, 2013), were linked to freshwater snails in their natural environments.

Coleps can consume algae, ciliates, flagellates, and bacteria, as well as live plant and animal matter, including fish, rotifers, and crustaceans (Foissner & Berger, 1996; Foissner, 1997; Mazanec & Trevarrow, 2001). In addition, it consumes young fish (Eversham, 2013). Azzam (2011) reported for the first time coleps attack embryos and newly hatched snails. In irrigation ditches in the municipality of Juiz de Fora, Minas Gerais, Brazil, Dias *et al.* (2006) found two suctorians (*Acineta* and *Tokophyra*) and five peritrichs (*Carchesium, Epistylis, Opercularia, Vaginicola,* and *Vorticella*), which are epibionts on ampullariid snails of the species *Pomacea lineata* (Spix, 1827). To determine the role of the microfauna as biocontrol agents of these harmful pests in the Egyptian environment, the current study evaluated the microfauna associated with these snails and their interactions to know if they have a role of snail biological control.

MATERIALS AND METHODS

Aquatic snails were collected from Qualyobyia and Giza governorates' irrigation systems using a metallic strainer with a 22cm diameter and a 70cm aluminum handle that could be extended to 200cm. Plant shacked, the net was quietly pressed into the water. The collected samples were then evacuated into tiny plastic aquaria after the net was repeatedly cleaned in the water to get rid of the mud (Azzam & Belal, 2006). All snails were identified according to the studies of Godan (1983), Ibrahim *et al.* (1999) and Eversham (2013).

Following snail identification, each species' individual was housed in an aquarium that was the right size for its population. Suspected and moribund snails were kept individually in plastic pots containing little of dechlorinated water for isolation of associated nematodes, or other pathogenic microorganisms and it is covered to keep malacophagous insects out. The water checked daily for presence of parasitic nematodes and other associated microorganisms. Aquatic snail B. alexandrina was reared in 45x25x22cm plastic aquaria with 12 liters of dechlorinated water. Each aquarium can hold no more than 60 snails (5 snails per liter). As a food source, some fresh lettuce leaves were provided. Little plastic sheets were used to deposit egg masses, which were then moved to a small pot. Before transferring, a microscopic inspection should be performed to ensure that there are no rotifers or coleps. A tiny portion of fresh lettuce leaves is provided for sustenance after hatching (Azzam, 2023). Some of the recovered nematodes were mounted in lactophenol or glycerol after being killed and fixed by hot Triethanolamine Formalin (TAF) or directly removed from the dead infected snails or from the collected suspension for microscopic analysis (Azzam, 2023). According to Andrassy (1976, 1983), Soliman (1996) and Hodda (2022), adults were identified. Other associated microorganisms, i.e.,

ciliates, ostracods, gastrotrichs, and rotifers, were separated by taking some of the water from the aquaria in which snails were placed, shaking it, and taking the sediment with a pasture pipette, placing it on a small petri dish, and examining it under a microscope. Some of them mounted for xonomic studies by placing 5μ l on a glass slide, adding a drop or equal amount of glycerol, and placing a cover slip over the sample after staining with methyl orange, methylene, or hematoxylin (Azzam, 2011). The organisms that hadn't been identified in previous articles of the authors were identified by DNA.

Ciliates were identified according to Carey (1992), Mikrjukov and Patterson (2001), Lynn (2008), Coats and Clamp (2009), Berger and Foissner (2014), Pan *et al.* (2016), Liu *et al.* (2020) and Nguyen *et al.* (2020). Gastrotrichs were identified based on Evans (1993), Leasi and Todaro (2010), Todaro *et al.* (2019) and André *et al.* (2020); rotifers according to Ricci and Melone (2000), Rochelle (2004), Fontaneto *et al.* (2008) and Fathibi *et al.* (2020). Ostracods followed the indentification outlined in the studies of Martens *et al.* (2008), Karanovic (2012, 2013) and Lord (2020).

Each isolated microorganism species was confirmed with lab-bred freshwater snails, *Biomphalaria alexandrina* (Ehrenberg, 1831) (Mollusca, Gastropoda, Basommatophora, Planorbidae) snails and their egg masses were placed in petri dishes to investigate the relationship between them. All organisms that caused mortality in the snails or their egg masses were tested using Koch's postulates to confirm whether they were pathogenic or parasitic species. (Azzam, 2003).

The relationships between associated microorganisms and each other were conducted by microscopic examination. A small drop of water contained the association of organisms put on a microscopic slide with 1-3 cavities and observed with low magnification, then higher magnification, and video-graphed by the Microscope XSZ-107T and Galaxy–A03S Camera, then photos were cut from videos by the Movie Maker program.

Molecular analysis

DNA for previously unidentified protozoa was extracted from 60 individuals placed in three Eppendorf tubes, 20 individuals/tube, which contained 25 microliters (μ l) of extraction buffer. According to Azzam (2023), this buffer is made up of 12.5µl DreamTaq Green PCR Master Mix (2X), 1µl forward primer, 1µl reverse primer, 2µl template DNA (nuclease (50ng: 500ng), and 8.5µl water free). Primers 18S: 5'-TTGATTACGTCCCTGCCCTTT-3' (forward) and 26S: 5'-TTTCACTCGCCGTTACTAAGG-3' (reverse) were used to amplify a portion of rDNA that contained the internal transcribed spacer regions (ITS1, 5.8S, ITS2) (Vrain et al., 1992). The samples were vortexed gently. PCR cycling involved five minutes of initial denaturation at 95°C for 5 min, forty cycles of 30s denaturation at 95°C, forty cycles of 30s annealing at 57°C, a cycle of extension at 72°C for 30 min, and a final extension at 72°C for 10min (**Nguyen, 2007**).

Sizes of the amplification products were assessed by electrophoresis in an agarose gel, and Wizard SV Gel and PCR Clean-Up System (Promega) were used to remove relevant bands for DNA (Azzam, 2023). The Big Dye terminator cycle sequencing kit v.3.1 (Applied BioSystems, USA) was used to sequence the purified PCR after the PCR product had been precipitated with ethanol and sodium acetate solution. An Applied BioSystems model 3730XE automated DNA sequencing system (Applied BioSystems, USA) at Macrogen, Inc. in Seoul, Korea, was used to resolve the sequencing products. BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to compare the resultant sequences with ITS rDNA 18S sequences of related sequences in NCBI GenBank. The software Multalin version 5.4.1 (Corpet 1988), which may be accessed at http://multalin.toulouse.inra.fr, was used to perform the alignments. The phyliogenitic tree was constructed using MEGA11 after the aligned 18S sequences were molecularly analyzed and inferred using neighborjoining (NJ) (Tamura *et al.*, 2021). *Euplotes incysticus* and *Euplotes elhusseini* n.sp. product sequences were deposited in the NCBI GenBank database as PQ637376 and PQ637379, respectively.

RESULTS

1. Freshwater snails

Freshwater snails and the different organisms that are associated with them are described in Tables (1– 5). There were five known species of prosobranchiats and pulmonates (Tables 1- 5). *Planorbis planorbis* (Linnaeus, 1758), *Helisoma duryi* (Wetherby, 1879), *Bulinus truncatus* (Audouin, 1827), *B. alexandrina* (Mollusca, Gastropoda Basommatophora, Planorbidae) and *Physa acuta* (Draparnaud, 1805) (Mollusca, Gastropoda Basommatophora, Physidae) were the Pulmonates. *Bellamya unicolor* (Olivier, 1804) (Gastropoda: Vivparidae), *Gabbiella senaariensis* (Küster, 1852) (Gastropoda: Bithyniidae), *Melanoides tuberculata* (Müller) (Gastropoda: Thiaridae), *Cleopatra bulimoides* (Olivier, 1804) (Gastropoda: Paludomidae), and *Lanistes carinatus* (Olivier, 1804) (Gastropoda: Ampullariidae) were the prosobranchiate.

2. Associated microfauna

2.1 Nematoda

Two species of snail parasitic nematodes were recorded, as shown in Table (1). *Rhabditis* sp. (Fig. 1a) (Nematoda, Chromadorea, Rhabditida, Rhabditidae) is associated with nine snail species from both Prosobranchiate (*Bellamya unicolor, C. bulimoides, L. carinatus,* and *M. tuberculata*) and Pulmonate snails (*B. alexandrina, B. truncatus, H.*

duryi, *P. planorbis*, and *P. acuta*). *Phasmarhabditis* sp. (Fig. 1b) (Nematoda, Chromadorea, Rhabditida, Rhabditidae) is associated with four Pulmonate species only (*B. alexandrina, B. truncatus, P. planorbis, and P. acuta*) (Table 1).

The two nematode species showed parasitic activity on the snails and their egg masses (Table 1).





b

Fig. 1. (a-b): Snail parasitic nematodes associated with freshwater snails in Giza and Qualiobyia governorates, Egypt

- a- Rhabditis sp.
- b- Phasmarhabditis sp.

Table 1. Aquatic snails and their associated nematodes in different locations in Egypt with their capability to control snails

Snail species	Locality	Associated	Capability to	Type of
		Nematodes	control snails	relationship
Prosobranchiate				
B. unicolor	Giza &	Rhabdihis sp.	+	Parasite on
	Qulyobyia			snails and egg
L. carinatus	Giza &	Rhabdihis sp.	+	masses
	Qulyobyia			
C. bulimoides	Qualyobyia	Rhabdihis sp.	+	-
M. tuberculata	Qualyobyia	Rhabdihis sp.	+	
G. senaariensis.	Qualyobyia	-	-	
Pulmonate Snails				
B. alexandrina	Giza &	Rhabdihis sp.	+	Parasite on
	Qulyobyia	Phasmarhabditis		snails and egg
B. truncatus	Qualyobyia	Rhabdihis sp.	+	masses
		Phasmarhabditis		
		sp.		
H. duryi	Giza &	Rhabdihis sp.	+	
	Qulyobyia	_		
P. planorbis	Giza	Rhabdihis sp.	+	
		Phasmarhabditis		
P. acuta	Giza &	Rhabdihis sp.	+	
	Qulyobyia	Phasmarhabditis		
		sp.		

2.2. Rotifera

Nine species of inquiline rotifers were recorded as being associated with Prosobranchiate and Pulmonate snails (Table 2). These species included Colurella adriatica Ehrenberg, 1831 (Fig. 2a) (Rotifera, Eurotatoria, Monogononta, Ploima, Lepadellidae); Lecane closterocerca (Schmard, 1859) (Fig. 2b) and Lecane luna (Müller, 1776) (Lecanidae); Mytilina ventralis Ehrenberg, 1830 (Fig. 2c) (Fig. 2d) (Brachiomidae); Trichocerca sp. (Fig. 2e) (Trichocercidae); Philodina brevipes Murray, 1902 (Fig. 2f); P. acuticornis Murray, 1902 (Fig. 2g); P. roseola Ehrenberg, 1832 (Fig. 2h); and Rotaria rotatoria Pallas, 1766 (Fig. 2i) (Rotifera, Eurotatoria, Bdelloidea, Philodinida, Philodinidae).



Fig. 2 g

Fig. 2 h

Fig. 2 i

Fig. 2. (a-i): Rotifers associated with freshwater snails in Giza and Qualiobyia governorates, Egypt

- a- Colurella adriatica Ehrenberge 1831
- b- Lecane closterocerca (Schmard, 1859)
- c- Lecane luna (Müller, 1776)
- d- Mytilina ventralis (Ehrenberg, 1830)

- e- Trichocerca sp.
- f- Philodina brevipes Murray, 1902
- g- Philodina acuticornis Murray, 1902
- h- *Philodina roseola* Eherenberg, 1832
- i- Rotaria rotatoria Pallas, 1766

Table 2. Aquatic snails and their associated rotifers in different locations in Egypt with their capability to control snails

Snail species	Locality	Associated Rotifers	Capability to control snails	Type of relationship
Prosobranchiate snails				
B.unicolor	Giza & Qulyobyia	L. closterocerca, L. luna, Mytilina sp., Trichocerca sp., P. brevipes, P. roseola	+	Inquiline To snails and their egg masses
L. carinatus	Giza & Qulyobyia	L. closterocerca, L. luna, Mytilina sp., Trichocerca sp., P. brevipes, P. acuticornis, P. roseola	+	
C. bulimoides	Qualyobyia	C. adriatica, L. closterocerca, L. luna, Mytilina sp., Trichocerca sp., P. brevipes, P. acuticornis, P. roseola	+	
M. tuberculata	Qualyobyia	<i>Trichocerca</i> sp., <i>Mytilina</i> sp., <i>P. brevipes</i> , <i>P. acuticornis</i> .	+	
G. senaariensis	Qualyobyia	C. adriatica, L. luna, L. closterocerca, Mytilina sp., Trichocerca sp.		
Pulmonate Snails				

B. alexandrina	Giza & Qulyobyia	L. closterocerca, L. luna, P. brevipes, P. acuticornis , P. roseola, R. rotatoria	+	Inquiline To snails and their egg masses
B. truncatus	Qualyobyia	C. adriatica, L. closterocerca, L. luna, P. brevipes, P. acuticornis, P. roseola, R. rotatoria	+	
H. duryi	Giza & Qulyobyia	C. adriatica, L. closterocerca, L. luna, P. brevipes, P. acuticornis, P. roseola, R. rotatoria , R. rotatoria	+	
P. planorbis	Giza	P. brevipes, R. rotatoria	+	
P. acuta	Giza & Qulyobyia	C. adriatica, L. closterocerca, L. luna, P. brevipes, P. acuticornis, P. roseola, R. rotatoria	+	

2.3. Ciliates

Two species of *Coleps (Coleps hirtus* (Müller, 1827) (Fig. 3a) and *Coleps* sp. (Fig. 3b)) (Ciliophora, Prostomatea, Prorodontida, Colpidae) were recorded, exhibiting gregarious predation behavior toward snail embryos and newly hatched snails (Fig. 3c, d) (Table 3). They were also observed successfully attacking rotifers, though sometimes the rotifers' defense mechanisms, such as changing to a loricate or ball form (Fig. 3e)

prevented capture. Once the *Coleps* retreated, the rotifer would return to an active state and move away. Additionally, many *Coleps* were involved in attacking gastrotrichs (Fig. 3f), and after the prey surrendered, some *Coleps* would leave, while a few remained attached to the prey (Fig. 3g). Finally, one individual *Coleps* that captured the head of the gastrotrich claimed the prey, with the others leaving after disintegrating the prey's tissues (Fig. 3h).

Dileptus anser (Müller, 1773) (Fig. 3i) (Table 3) (Ciliophora, Litostomatea, Dileptida, Dileptidae) was observed inside the umbilicus region of *B. alexandrina* snails, alongside rotifers. It did not appear to attack snails or their egg masses but likely excretes its waste products inside the snail. As it feeds on bacteria and other organisms, it may contribute to snail mortality, possibly in collaboration with rotifers. *Dileptus* was also seen consuming organisms larger than its neck width, causing the neck to swell like a crop (Fig. 3j).

Two species from the class Spirotrichea, *Stylonchia* sp. (Hypotrichida, Oxytrichidae) (Fig. 3k) and *Pseudokeronopsis* sp. (Urostylida, Pseudokeronopsidae) (Fig. 3l & m), were reported as commensals of snails, feeding on bacteria and small protozoa. Additionally, two species of *Euplotes* were recorded: the new species *Euplotes elhusseini* and *Euplotes encysticus* Yonezawa, 1985 (Fig. 3n, o) (Ciliophora, Spirotrichea, Euplotida, Euplotidae).

Two species of *Paramecium* were recorded as bacterial feeders: *Paramecium caudatum* Ehrenberg, 1833 (Fig. 3p) and *Paramecium* sp. (Fig. 3q), both belonging to Ciliophora, Oligohymenophorea, Peniculida, and Parameciidae. Additionally, one species of *Vorticella* (Fig. 3r) (Ciliophora, Oligohymenophorea, Sessilida, Vorticellidae) was identified as an epibiont (Table 4).

Snail species	Locality	Associated ciliate	Capability to control snails	Type of relationship
Pulmonate Snails				
B. alexandrina	Giza & Qulyobyia	Coleps hirtus Coleps sp. Deliptus anser	+	Predator of embryos and newly hatching snails. Predate on other protozoa and
B. truncatus	Qualyobyia	C. hirtus Coleps sp. D. anser	+ _	bacteria
H. duryi	Giza & Qulyobyia	C. hirtus Coleps sp. D. anser	+ -	

Table 3. Aquatic snails and their associated predator's ciliates in different locations in

 Egypt with their capability to control snails

P. planorbis	Giza	Coleps hirtus	+	
		D. anser	_	
P acuta	Giza &	Colens hirtus	+	
1. donta	Qulyobyia	D. anser	_	
Prosobranchiate snails				
B.unicolor	Giza & Qulyobyia	<i>Coleps hirtus</i> <i>Coleps</i> sp.	+	Predator of embryos and newly hatching snails.
L. carinatus	Giza & Qulyobyia	<i>Coleps hirtus</i> <i>Coleps</i> sp.	+	
C. bulimoides	Qualyobyia	Coleps sp.	+	
M. tuberculata	Qualyobyia	Coleps sp.	+	
G. senaariensis	Qualyobyia	Coleps sp.	+	



Fig. 3a











Fig. 3g





Fig. 3h



Fig. 3 j



40 Fig. 3 l



Fig. 3i





Fig. 3. (a-r): Ciliates associated with fresh water at Giza and Qualiobyia Governorates, Egypt

- a. Coleps hirtus (Muller, 1827)
- b. Coleps sp.
- c. Many individuals of *Coleps* while attacking the *B. alexandrina* snail embryo.
- d. Numbers of *Coleps* attack newly hatched *B. alexandrina* snails.
- e. *Philodina* rotifers, which change like a ball as a defense mechanism, while attacked by a group of coleps.
- f. Group of *Coleps* attack the gastrotrich
- g. Three individuals of *Coleps* remain attached to the gastrotrich prey.
- h. Finally, one *Coleps* remained attacking the gastrotrich and swallowing the prey head; the arrows are pointing to disintegration in the tissues as a result of predator attackin
- i. Dileptus anser (Müller, 1773)
- j. Swelling of the neck of *D. anser* as a result of swallowing some organism larger than its diameter
- k. Stylonchia sp.
- 1. Pseudokeronopsis sp.
- m. Pseudokeronopsis sp. high magnification
- n. Euplotes elhusseini n sp.
- o. Euplotes encysticus (Ehrenberg, 1833).
- p. Paramecium caudatum (Ehrenberg, 1833).
- q. Paramecium sp.
- r. Vorticella sp.

Snail species	Locality	Associated ciliates	Capability to kill snails	Type of relationship
Prosobranchiate snails				
B. unicolor	Giza & Qulyobyia	Stylonchia sp. Pseudokeronopsis sp. Paramecium caudatum Paramecium sp.	_	Commensal Bacterial feeder

Table 4. Aquatic snails and some associated ciliates in different locations in Egypt with their capability to control snails

L. carinatus	Giza & Qulyobyia	Stylonchia sp. Pseudokeronopsis sp. Paramecium caudatum Paramecium sp.	-	
C. bulimoides	Qualyobyia	Vorticella sp. Euplotes elhusseini Euplotes encysticus	_	Epibionts
M. tuberculata	Qualyobyia	Vorticella sp. Euplotes elhusseini	-	Epibionts
G. senaariensis.	Qualyobyia	Vorticella sp. Euplotes elhusseini Euplotes encysticus	-	Epibionts
Pulmonate Snails				
B. alexandrina	Giza & Qulyobyia	Stylonchia sp. Pseudokeronopsis sp. Paramecium caudatum Paramecium sp.	_	Commensal Bacterial feeder
B. truncatus	Qualyobyia	Stylonchia sp. Pseudokeronopsis sp. Paramecium caudatum Paramecium sp.	-	
H. duryi	Giza & Qulyobyia	Stylonchia sp. Pseudokeronopsis sp. Paramecium caudatum Paramecium sp.	_	-
P. planorbis	Giza	Euplotes elhusseini Euplotes encysticus	-	-
P. acuta	Giza & Qulyobyia	Stylonchia sp. Paramecium caudatum Paramecium sp. Pseudokeronopsis sp.	-	

2.4. Heliozoa

Table (5) lists one heliozoan species, *Actinosphaerium* sp. (Fig. 4a) belonging to the Gyrista, Raphidomonadea, Actinophryida, family Actinophryidae. It exhibits predatory behavior toward several organisms, i.e., different types of rotifers (Fig. 4b, c). Also, caught *Coleps* using their axopodia while the last attacking small protozoa (Fig. 4d, e) and preying on larger protozoa (Fig. 4f). *Actinosphaerium* sp. attacks rotifers, a natural enemy of snails. However, it also attacks coleps, which attack rotifers. Thus, it plays a complex role in snail control.



Fig. 4d

Fig. 4e

Fig. 4. (a-f): Heliozoa, associated with fresh water at Giza and Qualiobyia Governorates, Egypt

- a. The heliozoan Actinosphaerium sp.
- b. The heliozoan Actinosphaerium sp. attacks rotifer lorica.
- c. Two heliozoan Actinosphaerium attack two Trichocerca sp. rotifers.
- d. Coleps try to attack a small protozoa in the range of Actinosphaerium axopodia.
- e. The Actinosphaerium catches Coleps by axopodia.
- f. The heliozoan Actinosphaerium caught big protozoa

2.5. Gastrotrichs

Two species of gastrotrichs (Table 5), Lepidodermella squamata (Dujardin, 1841) (Fig. 5a) and Lepidodermella sp. (Fig. 5b) (Gastrotricha, Chaetonotida, Chaetonotidae), were recorded and fed on debris of dead snails and egg masses and bacteria.



Fig. 5a



Fig. 5. (a-b): Gastrotrichs associated with fresh water at Giza and Qualiobyia Governorates, Egypt

b- The gastrotrich Lepidodermella sp.

2.6. Ostracods

One species (Table 5), *Cypridopsis vidua* (Müller, 1776) (Arthropoda, Ostracoda, Ostracoda Cyprididae) (Fig. 6a & b), feeds on algae.



Fig. 6 a

Fig. 6 b

Fig. 6 (a-b): Ostracod associated with fresh water in Giza and Qualiobyia governorates, Egypt

- a- The ostracod Cypridopsis vidua (Müller, 1776) dorsal view.
- b- The ostracod Cypridopsis vidua (Müller, 1776) lateral view.

Table 5. Aquatic snails and some associated heliozoan, gastrotrichs, and ostracods in different locations in Egypt

Snail species	Locality	Associated microorganisms	Capability to kill snails	Type of relationship
Prosobranchiate snails				
B. unicolor	Giza & Qulyobyia	Actinosphaerium sp.	_	Prey on several organisms
		<i>Lepidodermella</i> sp., <i>L. squamata</i>	-	Saprophagous and bacterial feeder
L. carinatus	Giza & Qulyobyia	Actinosphaerium sp.	_	Prey on several organisms
		Lepidodermella sp., L. squamata	-	Saprophagous and bacterial feeder
C. bulimoides	Qualyobyia	<i>Actinosphaerium</i> sp.	_	Prey on several organisms

		Lepidodermella sp., L. squamata	-	Saprophagous and bacterial feeder
M. tuberculata	Qualyobyia	<i>Lepidodermella</i> sp., <i>L. squamata</i>	_	
G. senaariensis	Qualyobyia	Lepidodermella sp., L. squamata	_	-
Pulmonate Snails				
B. alexandrina	Giza & Qulyobyia	Actinosphaerium sp.	_	Prey on several organisms
		<i>Lepidodermella</i> sp., <i>L. squamata</i>	-	Saprophagous and bacterial feeder
		Cypridopsis vidua	-	Feed on algae.
B. truncatus	Qualyobyia	Cypridopsis vidua	_	
		Actinosphaerium sp.	-	Prey on several organisms
		<i>Lepidodermella</i> sp., <i>L. squamata</i>	-	Saprophagous and bacterial feeder
H. duryi	Giza & Qulyobyia	Actinosphaerium sp.	_	Prey on several organisms
		<i>Lepidodermella</i> sp., <i>L. squamata</i>		Saprophagous and bacterial feeder
P. planorbis	Giza	Lepidodermella sp., L. squamata	-	
P. acuta	Giza & Qulyobyia	<i>Lepidodermella</i> sp., <i>L. squamata</i>	-	
		Actinosphaerium sp.		Prey on several organisms

3. Classification and description of a new species, Euplotes elhusseini

3.1. Classification

Phylum: Ciliophora Doflein, 1901.
Class: Spirotrichea Bütschli, 1889.
Subclass: Hypotrichia Jankowski, 1979.
Order: Euplotida Small and Lynn, 1985.
Family: Euplotidae Ehrenberg, 1838.
Genus *Euplotes* Ehrenberg, 1831.
Species *elhusseini* n. sp.
Author: Azzam

Type locality: associated with freshwater snails.

Etymology: This new species of *Euplotes* was dedicated to the name of my professor, Dr. Monir Elhusseini, Professor of Biological Control, Insect Diseases, and Environmental Protection, as gratitude to him.

Type material: Specimens were deposited in the collections of animals of the Department of Harmful Animals, Plant Protection Research Institute, Agricultural Research Center.

GenBank accession numbers: PQ637376

ZooBank acess number LSID:

urn:lsid:zoobank.org:pub:5A5A875C-3EB0-4203-9F41-7632B6C760EF

3.2. Morphological description

In vivo, the body is oval-shaped and dorsoventrally flattened, with anterior portion little wider than the posterior (Fig. 7a & b). Body length is about $48.5\pm5.11\mu$ m, and width is $31.85\pm3\mu$ m (Table 6). The length/width ratio was 1.54 ± 0.23 . One marginal cirri, three caudal cirri, five transverse cirri, and six frontoventral cirri (Fig. 7a). The dorsal side has five prominent ridges. Five kinety with 28–30 dikinetids (Fig. 7b). One macronucleus is almost C-shaped, with noticeable curvature in the anterior and posterior regions, and one micronucleus is almost spherical in shape in the anterior end. Buccal cavity covering the anterior part without frontal plate. Contractile vacuole near the transverse cirri (Fig. 7a).

Character	Min	Max	Mean	SD	SE	V	CV	n
Body length in vivo	40	55	48.5	5.11	1.14	26.16	10.53	20
Body width in vivo	28	37	31.85	3	0.67	8.98	9.42	20
Body length/ width ratio	1.17	1.96	1.54	0.23	0.05	0.05	14.94	20
Adoral zone, length	26	38	31.73	3.56	0.92	12.64	11.21	15
Adoral zone length/ body length ratio	57.14	76.20	64.73	5.53	1.43	30.63	8.54	15
Adoral membranelles, number	26	34	30	2.42	0.62	5.86	8.07	15

Table 6. Morphometric data of *Euplotes elhusseini* n. sp. expressed as µm

Frontal cirri, number	6	6	6	0	0	0	0	15
Transverse cirri, number	5	5	5	0	0	0	0	15
Caudal cirri, number	3	3	3	0	0	0	0	15
Marginal cirri, number	1	1	1	0	0	0	0	15
Dorsolateral kineties, number	5	5	5	0	0	0	0	15
Dikinetids in DK1, number	28	30	29	0.85	0.22	0.71	2.93	15







Fig. 7b

Fig. 7. (a-b): Illustration of the new species *Euplotes elhusseini* a-Illustration of ventral view of *Euplotes elhusseini* n. sp. b- Illustration of dorsal view of *Euplotes elhusseini* n. sp.

3.3. Phylogenetic and molecular analysis of a new species Euplotes elhusseini

The PCR product of ITS rDNA of *Euplotes elhusseini* n. sp. produced 464 bp. *E. elhusseini* n. sp. is compatible with a high bootstrap value of 93.32-94.14% with *E. encysticus* and 93.22-94.36% with *E. magnicirratus*. It also coincided in 92.51% with *E. sinicus*, 94.12% and 93.43% with *Euplotes* spp., 91.45% with *E. dammamensis*, 91.3% with *E. antaracticus* and 89.61% with *E. rariseta*.

E. elhusseini n. sp. appeared in the tree closely related to a group of *E. encysticus*, forming a well-supported monophyletic subclade composed of *E. encysticus* and *E. muscorum*. This subclade is sister to another one mainly composed of two sub-subclades,

the first composed of *E. magnicirratus* and the second composed of two sub- sub clads, the first composed of different species, including *E. crassus, E. minuta vannus*, and *E. rariseta* (Fig. 8), and the second composed of *E. sinicus, E. antaracticus, R. alatus* and *E. orientalis.* The phylogenetic tree of the Egyptian *E. encysticus* (Fig. 9) placed it between *E. encysticus* (KX516559) and (KX516560).

DISCUSSION

Many microorganisms were reported in association with gastropods in different relationships. The associations between nematodes and gastropods range from accidental phoresis to parasitic or pathogenic relationships (Grewal *et al.*, 2003). Azzam and Belal (2006) reported three species of parasitic nematodes associated with freshwater snails. *Phasmarhabditis eagyptiaca* could kill 70% of *B. alexandrina* exposed to its infection (Azzam, 2023), while *Phasmarhabditis eobaniae* perish 80% from each of *B. alexandrina* and *B. truncatus* and 90% from *P. acuta* (Azzam, 2025). In the present investigation, two species of snail parasitic nematodes were recorded. *Rhabditis* sp. was associated with nine snail species from both Prosobranchiate and Pulmonate snails. While *Phasmarhabditis* sp. associated with four pulmonate snails only, the two nematode species showed parasitic activity to snails.

Nine species of inquiline rotifers were recorded as being associated with prosobranchiate and pulmonate snails. This is in accordance with Azzam (2011), which recorded the 13 rotifer species associated with freshwater and terrestrial snails and recorded for the first time that rotifers could kill mature snails, not only egg masses and newly hatching. She attributed the cause of mortality to disturbance and irritation caused by the rotation of the rotifer's corona that might affect the nervous system, accumulation of the excretory products inside the snail, which polluted it, cracking the shell, and abrasion of the mantle, which led to secondary infection by other pathogens. Azzam (2013) mentions that the egg masses that were exposed to rotifers in the early stage of development (one to four cells) had 98-99% of them rapidly die. While embryos in eight cells to gastrula recorded a mortality range of 92-95%. Deformation occurred in each of the late trochophore, early veliger, and pre-hatching stages, leading to death.

Line et al. (2021) found that *Philodina* killed newly hatched *Biomphalaria* straminea. A colony of *Epistylis plicatilis* Ehrenberg, 1838, epibiont on the gastropod *Pomacea bridgesi* (Reeve, 1856), was discovered to have *Philodina megalotrocha* Ehrenberg, 1832 attached to it (Chatterjee et al., 2021). Any contamination of *B. alexandrina* snails with *Philodina* sp. two weeks prior to miracidial exposure inhibited the development of *Schistosoma mansoni* Sambon, 1907 (Platyhelminthes, Trematoda, Diplostomatida, Schistosomatidae), cercariae in snail tissues (Mossallam et al. 2013).



Fig. 8. *Euplotes elhusseini* n. sp.'s evolutionary relationships with other *Euplotes* species, as deduced from NJ analysis of 464 (ITS) rDNA (18S) genes. Nodal support is represented by a bootstrap value as a percentage





Two species of *Coleps (Coleps hirtus* and *Coleps* sp.) were recorded and showed gregarious predation behavior toward snail embryos and newly hatched snails as many as other organisms.

The presence of *Coleps* and its attacking snail embryos and newly hatched snails in gregarious behavior may be in favor of snail control. On the other hand, it was also

observed attacking rotifers, which are considered natural enemies of snails. Thus it has a complicated role in its effect on the snails control bioagents. In addition, *Coleps hirtus* is histophagous, meaning it consumes live plant and animal tissue, including rotifers, crabs, and fish, in addition to feeding on bacteria, algae, flagellates, and ciliates (Foissner *et al.* 1999). Using several ciliate species as prey, similar predatory behavior was also noted (Buonanno *et al.*, 2014). Thus the *Coleps* couldn't be deemed a good agent for snail management.

In addition to one species of gastrotrich and another of ostracod, seven species of commensal ciliates were found to be connected to both prosobranchiate and pulmonate snails.

Seven taxa of ciliates associated with *Pomacea canaliculata* (Mollusca: Gastropoda) were originally identified in southern Brazil by **Dias** *et al.* (2006). The species *Epistylis licatilis* (Peritrichia, Epistylididae) was reported by Utz (2007) as being attached to the shell of *Pomacea canaliculata* (Lamarck). *Tokophrya fasciculate* was linked to *Lymnaea attenuata* Say, according to **Dias** *et al.* (2008). The first documented instance of peritrich ciliates exhibiting epibiosis on *Physa acuta* was reported by **Sartini** *et al.* (2018).

Significant predation pressure on all animal species was demonstrated by interactions between a predatory protist (Actinophrys sol, Heliozoa) and its prey, which included the ciliate *Urosomoida* sp., the mixotrophic protist *Chlamydomonas acidophila*, and the rotifers *Elosa woralli* and *Cephalodella* sp. (Weithoff & Bell, 2022).

In the present investigation, *Actinosphaerium* sp. exhibits predatory behavior towards several organisms, i.e., different types of rotifers, coleps and protozoa

The presence of bacterial feeder ciliates causes depletion of bacteria in the snail ecosystem; this encourages the parasitic nematodes to form the dauer infective stages, which are capable of infecting other individuals of gastropods.

The closest and most similar species (*E. encysticus, E. muscorum, E. magnicirratus, E. sinicus, E. antaracticus* and *E. rariseta*) are compared with the new species, *E. elhusseini*. In order to compare these species, common characteristics including small size, frontoventral cirri, caudal cirri, and marginal cirri were used. *E. vannus* and *E. encysticus* are different from *E. elhusseini* in that they have larger bodies in vivo *E. elhusseini* (40–50µm) as opposed to 80–90µm for *E. encysticus*, 60–95 for *E. sinicus*, 90–140 for *E. vannus*, and 63–78 for *E. muscorum* each.

E. elhusseini has one marginal cirri, whereas *E. encysticus, E. magnicirratus,* and *E. wuahanensis* have two (Liu *et al.,* 2020). Despite differences in certain morphological criteria, the phylogenetic tree revealed a strong link between *E. elhusseini* and other species within the genus with high bootstrap support values. Therefore, the fact that it is a new species from the genus *Euplotes* could be verified.

CONCLUSION

From the above results, it is clear that the parasitic nematodes and rotifers could be considered control bio agents for harmful freshwater snails. Since the *Coleps* attack embryos and newly hatched snails but also attack other organisms, including fish and plants, it couldn't be considered a suitable control bio agent to gastropods. The presence of *Actinosphaerium* sp. may have a double effect on snail control. *E. elhusseini* is a new species of the genus *Euplotes*.

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