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Ultra Structural Comparison of Trophi in Species of the Genus *Brachionus* Pallas, 1766 in Aguascalientes State, Mexico

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Abstract

Trophi is only found in rotifers. The trophi has been used as a tool to identify species as well as an instrument for reconstructing the phylogeny of the organism, morphometric analysis, etc. Twelve species of rotifers of the genus *Brachionus* Pallas, 1766 were collected from different regions of the state of Aguascalientes, Mexico. An ultra-structure analysis was performed to examine the structural composition of the trophi of each species using scanning electron microscopy (SEM). The SEM observations show significant differences in the structural composition of each species, with the most considerable differences found in the trophi of *B. budapestinensis*.

Keywords

Dorsal View, Ventral View, Rotifers, Taxonomy

1. Introduction

Rotifers are microscopic aquatic organisms of the phylum Rotifera. The mastax of the rotifer is a compound structure comprised of a complex set of jaws consisting primarily of four skeletal parts: the fulcrum, rami, unci and manubria, called trophi [1]. Their structure also consists of secondary structures such as the satellites, membranes and basifenestras (**Figure 2**), previously called anterior process, membranes and opening of cavities in rami [2]. Features like trophi are only found in rotifers; the only species that appears closely related in regard to the complex masticatory apparatus is *Gnathostomulid*, which also possesses a complex structure with a set of jaws. Nevertheless, only in rotifers, the trophi is used as a tool to identify species as well as an instrument for reconstructing the phylogeny of the organism [3] [4]; furthermore, trophi is the key structure in rotifer taxonomy

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[5] [6] in genetics with cryptic speciation [7], and morphometric analysis [8].

The ultra-structure of the trophi is used as an identification tool because the trophi is the strongest part of the rotifer and it never changes its shape and size after hatching [9]. They are made of chitin [10] which makes it more difficult to suffer structural damage or undergo modification after fixed, in comparison with the lorica. A whole analysis of the components of the trophi can provide the taxonomist specific information that will strongly aid in the identification of the complex species of rotifers.

In a past study conducted by Silva-Briano [11], the external morphology (anterior and posterior spines, lorica facets, foot, etc.) was used as the primary focus of the research. In this study, we analyzed 12 trophi of the genus *Brachionus* from various regions in the state of Aguascalientes, as described in past research [11] [12]. Now, with the help of the JEOL LV 5900 Scanning Electron Microscope, we took micrographics and measurement of the trophi and its components. We observe several differences.

Because trophi is important for taxonomic classification, we are trying to develop a tool that will help with the identification of complex species using all the structures of the trophi. This process will provide a more accurate identification method.

2. Material and Methods

In this study the SEM JEOL LV 5900 was used to compare the ultra-structure of the trophi between the different species of the genus *Brachionus*. We gathered a sample of 10 females per species; each sample was dissolved in NaOCl 5% formula. Then with a micropipette the trophi were washed several times using distilled water to remove the remaining materials [2]. Once completely cleaned, each trophi was covered in gold, and the micrographics were taken. All the species from **Table 1** are from Aguascalientes State, México.

We used the scale bars of each individual image taken by SEM to measure the longest and widest trophy and its component part. After image files were obtained, the first step was to transfer the image files into the computer graphics program, Microsoft Paint. The second step was to draw the lines on the images to facilitate their measurement in centimeters (see **Figure 1**). The third step was to measure the scale bar with a ruler to obtain the values in centimeters. Finally, to get values in microns, we used a rule of three, the value of the line drawn in centimeters was multiplied against the microns value provided by the scale bar, and then the result was divided with the value of the scale bar in centimeters measured with the ruler.

3. Results

Looking at 12 species malleate trophi, we use different components of trophi to see differences between them (**Figure 2**). They presented significant differences in the length and width of the trophi, the manubria, and unci

Table 1. List of species of *Brachionus* in Aguascalientes State.

Species	Origen of the sample
Brachionus angularis	Small pond on Hw. Aguascalientes—Calvillo. Aguascalientes, Ags. 21˚53'30"N. 102˚26'20"W.
B. araceliae	Temporal pond 2 Km away from Sierra Fría San José de Gracia, Aguascalientes 22˚10'4.7"N. 102˚24'58.5"W.
B. bidentatus	Near Buena Vista, Jesús María, Ags. 21˚53'N. 102˚28'W.
B. budapestinensis *B. calyciflorus*	Near highway town El Polvo—Villa Juárez, Asientos, Ags. 22˚06'N. 102˚04'W.
B. caudatus	Near road to Tapias Viejas, Jesús María, Ags. 21˚20'N. 102˚29'W.
B. falcatus	Media luna Calvillo, Ags. 21˚51'N. 102˚43'W.
B. havanaensis	La ordeña vieja, Calvillo, Ags. 21˚57'N. 102˚ 43'W.
B. josefinae	Los viñedos, Jesús María, Ags. 21˚53'18"N. 102˚ 29'46"W.
B. quadridentatus	Near road to Tapias Viejas, Jesús María, Ags. 21˚53'20"N. 102˚29'55"W.
B. Rubens	Near highway Tepezalá—Rincón de Romos, Tepezalá, Ags. 22˚13'N. 102˚11'W.
B. urceolaris	Los cuervos, El Llano, Aguascalientes, Ags. 21˚44'N. 102˚10'W.

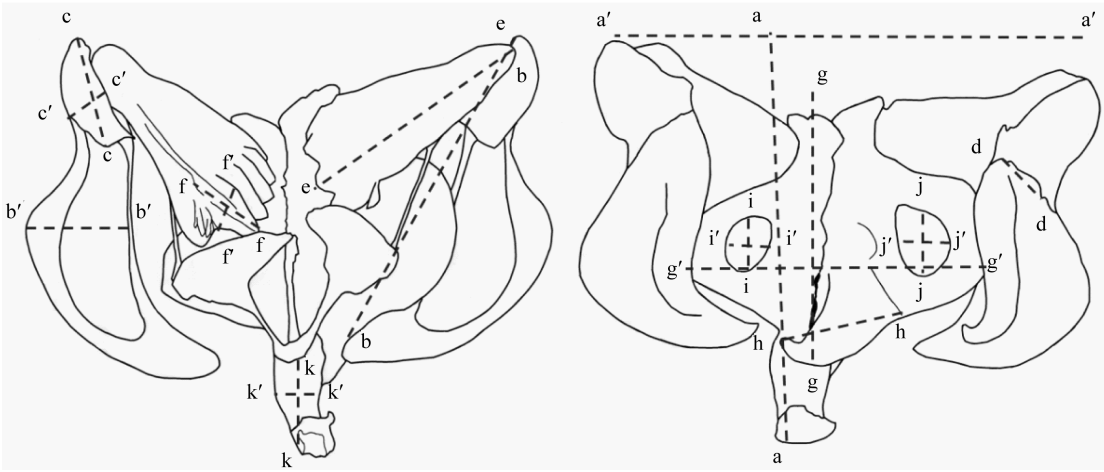
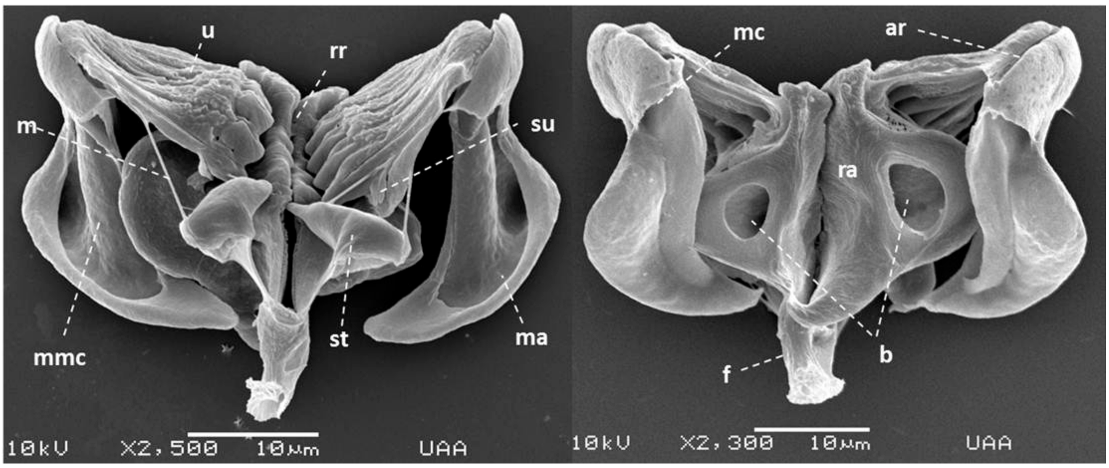


Figure 1. Ventral and dorsal view of a trophi with lines which shows how measurements were taken.



(a) (b)

Figure 2. Components of *Brachionus* trophi. (a) Ventral view: m: membrane; ma: manubrium; mmc: manubrium middle crest; rr: reinforced ridge; st: satellites; su: sub uncus; u: uncus. (b) Dorsal view: ar: articulation of manubrium; b: basifenestras; f: fulcrum; mc: manubrium cavities; ra: rami.

in the ventral view, and the basifenestras, manubria and the rami in dorsal view. The satellites were not measured because the differences were presented in the shapes and also in specifics characters.

3.1. Trophi

According to the measurements all the trophi have a rectangular shape except *B. budapestinensis* (length 28.3 ± 2 µm; width 27 ± 3.7 µm; N = 5), it has a square shape comparing with the other ten species, which have rectangular shape. *B. araceliae* presented the biggest trophi (length 48 ± 2.5 µm; width 61.5 ± 4.8 µm; N = 4), while the smallest was of *B. angularis* (length 21.1 ± 0.98 µm; width 28.3 ± 1.5 µm; N = 6) (see **Table 2**, **Figure 3** and **Figure 4**).

3.2. Manubria

The manubria presented four remarkable features to recognize the differences between them. We analyzed four of them separately: the shape of the manubrium, articulation of manubrium, manubrium middle crest in ventral view and manubrium cavities in dorsal view (**Figure 5** and **Table 2**).

The shape of the manubrium has variations in the edge, length and width. The edge could be rounded or sharp.

Table 2. Measurements of the trophi and its different components, all the values are in μm.

Species	Trophi		Components of trophi																
			manubrium					uncus			rami			basifenestras				fulcrum	
			manubrium		articulation		hole	uncus	fist tooth		rami		Right projection	right		left			
	length	width	length	width	length	width	width	length	length	width	length	width	length	length	width	length	width	length	width
Brachionus angularis	21.1 ± 0.98 N = 6	28.3 ± 1.5 N = 6	23 ± 1.2 N = 6	7.8 ± 0.79 N = 6	7.2 ± 1.9 N = 5	1.9 ± 0.92 N = 5	2.4 ± 0.14 N = 2	13.5 ± 1.2 N = 3	3 ± 1.2 N = 3	1.9 ± 0.1 N = 2	17.4 ± 0.6 N = 3	23 ± 1 N = 3	3.5 ± 0.5 N = 3	3.9 ± 0.3 N = 3	3.3 ± 0.2 N = 3	2.2 ± 0.3 N = 3	3.3 ± 0.2 N = 3	4 ± 0.4 N = 4	1.5 ± 0.1 N = 4
B. araceliae	48 ± 2.5 N = 4	61.5 ± 4.8 N = 4	37.9 ± 2.4 N = 6	14.2 ± 1.5 N = 6	12.8 ± 0.5 N = 3	3.3 ± 0.3 N = 3	7.3 N = 1	27.1 ± 1.3 N = 5	4.7 ± 0.2 N = 3	3.9 ± 0.6 N = 3	27.8 ± 0.8 N = 4	35.7 ± 2.8 N = 4	9.2 ± 1.4 N = 2	8.3 ± 0.8 N = 3	5 ± 0.2 N = 3	3.6 ± 0.3 N = 3	2.1 ± 0.1 N = 3	10.3 ± 1.5 N = 3	3.1 ± 0.5 N = 3
B. bidentatus	34.2 ± 6.5 N = 2	48.5 ± 2.1 N = 2	30.3 ± 2.6 N = 6	11 ± 1.8 N = 6	9 N = 1	2.8 N = 1	6.4 ± 0.7 N = 2	21 ± 1.4 N = 4	3.1 ± 0.2 N = 3	2 ± 0.2 N = 3	24.4 ± 1.6 N = 3	27.4 ± 2.8 N = 3	3 ± 0.7 N = 2	7 N = 1	4.4 N = 1	5 N = 1	3.5 N = 1	6.2 ± 1 N = 4	1.4 ± 0.2 N = 4
B. budapestinensis	28.3 ± 2 N = 5	27 ± 3.7 N = 5	20.8 ± 1 N = 4	2.1 ± 0.4 N = 4	hidden	hidden	1.8 ± 0.2 N = 3	15.3 ± 1.3 N = 3	4.3 ± 0.3 N = 3	1.9 ± 0.3 N = 3	21.3 ± 0.5 N = 5	17.8 ± 1.3 N = 5	absent	2.4 N = 1	1.4 N = 1	2 N = 1	1.2 N = 1	7 ± 0.3 N = 4	0.8 ± 0.09 N = 4
B. calyciflorus	37.4 ± 3.3 N = 6	52.6 ± 1.7 N = 6	29.7 ± 1.4 N = 9	10 ± 1.2 N = 9	9.1 ± 0.5 N = 3	4.3 ± 0.4 N = 3	4.7 ± 0.1 N = 6	21.6 ± 2.4 N = 4	7.6 ± 0.8 N = 4	2.7 ± 0.6 N = 4	23.7 ± 2.8 N = 7	31.3 ± 2.6 N = 7	11.5 ± 1.4 N = 6	8 ± 0.7 N = 5	4. ± 0.5 N = 5	7.8 ± 0.1 N = 5	3.8 ± 0.8 N = 5	10.7 ± 1.5 N = 6	1.8 ± 0.2 N = 6
B. caudatus	26.9 ± 1.7 N = 3	31.7 ± 1 N = 3	21.5 ± 0.8 N = 3	6.6 ± 0.1 N = 3	7.9 ± 0.4 N = 2	2.4 ± 0.2 N = 2	4.2 N = 1	15.9 N = 1	4.6 N = 1	2.3 N = 1	17.2 ± 1.2 N = 3	20.5 ± 1.1 N = 3	4.6 ± 0.2 N = 2	4 N = 1	2.9 N = 1	2.7 N = 1	1.4 N = 1	4.7 ± 1 N = 3	2.3 ± 0.1 N = 3
B. falcatus	25.5 ± 1.9 N = 2	30.4 ± 1.1 N = 2	22.9 ± 1.2 N = 3	7.6 ± 0.1 N = 3	8.2 N = 1	2.3 N = 1	2.5 ± 0.2 N = 2	16.3 ± 0.5 N = 2	3.7 N = 1	2.5 N = 1	16.2 ± 1.6 N = 2	20.2 ± 2.3 N = 2	4 N = 1	3.6 N = 1	2.8 N = 1	1.7 N = 1	2.8 N = 1	4.5 ± 0.6 N = 2	2.7 ± 0.1 N = 2
B.havanaensis	21.1 ± 2 N = 6	34.4 ± 2.9 N = 6	24.4 ± 1.1 N = 6	7.3 ± 0.5 N = 6	8 ± 0.07 N = 2	3.4 ± 0.4 N = 2	2.1 ± 0.3 N = 4	15 ± 0.2 N = 3	4.2 ± 0.2 N = 2	2.5 ± 0.2 N = 2	13.9 ± 0.8 N = 5	20.4 ± 1.2 N = 5	absent	1.5 ± 0.5 N = 4	1 ± 0.2 N = 4	1.4 ± 0.1 N = 4	0.9 ± 0.1 N = 4	3.6 ± 0.2 N = 4	2.7 ± 0.2 N = 4
B. josefinae	30.3 ± 3.2 N = 4	41.1 ± 2 N = 4	26.3 ± 1.3 N = 4	7.3 ± 8 N = 4	8 ± 0.1 N = 3	2.9 ± 0.2 N = 3	3.8 N = 1	19.1 ± 1 N = 3	5 ± 0.3 N = 3	3.4 ± 0.6 N = 3	21.3 ± .0.8 N = 4	24 ± 0.6 N = 4	10 N = 1	4.9 N = 1	4.1 N = 1	3.3 N = 1	1.5 N = 1	7.4 ± 0.2 N = 4	3.2 ± 0.7 N = 4

Continued

B. quadridentatus	32.9 ± 4.8 N = 6	40.7 ± 4.6 N = 6	25.3 ± 2.5 N = 7	8.5 ± 0.8 N = 7	8.8 ± 1.6 N = 4	2.5 ± 0.2 N = 4	4 ± 0.8 N = 3	19.4 ± 2.9 N = 4	3.8 ± 0.4 N = 4	2.6 ± 0.1 N = 4	19.5 ± 1.9 N = 6	24.5 ± 3.4 N = 6	7.9 ± 2.5 N = 2	5.6 ± 0.7 N = 3	4.3 ± 0.6 N = 3	4 ± 0.8 N = 3	2.9 ± 0.7 N = 3	5.9 ± 0.6 N = 5	3.2 ± 0.7 N = 5
B. rubens	30.6 ± 2.8 N = 4	38.5 ± 4.7 N = 4	23.2 ± 2 N = 8	8.3 ± 0.5 N = 8	8.7 ± 0.6 N = 3	2.9 ± 0.6 N = 3	3.5 ± 0.2 N = 3	19.2 ± 1.1 N = 2	4.2 ± 03 N = 3	2.1 ± 0.4 N = 2	19 ± 1.2 N = 5	23.1 ± 1.7 N = 5	7.7 ± 0.3 N = 3	4.6 ± 1 N = 3	2.3 ± 0.4 N = 3	1.3 ± 0.5 N = 3	1.1 ± 0.2 N = 3	6.3 ± 0.6 N = 5	3.1 ± 0.3 N = 5
B. urceolaris	38 ± 1.1 N = 6	44.1 ± 2.5 N = 6	28 ± 3.1 N = 7	9.5 ± 0.8 N = 7	9.6 ± 0.8 N = 3	3.3 ± 0.1 N = 3	2.9 ± 0.3 N = 4	20 ± 0.7 N = 3	4 ± 0.3 N = 3	2.4 ± 0.2 N = 3	17.5 ± 2.6 N = 5	24.8 ± 1.3 N = 5	7.1 ± 1.6 N = 4	5.3 ± 0.2 N = 4	4.1 ± 0.2 N = 4	2.3 ± 0.3 N = 4	2.1 ± 0.4 N = 4	7.2 ± 0.9 N = 7	1.8 ± 0.2 N = 7

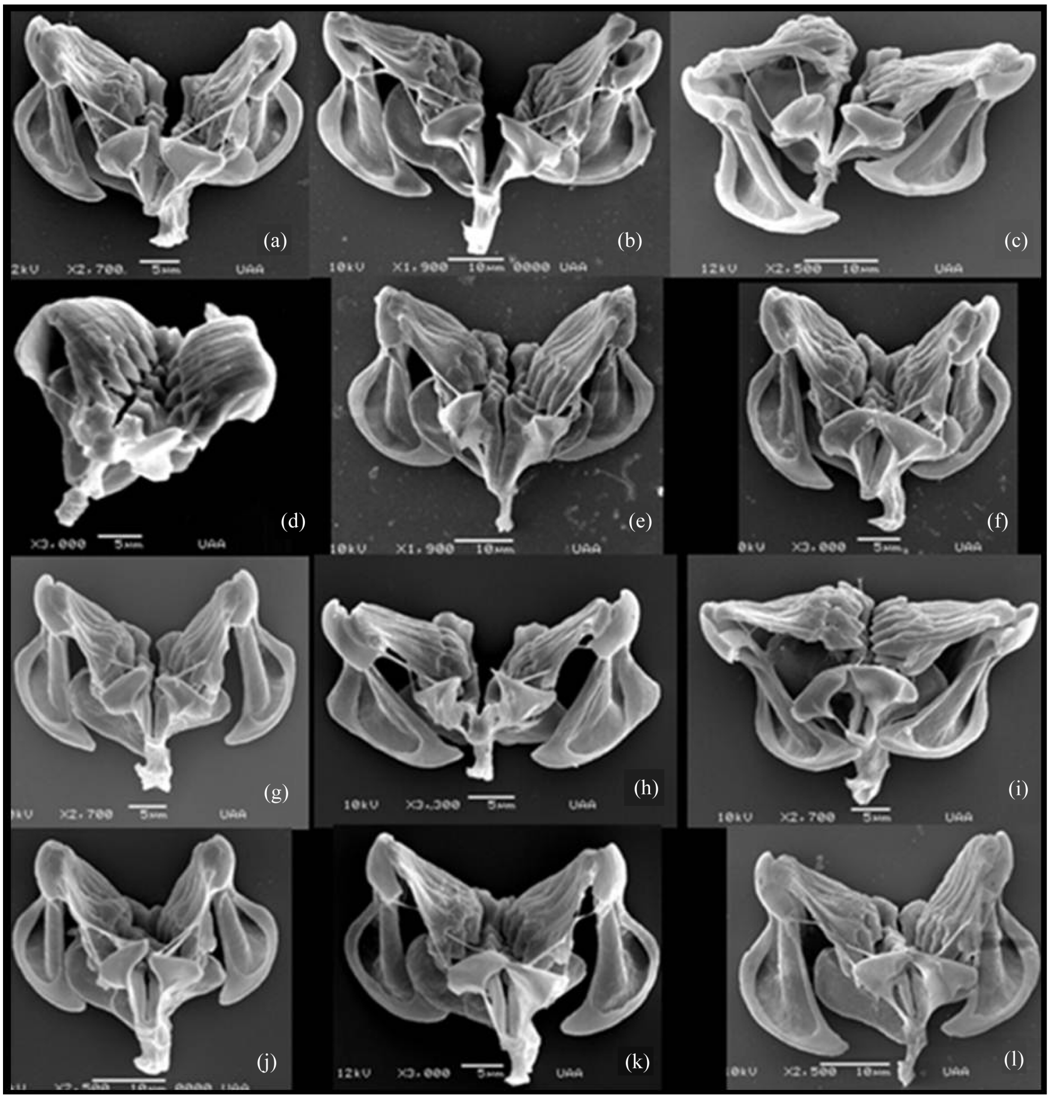


Figure 3. Ventral view of the 12 *Brahionus* trophi. (a) *B. angularis*; (b) *B. araceliae*; (c) *B. bidentatus*; (d) *B. budapestinensis*; (e) *B. calyciflorus*; (f) *B. caudatus*; (g) *B. falcatus*; (h) *B. havanaensis*; (i) *B. josefinae*; (j) *B. quadridentatus*; (k) *B. rubens*; (l) *B. urceolaris*.

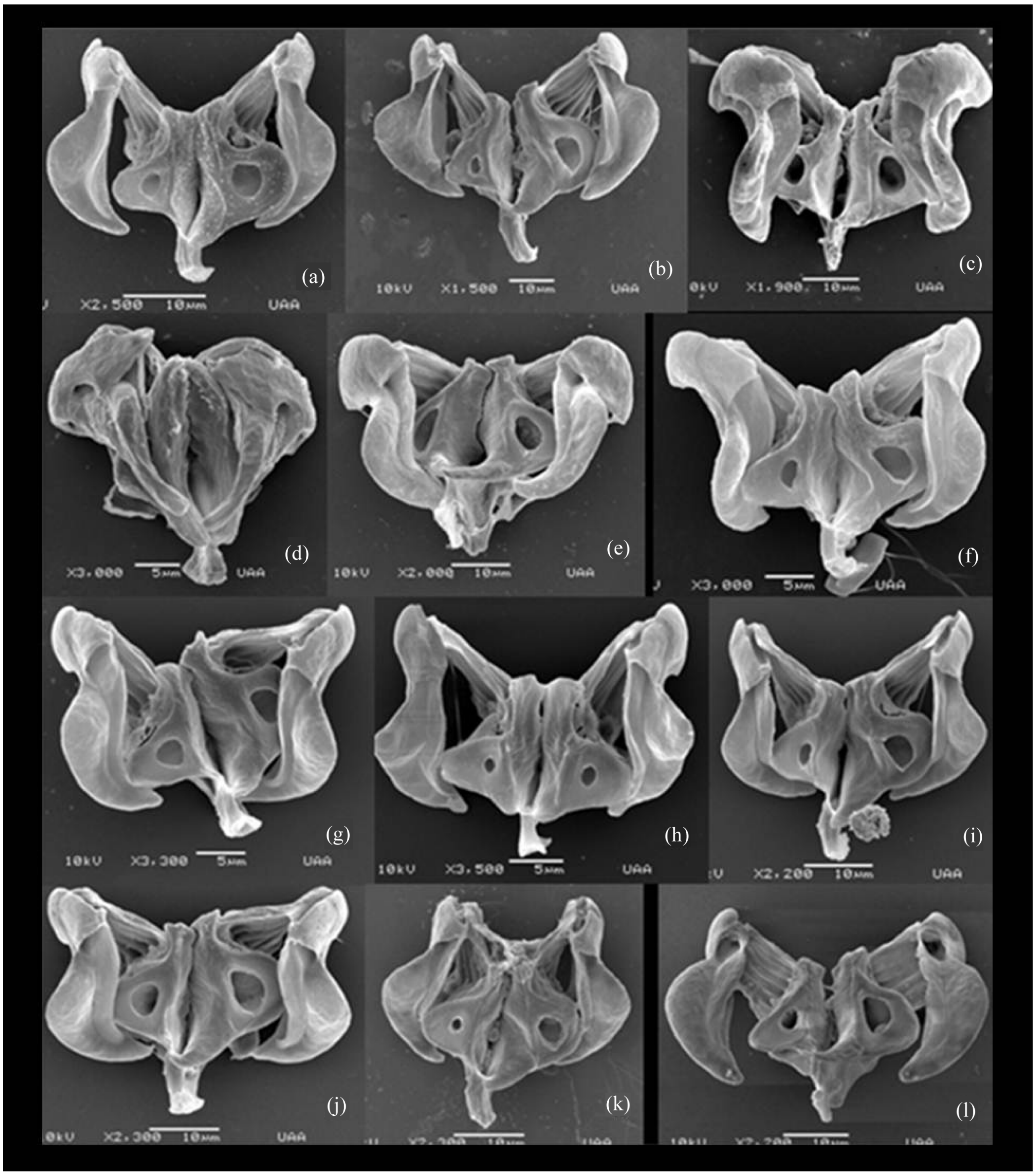


Figure 4. Dorsal view of the 12 *Brahionus* trophi. (a) *B. angularis*; (b) *B. araceliae*; (c) *B. bidentatus*; (d) *B. budapestinensis*; (e) *B. calyciflorus*; (f) *B. caudatus*; (g) *B. falcatus*; (h) *B. havanaensis*; (i) *B. josefinae*; (j) *B. quadridentatus*; (k) *B. rubens*; (l) *B. urceolaris*.

There measurements of the manubrium show that *B. araceliae* has the lengthiest and withiest values (length 37.9 ± 2.4 µm; width 14.2 ± 1.5 µm; N = 6); the thinner manubrium were of *B. caudatus* (length 21.59 ± 0.8 µm; width 6.6 ± 0.1 µm; N = 3) and *B. josefinae* (length 26.3 ± 1.3 µm; width 7.3 ± 8 µm; N = 4), while *B. havanaensis* shows robust manubrium (length 24.4 ± 1.1 µm; width 7.3 ± 0.5 µm; N = 6), furthermore it is possible to see the sharp of the outline of *B. havanaensis* (**Figure 5(a)**) compared with the rounded of the outline of *B. quadridentatus* (**Figure 5(d)**). *B. budapestinensis* shows different shape and between the others species.

The articulation of the manubria is represented by a proximal structure that attaches the manubria with the unci. The thinnest articulation was of *B. araceliae* (12.8 ± 0.5; 3.3 ± 0.3 µm; N = 3), while the widest articulation was of *B. havanaensis* (8 ± 0.07; 3.4 ± 0.4 µm; N = 2). Articulation of *B. budapestinensis* was not able to see it (**Figures 5(a)-(d)**).

The manubrium middle crest goes across vertically in ventral view (see arrows **Figures 5(a)-(d)**). Its edge is much defined in most cases, however it is possible to see it dissipated such in the case of the comparison be-

Figure 5. Ventral view of the right manubrium: (a) *Brachionus havanaensis*; (b) *B. urceolaris*; (c) *B. caudatus*; (d) *B. quadridentatus*. Dorsal view of the left manubrium: (e) *B. budapestinensis*; (f) *B. angularis*; (g) *B. quadridentatus*.

tween *B. angularis* which is dissipated against *B. quadridentatus* which is marked, with cylindrical shape; in *B. budapestinensis* it is not present.

The manubrium cavities are presented in dorsal view. They are small holes with different diameters between species in the proximal portion of the manubrium. *B. araceliae* has the highest diameter (7.3 µm; N = 1), whereas *B. budapestinensis* has the smallest diameter (1.8 ± 0.3 µm; N = 3); the rest of the species have hole sizes between 2 and 4 µm (**Figures 5(e)-(g)**).

3.3. Uncus

Only two differences were observed for the uncus in ventral view. The first distinction involves the length of the uncus and second distinction is about the length and width of the first tooth (**Figure 6(A)** and **Figure 6(C)**). Furthermore, were observed some differences in the location of the attachment between the uncus and the membranes, but they were not measured (see arrows **Figure 6(B)**). The general length of the uncus in the 12 species is around of 20 µm; *B. angulars* has the smallest measure (13.5 ± 1.2 µm; N = 3), while *B. araceliae* has the biggest measure (27.1 ± 1.3 µm; N = 3). The first tooth presented different sizes between the species in the length and width. However, the length was around 4 µm while width was around 2 µm. *B. calyciflorus* presented the lengthiest tooth (7.6 ± 0.8 µm; N = 4), and *B. araceliae* presented the widest tooth (3.9 ± 0.6 µm; N = 3), to see the differences, the scale bar of the pictures between *B. calyciflorus* and *B. budapestinensis* in **Figure 6(A)** and **Figure 6(C)** shows it.

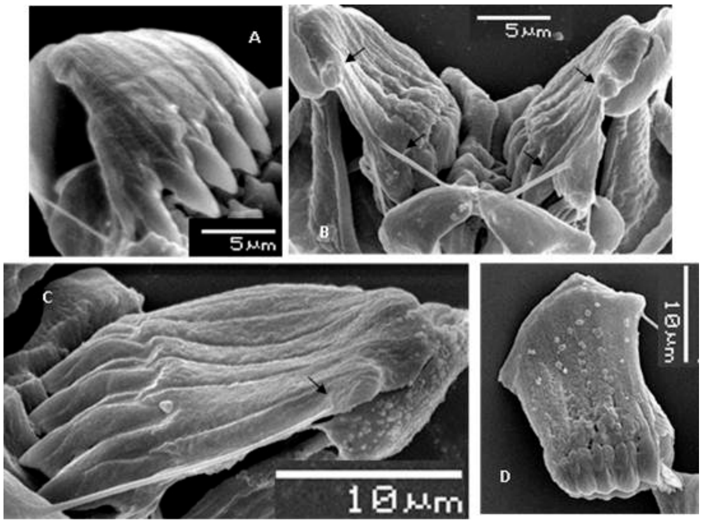


Figure 6. Unci characters in ventral view: Teeth of *B. budapestinensis* and *B. calyciflorus* (**Figure 4(a)** and **Figure 4(c)**); attachment with the membranes in *B. caudatus* and *B. calyciflorus* (see arrows **Figure 4(b)** and **Figure 4(c)**); uncus of *B. bidentatus,* frontal (**Figure 4(d)**).

3.4. Satellites

In ventral view, the satellites are placed in front of the trophi; they are not symmetric. Thin membranes connect the satellites to the unci on the top and to the rami on the bottom.

The satellites can be cylindrical similar to *B. angularis* and *B. budapestinensis* or triangular in shape resembling *B. calyciflorus* and *B. havanaensis*; species presented in the left satellite protruding projections in *B. araceliae* and *B. josefinae*, gems like *B. falcatus* or distinctive wrinkles like *B. urceolaris,* which help to distinguish the species; they were not measured (see arrows **Figure 7**).

3.5. Rami

The rami are compounded by two halves that are fused in the basal portion by the fulcrum; each half shows one circular hole at the center in the dorsal view. They are called basifenestras (**Figure 8(a)** and **Figure 8(b)**). We also observed a right projection in the right halve in dorsal view (see arrow **Figure 8(a)**); the reinforced ridge in ventral view (**Figure 8(c)**), and the rami scleropilia in frontal view (**Figure 8(d)**). We measured the length and width of the complete rami and also measure the length of the right projection in the right halve. *B. araceliae* has the highest values in length 27.8 ± 0.8 µm and width 35.7 ± 2.8 µm; N = 4, while B. havanaensis has the lengthiest value 13.9 + 0.8 µm; N = 5, but *B. budapestinensis* has the withiest value 17. 8 ± 1.3 µm; N = 5, of the rami. The measurements of the right projection show three different possibilities. The first is the absence of it like *B. budapestinensis* and *B. havanaensis*; the second is a small right projection around 3 µm like *B. angularis* and *B. bidentatus*; the third is a very prominent right projection in the range of 7 - 11 µm for the rest of the species (see **Table 2**).

3.6. Basifenestras

The basifenestras are two holes in the dorsal view of the rami. They could be symmetric like *B. calyciflorus* (length 8 ± 0.7 µm; 7.8 ± 0.1 µm and width 4 ± 0.5 µm; 3.8 ± 0.8 µm; N = 5) and *B. havanaensis* (length 1.5 ± 0.5 µm; 1.4 ± 0.1 µm and width 1 ± 0.2 µm; 0.9 ± 0.1 µm; N = 4), or they could be asymmetric like the rest of the species. When they are asymmetric the left hole is smaller than the right, for example *B. angular*, *B. araceliae*, and *B. ru*bens (see **Figure 9**).

3.7. Fulcrum

The fulcrum is a basal part attached to the rami with cylindrical in form. We measured fulcrums in the 12 spe-

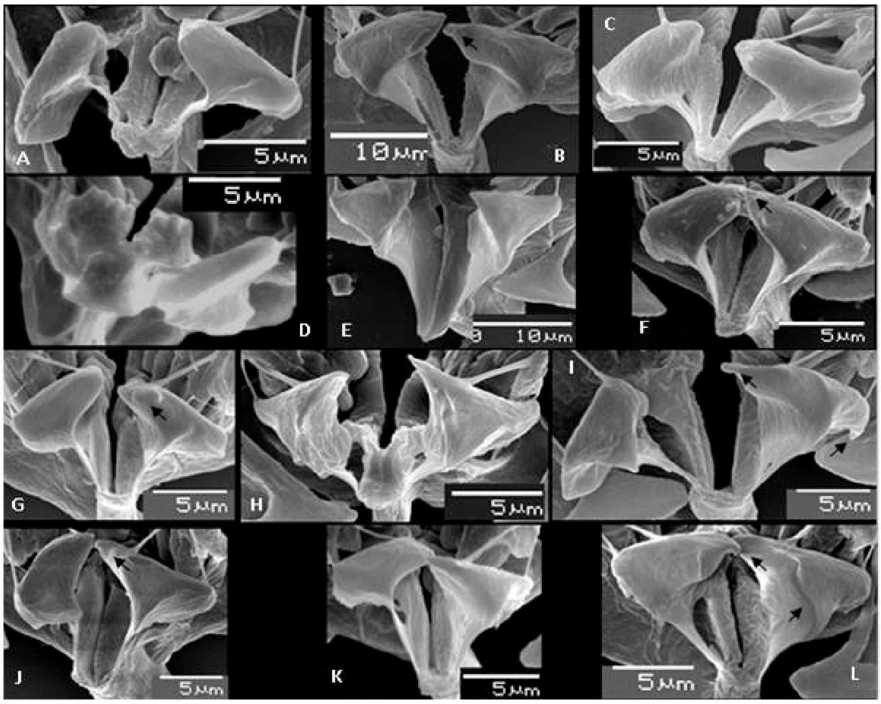


Figure 7. Satellites of: (A) *B. angularis*; (B) *B. araceliae*; (C) *B. bidentatus*; (D) *B. budapestinensis*; (E) *B. calyciflorus*; (F) *B. caudatus*; (G) *B. falcatus*; (H) *B. havanaensis*; (I) *B. josefinae*; (J) *B. quadridentatus*; (K) *B. rubens*; (L) *B. urceolaris*.

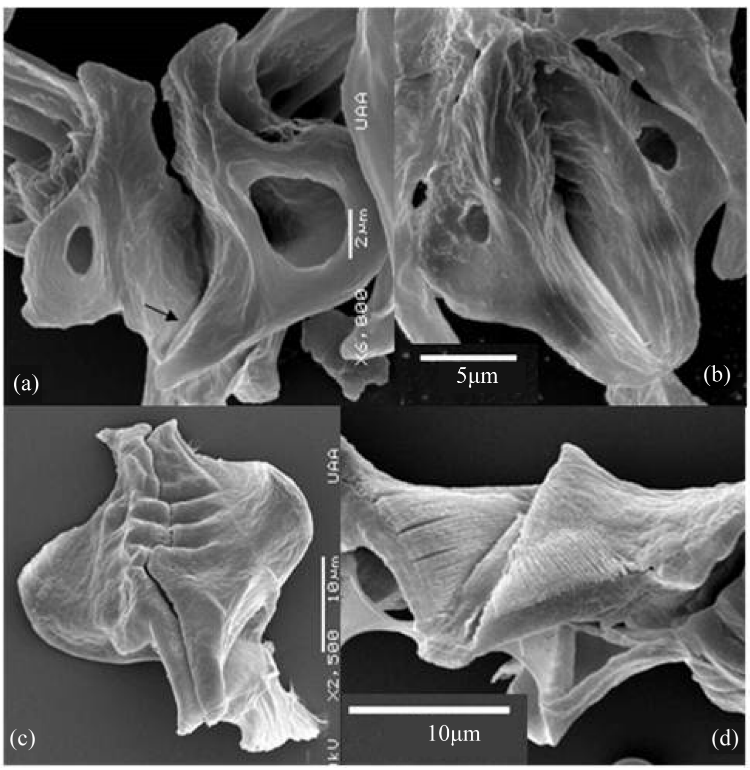


Figure 8. Rami structures: (a) Projection of the right halve in *B. angularis*, dorsal view; (b) Basifenestras in *B. budapestinensis*, dorsal view; (c) Reinforced ridge in *B. calyciflorus*, ventral view; (d) Rami scleropilia in *B. josefinae*, frontal view.

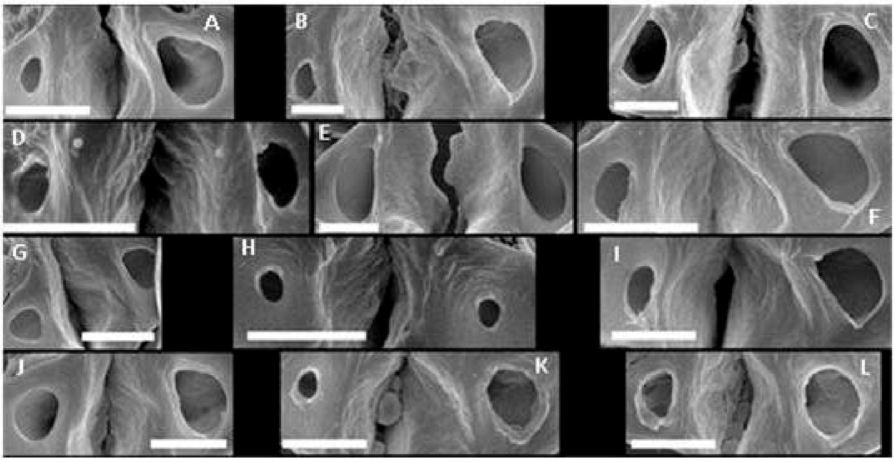


Figure 9. Basifenestras: (A) *B. angularis*; (B) *B. araceliae*; (C) *B. bidentatus*; (D) *B. budapestinensis*; (E) *B. calyciflorus*; (F) *B. caudatus*; (G) *B. falcatus*; (H) *B. havanaensis*; (I) *B. josefinae*; (J) *B. quadridentatus*; (K) *B. rubens*; (L) *B. urceolaris*. The white bar at the posterior margin of each figure equals 5 µm.

cies and we got length values from 3.6 ± 0.2 µm; N = 4 for *B. havanaensis* to 10.7 ± 1.5 µm; N = 6 for *B. calyciflorus*, whereas width values were from 0.8 ± 0.09 µm; N = 4 for *B. budapestinensis* to 3.2 + 0.7 µm; N = 5 for *B. quadridentata* when they were in ventral view. Furthermore, we observed four different conditions in the appearance of the fulcrum in the basal portion. First, it was observed that no holes, and a basal plate were present (**Figure 10(A)**), the second presented a symmetrical hole in the center (**Figure 10(B)**), third was hollow (**Figure 10(C)**) and fourth showed multiple small holes (**Figure 10(D)**). According with all images observed *B. budapestinensis*, *B. calyciflorus*, and *B. urceolaris* show a remarkable basal plate, while the rest of the species present the cylindrical shape with the modification in the holes structure.

4. Discussion

The trophi plays an important part in the rotifer, as it has been used as a general tool for identification of the species. We believe that using each small component of the ultra-structure of the trophi, coupled with the trophi itself will help establishing a more accurate identification process. All components should be used in the identification of complex species because every small change is very important for morphometric analysis. For instance, even when rotifers are fixed well some structures could suffer cellular structural alteration, such as the lorica [13]. These alterations could affect measurement accuracy, but the trophi is less susceptible to suffer alteration due to its chitin composition which is composed of calcium carbonate and proteins [14]. This molecular composition gives the material a strong resistance and therefore makes the structure of the trophi a better identification tool. Paleontological findings that date back to 570 million years support that the composition in chitin contains a strong molecular structure [15] which provides the molecular stability to preserve the structure that helps identify rotifers by the trophi.

When analyzing the trophi, there were several differences found in the components of the 12 species of *Brachionus*; some of these differences were consistent while others were not (see **Table 2**). The most reliable and consistent characteristics to aid in the identification process of rotifers by trophi were the basifenestras, satellites and the right projection in the posterior portion in the right halve of the rami. Basifenestras and satellites have been separately used to support previous studies, as in the case of the basifenestras in the cryptic speciation [7], and the satellites in taxonomical features in *B. plicatilis* [16]. However, nobody has analyzed the right projection in the rami. We want to emphasize the significance of utilizing the basifenestras, satellites, and right projection in the rami as reliable tools in the identification process of the *Brachionus* species. Although Kleinow [17] provided a 3D technique using low-power stereo-microscope to compare components of trophi, we believe that the identification process should begin by analyzing the basifenestras, satellites and the right projection in the rami.

Due to the limited population samples of trophi available for comparison, we examined images from our own findings against images found in previous researches. When comparing our data to previously published work,

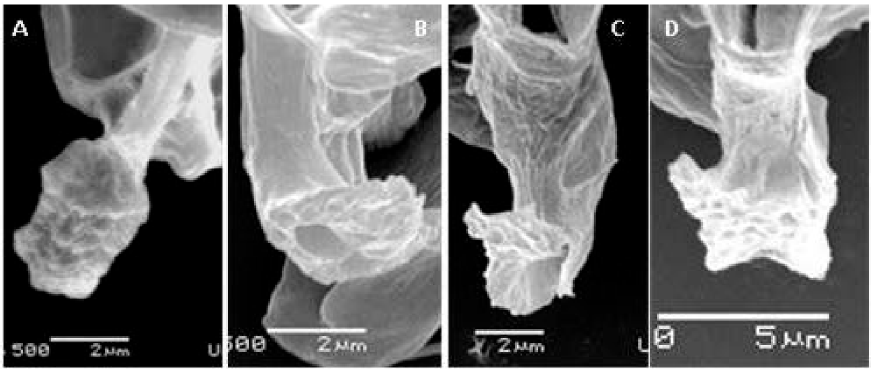


Figure 10. Different forms of fulcrum: (A) *B. budapestinensis*; (B) *B. caudatus*; (C) *B. falcatus*; (D) *B. josefinae*.

we found similitudes in various components of the trophi. For instance, in *B. havanaensis* we observed close similarities in the shape of the satellites; the symmetrical structure of the basifenestras; the shape of the manubria with the visible crest in the center; and a broad fulcrum [2]. Similarly, *B. calyciflorus* presented similarities in the shape of its satellites; the basifenestras were symmetrical; there were two projections in the rami, a sizable one on the right and a smaller on the caudal portion of the left ramus; and the manubrium had the same middle crest [2] [17] [18]. In *B. rubens* we observed the same fulcrum; the basifenestras were not visibly clear but the portion that was visible presented a high resemblance, which leads us to believe that the rest is also similar [17]. *B. angularis* had no symmetrical basifenestras, the right appeared bigger than the left; there was a similar caudal projection in the rami; the cavities in the manubrium were the same shape; and so was the fulcrum [2]. *B. falcatus* presented the same satellites; equal basifenestras; same shape of the rami; an apical and caudal projection in the right ramus; the shape of the manubrium, articulation of manubrium, middle crest in ventral view and manubrium cavities in dorsal view were extremely similar [2].

The similarities and changes between trophi among species have occurred because of the organism's adaptation to the various aquatic systems to which it is exposed. The feeding habits of the rotifers have transformed the morphology of the trophi even among the same species. These changes have become more noticeable among species that are phylogenetically distant.

In this work the trophi of *B. budapestinensis* has a completely different physiognomy than the other 11 species. Sanoamuang [19] described the *B. budapestinensis* trophi as similar with *Plationus* genus described by Seguers [2]. However, we gathered some trophi of the genus *Keratella* and we compared with them because the group is closely related with *Brachionus* group. After careful examination, the measurements of the *B. budapestinensis* trophi components show significant differences (see **Figure 3**, **Figure 4** and **Table 2**). We observed more similarities with the *Keratella* and *Plationus* genus than *Brachionus* genus.

B. budapestinensis trophi has five remarkable teeth of the same size; four transverse grooves located in the unci; a squared uncus; wide satellites in the caudal portion; a reinforced ridge with esthetical teeth decreasing from the posterior portion to the anterior; the manubria is placed behind the rami; an arrow like shaped rami; the basifenestras are covered by the manubria; the manubrium is robust and has a hole in the anterior portion, the posterior portion is thin, and a thin fulcrum with a basal plate (**Figure 3(d)** and **Figure 4(d)**).

Trophi undergo minor changes; these changes could be caused by natural processes or through manipulation during the mounting process. Once the changes caused by manipulation are discarded, the changes that occur naturally are of great significance for resolving issues surrounding taxonomy. This is why it is important to be familiarized with the structures that yield significant details surrounding differences.

We will continue to add more samples into our results because we aim to clarify which are the best characteristics to use in the identification of each species.

5. Conclusions

The components of the trophi served as a good tool to help in the identification of rotifers. Nonetheless, it is necessary to see the structure of the lorica, and the genetics in order to add more evidence for the analysis of rotifers complex species.

There are various differences among the components of all 12 species of trophi. However, in the satellites, the basifenestras and the right projection in the rami exposed more evidence and they exhibited morphological details more consistent for identification.

The trophi of *B. budapestinensis* clearly shows a different morphology when compared to the other species of *Brachionus*; the trophi is more similar to the *Keratella* and *Plationus* genus.

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