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New World Record of *Keratella quadrata* Rotifers Isolated from Shatt Al-Basrah, South of Iraq

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ABSTRACT

This study focused on identifying *Keratella quadrata* (*K. quadrata*) collected from Shatt Al-Basrah, South of Iraq. After sampling and isolation, the extracted DNA from isolates of *K. quadrata* was subjected to PCR technique to amplify the 18S rRNA gene which amplified 1759 bp fragment. Those sequences with similarity rate of ≥99% of *K. quadrata* were considered as the common species. The phylogenetic tree unraveled the distribution and phylogenetic relationships among the studied *Keratella* species and their identical reference strains according to CLUSTAL W program. The extracted DNA from *Keratella* isolates identified one *K. quadrata* species, which was different from those in previous studies in Basrah province and Iraq and the global isolates of *K. quadrata* sequence registered in the NCBI of USA, ENA of Europe and DDBJ of Japan.with KX358062.1 accession number. It was concluded that more sampling studies are required to identify and classify the taxonomy of Rotifera species.

INTRODUCTION

Copepoda, Cladocera and Rotifera are main zooplankton species and which defined as wheel animals or wheel animalcules residing in freshwater **(Forró *et al*., 2008)**. Many studies have focused on the Rotifera species and their taxonomy in various water bodies such as **(Al-Saboonchi *et al*., 1986; Mohammed, 1986; Sabri, 1988; Abdul-Hussein *et al*.,1989; Sabri and Maulood, 1989; Ali and Abdullah, 1999; Al-Lami *et al*.,1999; Salman *et al*., 2008; Ahmed and Ghazi, 2009; Hammadi, 2010; Abd Al-Rezzaq, 2014)**. Data from the Shatt Al-Arab River. Various species up to 105 Rotifera spp has been isolated from the same river **(Ali and Ghazi, 2008; Rabee, 2010; Ajeel *et al*., 2015)** Southern Iraq is in scarcity regarding the Rotifera existence similar to other parts of the country remaining unknown **(Sabri *et al*., 1990)**. However, 11 species of Brachionid rotifers in the Tigris river in the current study of the Rotifera ecology from the year 1989 **(Abdul-Hussein *et al*.,1989; Sabri, *et al*., 1990; Ghazi and Ahmed, 2008)** which also reported the vertical distribution of zooplankton species in Samarra Impoundment. Nutrients have a role in the distribution of species in the river. **Al-Doori, (2012)** recorded

32 species in Al-Tharthar-Euphrates Canal. While **Al-Ameen *et al*., (2017)**. recorded 10 species of Zooplankton in the Lake of Baghdad City Games and **Akbar, (2013)** recorded 9 taxa of Zooplankton in Al-Kufa River also **Yoshimura *et al*., (1996)** recorded 20 species of Rotifers in Al-Gharaf Canal at Thi Qar province. Moreover, fish larvae affect their population and a wide variety of fish and crustacean species larvae are dependent on the sufficient amount of qualified rotifers (reproduction rate and density and microbiota **(Lavens and Sorgeloos, 1996; Vestrum *et al*., 2018)**. Those intrigued scientists around the world have assessed various aspects of rotifers **(Snell and Janssen 1995; Lubzens *et al*., 2001)**. These creatures feed on algae and bacteria, and are considered as main freshwater zooplankton species used in immunological studies **(Segers and De Smet, 2008; Chandrasekhar, 2012)**. Molecular identification, rapid, sensitive and specific detection, load determination and sequence analysis of these species have been more rapid and reliable where the culture has not been possible **(Choi *et al*., 2020)**. The entire and exact phylogenetic assessment of Rotifers has not been implemented previously **(Keller and Zengler, 2004; Spiegelman *et al*., 2005; Heiri, 2013)**. Rotifers carry bacterial probiotics and are used as food of fish larvae. Application of biochemical approaches alongside phylogeny determination uncovers the probiotics features and interactions in complex microbial communities. Various amplification and identification methods have been applied for the distinguishing of probiotics and zooplankton species from aquaculture and ecological tools such as restriction fragment length polymorphism (RFLP) and randomly amplified polymorphic DNA (RAPD) using 16S or 18S rRNA fingerprinting **(McCaig and Prosser, 2001)**.

MATERIALS AND METHODS

Samples collection

Routine monthly sampling was carried out from eight selected stations. Two hundred specimens were obtained from Shatt al-Arab River using aconical net of 1meter length and 40 cm diameter with amesh size of 50 µm .Its thrown into the water and pull to a distance of 3 meters by tied rope and then the collected 250 ml of water were poured in a plastic bottles **(Hassan *et al*., 2021)**.

Identification of *Keratella quadrata* using specific 18S rRNA amplification

The amplification of the 18S rRNA gene conserved regions was fulfilled using specific primers (F: 5-AGATTAAGCCATGCATGCGTAAG-3 and R: 5-TGATCCTTCTGCAGGTTCACCTAC-3) which give 1759bp PCR product **(Winnepenninckx *et al*.,1995)**.After samples were identifed morphologically using microscope (see fig.1). These primers were used as per existing data of 18S rRNA sequence (Genbank:U29235). The PCR Master Mix and reaction conditions are included as Tables 1 and 2.

Table 1. Reagents of PCR amplification (50 µL) for 18S rRNA

No	Reagent	Volume
1	DNA	10µl
2	Forward Primer	2µl
3	Reverse Primer	2µl
4	Master Mix .2x	11µl
5	Nuclease-free water	25µl
	Total	50µl

Table 2. Touch down PCR Condition for Specific 18S rRNA gene.

Stages	Temperature	Time
Stage 1	95 ºC	2 min
Stage 2	95º C	30 sec
Stage 3	61.3 ºC decrease 0.5 ºC per cycle	30 sec
Stage 4	72 ºC	20.0 sec
Stage 5	Repeated stages 2-4	14 cycles
Stage 6	95 ºC	30 sec
Stage 7	54.3 ºC	30 sec
Stage 8	72 ºC	20 sec
Stage 9	Repeated stages 6-8	19 cycles
Stage 10	72 ºC	5 min

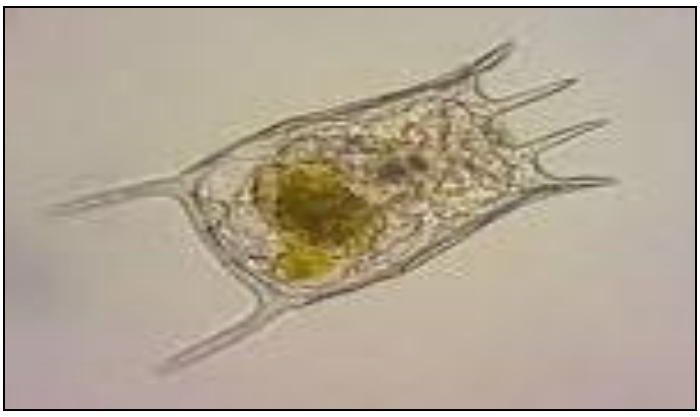

Fig.1. Microscopic image of *K. quadrata*

RESULTS AND DISCUSSION

Identification of *K. quadrata* using 18S ribosomal RNA

The extracted DNA samples from the isolates were subjected to PCR for amplifying 18S rRNA (Fig.2) which gave a 1759 bp product and using a 2kb DNA marker for comparison.

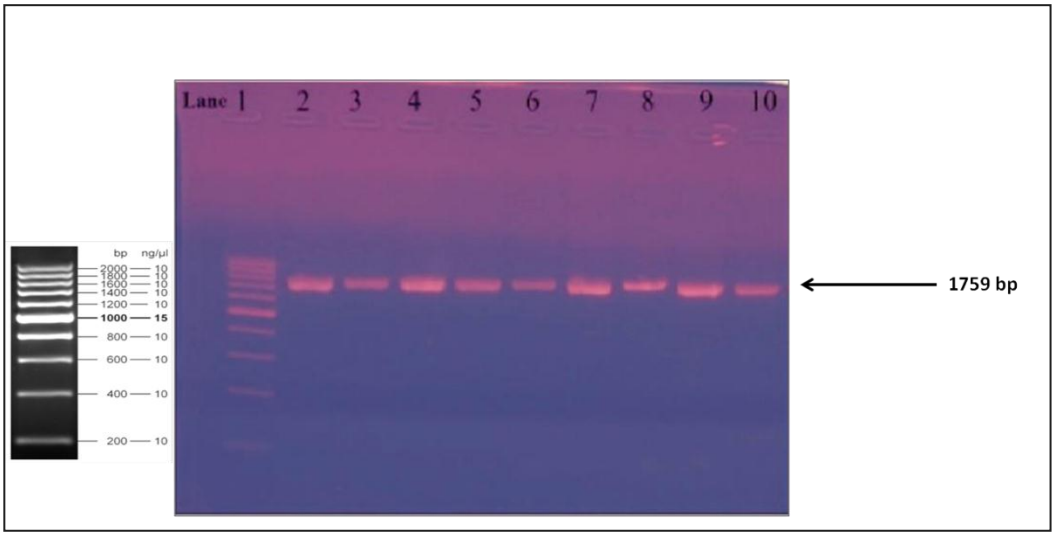

Fig .2. Agarose gel electrophoresis (2% gm and 60V, 2MA) patterns depicting PCR amplified products of the 18S rRNA gene. Well 1: 2kb DNA marker, wells 2- 10: 18S rRNA gene products of *K. quadrata* .

Sequencing for 18S rRNA and identification of *K. quadrata*

The results of 18S rRNA gene sequencing for each isolate have been presented in Table (3) .The identification with similarity ≥99% *K. quadrata* was the common species.

Identification of new global isolates

There is one isolate of *K. quadrata* (No.1) which has different residues/nucleotides sequence from the reference strains. Considering these differences, the new isolate was published in The National Center for Biotechnology Information (NCBI) with KX358062.1 accession number, DNA Data Bank of Japan (DDBJ) and The European Nucleotide Archive (ENA).

Table 3. Sequencing results of 18S rRNA gene for *Keratella quadrata*.

Size (bp)	**Sequence**	**Species**
1759	TCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCTAAGTACATAC TTTTGCACGGTGAAACCGCGAATGGCTCATTAAATCAGTTATGGTT CCTTAGATCGTTCATATTACATGGATAACTGTGGTAATTCTAGAGCT AATACATGCAAAAAGCTCCGACCGTATGGGAAGAGCGCTTTTATTA GACCAAAACCAATGGGGTCGCAAGATCCCTTTGTATGGTGACTCTG GATAACTTTGTGCTGATCGCATGGCCTCGAGCCGGCGACGTATCTTT CAAATGTCTGCCCTATCAACTTTCGATGGTAAGCGATTTGCCTACCA TGGTTGTAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGG GAGCATGAGAAATGGCTACCACATCTACGGAAGGCAGCAGGCGCG CAAATTACCCACTCCTAGATCGGGGAGGTAGTGACGAAAAATAAC AATACCGGACTCTATGAGGCTCGGTAATTGAAATGAGTACAGTTTA AAACCCTTAACGAGGATCTATTGGAGGGCAAGTCTGGTGCCAGCAG CCGCGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCAGTT AAAAAGCTCGTAGTTGGATCTCGGGTACTAGCATGTGGTACGCTTA ATTGCTGTTACTACATGTCTGGTCCTAATCAATCGGTTGGCTGTTAA TGCCCTTTATTGGGTGTTTTCAGTAGCCGGTACGTTTACTTTGAGAA AATTAGAGTGCTTAAAGCAGGCCATACGCCTGAATAATGTTGCATG GAATAATAGAATAGGACCTCGGTTCTATTTTGTTGGTTTTGTAGAGC ATTGAGGTAATGATTAATAGGGACAGACGGGGGCATTCGTATTGCG GTGTTAGAGGTGAAATTCTTGGATCGCCGCAAGACGAACAACTGCG AAAGCATTTGCCAAGAATGTTTTCATTAATCAAGAACGAAAGTTGG AGGTTCGAAGACGATTAGATACCGTCCTAGTTCCAACCATAAACGA TGCCAACTAGCGATTAGCTGCTGTTAATTAAACGACACAGCTAGCA GCTTCCGGGAAACCAAAGTTTTTGGGTTCCGGGGGAAGTATGGTTG CAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAG TGGAGCCTGCGGCTTAATTTGACTCAACACGGGAAATCTCACCCGG CCCGGACACTGTAAGGATTGACAGATTGAGAGCTCTTTCTTGATTC GGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGT CTGGTTAATTCCGATAACGAACGAGACTCTAGCCTGCTAAATAGTA CGCCGATCTTTAGTCGGCGCGTACTTCTTAGAGGGACAAGTAGCGG ATAGCTACACGAAATTGAGCAATAACAGGTCTGTGATGCCCTTAGA TGTTCGGGGCCGCACGCGCGCTACACTGAAGGGATAAGCGTGTTTT TCCTGCTCCGAAAGGAGTGGGTAACGGGCTGAAACCCCTTCGTGAT TGGGATCGGGGCTTGAAATTATTCTCCGTGAACGAGGAATTCCCAG TAAGCGCGAGTCATAAGCTCGCGTTGATTACGTCCCTGCCCTTTGTA CACACCGCCCGTCGCTACTACCGATTGAATGGTTTAGTGAGATCCT CGGATTGGACCGGAGGTGGCCGCAAGGTTGCCTCTAGGTTCCGAGA AGACGCAAACTTGATCATTTAGAGGAAGTAAAAGTCGTAACAAGG TTTCC	***K. quadrata***

Neighbor Joining (NJ) Phylogenetic tree of *K. quadrata*

The Nucleotide sequences data of all *K. quadrata* isolates were concatenated producing a sequence length of 1759 bp for all the species. The rooted phylogenetic tree was constructed and visualized in Figure 3 for *K. quadrata*. Individual NJ tree were drawn for each isolate. All data which share the same sequences in the table number (3) were pasted together in the "Clustal W" and "TREE RVIEW" was employed to draw the tree. This tree shows the distribution and phylogenetic relationships among the studied *K. quadrata* and their identical reference strains.

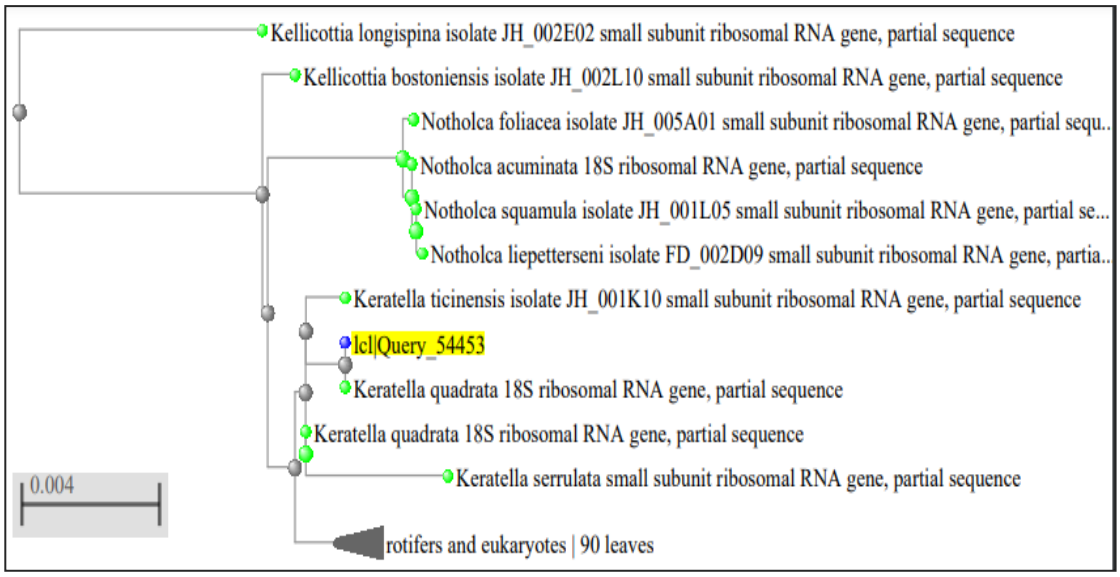

Fig.3. Rooted Neighbor Joining phylogenetic tree constructed from concatenated sequences of 1759 bp for each isolate (derived from an alignment of 18SrRNA sequences) then produced from a MAFFT alignment version 8 and visualized using forester version1027 and CLUSTAL W program.

CONCLUSION

The present study focused on the amplify the 18S rRNA gene of *K. quadrata* which were collected from the area of study (Southern Iraq) as mentioned in the methodology. Ten isolates were identified as the *K. quadrata* using 18SrRNA sequence and size of PCR products. While the others did not give the results due to the DNA sequence does not match.

The present study approves the results of the previous studies which showed the same domains of this species such as **(Near *et al*., 1998; Reid *et al* 2008)**. Also the current results are in agreement with many different studies in this field**.(Michaloudi, 2018)** Therefore, the aim of the present study was to detect the genetic differences of the isolates of *K. quadrata* using phylogenetic tree **(Tam *et al*., 2019)**.

The results of 18S rRNA gene sequencing for each isolate has been presented in Table (3).The identification with similarity ≥99% *K. quadrata* was the common species.
We identified one isolate of *K. quadrata* (No.1) which had different residues nucleotides sequence from the reference strains. Considering these differences, the new isolate sequence was published in NCBI with KX358062.1 accession number. Major limitations of this study included low number of samples, restricted area of study, lack of gene expression profile of isolates and interactions **(Thakong *et al*., 2019)**.

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