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Application of DNA barcoding for identifying potential biotechnological candidate organisms from the River Nile, Egypt.

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ABSTRACT

Ciliated protozoa play many fundamental roles in the aquatic environments where they thrive. They mediate the microbial loop of the universal food web, control the surrounding organisms, and act as biomarkers for water quality, beside some capability for the production of bioactive compounds. Identification of different ciliate species is chiefly based on morphological taxonomy. In order to develop molecular tools that facilitate the exploration of freshwater Nile ciliates, a direct, 18srDNA-based PCR assay was tuned up. This assay was tested for DNA-barcoding of some species that prevail the Nile in Mansoura City in the North of Egypt. The resulting sequences were applied to design new species-specific PCR assays for detection of density of these ciliates in River Nile. Using both newly-designed primers and assays, the top identified species were *Euplotes woodurffi*, *Halteria grandinella*, and *Coleps hirtus.* The species-specific primers produced variable-sized PCR amplicons, with densities that were at least correlated by 95 % with the real species count in the riverine water. The newly designed molecular tools are expected to facilitate the exploration and quantification of those species in different aquatic habitats where they exist, adding a major contribution to future efforts for exploiting them as resources for blue biotechnology.

INTRODUCTION

Ciliates are one of the most abundant taxa in the biosphere, representing key rate-controllers for sympatric bacteria, algae, and other protist populations. They also form a key part of the microbial loop of the universal food web, ultimately affecting its top level, viz. mammals including human (Corliss, 2002). Directly in relation to human benefits, they are gaining an increasing attention as promising experimental models, due to their short-time life cycles, rapid population resilience time, relative ease in laboratory culture, and a cosmopolitan distribution in almost all aquatic habitats on earth (Gutiérrez, *et al.*, 2008). Even there are some recent studies that assessed the role of their pheromones and secondary metabolites as potential anti-cancer derivatives (Catalani *et al.*, 2016). They have been elucidated as biomarkers for improved environmental quality, such as in the soil (Abraham *et al.*, 2019). Moreover, some species actively reduce bacterial flocs and increase ammonia removal from activated sludge, leading to a long-term enhancement of sewage-treatment

through activated sludge (Walczyńska *et al.*, 2018). Also, they are key aquatic biosensors towards heavy metals due to the absence of a rigid cell wall, exhibiting in many cases the loss in cells´ morphological integrity; roundness, intracellular vacuolization, morphological deformities and cellular rupture (Pudpong and Chantangsi, 2015 and Madoni and Romeo, 2006; Somasundaram *et al.*, 2018). Even ciliates´ superoxide dismutase exhibited clear heavy metal concentrations-dependent activities (Toteja *et al.*, 2017).Freshwater ciliates contribute much to the environmental bioremediation from the heavy metals (Abraham *et al.*, 2017). Ciliates also play a key role in water purification systems (Debastiani *et al.*, 2016 and Radhakrishnan and Jayaprakas, 2015).

The early works on ciliate´s taxonomy solely based on living morphology (Kahl; 1932 and Müller, 1786), then silver staining (Curds, 1975). However, with the advent of the molecular taxonomy era, there is a clearly increasing number of works that use molecular information to define species (Alekperov *et al.*, 2006; Chen *et al.*, 2013; Schwarz and Stoeck 2007*;* Schwarz *et al.*, 2007*;* Syberg-Olsen *et al.*, 2016; Zhao *et al.*, 2018). Yet, still the classification of microbial eukaryotes using DNA barcoding and molecular phylogenies is hindered by the difficulty of isolating specific species, and it kept almost restricted to those that are easily cultured in the laboratory. Studies on the 18srDNA, also termed the small ribosomal subunit SSU rDNA, exhibited that this gene in ciliates have a high level of interspecific sequence variability upon its amplification by Polymerase chain reaction (PCR) and sequencing. Therefore, it gained a specific popularity as an important tool for ciliates´ barcoding, phylogenies and other taxonomy-based analyses (Wang *et al.*, 2017). It could be successfully applied to identify the seasonality of planktonic freshwater ciliates, the diversity of microbial communities hosted by some plants and colonizing some specific coastal systems like mangroves, systematic positioning of some ciliates species, as well as surveilling some ciliate species related to biomonitoring and ecological impacts-assessment of aquaculture of key fish species (Grimonprez *et al.*, 2018; Yan *et al.*, 2018; Forster *et al.*, 2019; Grothjan and Young, 2019; Pitsch *et al.*, 2019). Still DNA barcoding for free living ciliates a poorly researched discipline, despite the very well-known importance of this group in potable and waste water treatment (Galal *et al.*, 2011; Galal, 2013).

The current work aims to: i) identify, at the molecular level, the most abundant River Nile ciliates, especially those having roles in environmental biotechnology; ii) provide a phylogenetic and DNA-barcoding-based evidences for their accurate species designation; and iii) develop different DNA barcoding primers based on their sequences to provide the possibility of their detection in water through PCR and QPCR.

MATERIALS AND METHODS

Sampling and samples preservation

Freshwater samples were collected from River Nile at Mansoura city Dakhleyia province, North Egypt (in front of EL-Sharq station for water treatment). The samples were transferred to the Lab of Molecular Genetics and Biotechnology in the Faculty of Science of Menoufia University (Shebin El-Kom City, Egypt). The samples were subjected to a rapid microscopic examination for identification of different kinds of ciliates according to Patterson and Hedley (1992).

Isolating Nile Ciliates

Singular cells from the most abundant ciliates were collected manually using a 10 µL micropipette, and each was placed in a single, 0.2 mL tube. Each cell of the selected species; *Euplotes* (Spirotrichea), *Halteria* (Oligotrichea), and *Coleps* (Prorodontida); was placed in 2 µL of deionized water and then stored in a -20 ºC freezer until running the 18srDNA-based PCR. This was carried out in triplicates for each species. At the same time, the rest of cells were sorted and placed in tubes containing only either of the three species, and stored in the -20 ºC deep freezer until subsequent genetic analyses.

18srDNA primers design

18srDNA sequences of many species belonging to the species that are mostly abundant in the area of study were retrieved from the GenBank database, for example *E. euryhalinus* (accession number MG994991.1), *E. woodruffi* (acc. No. MG994981.1), *E. parawoodruffi* (acc. No. AF452708.1), *E. muscicola* (acc. No. MH795290.1), *E. harpa* (acc. No. KX138651.1), *Levicoleps biwae* (acc. No. KT0726321), *C. hirtus* (acc. No. KF1772781.1), Uncultured *Halteria* sample (acc. No. GU067995.1) and *Halteria grandinella* (acc. No. AY007444.1). These sequences were aligned using ClustalW algorithm (Thompson *et al.*, 1994) integrated to Mega6 (Tamura *et al.*, 2013). The sequences were then trimmed to obtain a final common zone of about 600 base pairs. A single sequence from each species was uploaded to primer3plus algorithm (http://primer3plus.com/cgi-bin/dev/primer3plus.cgi) in order to design a pair of primers with annealing temperature between 55-60 ºC, maximum repetitions of the same nucleotides less than 3, and expected amplicon size of 800 bp. Also, the primers were checked to assure the least self-complementation of bases in each given primer alone and between the two primers in the selected primer pairs (not exceeding 3 complementary bases adjacent to each other) for avoiding formation of hair-pin loops.

PCR amplification for 18s ribosomal RNA gene

A direct PCR was carried out for the isolated single ciliates´ cells, using the newly designed, universal primer pairs (EupF- EupR) that flank the hypervariable barcode region in the 18s ribosomal RNA gene. The amplification reactions were performed in a total volume of 25 µL. The reaction mixture consisted of 2 µL of deionized H_2O containing the single ciliate organism as a template DNA, 12.5 µL of MyRedTaq™ PCR Master mix (BIOLINE), 0.4 µM of each primer , and 5 µg of Bovine serum albumin (BSA). A blank sample containing only PCR reagents and deionized water was included with that PCR as a negative control. PCR amplifications were run in the thermal cycler Biometra (T-personal 48, Germany). The PCR protocol included an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of 95 °C for 45 s, 55.9 °C for 45 s, 72 °C for 1 min, and a final extension of 72 °C for 10 min. PCR products were electrophoresed in a 1% agarose gel. with a 1kbp DNA ladder(Thermo Scientific Cat No. SM0314). After completion of the electrophoresis, the agarose gel was visualized using a UV- Transilluminator (Transilluminator Ti 1, Biometra, Germany). Accurate sized PCR products (~ 800bp) were purified from the Gel using Mega quick-Spin™plus Fragment DNA purification Kit (Intron Biotechnology, South Korea), then sent to Macrogen Inc. (Seol, South Korea) for conventional Sanger sequencing.

Genetic and phylogenetic Sequences analyses

The quality of the obtained sequences was manually checked using the freeware Chromas 6.2.5. Sequences were manually checked and corrected for nucleotide

deletions/insertions whenever necessary. Each sequence was individually compared to GenBank database using basic local alignment search tool, BLAST (http:// blast.ncbi.nlm.nih.gov/Blast.cgi; Altschul *et al.*, 1990). Sequences of different species of the same genera were retrieved from the GenBank database, aligned using CLUSTALW as before. The file was saved as .nxs one. Bayesian inference (BI) for phylogenetic relations among assessed species was carried out using MrBayes 3.2.1 (Ronquist *et al.*, 2018), Four Markov Chains Monte Carlo (MCMC) chains were analyzed for 10 million (ngen=10,000,000) generations, saving a tree each 1,000 generations. The subsequent analysis started when the average standard deviation of split frequencies reached 0.002. Tracer 1.7 (Rambaut *et al.*, 2018) was applied for calculating effective samples size and number of burn-ins. Tracer 1.7 exhibited that 25 % of the saved trees are to be discarded as burn-ins. This information was transferred to MrBayes 3.2.1. for constructing the summarized tree, which was later opened also using FigTree v 1.3.1.

Design, testing, and application of species-specific primers

Design of specific primers for *Coleps*, *Euplotes* and *Halteria* followed the same procedure detailed in section 2.1 before, but based on the specific 18srDNA sequence obtained for each species using the 18srDNA universal primers designed. Another critical criteria that was considered for the design of species-specific 18srDNA primers is to provide a tool for facilitating ciliate detection using a simple agarose gel electrophoresis is to produce PCR amplicons with different sizes among the three species. Hence, the primer3plus algorithm was set to produce PCR amplicon with sizes of 400 bp for *Euplotes*, 250 bp for *Coleps*, and 180 bp for *Halteria.*

Upon arrival, the primers were applied in a PCR program for each species, using a direct PCR and the same samples stored separately in the -20 ºC deep freezer. PCR protocol and conditions followed the same as mentioned in section 2.3. herein, but using a gradient of annealing temperatures between 50-60ºC to detect the best for each organism. The PCR products were resolved in 1 % agarose gel stained with 0.5 µg ethidium bromide and visualized using a UV transilluminator.

Later on, field water collected and counted for the target organisms. From a 10 mL sample containing 2,450 *Euplotes*, 4,800 *Coleps*, and 8,050 *Halteria* individuals; 1 mL in triplicates was placed in a 1.5 mL sterile Eppendorf tubes, then used to make 3 half-serial dilutions with deionized water. All tubes were centrifuged at 12,000 g for 10 min and the supernatant was completely removed. 250 µL of 5 % Chelex® 100 sodium form resin (Sigma-Aldrich, Madrid, Spain) in TE buffer (pH 8) were added to the precipitate, together with 2.4 U of Proteinase K solution (Thermo Scientific Cat. No. E00491). The samples were incubated at 55°C for 90 min, then at 100 ºC for 20 min. DNA samples were then stored in a 4 °C fridge and PCR amplifications were done in a period not exceeding 1 week. The rest was stored at - 20°C for archival storage. A final PCR was carried out to quantify the intensity of each organism in relation to its count in the serially diluted samples, using the same PCR program and protocol mentioned in section 2.3 herein, but applying only 25 PCR cycles instead 40, and using the annealing temperatures adequate for each species-specific primer pair. PCR amplicons were resolved in a 1% agarose gel stained with 0.5 µg ethidium bromide. Band intensities were quantified using ImageJ software. Finally, the regression coefficient (r^2) was calculated for each organism´s dilutions vs. intensity of PCR bands to assess the robustness of quantitative detection by PCR. Finally, the PCR amplicons produced for each sepcies from the highest concentration samples were sequenced to confirm the identity of the amplified products.

Statistical analysis

For all measurements, the results were analyzed using one-way ANOVA in the program Statgraphics Centurion IX. Least significant Difference (LSD) was used as a post-hoc test to estimate statistical significance among band intensities in different serial dilutions for each organism. Differences were considered significant at $P< 0.01$.

RESULTS

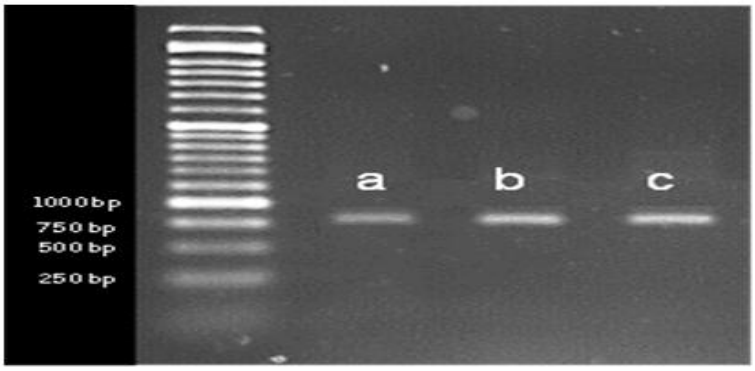
PCR amplification for 18s ribosomal RNA gene.

18srDNA universal ciliate primers (EupF- EupR) were designed for ciliates. Sequences and PCR protocol for these primers are shown in Table 1. The gene coding for 18srDNA could be successfully amplified, showing the expected 800 bp-bands for the three tested organisms (Figure 1).

Table 1: Sequences of primers used for amplification of partial fragment of ciliates

Category	Primer designation	Primer sequence (5´ →3´)	No Cycles	Cycling conditions		
				1	2	3
Universal	Eup F	5´-GGTTCGATTCCGGAGAGGGAGC-5´	40	95°C, 45 sec	55.9°C, 45 sec	72°C, 1 min
	Eup R	5´-CTTTAAGTTTCAGCCTTGCGACC-5				
Species-specific	Euplotesfw	5´-AAACACTTAGCGAGGAACAA-5´	25		57.6°C, 45 sec	
	Euplotesrv	5´-TATTAACCATCTCCGTGTCC-5´				
	Halteriafw	5´-GCCCTTCCATCCTTCTGTTA-5´			57.6°C, 45 sec	
	Halteriarv	5´-TCAATCCCTCAAACCAACAA-5´				
	Colepsfw	5´-TCTTACGAACTTTGGGATTG-3´			60.1°C, 45 sec	

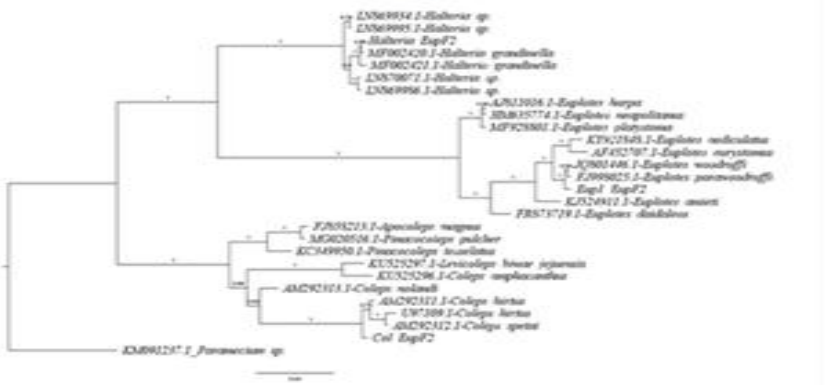
Figure 1. Agarose gel (1%) electrophoresis showing a 800 bp fragment of the 18srDNA of (a) *Coleps*, (b) *Halteria*, and (c) *Euplotes*. Sizes of ladder bands are shown in base pairs (bp) beside each band.



Sequencing resulted in excellent quality sequence chromatograms, indicating the efficiency of the direct PCR applied for amplification of the 18srDNA of each organism. Manual trimming of the sequences for removal of initial low quality sequences resulted in about 750 base pairs-long sequences that exhibited 99-100 % identities with *E. woodruffi* and *E. parawoodruffi* for the *Euplotes* samples (accession numbers JQ801446 and FJ998025), *H. grandinella* for *Halteria* samples (acc. No. MF002420, MF002421), and 99.42 % with *C. hirtus* for *Coleps* samples (acc. No. AM292311.1). River Nile samples of *Euplotes*, *Coleps*, and *Halteria* with the accession numbers MN535693, MN535694, MN535695, respectively.

Maximum likelihood phylogenetic tree (Figure 2) coincided with the results of BLAST comparison. It exhibited a clear clustering of Mansoura´s Nile samples with their counterparts whose 18srDNA sequences were previously obtained and deposited in the GenBank database. Taking *Paramecium* species as an outgroup, three clusters could be clearly identified with a high bootstrap support values, in which each one of Nile species fitted perfectly. The first one encompassed *Euplotes* species coming from freshwater and brackish water bodies in Egypt, the country where current study was carried out, China, USA, Germany, Italy, Denmark, Korea, and Colombia.

Figure 2. BI phylogenetic tree for the ciliated protozoans assessed in the current studies. Bootstrap values are shown above the clades. 10,000,000 Markov Chains were applied and the first 25 % of this were scrapped-off as burn-ins.



The second one encompassed *Halteria* samples from Egypt, China, Korea, and UK. The third one included different *Coleps* species from Egypt, China, Korea, and Germany. Unfortunately, No 18srDNA sequences for Mansoura´s Nile ciliates were available in the GenBank database for the Middle East region, hence no clear phylogenetic relation among these species and their relatives in the nearby geographical regions could be deduced.

Species-specific primers designed and used for targeted identification of Nile ciliates collected in the current study resulted in band sizes as expected and mentioned in section 2.3 (Figure 3). A annealing temperature of 57.6 °C for *Euplotes* and *Halteria*, but 60.1 °C for *Coleps*, were the best. Primers´ sequences and PCR conditions are shown in Table 1. Serially diluting samples containing targeted ciliates resulted in a significant decrease in the amount of DNA available from each organism, as identified by the decreased PCR product intensity (Figure 4). PCR products´ intensities and the number of ciliate cells from all studied species showed a highly positive and significant correlation ($r^{2=}$0.99 for *Halteria*, 0.95 for *Coleps*, and 0.94 for *Euplotes)* as could be seen in Figure (5a,b).

Figure 3. Agarose gel (1%) electrophoresis showing the results of amplification of (a) *Coleps* (b) *Halteria*(c) *Euplotes* gene by the designed specific primers. Sizes of ladder bands are shown in bp beside each band.

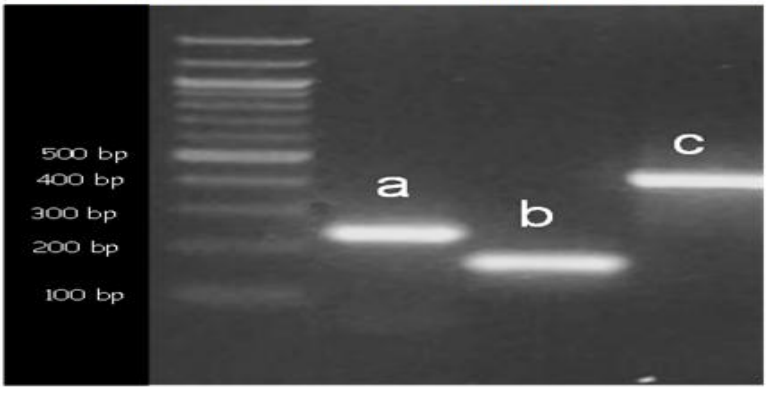


Figure 4: Agarose gel (1%) electrophoresis showing the results of amplification of (a) *Euplotes woodruffi*, (b) *Halteria grandinella* and(c) *Coleps hirtus* 18srDNA by the designed specific primers after DNA extraction with descending half serial dilutions (left to right). Sizes of ladder bands are shown in bp beside each band.

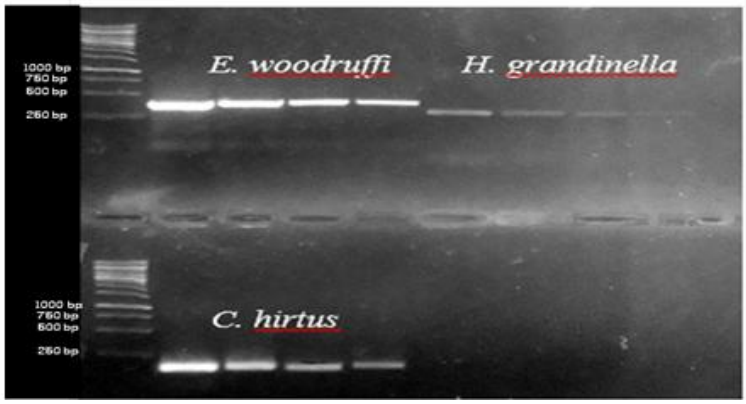
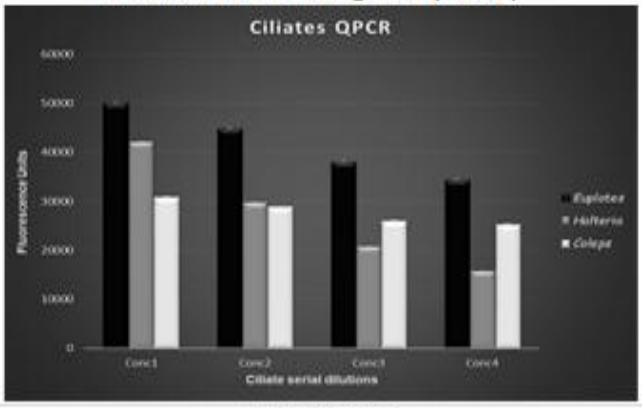
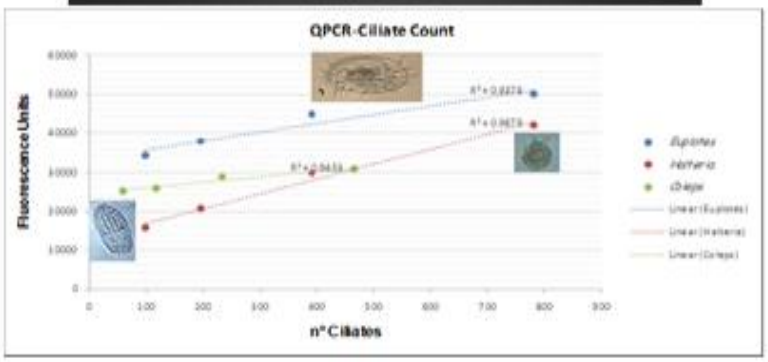


Figure 5: PCR products concentrations in relation to the number of ciliates' in water (Fig. 5a) and regression coefficient for all the three species (Fig. 5b). Small letters in Fig. 5a refer to significant difference among the serial dilutions of the each organism (P<0.01).





Finally, the sequenced QPCR amplicons were 100 % identical to those obtained by sequencing the results of the direct PCR over the single-cell using the species-specific 18srDNA primers.

DISCUSSION

For the best of authors'´ knowledge, the current study is the first trial in River Nile to apply molecular genetic approaches for identifying and assessing the

abundance of free living freshwater ciliates. The newly designed 18srDNA primers could be efficiently applied in a direct PCR to barcode different ciliate species. Moreover, the species.-specific 18srDNA primers exhibited a clear quantitative capacity. The highly significant, positive correlation between organism count and PCR profiles for the three assessed ciliate specie indicate the efficiency of these primers to detect changes in *Euplotes, Halteria,* and *Coleps* abundances in a given freshwater body, especially since the serial dilutions were made from the Nile water that contained other plankton species. The sequencing step appended to the QPCR measurement confirmed the high specificity of the species-specific designed primers.

Furthermore, the investigated species in the Mansoura Nile herein showed very high sequence identity with fresh- and brackish water representatives from different areas in the world. The *Euplotes* species present in our samples showed the closest sequence identity and phylogenetic relation with *Euplotes woodruffi* and *Euplotes parawoodruffi*. Identification of *Euplotes* species using only morphological appro-aches is not completely accurate. Application of 18srDNA seqeuecning has been proven, even with very slight sequence differences, to be adequate for characterization of different morphotypes of the same species, as well as to characterize cryptic species in the world of euplotid ciliates (Fotedar *et al.,* 2016; Yan *et al.,* 2018). For example, *E. woodruffi* was originally isolated from a freshwater pond in China (Gaw 1939) and subsequently found in fresh- and brackish water habitats in Asia, Europe, North America, and Africa (e.g. Fokin *et al.,* 2008; Gaw, 1939; Pierson, 1943; Vannini *et al.,* 2012). It is a common model organism in many fields of protistology (e.g., Song and Bradbury, 1997; Fokin *et al.,* 2008). *E. parawoodruffi* was originally isolated from brackish waters in the USA (Song and Bradbury 1997). *E. parawoodruffi* is separated from *E. woodruffi* by several morphological differences in body shape, pre-oral pouch, adoral zone of membranelles, macronucleus arm length, ciliary pattern and silver line system, and in particular the habitat (Song and Bradbury, 1997). However, since short time, Schwarz *et al.,* (2007) referred to molecular data for *E. woodruffi* and *E. parawoodruffi* that cast doubt on the separation of the two species from each other. Dai, *et al.,* (2013) isolated *E. parawoodruffi* and *E. woodruffi* from brackish waters and referred to *E. parawoodruffi* as a junior synonym for *E. woodruffi*. The 99 % sequence identity between the Mansoura's Nile species and both *E. woodruffi* and *E. parawoodruffi* indicated that our species is then *E. woodruffi*. Another example but for characterization of cryptic species of the genus *Euplotes* is the case of *E. estuarinus* and *E. curdsi.* They are very close together in morphology and ciliary pattern but differ in the sequence of the 18srDNA by only by 1 bp. Likewise, *E. harpa* and *E. neapolitanus* are also morphologically similar but differ in only by 3 - 4 bp in their 18srDNA sequences (Yan. *et al.,* 2018).

Besides being an active predator for smaller planktonic organisms, bacteria, and algae (Tarangkoon *et al.,* 2018), *Euplotes* capability to tolerate heavy metals concentrations seems considerable. Rehman *et al.,* (2009) isolated *E. mutabilis* from industrial wastewater, where it survived Zn levels up to 33 mg L^{-1}, Cd up to 22 mg L^{-1} and Ni up to 18 mg L^{-1}. It was suggestd as a possible bioremidation agent as it has absorption ability to 85% Zn, 84% of Cd and 87% of Ni after 96 h (Rehman *et al.,* 2009). Similarly, *E. crassus* was suggested as a fundamental biomarker organism for monitoring the effects of heavy metals, as it could tolerate Cu level of 1.58 mg L^{-1}, Pb level of 4.13 mg L^{-1}, and Zn level of 4.97 mg L^{-1}; after 48 h exposure (Kim, *et al.,* 2011).

Coleps obtained in the current study from river Nile showed 99% COI sequence identity with *C. hirtus*. *Coleps* is a widely distributed genus present in many habitats

as fresh-, brackish and saline waters, being distributed in both benthos and plankton (Dragesco and Dragesco-Kerneis, 1991; Foissner 1980, 1984). Its usual feeds include bacteria, algae, flagellates and living or dead plant and animal tissue (Foissner *et al.*, 1999; Mazanec and Trevarrow, 1998). Such like *Euplotes*, the advent of DNA barcoding aided much the taxonomy of this genus and the allied ones. Phylogenetic analyses of different colepidae species exhibited that *Coleps amphacanthus* is closer to the clade encompassing *Levicoleps* spp., especially *L. biwae jejuensis* than to the other clade encompassing all other *Coleps* spp. (Lu *et al.*, 2016). Similarly, 18srDNA based phylogeneies exhibited the sister relationships between genera *Apocoleps* and *Pinacocoleps* (Moon *et al.*, 2017). Hence, still many aspects of taxonomy in that group are in need for more research.

The obtained Mansoura´s Nile *Halteria* species showed 100 % sequence identity with *H. grandinella* (Müller, 1786). The collected sample was closer to the ones isolated from China and UK freshwater ponds (Riley *et al.*, 2001; Wang *et al.*, 2017). In general this species is a cosmopolitan one, being found in most freshwater environments all over the world (Finlay, 2002). It feeds on bacteria, nanoprotists, algae and detritus (Jürgens *et al.*, 2000). However, and in contrast to the two previously assessed species in the current study, it needs variety of food because it cannot be feed on a pure bacterial diet. Another key criterion in *H. grandinella* is that it exhibits the highest record for rDNA copy number in ciliates, being about 567,893 per cell, regardless to the cell or genome size (Wang *et al.*, 2017).

CONCLUSION

Direct PCR over individual freshwater ciliates was proven to be very efficient tool for molecular investigations of these uni-cellular organisms. Application of DNA barcoding in investigating the free living ciliates seem to be fundamental to identify, assess and control the presence and abundance of these organisms. Specially, since they represent a cornerstone for lower and higher organisms´ biodiversity, water treatment and heavy metals bioremidiation processes, and consequently, the ecosystem health and performance in general.

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ARABIC SUMMARY

تطبيق التشفير اللوحي الجيني لتحديد انواع الكائنات الهدبية المرشحة للاستعمالات التكنولوجية الحيوية من نهر النيل ، مصر.

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تلعب الاوليات الهدبية العديد من الأدوار الأساسية في البيئات المائية حيث تزدهر، فهي تتوسط الحلقة الميكروبية لشبكة الغذاء العالمية ، وتتحكم في معدل الكائنات الحية الاولية المحيطة ، وتعمل كمؤشرات حيوية لجودة المياه ، إلى جانب القدرة على إنتاج مركبات نشطة بيولوجيًا. هذا و يعتمد تحديد الأنواع المختلفة للهدبيات على التصنيف المورفولوجي أساسًا. من أجل تطوير الأدوات الجزيئية التي تسهل استكشاف الهدبيات النيلية، تم وضع تفاعل سلسلة بلمرة متسلسل مباشر، قائم على تكثير الوحدة الريبوسومية الصغيرة 18srDNA لتحديد الشفرة اللوحية الجينية لبعض الهدبيات التي تسود نهر النيل في مدينة المنصورة في شمال مصر، ثم تم تطبيق النتائج لتصميم برايمرات جديدة خاصة بتلك الانواع الاكثر انتشارا في مياه النيل للكشف عن كثافتها العددية، وقد وجدت الانواع الهدبية الثلاثة الاكثر انتشارا في نيل المنصورة هي *Euplotes woodurffi, Halteria grandinella, Coleps hirtus*. ونتج عن البرايمرات الخاصة بتلك الانواع تفاعلات بلمرة متسلسلة بكفاءة لاتقل عن ٩٥ %، و من المتوقع أن تسهل تلك الاختبارات الجزيئية المصممة حديثًا في استكشاف تلك الأنواع وقياسها في الموائل المائية المختلفة حيثما وجدت ، مما يضيف مساهمة كبيرة في الجهود المستقبلية لاستغلالها كموارد للتكنولوجيا الحيوية الزرقاء.