International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

E-ISSN 2347-2677
P-ISSN 2394-0522
www.faunajournal.com
IJFBS 2021; 8(2): 40-53
Received: 09-01-2021
Accepted: 14-02-2021

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First record of a seed shrimp (Ostracoda: Podocopida) *Cypretta campechensis* (Cyprididae) in a perennial lake (Coimbatore, India): Its molecular identification

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DOI: https://doi.org/10.22271/23940522.2021.v8.i2a.810

Abstract
This study represents diversity of zooplankton species in a perennial freshwater lake located in Singanallur (Lat. 10.59° N and Long. 77.88° E) at Coimbatore, Tamil Nadu, India for a period of one year (June, 2017-May, 2018). Fifty three zooplankton species including rotifera-18, cladocera-10, copepoda-13 and ostracoda-12 were observed. The first record of an ostracoda species *Cypretta campechensis* was observed during summer. This species was recognized according to its morphological characteristics of anterior view, posterior view, dorsal margin, lateral view (triangular shape), maxillular palp, posterior seta, posterior claw, uropodal ramus and hemipenis. This was mass cultured in mixed phytoplankton as feed. The molecular identification was done via analysis of the mitochondrial cytochrome c oxidase subunit I (mt-COI). The gene was amplified with universal primers (LCO1490 and HCO2198), sequenced and authenticated with NCBI GenBank (MN641913). The BLAST showed 100% similarity with *C. campechensis* of Mexico (MF076727). The sequence showed more number of identical amino acid residues than that of variable amino acid sites. The noticed higher AT biases (62.4-63.0%) than GC biases (36.1-37.5%) indicated that lower abundance of nuclear copies of mt-DNA (NUMTs) genes. The divergence rate was very low between the subjected and retrieved species (0.000-0.290%). The endemism of *C. campechensis* can be used as a bio-indicator of water pollution status of this lake and may afford an opening to assess its importance in aquaculture production.

Keywords: Zooplankton, *Cypretta campechensis*, mt-COI gene, divergence, phylogeny

1. Introduction
Plankton are free-floating characteristic provide a crucial source of food to many small and large aquatic organisms, such as prawns, bivalves and fishes. Phytoplankton (earth's most critical organisms are microscopic in nature, such as diatoms and dinoflagellates as well as blue-green algae) possess photosynthetic capacity. Therefore, they are responsible for half of the atmospheric oxygen. Zooplankton diet consists of various phytoplankton species, which differ in their grazing resistance (e.g., size, shape, and toxins) and nutritional quality (Taipale *et al*., 2013; Taipale *et al*., 2016) [1, 2]. Zooplankton species have different types of life histories influenced by seasonal variations of biotic factors, feeding ecology and predation pressure. The composition, abundance, and distribution of zooplankton species in any particular aquatic habitat usually provide information on the prevailing physical and chemical conditions in that habitat and hence, they are of great ecological importance (Abdul *et al*., 2016) [3].
The presence and abundance of zooplankton represents one of the ecological indicators of water quality. Zooplankton constitutes an important food sources for many omnivorous and carnivorous fishes and support the necessary amount of protein for their rapid larval growth (Bhavan *et al*., 2017) [4]. In any freshwater pond or lake, generally there are four zooplankton groups, namely Rotifers and crustacean zooplankton of Cladocera, Copepoda and Ostracoda. Among these, Ostracoda are of great interest as a model group in various ecological and evolutionary studies. This consists of lateral compressed bivalve carapace closing appendages and soft parts (Yousef, 2014) [5].
Ostracods can be found worldwide both in fresh and marine water bodies at different depths (Cohen *et al*., 2007) [6]. They are found in polluted water and serve as indicator species of climate and ecosystem changes (Martens *et al*., 2008) [7]. Globally, freshwater ostracods were documented and classified under the order Podocopida, which contains 15 families, 209 genera and 2103 species (Martens *et al*., 2008; Martens and Savatenalinton, 2011; Karanovic, 2012)

2012) [7-9]. They are playing a vital role in food chain and energy flow in the aquatic ecosystem (Li *et al*., 2018) [10]. The freshwater ostracods are the most important proxies in lacustrine environments, because of their high abundance and good preservation in sediments (Cohuo-Durán *et al*., 2013) [11]. There are at least 25,000 extant species, of which around 12,000 have been described (Cohen *et al*., 2007) [6]. Nevertheless, only few DNA barcoding data analyses have been conducted on these small crustaceans (Bhavan *et al*., 2016, 2017; Kalpana *et al*., 2018) [12, 4, 13].

The identification of cryptic species is in endemic taxon and biome and, therefore, these species have significant implications for evolutionary, biogeography and conservation studies (Bickford *et al*., 2007) [14]. Morphological identification of very similar species disregards certain evolutionary and ecological aspects because neither, does close morphological similarity come along with identical ecology nor with the same genetic background (Giere, 2009) [15]. Among various gene regions available for correct and quick discrimination of species, the mitochondrial cytochrome oxidase subunit-I (COI) gene region is unique, because its haplotypes are often used for studying the molecular ecology/taxonomy of animals (Mills *et al*., 2017) [16]. Actually, mt-COI gene has offered the most efficient and accurate barcoding method for species-level identification of animals including zooplankton regardless of their condition and life history stages (Bucklin *et al*., 2011; Li *et al*., 2011) [17, 18].

Morphological and molecular taxonomic analysis is particularly useful and necessary to develop accurate tools for species identification, and thereby to ensure valid estimates of their diversity, distribution, and abundance in routine taxonomic analysis of zooplankton samples (Bhavan *et al*., 2016; Bhavan *et al*., 2015a; Kalpana *et al*., 2017) [12, 19, 20]. In this study, we have studied the distribution, morphology and molecular aspects of an ostracoda species, *Cypretta campechensis* present in the Singanallur lake, Coimbatore, India.

2. Materials and methods

2.1 Description of the study area

The Singanallur (Lat. 10.59° N and Long. 77.88° E) Lake is located in Coimbatore city, Tamil Nadu, India, and is fed by canals derived from Noyyal River (Plate 1). The lake also receives water from Sanganur drain and sewage water. The water can be released through two sluice gates on the lake. In 2010, pipes were laid to connecting the lake to Valankulam Lake (Coimbatore, Tamil Nadu, India) to drain the excess water during floods. Various birds including grebes, painted storks and purple moorhen can be spotted in this lake. Fishing is carried out by neighboring fishermen and enthusiasts.

a. Satellite image taken from Google maps b. The lake view

Plate 1: Images of the Singanallur Lake (Lat. 10.59 ° N and Long. 77.88 ° E), Coimbatore, Tamil Nadu, India.

2.2 Qualitative analysis of the zooplankton

The surface water sample was collected during the early morning hours (6.00 AM - 8.00 AM). For qualitative analysis of zooplankton, water samples were collected by Towing method using Henson's standard plankton net (150 µm mesh) in zigzag fashion horizontally at a depth of 50 to 100 cm for about 10 min with a uniform speed of boat. The identification of zooplankton is made referring the standard manuals, text books and monographs (Sharma and Michael, 1987; Santhanam *et al*., 1989; Battish, 1992; Reddy, 1994; Shiel, 1995; Murugan *et al*., 1998; Altaff, 2005; Cohuo-Duran *et al*., 2013) [21-27, 11] with the help of a compound microscope. The photomicrographs were taken using Inverted Biological Microscope (Model Number INVERSO 3000 (TC-100) CETI) attached with a camera (Model IS 300). General elements that have been taken to assess all zooplankton groups were body shape and size, relative length of various appendages, including antennae, legs and setae, and presence and relative sizes of spines.

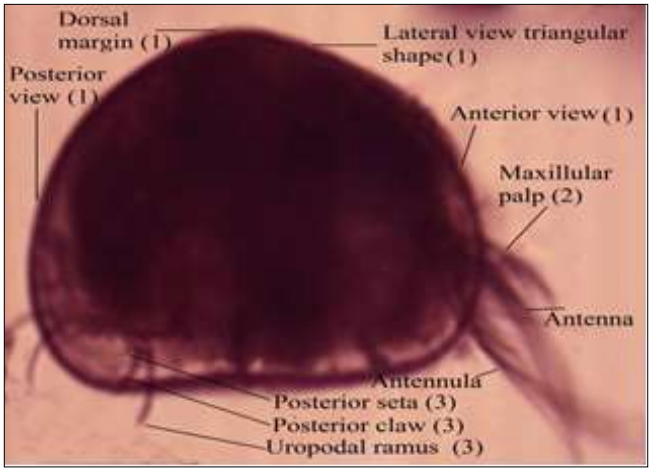
2.3 Mass culture of zooplankton

The collected zooplankton species were identified and segregated. The ostracoda species, *C. campechensis* especially seen in summer was subjected to mass culture individually for 21 days and fed *ad libitum* with a mixture of phytoplankton culture, which contains Spirulina (*Arthrospira platensis, Spirulina meneghiniana, Labyrinthiformis* and *Arthrospira maxima*), Chlorophyceae (*Pediastrum tetras, Pediastrum duplex, Ulothrix zonata, Tabellaria fenestrata* and *Spirogyra hyalina*) and Cyanophyceae (*Chroococcus minutus, Aphanocapsa pulchra, Phormidium granulatum* and *Oscillatoria brevis*). The zooplankton culture medium was maintained under the following conditions: temperature (°C), 28.40±2.10; pH, 7.17±0.46; salinity (ppt), 0.772±0.36;

dissolved oxygen, DO (mg/l^{-1}), 7.65±0.16; total dissolved solids, TDS (mg/l^{-1}), 1038±12.09; electrical conductivity, EC (µS/ cm), 2.113±0.12 and NH_3 (mg/l^{-1}), 0.024±0.004 with continued aeration. At the end of mass culture it was harvested and the number of individuals grown was counted by using counting chamber mounted on a microscope at a magnification of 10X and 40X.

2.4 Morphological characterization

The morphological characterization in *C. campechensis* was done based on anterior view, posterior view, dorsal margin, lateral view (triangular shape), maxillular palp, posterior seta, posterior claw, uropodal ramus and hemipenis. (Plate 2). Its SEM was also performed (Plate 3) using out sourcing service (Department of Nanoscience and Technology, Bharathiar University, Coimbatore, India).



Taxonomic position

Phylum	: Arthropoda
Subphylum	: Crustacea (Brunnich 1772) [28]
Class	: Ostracoda (Latreille 1802) [29]
Order	: Podocopida (Sars 1866) [30]
Suborder	: Podocopina (Sars 1866) [30]
Family	: Cyprididae (Baird 1845) [31]
Subfamily	:Cyprettinae (Hartmann 1964) [32]
Genus	: *Cypretta* (Vavra 1895) [33]
Species	: *campechensis* (Cohuo-Duran *et al*., 2013) [11]

Plate 2: Morphology (400x) and taxonomic positions of first recorded zooplankton species, *Cypretta campechensis* from the Singanallur Lake, Coimbatore, Tamil Nadu, India.

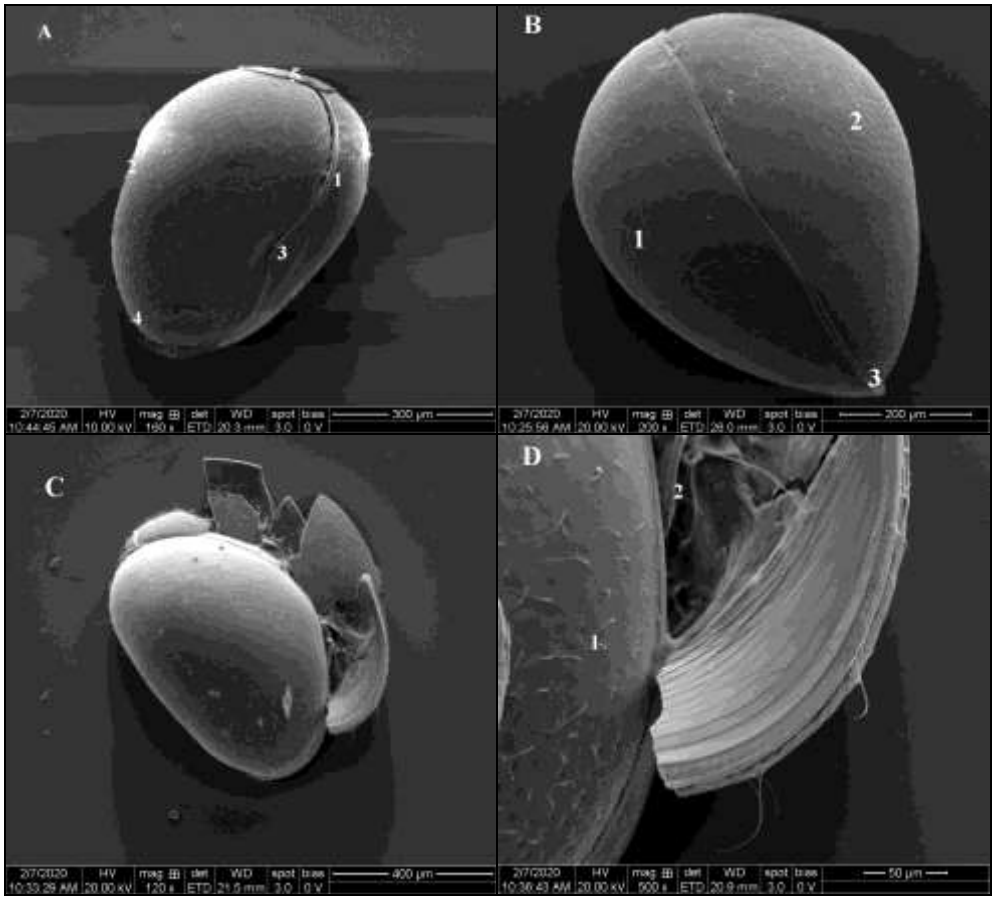


Plate 3: SEM views of *Cypretta campechensis*. **A:** ventral view (1), dorsal view (2), hinge (3), anterior view (4), posterior view (5); **B:** right valve of external view (1), left valve of external view (2), dorsal margin strongly arched (3); **C:** hemipenis with two conspicuous terminal lobes (1); **D:** anterior marginal pore canals (1), antennula 1st and 2nd segment (2).

2.5 Specific features of the class, ostracoda

Ostracods are commonly called as 'seed shrimps' or 'mussel shrimps' and are very small, and the freshwater forms are usually smaller than a millimeter. Ostracods are equipped with a low Mg-calcite carapace attached by a dorsal hinge and a ligament. Members of ostracoda are separated from other

crustaceans by a laterally compressed body, undifferentiated head, and seven or less limbs and a bivalve carapace with no growth lines.

2.6 Specific features of the sub-class, podocopa

Carapace ovoid, inflated sub-triangular, oblong elongate, or compressed; no rostrum or incisura; valves overlap around free margin; 2nd antenna geniculate, pediform; very small exopod with no more than two podomeres; much larger propulsive endopod with up to four podomeres; variable furca anterior to anus; no lateral eyes or Bellonci organ.

2.7 Specific features of the order, podocopida

Carapace straight or concave in the oral region, valves strongly calcified, smooth or ornamented. Six or seven pairs of appendages, antenna biramous; exopodite often reduced to a single scale or seta, endopodite generally with four podomeres and with stout terminal chelate setae. Maxilla is with a branchial plate. Fifth limb with or without a branchial plate, either a maxilliped or a walking leg, or transformed (in males) into a clasping organ. Furca narrow, lamelliform bearing a few setae, or reduced, or absent (Sars, 1866) [30].

2.8 Specific features of the family, cyprididae

Bivalved carapace, jointed in dorsal region, encloses whole animal. Anterior margin of both valves are with a parallel row of radiating septa or funnel-shaped radial pore canals (reduced septa), located perpendicular to the margin. Terminal segment elongated and cylindrical, developed claws and two stout setae. Surface ornamentation punctuate, reticulate or smooth. Right valve usually overlapping left one, although in some species the left valve overlaps the right one (Baird, 1845) [31].

2.9 Specific key characters of *Cypretta campechensis* (Cohuo-Duran *et al*., 2013) [11]

1. Lateral view sub triangular, anterior and posterior margins sub equally rounded, dorsal margin strongly arched, greatest height situated at mid-length …2
2. Terminal segment of maxillular palp cylindrical and armed with two or three claws and two or three setae …3
3. Posterior seta less than half length of posterior claw, attachment of uropodal ramus long and narrow, distally bifurcated …4
4. Hemipenis with two conspicuous terminal lobes, small lobule between them, without spine-like process, internal canal double coiled…*Cypretta campechensis*.

2.10 Molecular analysis

The molecular analysis was performed using mt-COI gene. The genomic DNA was isolated from 10 individuals as a whole by using Qiagen Dneasy Blood and Tissue Kit (Germany). Agarose gel electrophoresis (AGE, 1%) was performed and the genomic DNA was detected in a gel documentation system (Medicare, India). DNA amplification of mt-COI gene was carried out in Applied Biosystem (ABI) Thermo Cycler (Veriti™ 96-Well Thermal Cycler) with universal primers of forward and reverse in nature, LCO1490 and HCO2198, respectively (Folmer *et al*., 1994) [34]. These primers set were worked well with crustaceans, crabs, zooplanktons and prawns (Udayasuriyan *et al*., 2017; Bhavan *et al*., 2016, 2017; Kalpana *et al*., 2018) [35, 12, 4, 13].

Amplification was performed in a total volume of 100 µl reaction mixture contain 1 µl of DNA template, 400 ng of each primer (Forward primer, 400 ng, 0.5 µl; Riverse primer, 400 ng, 0.5 µl), 4 µl each dNTPs (10mM), 10µl of 10X ChromTaq DNA polymerase Assay Buffer, 1µl of ChromTaq DNA Polymerase Enzyme (3U/µl) and milli q water (83µl). The thermo cycler condition was as follows: 5 min at 95°C for prerunning, 35 cycles of 30 s each at 95°C for denaturation, 45 s at 57°C for annealing,1 min at 72°C for extension and followed by 7 min at 72°C for a final extension. The amplified product was resolved with AGE (2%).

Sequencing was performed with ABI 3500 XL Genetic Analyzer by using outsourcing service with a total volume of 20µl reaction mixture, which contains 3µl of template DNA, 3.2pM/µl of primers (forward, 0.50 µl and reverse, 0.50 µl), 2µl of 5X big dye sequencing buffer and 4µl of 2.5X ready reaction premix and 10µl of DNase-RNase free water. The other conditions were as per company protocol (Chromous Biotech, Bengaluru, India).

The forward and reverse sequences were aligned pair wise using CAP3. The sequence similarity available with NCBI database was identified and the internal stop codon was removed by BLAST. The reading frame shift was deducted by open reading frames (ORF) finder. The trimmed sequence was authenticated with GenBank. The multiple sequence alignment was done using T-Coffee and the aligned sequence was highlighted with multiple align show (MAS) as identical, similar and variable sites of amino acids. The nucleotide composition (AT and GC biases), nucleotide divergence (Kimura two-parameter (K2P) substitution model; Kimura, 1980) [36] and some phylogenetic information were calculated by using MEGA v. 6.01.

Assessment of synonymous (Ks) and non-synonymous (Ka) substitutions for 3rd codon positions was calculated by Li93 method using DAMBE (Xia, 2000) [37]. The transitional (Ts) and transvertional (Tv) substitutions of nucleotides were determined (Felsenstein, 1981) [38]. Analysis of sequence saturation, index of substitutional saturation (Iss) and critical value of index of substitutional saturation (Iss.c) was done by Xia method using DAMBE (Xia, 2000; Xia *et al*., 2003) [37, 39]. Finally, the phylogenetic tree was reconstructed by Maximum Likelihood model (Tamura, 1992; Kumar *et al*., 2016) [40, 41].

3. Results

3.1 Identified zooplankton species

In this study, 53 zooplankton species were recorded under 13 families and 27 genera in the Singanallur lake, which include 18 species of Rotifer, 10 species of Cladocera, 13 species of Copepoda and 12 species of Ostracoda (Table. 1). Among the rotifers, *Brachionus calyciflorus, Brachionus caudatus personatus, Brachionus durgae, Brachionus quadridentatus, Brachionus leydigii, Brachionus srisumonae, Brachionus urceolaris* and *Notholca laurentiae* were present in all seasons; *Brachionus rotundiformis* and *Brachionus diversicornis* were absent in pre-monsoon season; *Brachionus plicatilis* and *Brachionus ibericus* absent in post-monsoon; *Filinia longiseta* absent in pre-monsoon and summer; *Asplanchna intermedia* and *Asplanchna brightwelli* absent in monsoon season; *Brachionus variabilis, Brachionus rubens* and *Brachionus nilsoni* were absent in summer season. Among the cladocerans, *Diaphanasoma sarsi, Pseudochydorus globosus* and *Moina brachiata* were present in all seasons; *Moina macrocopa* and *Latonopsis australis*

were absent in pre-monsoon; *Leydigia leydigia* was absent in pre-monsoon and post-monsoon; *Leydigia lousi* was absent in post-monsoon and summer season; *Ceriodaphnia cornuta* was absent in monsoon season; *Moina micrura* and *Macrothrix spinosa* were absent in summer season. Among the copepods, *Thermocyclops inversus*, *Thermocyclops crassus*, *Mesocyclops leuckarti* and *Macrocyclops albidus* were present in all seasons; *Acanthocyclops vernalis*, *Thermocyclops decipiens* and *Mesocyclops ogunnus* were absent in pre-monsoon; *Heliodiaptomus viduus* was absent in post-monsoon; *Thermocyclops consimilis* was absent in monsoon season; *Cyclops strenuus*, *Eucyclops speratus*, *Mesocyclops edax* and *Mesocyclops pehpeiensis* were absent in summer season. Among the ostracods, *Cyprinotus nudus* was present in all seasons; *Cypris decaryi*, *Candona candida* and *Prionocypris glacialis* were absent in summer season; *Heterocypris dentatomarginatus*,*Cypris protubera* and *Eucypris virens* were absent in monsoon and summer; *Heterocypris incongruens* was absent in post-monsoon; *Chrissia formosa* was absent in pre-monsoon; *Tanycypris pellucida* was absent in pre-monsoon and summer*; Eucypris bispinosa* was absent in post-monsoon and summer; the presence of *C. campechensis* only in summer season was the first record in this lake. The zooplankton abundance in this lake was as follows: Rotifera > Ostracoda > Copepoda > Cladocera.

Table 1: List of zooplankton species recorded in the Singanallur lake during the study period (June, 2017 – May, 2018)

Group Phylum/ Class/Order	Family	Genus	Species	Pre-Monsoon	Monsoon	Post-Monsoon	Summer
Rotifera 18-species	Brachionidae (Ehrenberg 1838)	*Brachionus* (Pallas 1776)	*Brachionus rotundiformis* (Tschugunoff 1921)	-	+	+	+
			Brachionus calyciflorus (Pallas 1776)	+	+	+	+
			Brachionus caudatus personatus (Ahlstrom 1940)	+	+	+	+
			Brachionus diversicornis (Daday 1883)	-	+	+	+
			Brachionus rubens (Ehrenberg 1838)	+	+	+	-
			Brachionus durgae (Dhanapathi 1974)	+	+	+	+
			Brachionus plicatilis (Muller 1786)	+	+	-	+
			Brachionus quadridentatus (Hermann 1783)	+	+	+	+
			Brachionus leydigii (Cohn 1862)	+	+	+	+
			Brachionus nilsoni (Ahlstrom 1940)	+	+	+	-
			Brachionus srisumonae (Segers, Kotethip and Sanoamuang 2004)	+	+	+	+
			Brachionus urceolaris (Muller 1773)	+	+	+	+
			Brachionus ibericus (Ciros-Perez, Gomez and Serra 2007)	+	+	-	+
			Brachionus variabilis (Hempel 1896)	+	+	+	-
		Notholca (Gosse 1886)	*Notholca laurentiae* (Stemberger 1976)	+	+	+	+
	Trochosphaeridae (Harring 1913)	*Filinia* (Bory de St. Vincent 1824)	*Filinia longiseta* (Ehrenberg 1834)	-	+	+	-
	Asplanchnidae (Eckstein 1883)	*Asplanchna* (Gosse 1850)	*Asplanchna intermedia* (Hudson 1886)	+	-	+	+
			Asplanchna brightwelli (Gosse 1850)	+	-	+	+
Cladocera 10-species	Sididae (Baird 1850)	*Diaphanasoma* (Fischer 1850)	*Diaphanasoma sarsi* (Richard 1895)	+	+	+	+
	Chydoridae (Dybowski and Grochowski 1894)	*Leydigia* (Kurz 1875)	*Leydigia leydigia* (Schodler 1863)	-	+	-	+
			Leydigia lousi (Elias-Gutierrez and Nieto 2003)	+	+	-	-
		Pseudochydorus (Fryer 1968)	*Pseudochydorus globosus* (Baird 1843)	+	+	+	+
	Daphnidae (Straus 1850)	*Ceriodaphnia* (Dana 1853)	*Ceriodaphnia cornuta* (Sars 1853)	+	-	+	+
	Moinidae (Goulden 1968)	*Moina* (Baird 1850)	*Moina micrura* (Kurz 1874)	+	+	+	-
			Moina macrocopa (Straus 1820)	-	+	+	+
			Moina brachiate (Jurine 1820)	+	+	+	+
	Sididae	*Latonopsis* (Sars 1888)	*Latonopsis australis* (Sars 1888)	-	+	+	+

	(Baird 1850)						
	Macrothricidae (Norman and Brady 1867)	*Macrothrix* (King 1853)	*Macrothrix spinosa* (King 1853)	+	+	+	-
Copepoda 13-species	Diaptomidae (Baird 1850)	*Heliodiaptomus* (Kiefer 1932)	*Heliodiaptomus viduus* (Gurney 1916)	+	+	-	+
	Cyclopoidae (Dana 1853)	*Cyclops* (Muller 1785)	*Acanthocyclops vernalis* (Fischer 1853)	-	+	+	+
			Cyclops strenuus (Fischer 1851)	+	+	+	-
		Eucyclops (Claus 1893)	*Eucyclops speratus* (Lilljeborg 1901)	+	+	+	-
		Thermocyclops (Kiefer 1927)	*Thermocyclops inversus* (Kiefer 1936)	+	+	+	+
			Thermocyclops crassus (Fischer 1853)	+	+	+	+
			Thermocyclops consimilis (Kiefer 1934)	+	-	+	-
			Thermocyclops decipiens (Kiefer 1929)	-	+	+	+
		Mesocyclops (Sars 1914)	*Mesocyclops leuckarti* (Claus 1857)	+	+	+	+
			Mesocyclops edax (Forbes 1891)	+	+	+	-
			Mesocyclops ogunnus (Onabamiro 1957)	-	+	+	+
			Mesocyclops pehpeiensis (Hu 1943)	+	+	+	-
		Macrocyclops (Claus 1893)	*Macrocyclops albidus* (Jurine 1820)	+	+	+	+
Ostracoda 12-species	Cyprididae (Baird 1845)	*Cypris* (Muller 1776)	*Cypris decaryi* (Gautier 1933)	+	+	+	-
		Candona (Baird 1845)	*Candona candida* (Muller 1776)	+	+	+	-
		Cyprinotus (Brady 1886)	*Cyprinotus nudus* (Brady 1885)	+	+	+	+
		Heterocypris (Claus 1892)	*Heterocypris dentatomarginatus* (Baird 1859)	+	-	+	-
			Heterocypris incongruens (Ramdohr 1808)	+	+	-	+
		Prionocypris (Sars 1928)	*Prionocypris glacialis* (Sars 1890)	+	+	+	-
		Cypris (Muller 1776)	*Cypris protubera* (Muller 1776)	+	-	+	-
		Tanycypris (Triebel 1959)	*Tanycypris pellucida* (Klie 1932)	-	+	+	-
		Chrissia (Hartmann 1957)	*Chrissia formosa* (Klie 1938)	-	+	+	+
		Eucypris (Vavra 1891)	*Eucypris virens* (Jurine 1820)	+	+	+	-
			Eucypris bispinosa (Victor and Michael 1975)	+	+	-	-
		Cypretta (Vavra 1895)	*Cypretta campechensis* (Cohuo-Duran 2013)	-	-	-	+
Total	Families-13	Genera-27	Species-53	41 sp.,	46 sp.,	45 sp.,	33 sp.,

Pre-Monsoon (June, 2017-August, 2017), Monsoon (September, 2017-November, 2017), Post-Monsoon (December, 2017-Feberuary, 2018) and Summer (March, 2018-May, 2018). +, Present; -, Absent

3.2 Mass cultured zooplankton

The ostracoda species, *C. campechensis* was subjected to mass culture for 21 days during the months of March-May, 2018 with mixed phytoplankton (Spirulina: *Spirulina meneghiniana*, *Arthrospira platensis*, *Arthrospira maxima* and *Labyrinthiformis*; Chlorophyceae: *Pediastrum duplex*, *Pediastrum tetras*, *Spirogyra hyalina*, *Ulothrix zonata* and *Tabellaria fenestrata*; Cyanophyceae: *Aphanocapsa pulchra*, *Chroococcus minutus*, *Oscillatoriasub brevis* and *Phormidium granulatum*) as feed, and found to be grown well. The details are given in Table. 2.

Table 2: Number of individual (ind./L^{-1}) zooplankton species of *C. campechensis* grown under mass culture for 21-days with mixed phytoplankton as a feed during summer months

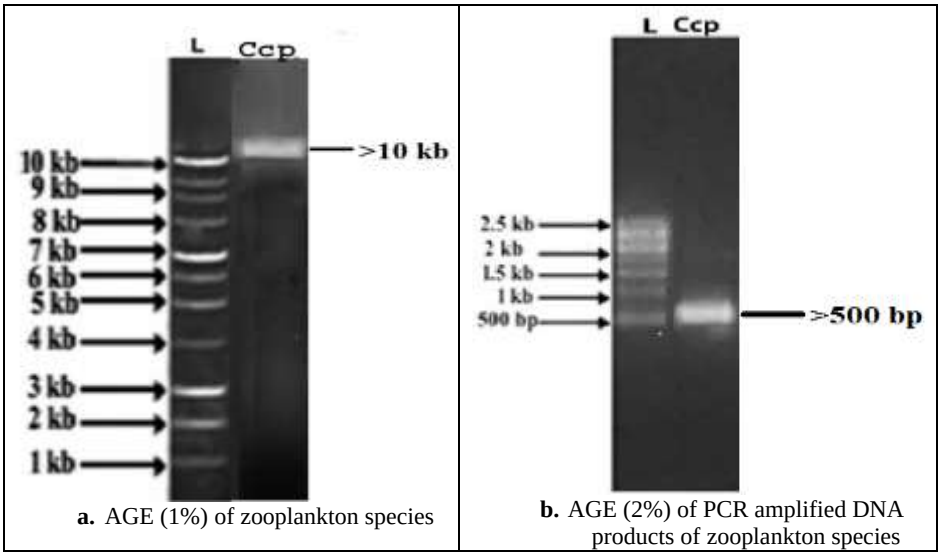
Ostracoda Species	March, 2018		April, 2018		May, 2018	
	Introduced (ind./L^{-1})	Harvested (ind./L^{-1})	Introduced (ind./L^{-1})	Harvested (ind./L^{-1})	Introduced (ind./L^{-1})	Harvested (ind./L^{-1})
Cypretta campechensis	11±4	40±5	7±1	36±2	13±4	41±6

3.3 Diagnosis of *C. campechensis*

Relatively big animal, the surface of the carapace is smooth and covered by short and sparsely located setae, except at the dorsal region. Both, anterior and posterior calcified inner lamella narrow. Funnel-shaped radial pore canals present at lower anterior margin on both valves. Anterior seta short and length of anterior claw, posterior claw thin and distally serrated and attachment of uropodal ramus long and narrow distally bifurcated. Hemipenis with two conspicuous terminal lobes, internal one anvil-like. A small lobule between them, without spine-like process, internal canal double coiled (Plates 2 and 3).

3.4 Genomic DNA and its amplification

The size of isolated genomic DNA from *C. campechensis* was >10 kb and its PCR amplified product was >540 bp (Plate 4 (**a** and **b**, respectively). Actually the size of the aligned sequence was 544 bp, which was authenticated with Gen Bank (MN641913) and the data of the specimen, photograph and their sequences were submitted in the BOLD database as well.



a. AGE (1%) of zooplankton species

b. AGE (2%) of PCR amplified DNA products of zooplankton species

Plate 4: DNA of zooplankton species. L, Ladder; Ccp, *C. campechensis*.

The BLAST of *C. campechensis* sequence revealed 100% similarity with its matched sequence available in NCBI data base (Table. 3). The results of multiple sequence alignment with MAS for identification of identical, similar and variable sites of amino acids for *C. campechensis* with retrieved species showed 326 identical amino acids residues, 17 similar amino acids residues and 201 variable amino acids sites (Table. 4; Plate 5). These data showed less numbers of variable amino acid sites and similar amino acid residues, and more number of identical amino acid residues. In this study, the base composition of the COI gene fragment showed 63.0% AT biases and 37.5% GC biases (Table. 5).

Table 3: BLAST identification of COI partial gene sequences of subjected and retrieved zooplankton species with their Gen Bank accession numbers

Queried sequences	Author, Country and Accession Number	I (%)	G (%)	Retrieved/ Matched species	Author, Country and Accession Number
Cypretta campechensis	Paper authors, India, MN641913	100	0	*Cypretta campechensis*	Macario-Gonzalez *et al*., 2018 [42]; Mexico MF076727

I, Identity; G, Gap; M.S, Matched strand; COI, Cytochrome C oxidase subunit I gene

Table 4: Number of identical and similar amino acid residues, and number of variable amino acid sites of the COI gene partial sequences generated for subjected zooplankton species

Comparison of zooplankton species	Number of identical amino acid residues	Number of similar amino acid residues	Number of variable amino acid sites
Cypretta campechensis with retrieved species	326	17	201

COI, Cytochrome C oxidase subunit I gene

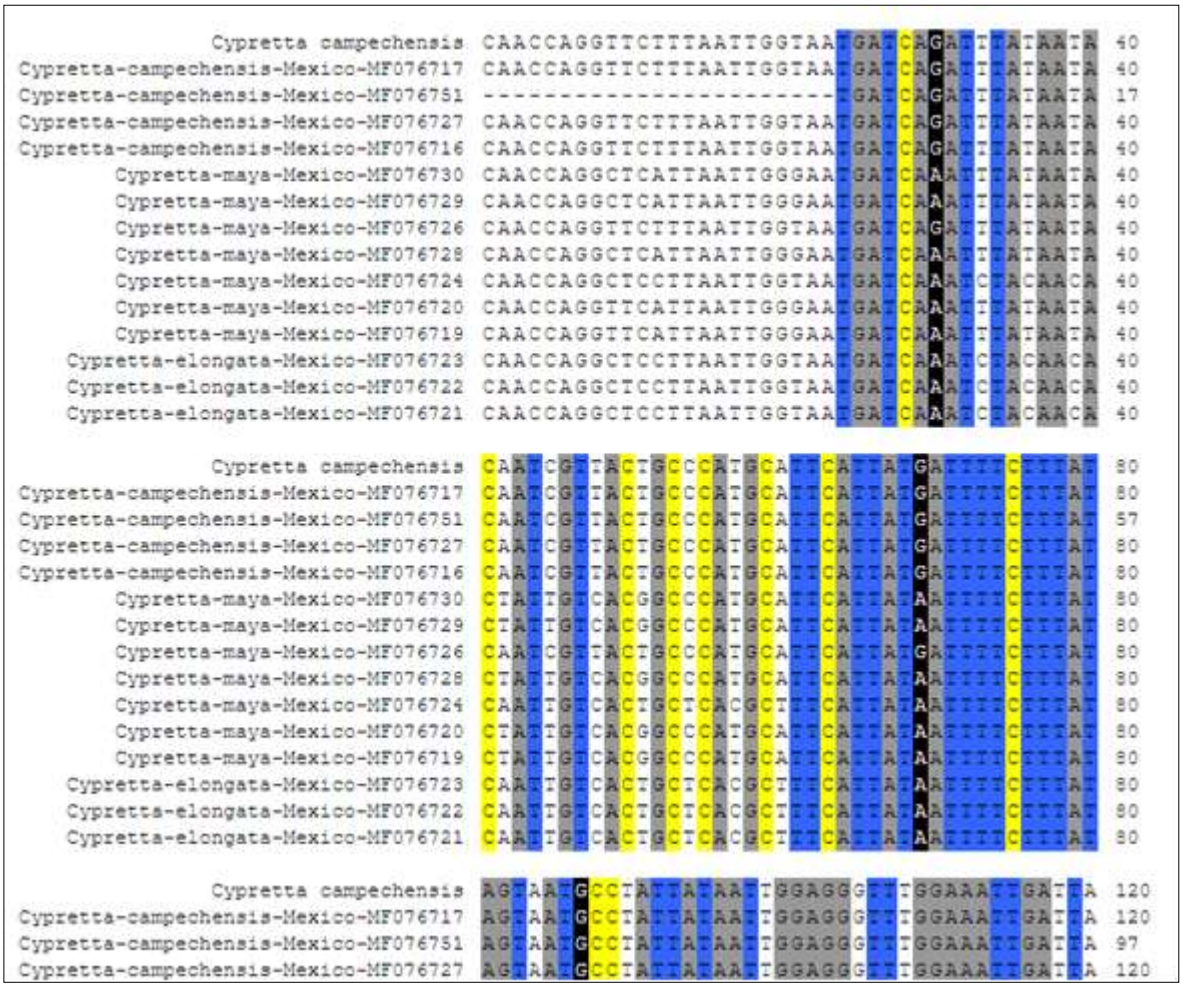


Plate 5: Multiple sequence alignment of COI gene sequences generated for subjected zooplankton. An alignment is formatted by using multiple align show (MAS) with coloured background and a consensus setting of 100%. Identical residues are indicated by amino acid colour and similar residues are black in colour. Gaps and other residues are given in white background

Table 5: Nucleotide composition of COI gene partial sequences for subjected zooplankton species, *C. campechensis*

Species Name	Nucleotide %					
	A	G	T	C	AT	GC
Cypretta campechensis, Paper Authors, MN641913	28.3	16.0	34.2	21.5	63.0	37.5
Cypretta campechensis, Mexico, MF076751	28.7	16.1	34.7	20.5	63.4	36.1
Cypretta campechensis Mexico MF076727	28.3	16.0	34.2	21.5	62.5	37.5
Cypretta campechensis Mexico MF076717	28.7	15.9	34.5	20.9	63.2	36.8
Cypretta campechensis Mexico MF076716	28.4	16.2	35.5	19.9	63.9	36.1
Cypretta maya Mexico MF076730	26.1	15.8	36.5	21.6	62.6	37.4
Cypretta maya Mexico MF076729	26.1	15.8	36.5	21.6	62.6	37.4
Cypretta maya Mexico MF076726	28.3	16.0	34.6	21.1	62.9	37.1
Cypretta maya Mexico MF076728	26.1	15.8	36.5	21.6	62.6	37.4
Cypretta maya Mexico MF076724	26.5	15.8	36.2	21.5	62.7	37.3
Cypretta maya Mexico MF076720	26.7	15.6	35.7	22.1	62.4	37.7
Cypretta maya Mexico MF076719	26.7	15.6	35.7	22.1	62.4	37.7
Cypretta elongata Mexico MF076723	26.5	15.8	36.2	21.5	62.7	37.3
Cypretta elongata Mexico MF076722	26.5	15.8	36.2	21.5	62.7	37.3
Cypretta elongata Mexico MF076721	26.7	15.6	36.2	21.5	62.9	37.1
Avg.	27.2	15.8	35.5	21.4	62.7	37.2

COI, Cytochrome C oxidase subunit I gene; A, Adenocine tri-phosphate; G, Guanocine tri-phosphate; T, Thymidine tri-phosphate; C, Cytidine tri-phosphate.

3.5 Inter species nucleotide divergence

Between subjected and retrieved inter species category, the mean divergent rate of *C. campechensis* showed a minimum value of 0.000 (*C. campechensis* Vs. *C. campechensis*, Mexico) and a maximum of 0.290 (*C. campechensis* Vs. *C. maya*, Mexico and, Vs. *Cypretta elongate*, Mexico) (Table. 6).

Table 6: Inter-species divergence of subjected (*C. campechensis*, paper authors, MN641913) and retrieved zooplankton species

Subjected Vs. Retrieved Inter Species	Divergence (%)
Cypretta campechensis MN641913 Vs.*Cypretta campechensis*, Mexico, MF076751	0.002*
Cypretta campechensis MN641913 Vs. *Cypretta campechensis* Mexico MF076727	0.000*
Cypretta campechensis MN641913 Vs. *Cypretta campechensis* Mexico MF076717	0.009*
Cypretta campechensis MN641913 Vs. *Cypretta campechensis* Mexico MF076716	0.005*
Cypretta campechensis MN641913 Vs. *Cypretta maya* Mexico MF076730	0.260
Cypretta campechensis MN641913 Vs. *Cypretta maya* Mexico MF076729	0.260
Cypretta campechensis MN641913 Vs. *Cypretta maya* Mexico MF076726	0.005
Cypretta campechensis MN641913 Vs. *Cypretta maya* Mexico MF076728	0.260
Cypretta campechensis MN641913 Vs. *Cypretta maya* Mexico MF076724	0.290
Cypretta campechensis MN641913 Vs. *Cypretta maya* Mexico MF076720	0.256
Cypretta campechensis MN641913 Vs. *Cypretta maya* Mexico MF076719	0.256
Cypretta campechensis MN641913 Vs. *Cypretta elongata* Mexico MF076723	0.290
Cypretta campechensis MN641913 Vs. *Cypretta elongata* Mexico MF076722	0.290
Cypretta campechensis MN641913 Vs. *Cypretta elongata* Mexico MF076721	0.290

*****intra species divergence

3.6 Phylogenetic information

The subjected *C. campechensis* along with their respective retrieved species showed Ka value of 2.064 and Ks of 0.850, which indicated the possibility of occurrence of more deleterious mutation and less silent mutation. Similarly, the Tv value of 0.12 and the Ts of 0.09 were recorded, which indicated the fact that these sequences have more phylogenetic information (Table. 7). However, saturation might have not occurred in this sequence, which was confirmed by the predicted higher Iss.c values of 0.741 than that of the Iss value of 0.252 (Table. 7).

Table 7: Phylogenetic information of subjected zooplankton species along with retrieved

Zooplankton species	Phylogenetic information								
	Ks	Ka	Ks-Ka	Ts	Tv	Tv-Ts	Iss	Iss.c	Iss.c-Iss
Cypretta campechensis and retrieved species	0.850	2.064	1.214	0.09	0.12	0.03	0.252	0.741	0.489

Ks, Synonymous substitution; **Ka**, Non-synonymous substitution; **Ts**, Transitional substitution; **Tv**, Transversional substitution; **Iss**, Index of substitution saturation; **Iss.c**, Critical value of index of substitution saturation.

3.7 Phylogenetic tree topology

Plate 6 represent *Cypretta* phylogenetic tree topology, the subjected (*C. campechensis*) and retrieved (*C. campechensis*, *C. maya* and *C. elongata* from Mexico) species formed four clusters. The I-cluster was formed by three species of *C. elongata* and one species of *C. maya*. The II-cluster was formed by three species of *C. maya*. The III-cluster was formed by two species of *C. maya*. The IV-cluster was formed by subjected *C. campechensis*, and retrieved (three species of *C. campechensis* and one species of *C. maya*).

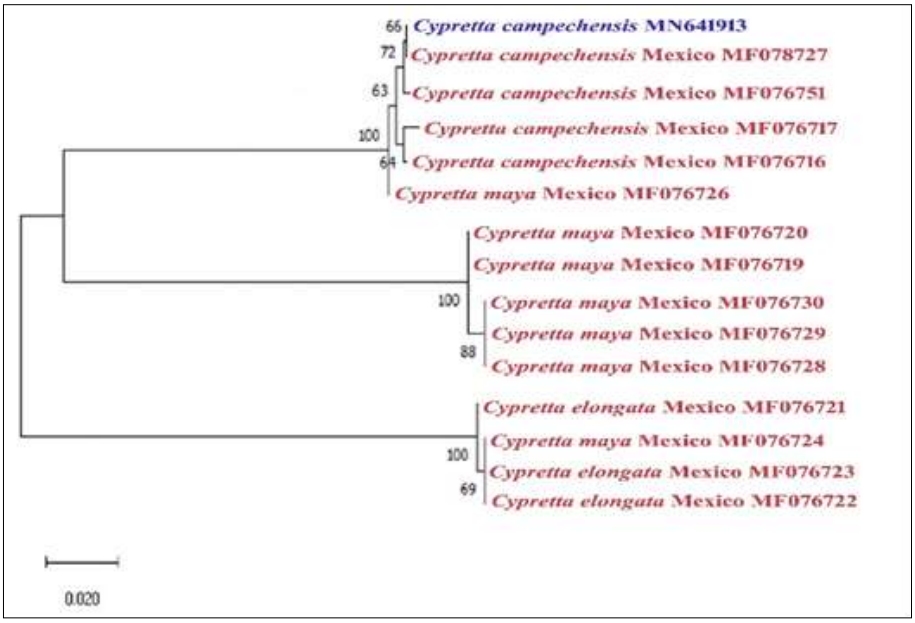


Plate 6: Phylogenetic tree topology of subjected zooplankton, *C. campechensis* along with retrieved species

4. Discussion

Planktonic organisms form the food for many aquatic animals including whales, fishes and prawns. The zooplankton serves as a main source of nutrition for freshwater and marine fishes and prawns larvae (Hamdy, 2019) [43]. They supply necessary amount of protein, lipid, essential amino acids and fatty acids,

which provide immune stimulation, pigment enhancement, physiological regulations, and growth and quality larval production (Mayzaud *et al*., 2016; Manickam *et al*., 2017) [44, 45]. Zooplanktons are highly sensitive to detect any environmental disturbances through changes in species composition, abundance, and body size, distribution and diversification (Xiong *et al*., 2020; Eskinazi-Sant'Anna *et al*., 2020) [46, 47]. Zooplankton species composition in a particular water body is controlled by several ecological factors, including nutritional load and pollution status (Bhavan *et al*., 2015a) [19]. Different species of Rotifera, Cladocera, Copepoda and Ostrocoda have been reported in few lakes and Dams in Coimbatore region, Tamil Nadu, India (Bhavan *et al*., 2015a; Kalpana *et al*., 2017; Kowthaman *et al*., 2019) [19, 20, 48]. Here, the observed species of planktonic groups suggestively provide good productivity in pre-monsoon, monsoon and post-monsoon seasons.

Abundance of Rotifera such as *Brachionus calyciflorus*, *Brachionus caudatus personatus*, *Brachionus diversicornis*, *Brachionus angularis*, *Brachionus forficula*, *Brachionus quadridentatus Keratella cochlearis*, *Conochilus unicornis*, *Kellicottia longispina*, *Bosmina longispina*, *Filina longiseta* and *Trichotria tetractis* have been reported as indicators species of pollution in few perennial pond and lakes of Madurai region, Tamil Nadu, India and Pinyin lake in Jiangxi region, China (Dutta *et al*., 2013; Xiong *et al*., 2020) [49, 46]. So also, in this study, we found few pollution indicator species of rotifera, such as *B. calyciflorus*, *B. caudatus personatus*, *B. diversicornis*, *B. angularis*, *B. quadridentatus Keratella spp*., and *Filina longiseta*. However, the number of individuals in each indicator species represents pollution status. Moreover, the rotifer species serve as feed for carp larvae.

There are few Cladoceran genera are planktonic in the freshwater, while majority of them are littoral, live among the weed and some of them live on the bottom mud; *Diaphanosoma*, *Daphnia*, *Ceriodaphnia*, *Bosmina longispina*, *Daphnia galeata*, *Moina macrocopa*, *Macrothrix spinosa* and *Moina* have been recorded in eutrophic lakes throughout the world, for instance, the Ganga River at Arrah from Bihar, India and Mula river in Pune, India (Balakrishna *et al*., 2013; Pandit *et al*., 2020; Padhye, 2020) [50-52]. Few species belongs to cladocera, such as *Diaphanosoma*, *Daphnia*, *Ceriodaphnia*, *Moina macrocopa*, *Macrothrix spinosa* and *Moina* have been recorded in this study. Most of the cladoceran species are feed for carps larvae. Thus, the presence of these species involves in energy flow and nutrient cycling.

Regarding Copepoda, *Sinodiaptomus* (*Rhinediaptomus*) *indicus*, *Heliodiaptomus*, *Mesocyclops leuckarti*, *Thermocyclops*, *Thermocyclops decipiens*, *Thermocyclops crassus*, *Tropocyclops*, *Tropocyclops confinis*, *Mesocyclops* and *Cyclops* have been reported in South India and Huangpu River, Baoshan region in Shanghai, China (Dutta *et al*., 2013; Ni *et al*., 2020) [49, 53]. Since copepods are excellent food with high nutritional value for aquaculture of fishes and prawns, the consistent presence of *Heliodiaptomus*, *M. leuckarti*, *Thermocyclops*, *T. decipiens*, *T. crassus*, *T. crassus*, *T. confinis*, *Mesocyclops* and *Cyclops* indicates the nutritional status of the lake and its suitability for aquaculture.

Ostracods are found in heavily polluted water and they serve as indicator species of climate and ecosystem changes (Martens *et al*., 2008) [7]. It has been reported that *Heterocypris incongruens*, *Candona neglecta*, *Prionocypris zenkeri*, *Ilyocypris bradyi*, *Cypridopsis vidua*, *Vestalenula boteai*, *Hemicypris inversus*, *Hemicypris congenera*, *Cypretta hirsute*, *Trajancypris clavata* and *Cypretta hirsuta* were suggested as indicators of organic pollution in Tibetan Plateau surrounded by the Himalayas and high-altitude shallow lakes in Brazil (Kulkoyluoglu *et al*., 2016; Eskinazi-Sant'Anna *et al*., 2020) [54, 47]. Here, the occurrences of such ostracoda species (*Heterocypris incongruens*, *Prionocypris* sp., *Candona* sp., and *Cypretta* sp.) have been recorded; in addition to these, the presence of *C. campechensis* indicates the water was getting polluted during the summer.

The mass culture of zooplankton, such as *Brachionus*, *Daphnia*, *Asplanchna intermedia*, *Ceriodaphnia*, *Eucyclops speratus*, *Mesocyclops edax*, *Moina*, *Cyclops*, *Lecanea*, *Keratella*, *Diaphanosoma* and *Diaptomus* with different feeds, such as chlorella, Yeast, cow-dung, mixed phytoplankton, condensed phytoplankton products, pulse bran water, poultry manure, snail faeces, chicken manure and fish waste diet have been reported (Bhavan *et al*., 2016; Kalpana *et al*., 2018; Ogello *et al*., 2019) [12, 13, 55]. In this study, the first reported zooplankton species *C. campechensis* have grown well in the laboratory with mixed phytoplankton diet.

The COI gene marker have been widely used to identify freshwater prawns and zooplankton and to clarify the phylogenetic relationships and genetic divergences between species (Bhavan *et al*., 2017; Kalpana *et al*., 2018; Blanco-Bercial, 2020) [4, 13, 56]. This gene marker has also been used to differentiate the cryptic species in *Oithona* spp., and *P. nana* (Burton *et al*., 2018) [57]. Penton *et al*. (2004) [58] discriminated two cryptic species within the *Daphnia obtusa* complex in North America using COI sequences. Similarly, Gilbert *et al*. (2005) [59] described ten genetically distinct cryptic species of *B. calyciflorus* in eastern China. The higher AT biases have been reported in crabs and prawns (Bhavan *et al*., 2015b; Umamaheswari *et al*., 2016; Udayasuriyan *et al*., 2017) [60, 61, 35], and in freshwater zooplankton (Bhavan *et al*., 2016, 2017; Kalpana *et al*., 2018) [12, 4, 13]. The higher A+T and lower G+C contents have also been reported by Wang *et al*. (2016) [62]. The highest GC content was reported in *Parartemia longicaudata* from saline lakes in Australia, while the lowest GC content was reported in the amphipod, *Hyperia galba* a parasite of jellyfish. Isopoda possessed the highest and lowest average GC content (Costa *et al*., 2018) [63]. Arisuryanti *et al*. (2020) [64] reported the highest percentage of nucleotide C and A+T, similar percentage of nucleotide A and G was observed in *Parhippolyte uveae* (red shrimp). The higher AT biases and lower GC biases have been reported in *Phyllodiaptomus tunguidus* (Zhang *et al*., 2020) [65]. The higher AT biases recorded in this study indicates the lower abundance of nuclear copies of mt-DNA (NUMTs) genes known as pseudogenes, homologs or paralogs in all the three species.

Generally, deep genetic divergence exists among allopatric populations of a single species. For example, five phylo groups of *Daphnia ambigua* (four in North America and one in South America) had been reported with >3% divergence (Hebert *et al*., 2003) [66]. In a study with six phylogroups of *Sida crystalline* with >5% divergence has also been reported by Cox and Hebert (2001) [67]. Zooplankton like rotifer, often have complex life cycles, high dispersal capacities and rapid local adaptations, which may facilitate inter specific gene flow and intra specific divergence (Gomez *et al*., 2002; Cristescu *et al*., 2012) [68, 69]. Barcode analysis enabled some forms and varieties of common species identified as separate

species; the cryptic species in *Ascomorpha ovalis*, *Lecane bulla*, *Lecane cornuta*, *Lecane curvicornis*, *Lecane crepida*, *Lecane lunaris*, *Lecane hastata*, *Platyias quadricornis*, *Keratella cochlearis*, *B. calyciflorus* and *Testudinella patina*, as well as in some forms and varieties such as *B. quadridentatus*, *Mytilina ventralis*, *Corbicula fluminea* and *Leptodiaptomus* (Elias-Gutierrez *et al*., 2008; Garc Ia-Morales and Elias-Guti Errez, 2013) [70, 71]. The biogeographic range of *Subeucalanus subtenuis*, *Subeucalanus mucronatus*, *Subeucalanus subcrassus* and *Pareucalanus langae* have been extended in the western Indian Ocean; the Indo-Pacific region consist of genetically divergent, allopatric populations of *Subeucalanus pileatus*, *Pareucalanus sewelli*, *Rhincalanus rostrifrons*, *Arietellus pavoninus*, *Codium pulvinatum* and *Boccardia proboscidea*; genetically divergent lineages of *Subeucalanus crassus* and *Rhincalanus nasutus* have been inadequately characterized (Suatoni *et al*., 2006; Zenetos and Galanidi, 2020) [72, 73]. The sequence divergence has also been reported in *Candacia*, *Pareucalanus*, *Rhincalanus* and *Temora* (Pitz *et al*., 2020) [74]. In this study, the recorded lower inter-species divergence suggests that the first recorded species are closely related species with each other.

The intra and inter specific genetic distances have considerably greater with larger proportions of closely related taxa (Funk *et al*., 2003; Moritz *et al*., 2004) [75, 76]. The smallest intra specific distances yield more consistent results (Meier *et al*., 2008) [77]. The identification success found to be declined when the overlap between intra- and inter specific distances increased (Ross *et al*., 2008; Virgilio *et al*., 2010) [78, 79]. Brandao *et al*. (2010) [80] and Schon *et al*. (2010) [81] have found a very small (0.0–0.8 %) intra specific COI distances between western Australian *Eucypris virens* and their closest European relatives. By using the distance-based approach, a species can be correctly identified when the mean distance to the most closely related species (nearest neighbor) is higher than the maximum intra specific distance (Aliabadian *et al*., 2009) [82].

The similarity between the sequences usually depends entirely on the similarity in nucleotide frequencies, which is based on level of substitutional saturation, which in turn decreases phylogenetic information (Xia and Lemey, 2009) [83]. The saturation of substitutions in sequences decreases phylogenetic information (Xia *et al*., 2003) [39]. When the sequences have experienced full substitutional saturation, the similarities between the sequences depend entirely on the similarity in nucleotide frequencies (Xia, 2000; Xia *et al*., 2003) [37, 39], which often does not reflect on phylogenetic relationships. The phylogenetic information have previously been established by us for species of crabs, prawns, shrimps and plankton (Bhavan *et al*., 2016, 2017; Udayasuriyan *et al*., 2017; Dasilva *et al*., 2019) [12, 4, 35, 84]. The possibility for occurrence of more transvertional substitutions have been reported when the sequences of *Macrobrachium* and *Caridina* were compared (Udayasuriyan *et al*., 2017) [35]. In this study, the recorded more transversional substitution in comparison with transitional substitution suggests more phylogenetic information. Therefore, there are possibilities for evolutionary changes in these species in due course of time.

5. Conclusions

In the present work, the first recorded ostracoda species, *C. campechensis* was largely found in the perennial standing freshwater of the Singanallur lake in summer season. This suggests that the water of this lake is being polluted during summer due to undiluted inflow of household let-outs and water evaporation. In other seasons, this effect may be diluted by rainwater inflow. Since this is the first report in India, we described by both morphological and molecular levels. The molecular analysis revealed that the species was distinct and showed significant variation. The mass culture of *C. campechensis* would provide a feeding option in summer months for maintenance of sustainable aquaculture.

6. Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

7. Acknowledgements

The authors would like to thank Department of Nanoscience and Technology, Bharathiar University, Coimbatore, India for providing SEM facility. We also thank Dr. Chithravel Vadivalagan for rendering certain help.

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