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The taxonomy of *Brachionus plicatilis* species complex (Rotifera: Monogononta) from the Southern Kerala (India) with a note on their reproductive preferences

P. S Anitha and Rani Mary George

Central Marine Fisheries Research Institute, P. B. No. 1603, Ernakulam North P. O., Cochin-682 018, India
E. mail: ranimary@yahoo.com

Abstract

This paper deals with the species of *Brachionus plicatilis* complex (Rotifera: Monogononta) found in a brackishwater lake,Veli-Aakulam, in Southern Kerala. Morphological and reproductive potential studies revealed that these rotifers have significant morphological distinction with regard to ecological preferences. The three related rotifers in our study were classified as *Brachionus plicatilis* Müller, 1786, *B. rotundiformis* Tschugunoff, 1921 and *B. murrayi* Fadeew, 1925. The fine morphology and morphometric data revealed that taxonomic characters were constant enough to recognize three well-defined morphologies and *B. murrayi* is redescribed. The reproductive potential studies in relation to ecological parameters showed that the 'r' values of these rotifers were significantly influenced by salinity as well as temperature, and was species specific. In the light of reproductive preferences, the reorganization of three distinct species has been confirmed by this study and this would allow further comparative work in these lines on this economically important species complex.

Keywords: *Brachionus plicatilis* species complex, reproductive preferences

Introduction

Among brachionid rotifers, *Brachionus plicatilis* Müller, 1786 (Monogononta) is probably one of the best-studied taxa because of its suitability as an initial live feed for various finfish and shellfish larvae (Lubzens, 1987). In *B. plicatilis*, the size and shape of lorica vary greatly according to the strain (Snell and Carrillo, 1984). Because of the morphological and physiological differences between the strains of this species, several comparative studies have been carried out by a number of workers (Fu *et al.*, 1991a & b; Hagiwara *et al.*, 1995). These studies have revealed that *B. plicatilis* is not a single species but a complex of at least two morphologically recognizable taxa, the so-called 'L' (large) and 'S' (small) types. On the basis of this evidence, Segers (1995) re-examined the existing available names and proposed that *B. plicatilis* Müller, 1786 and *B. rotundiformis* Tschugunoff, 1921 were correct names for the 'L' and 'S' types respectively. Since then, those names have been applied to several strains from all over the world. However, recent data (Ciros-Perez *et al.*, 2001) from the Spain waters revealed that the complex is in fact composed of three biological species instead of two and these were named as *B. plicatilis*, *B. rotundiformis* and *B. ibericus* (=*B. rotundiformis* SM type).

The occurrence of *B. plicatilis* in Indian waters was noted by a few numbers of workers (Rao and Mohan, 1984; Shakuntala and Singh, 1993; Gopakumar and Jayaprakash, 2003) whereas the halobiont rotifer *B. rotundiformis* (= *B. plicatilis* 'S' type) has been reported so far only from the estuaries and backwaters of Kerala (Gopakumar and Jayaprakash, 2003; Molly, 2003). Despite this body of evidence the taxonomy of this species complex has not been established in India. So here, we present a morphological analysis of three well-defined rotifers belonging to the *B. plicatilis* complex from Kerala with a remark on their reproductive rate ('r') to show the ecological preference of these taxa in their natural habitat. Similarly, the maximum lorica size attained by a female rotifer over her life span (from neonate to adult size) was also recorded for the morphometric data comparison between the species.

Materials and methods

Fortnightly zooplankton collections were made from Veli-Aakulam Lake (Lat. 8°25'-8°35'N; Long. 76°50'-76°58'E) during February 2000 to January 2001 to examine the morph variations in rotifers. The surface plankton samples were collected by horizontal hauls using

plankton net of 32cm mouth diameter with a mesh size of 70μm and immediately preserved in 4% formaldehyde. Specimens were sorted and examined using a stereo-dissecting microscope. All the illustrations given are Camera-Lucida drawings made with the aid of a compound microscope. Standard references (Sudzuki, 1987, 1995, 1996, 1999; Fu *et al.*, 1991a; Ciros-Perez *et al.*, 2001) were consulted for the identification and classification of rotifers.

For experimental studies, three morphologically distinct females of *B. plicatilis* were isolated from the Veli-Aakulam waters. They were classified as *B. plicatilis*, *B. rotundiformis* and *B. murrayi* based on the key given by Sudzuki (1999). Each species was cloned from a single parthenogenetic female and the stock culture was maintained in the laboratory by feeding them with *Isochrysis galbana* @ 1million cells per ml. The culture temperature and salinity was 28°C and 10 x 10^{-3} respectively for each clone. A factorial experimental design was used to investigate the effect of temperature and salinity on the reproductive potential of these animals. Females of three species were first allowed to grow exponentially for six days in the pre-experimental conditions. Five ovigerous (females with single egg stage) females from each type were isolated and then introduced into 10ml test tubes containing 5ml of *I. galbana* @ 1 million cells per ml at three temperatures (25, 28 & 35°C), 6 salinities and 1000 lux constant light. The salinity levels chosen for *B. plicatilis* and *B. murrayi* were 0.5 x 10^{-3}, 5 x10^{-3}, 10 x10^{-3}, 15 x 10^{-3}, 25 x 10^{-3} & 35 x 10^{-3} and those chosen for *B. rotundiformis* were 2 x 10^{-3}, 5 x10^{-3}, 10 x10^{-3}, 15 x 10^{-3}, 25 x 10^{-3} & 35 x 10^{-3}. The salinity levels were chosen according to the tolerance ranges of the organisms studied. After three days, the number of females per tube was counted, and 'r' value was calculated according to the formula: r = (ln Nt - ln No) / t; where, Nt = number of females after 't' days; No = initial number of females, t = duration of experiment (3 days). The size at birth and maximum size of a female over her life span was also examined. For this, a large number of egg carrying females were randomly selected from the acclimatized stock and checked at 15 minutes intervals to collect neonates. After that, twelve neonates of each type were isolated singly into small test tubes containing 1ml of *I. galbana* @ 1 million cells per ml and at six salinity levels as mentioned above. The experiments were carried out at room temperature based on the best performance of each taxon in the previous reproductive potential trials. The animals in the test tubes were observed at regular intervals (2-3 hr). Data on three-life table parameters namely size at birth, size at first egg production and maximum size attained by a female over her life span was recorded. The newly hatched neonates were measured and removed after each observation. These experiments were conducted in order to ascertain the identity of these species complex with ecomorphs, as there is so much confusion to delineate the species properly.

Results

Taxonomic placement: Phylum: ROTIFERA (Cuvier)

Class: MONOGONONTA Wesenberg-Lund

Order: PLOIMIDA (Hudson & Gosse)

Family: BRACHIONIDAE Ehrenberg

Genus: *Brachionus* Pallas

Type: *Brachionus urceolaris* Müller

Key to the species of plicatilis group

1. Lorica ovoid to elliptical; occipital spines saw-teethed; broad based; pectoral margin four lobed.....*plicatilis* Müller, 1786

   Lorica, occipital spines and pectoral margin are not as above.....2

2. Lorica vase-shaped; occipital spines small based; elevated lateral pectoral projections .....*rotundiformis* Tschugunoff, 1921

   Lorica not as above; inter-medians comparatively short; pectoral margin four lobed with variations.....*murrayi* Fadeew, 1925

Brachionus plicatilis Müller, 1786 (Figs. 2 – 10)

Brachionus plicatilis Müller, 1786, p. 344, pl. L, Fig.1

Brachionus mülleri Ehrenberg, 1838, pl. LXIII, Fig. V

Brachionus hepatotomus Gosse, 1851, p. 203

Material: Several parthenogenetic females from Veli-Aakulam Lake

Measurements: Total lorica length 171-277μm; maximum width of lorica 156-225μm; anterior lorica width103-156μm; distance from lateral spine to median spine 33.4-68.8μm; median sinus gape 12-22μm; antero-median spine length 20-43μm; antero-intermediate spine length 9-34μm; antero-lateral spine length 15-30μm; maximum width - total length ratio 0.81-0.91; anterior width - maximum width ratio 0.67-0.69; egg size 98 x 87μm (L x W)

Description of the female: Lorica rather flexible, oval, not sharply separated into dorsal and ventral plates, but little compressed dorso-ventrally; anterior dorsal margin

with six broad-based saw-toothed spines, nearly equal in length; pectoral margin rigid, separated into four lobes with distinct variations; lorica without posterior spines; smooth or lightly stippled; foot opening with small subsquare aperture ventrally.

A number of morphs were observed in the plankton samples as well as in the clones maintained in the laboratory at different culture conditions. The most frequently observed morphs are *f. mülleri* Ehrenberg (Fig. 3), *f. hepatotomus* Gosse (Fig. 4a-b), *f. decemcornis* Fadeew (Figs. 7-8), *f. ovalis* f. nov. (Figs.9-10). The significant diagnostic features of these eco-morphs are given in Table 1.

Reproductive rate: The highest 'r' value of 1.47 for *B. plicatilis* was recorded at a salinity of 5 x 10^{-3} and at temperature of 28°C. Above or below this level, the 'r' values showed a decreasing trend irrespective of temperature (Fig. 1). Similarly, at birth, *B. plicatilis* females ranged from 146-151μm and they attained a maximum adult size of 257-269μm at a salinity of 5 x 10^{-3} (Table 2)

Brachionus rotundiformis Tschugunoff, 1921

(Figs.11-16)

Brachionus mülleri var. rotundiformis Tschugunoff, 1921, p. 120, pl. 1, Fig. 12

Brachionus plicatilis rotundiformis Rodewald, 1937, p. 239, Fig. 5

Brachionus rotundiformis estoniana Haberman & Sudzuki, 1998, p. 335, Fig. 2

Brachionus rotundiformis Ciros *et al.*, 2001, p. 1318, Figs. E-F

Material: A few parthenogenetic females from Veli-Aakulam.

Measurements: Total length of lorica 125-156μm; maximum width of lorica 87-125μm; antero-width of lorica 51-73μm; distance from median to lateral spine 33-47μm; median sinus gape 17-22μm; antero-median spine length 11-22μm; antero-intermediate spine length 9-13μm; antero-lateral spine length 9-13μm; maximum width- total length ratio 0.80-0.89; anterior width-maximum width ratio 0.58-0.59., egg size 90 x 65 (L x W).

Description of the female: Lorica small, more rounded and not sharply separated into dorsal and ventral plates; occipital margin with small based acutely pointed spines; pectoral margin four-lobed; lateral ones roughly triangular; foot opening with subsquare aperture ventrally and rather ovoid aperture dorsally.

Reproductive rate: B. rotundiformis recorded its highest 'r' value of 1.83 at the best salinity of 15 x 10^{-3} and at 28°C (Fig. 1). The size at birth and maximum adult size of this taxon was recorded as 98 μm and 176 μm respectively at the best salinity of 15 x 10^{-3} (Table 2).

Brachionus murrayi Fadeew, 1925

(Figs. 17-25)

Brachionus mülleri Murray, 1913, p. 499-451, Figs. 47a-c

Brachionus mülleri var. murray Fadeew, 1925, p. 22, pl. IV, Figs. 4 – 5

Brachionus rotundiformis murrayi Sudzuki, 1987, p. 46, Fig.1.3

Material: Several parthenogenetic females from Veli-Aakulam.

Measurements: Total length of lorica 150-213μm; maximum width of lorica 116-144μm; anterior lorica width 88-108μm; distance from median to lateral spine 38-67μm; median sinus gape 22-36μm; antero-median spine length 22-36μm; antero-intermediate spine length 11-18μm; antero-lateral spine length 13-22μm; maximum width-total length ratio 0.67-0.77; anterior width- maximum width ratio 0.63-0.68; egg size 103 x 89μm (L x W).

Description of the female: Lorica small ovoid to elliptical and not sharply separated into dorsal and ventral plates; occipital spines six in number which are narrow markedly above the broad, inflated base and end in thin, acutely pointed tips or small based equilateral, equidistant triangular spines; the pectoral margin rigid and scalloped, shows considerable variations, irregularity of the four rounded projections; the occipital spines show considerable variations especially in the relative length of intermediate spines; posterior spines absent; foot opening with a small subsquare aperture ventrally.

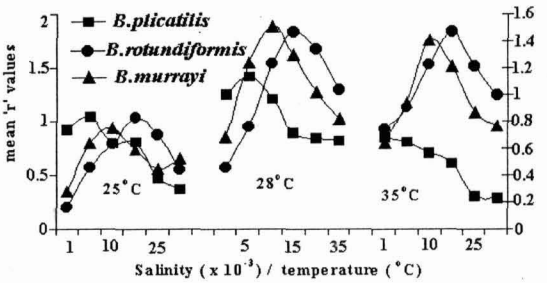
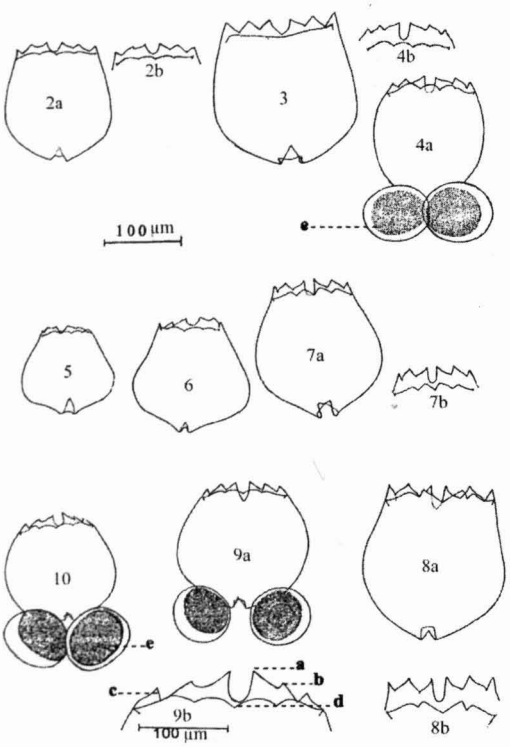
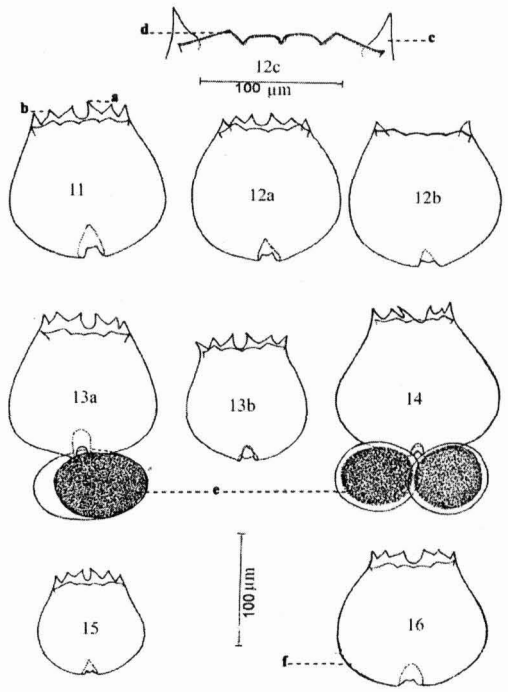


Fig. 1. Mean 'r' values of *Brachionus plicatilis*, *B. rotundiformis* and *B. murrayi* reared at different salinities at three temperatures



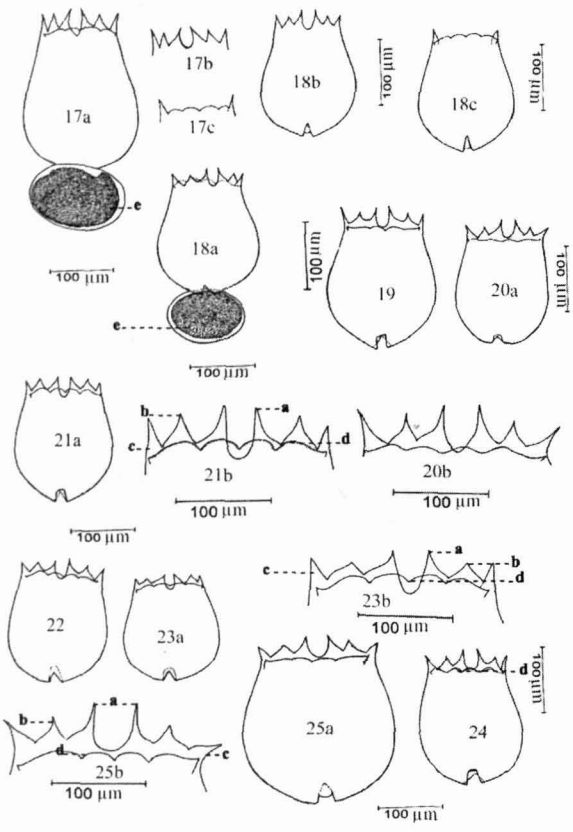
Figs. 2-10: *Brachionus plicatilis* Müller, Fig. 2a-b: *B. plicatilis f. typica*; Fig. 3: *f. mülleri* Ehrenberg; Fig.4a-b: *f. hepatotomus* Gosse; Figs. 5-6: small morphs of *plicatilis*; Figs. 7a-b, 8a-b: *f. decemcornis* Fadeew; Figs. 9-10: *f. ovalis* f.nov. a: occipital median spine; b: occipital intermedian spine; c: occipital lateral spine; d: pectoral margin; e: egg



Figs.11-16: *Brachionus rotundiformis* Tschugunoff, Fig. 12c: Enlarged view of pectoral margin showing lateral elevated lobes; Fig. 14 Shows the vase shaped lorica with irregularity in the occipital spines; a: occipital median spine; b: occipital intermedian spine; c: occipital lateral spine; d: pectoral margin; e: egg; f: swollen lorica

Table 1. *Diagnostic characteristics of different ecomorphs of* B. plicatilis *and* B. murrayi

Species/forms	Diagnosis
Brachionus plicatilis	
f. mülleri	Occipital spines broad shaped; pectoral margin obliterated
f. hepatotomus	Occipital spines with external swellings; pectoral margin not obliterated
f. decemcornis	Occipital spines without external swellings; median elevated pectorals
f. ovalis	Lorica roughly spherical in shape; occipital spines scalar in arrangement
Brachionus murrayi	
f. ecornis	Occipital spines long, short based; pectoral margin obliterated or nearly straight line
f. longicornis	Occipital spines equilateral, equidistant; scalloped pectoral margin
f. divergispinus	Occipital spines diverged especially the lateral ones; pectoral margin four lobed



Figs. 17-25: *Brachionus murrayi* Fadeew, Figs. 19, 20 & 23: *f. ecornis* Fadeew; Figs 17, 18 & 21: *f. longicornis* Fadeew; Fig. 25: *f. divergispinus* f. nov; Figs. 20b, 21b 23b & 25b: Enlarged view of occipital region showing variability in the pectoral margin and the length of the spines especially the intermediate; a: occipital median spine; b: occipital intermedian spine; c: occipital lateral spine; d: pectoral margin; e: egg

The morphs represented in the present collection are *f. ecornis* Fadeew, 1925 (Figs. 19, 20a-b & 24), *f. longicornis* Fadeew, 1925 (Figs. 21-23) and *f. divergispinus* f. nov. (Fig. 25a-b). The important diagnostic features are given in Table 1.

Reproductive rate: The highest 'r' value of 1.74 was recorded at the best salinity of 10 x 10^{-3} and at 28°C for this taxon (Fig. 1). Like wise, at birth, *B. murrayi* females ranged from 146-151µm and they attained a maximum adult size of 257-269µm at the above-mentioned salinity (Table 2).

Discussion

The morphological analysis of the *plicatilis* species complex revealed that three morphologically recognizable species co-existed in the Veli-Aakulam Lake instead of two and they were identified as *B. plicatilis*, *B. rotundiformis* and *B. murrayi* based on the key given by Sudzuki (1999). Of these, *B. murrayi* was originally established as a new variety of *B. mülleri* (= *B. plicatilis var. murray*) by Fadeew (1925) based on Murray's (1913) collection from S. America. Subsequently, Ahlstrom (1940) and Wiszniewski (1954) grouped this taxon as a variety of *B. plicatilis (f. longicornis)* in their reviews in rotifers, while Sudzuki (1987) classified it as *B. rotundiformis murrayi* due to the close morphological resemblance with *B. rotundiformis* Tschugunoff (1921). According to Sudzuki (1987, 1995, 1996), the striking morphological characteristic of the known species *B. rotundiformis murrayi* was the length of the intermediate spine, which is comparatively smaller than the median and lateral occipital spines. Similarly considerable variations in the length of the occipital spines particularly the intermedian was also observed in the rotifers collected from Veli-Aakulam Lake (Figs. 17-25) but the present taxon has showed some distinct morphological difference especially on the pectoral margin than that of Tschugunoff's *B. rotundiformis*. Likewise, the rotifers inhabiting in the Veli-Aakulam waters exhibited some morphological similarities with *B. ibericus* n. sp., (= *B. rotundiformis* 'SM' type) from Spain waters (Ciros-Perez *et al.*, 2001) and *B. rotundiformis* 'S' type from Japan (Hagiwara *et al.*, 1995) but they did not mention any morphological variability in their specimens particularly the pectoral margin. Moreover, according to Serra *et al.* (1998) the principal distinguishing character between *B. rotundiformis* and *B. ibericus* (= *B. rotundiformis* 'SM' type) was mode of conveying the resting eggs. In *B. plicatilis* and *B. rotundiformis*, resting eggs are carried outside the female lorica in the amictic or male eggs, while in *B. ibericus* they are retained within the lorica of the female. However, such females with resting egg inside the lorica were not observed in our study; because of the above mentioned morphological variability between the present taxon and *B. ibericus* (Ciros-Perez *et al.*, 2001) the rotifers that occurred in the brackishwater habitat of Kerala is named as *B. murrayi* Fadeew, 1925 based on the nomenclature preferences and we hope that this would facilitate the future reorganization of this taxon from the Indian waters.

The experimental studies on the reproductive potential of these species in relation to ecological parameters revealed that all the variables significantly influence the 'r' values of these rotifers. Of these, salinity showed a strong

Table 2. *Mean (± SD) values for the size at birth, size at first egg production and maximum size attained by* B. plicatilis, B. rotundiformis *and* B. murrayi *over their lifespan at different salinities at room temperature (28-30°C).*

Salinity (x 10^{-3})	Neonate (μm)	Size at first egg production (μm)	Maximum size (μm)	Life span (days)
Brachionus plicatilis				
0.5	148.69±3.91	188.67±3.28	261.94±2.91	5.72±0.01
5	148.92±0.21	189.92±2.15	262.74±2.91	5.77±0.11
10	146.46±0.65	186.87±0.33	259.80±5.62	5.52±0.02
15	146.31±1.19	186.74±1.48	258.83±7.39	5.27±0.02
25	146.06±1.69	186.03±1.97	257.47±7.99	4.17±0.04
35	145.64±0.84	186.11±0.48	257.10±9.62	3.89±0.18
Brachionus rotundiformis				
2	98.83±9.57	118.32±10.9	170.06±6.08	3.25±0.50
5	98.83±7.57	118.12±9.48	172.70±6.23	3.48±0.60
10	97.80±9.70	117.51±10.3	172.83±3.23	3.52±0.71
15	96.73±7.90	117.32±10.4	173.83±4.73	5.68±0.35
25	97.73±6.93	116.98±9.34	171.83±4.98	3.99±0.46
35	94.50±6.71	109.40±9.83	171.32±4.88	3.98±0.78
Brachionus murrayi				
0.5	137.06±4.60	171.70±2.80	221.04±2.23	4.12±0.15
5	137.33±5.24	172.80±3.82	221.60±3.55	4.31±0.24
10	137.40±2.13	172.80±1.80	223.50±2.61	5.65±0.27
15	136.72±2.93	172.70±3.33	221.77±3.22	4.47±0.13
25	136.70±2.92	172.30±2.22	220.90±4.30	3.62±0.11
35	136.11±2.22	171.20±1.80	219.41±5.44	3.37±0.36

influence on the 'r' values of these rotifer populations. *B. plicatilis*, *B. rotundiformis* and *B. murrayi* recorded their highest 'r' values at their optimum salinities of 5 x 10^{-3}, 15 x 10^{-3} and 10 x10^{-3} respectively, and above or below these levels their 'r' values decreased irrespective of the temperature (Fig. 1). Thus the present study is in a good agreement with the findings of Miracle and Serra (1989) and Serra *et al.* (1998) who concluded that the direct effect of salinity on 'r' values depended on the species and genotype. The influence of salinity on rotifers is directly correlated with their osmo-regulatory capacity which in turn is strongly dependent on their genotype. Thus the genotype of each species is adapted to an optimum salinity in which their 'r' is high. The size at birth and maximum adult size attained by the female of *B. plicatilis*, *B. rotundiformis* and *B. murrayi* were 151-265μm, 98-173μm and 137-225μm (laboratory clones) at the best salinities of 5 x 10^{-3}, 15 x 10^{-3} and 10 x 10^{-3} respectively and this suggests that each individual rotifer had a specific size range, which is directly correlated with the genotype of the respective taxon.

Therefore, the morphology and reproductive potential studies of the *plicatilis* species complex that inhabited the Veli-Aakulam Lake revealed that there are three morphologically and ecologically recognizable taxa co-existing in this estuary instead of two. Further, based on the iden-

tification key given by Sudzuki (1999) they are classified as *B. plicatilis*, *B. rotundiformis* and *B. murrayi*. The observations recorded here will provide significant tips to differentiate similar species when biological species are recognized on ecological, physiological and molecular data. Further, for these reasons, this study contributes to establish a reference base for further comparative works and elucidates the actual species identity of those related *B. plicatilis*-like rotifers from Indian waters.

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