Chapter 13

Ultrastructural and Morphological Description of the Three Major Groups of Freshwater Zooplankton (Rotifera, Cladocera, and Copepoda) from the State of Aguascalientes, Mexico

Marcelo Silva-Briano, Araceli Adabache-Ortiz,
Gerardo Guerrero-Jiménez,
Roberto Rico-Martínez and
Guadalupe Zavala-Padilla

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60659

Abstract

An ultrastructural and morphological description of the three major groups of freshwater zooplankton (Rotifera, Cladocera, and Copepoda) from the state of Aguascalientes using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) was performed. The main characteristics used for identification keys for each group were particularly investigated and also the cellular morphology of rods and spermatozoids in males of the rotifer *Brachionus bidentatus* has also been investigated. It is noteworthy to mention that in the state of Aguascalientes, three endemic species of rotifers new to science have been described: *Keratella mexicana, Brachionus araceliae*, and *Brachionus josefinae*. Regarding the suborder Cladocera, the analysis of the first and second pair of antenna, rostrum, cephalic pores, postabdomen, and the five pairs of swimming legs has resulted in the description of seven species new to science from the state of Aguascalientes: four species of *Macrothrix,* two species of *Alona*, and one species of *Karualona*. Regarding the subclass Copepoda, four species of Cyclopoida group new to science have been described from Aguascalientes. The taxonomical description of these species included the morphological analysis of the buccal parts and the five pairs of swimming legs with emphasis on the fifth pair of legs. The ultrastructural and morphological analysis of each characteristic has been an exhaustive task. The use of SEM and TEM was crucial to identify all these new species. SEM has allowed focusing in the study of new micro-details that have been used for taxonomical clarity, while TEM allows for studies of cellular composition and the physiological functioning of these zooplankton species. The state of Aguascalientes inventory today comprehends more than 100 rotifer species and about 50 cladoceran and 30 copepod species (of which 14 were new to science in all three groups), leading us to believe that the number of species for this inventory could be increased, adding new species to science, in the process.

Keywords: Rotifera, Cladocera, Copepoda, Scanning electron microscopy (SEM), Taxonomy, Transmission electron microscopy (TEM)

1. Introduction

Electronic microscopy (EM) has different elements for its applications; all of them have produced important contributions to the different branches of science. While transmission electron microscopy (TEM) has produced an enormous advance in the study of the different cellular components as well as their function, the scanning electron microscopy (SEM) has helped in the recognition of surfaces of cells, tissues, and structures, developing a new way of more detailed study [1].

In the case of limnology, the use of SEM has been of great importance for taxonomists as a tool that allows for a more detailed study of the different planktonic organisms that are mainly microscopic, and thus optical microscopy might not allow for a clear distinction of structures of taxonomical importance. In the other hand, nuclear ultrastructure in animals, plants, fungi, and Protoctista was studied by TEM to consider variations of RNP particles that may be related to the initial evolution of posttranscriptional processing [2].

In the state of Aguascalientes, Mexico, with the help of SEM, the ultrastructural study of the taxonomic groups Rotifera, Cladocera, and Copepoda has led to the discovery of species new to science, as well as the observation of new structures previously unnoticed. Fourteen new species of these three taxonomic groups were described in Aguascalientes (Table 1). In spite of the discoveries obtained so far, there are still many water reservoirs that have not been analyzed before which opens the probability of increasing the number of new species records for the State or even species new to science.

1.1. Sample preparation

To prepare any organism for SEM is an art. To calculate the concentrations of each substance to apply, to measure the exposure time for each substance on the desired organism, and to know the order in which to proceed through this methodology is an alchemic process. For

http://dx.doi.org/10.5772/60659

Species new to science from Aguascalientes, México		
Rotifera	**Cladocera**	**Copepoda**
Brachionus araceliae	*Alona aguascalentensis*	*Acantocyclops caesariatus*
B. josefinae	*A. anamariae*	*A. dodsoni*
Keratella mexicana	*Karualona penuelasi*	*A. marceloi*
	Macrothrix agsensis	*Paracyclops hirsutus*
	M. mexicanus	
	M. sierrafriatensis	
	M. smirnovi	

Table 1. Fourteen species new to science described from Aguascalientes, México.

rotifers, cladocerans, and copepods, we have used methodologies previously designed. However, each organism requires slight adequations in this methodology to obtain better results for its observation. In these cases, we have used the next two methodologies.

1.1.1. Preparation of Samples for SEM

Specimens were fixed in 4 % formaldehyde, dehydrated in graded series of ethanol, taken to critical point, mounted in an aluminum stub (1 cm high and 1.2 cm in diameter) and covered with gold. To study the trophi in rotifers, organisms of every species were prepared according to the protocol of [3] with slight modifications. Briefly, this protocol consisted of isolating ten females of rotifers in a Petri dish and then adding a drop of sodium hypochlorite and waiting until the lorica was dissolved. Then the females were washed three times with distilled water and mounted in a SEM cylinder. The specimens were observed in a JEOL 5900 LV scanning electronic microscope.

1.1.2. Preparation of Male Specimens for TEM

We cultured rotifers until we obtained 500 males. They were fixed in 2 % glutaraldehyde (GTA) and 4 % paraformaldehyde (PFA) with 0.16 M phosphate buffer (PBS). Then, they were post-fixated with 1 % osmium tetroxide (OsO_4). Later, the males were embedded in epoxy resin (EPON) and observed in a JEOL 1010 transmission microscope operated at 80 kv.

2. Rotifera

2.1. General information

Rotifers are a primary group of small invertebrates ranging from 53 μm to 2 mm that play an important role in freshwater ecosystems; they can also colonize marine and terrestrial ecosystems; they can even be found in plants (Bromeliaceae), mosses, and lichens [4]. They are recognized by three main characteristics: 1) the corona, which is a complex of cilia in the anterior part of the organism and allows the production of water currents that help the animal to capture food; 2) the trophi which is the chewing apparatus made of chitin [5], and its function is to grind food that has been captured; and 3) the foot which is found in the posterior part of the organism and its function is to secrete a sticky substance which allows the animal to adhere to a substratum. However, in some species, the foot can be absent, as in the case of the species of the genus *Asplanchna*.

This phylum is composed of three main classes: The Monogononta with 1,570 species, the Bdelloidea with 461 species, and the Seisonidae with a few marine species [6]. In Mexico, 300 species have been reported [7, 8], and for the state of Aguascalientes, there are 96 species belonging to 33 different genera (Biodiversidad de Aguascalientes 2009). In the state of Aguascalientes, the taxonomic studies so far has focused in the class Monogononta, specifically in the genera *Brachionus* and *Keratella*. These genera are very common and well represented in the state of Aguascalientes as well as worldwide; in both genera, cryptic species or species complexes have been described. Therefore, ultrastructural analysis represents an important tool to elucidate the relationship among species of these two genera. The emphasis in the use of Monogononta is related to the ease in its manipulation for ultrastructural analysis compared with Bdelloidea, where only a few specialists worldwide are able to correctly identify them. However, there are many other genera of rotifers that might be worth to study and to detail ultrastructural differences among cryptic species. That might be the case of the numerous genus *Lecane*.

2.2. Representative characteristics for identification

Rotifers are divided into loricated and illoricated, which means that the loricated rotifers possess a cover or carapace that protects them, while in the illoricated ones, the tegument is exposed to the medium. Due to this, the identification of rotifers is performed in two different ways:

1. For the study of illoricated organisms which is the case of the class Bdelloidea or the genus *Asplanchna*, it is necessary to locate the trophi (Figure 1) as it is the best structure to aid in the identification. However, we must record all the characteristics like body shape and count the number of ovaries, vitellarium, and other features in a live uncontracted animal previous to the fixation of the specimen.

2. For the loricated rotifers, it is necessary to fix them in 4 % formaldehyde. Identification is commonly based on the number and position of the spines as in the genus *Brachionus*; on the shape of the dorsal and ventral valves of the lorica, the different folds, and the shape and structure of the foot and the presence or absence of claws as in the genus *Lecane*; and on the structure and number of the facets of the lorica and the number and disposition of spines in the genus *Keratella* (Figure 1).

However, for a complete analysis in certain species, it is necessary to observe the male and the ornamentation of the cysts; and in cryptic species (like the *Brachionus calyciflorus*, *Brachionus plicatilis*, and *Keratella cochlearis* species complexes), a genetic analysis of coxI sequences [10] or data on cross-mating behavior tests [11] is necessary.

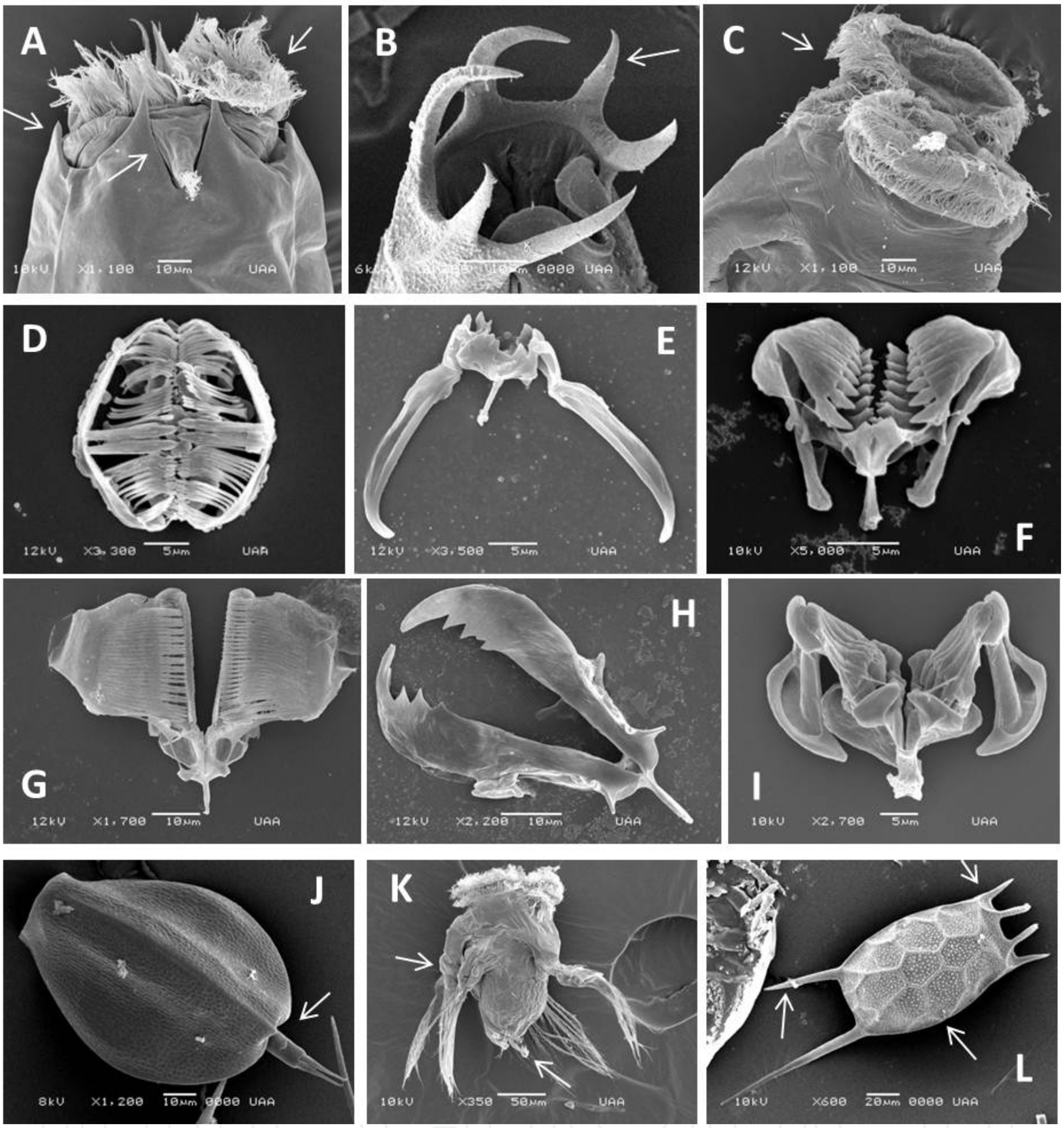

Figure 1. Several characteristics used for rotifer identification. The corona and anterior spines of *Brachionus* (A), anterior spines of *Keratella* (B), corona of *Hexarthra* (C), ramate trophi of a Bdelloid (D), malleate trophi of *Lecane* (E), malleate trophi of *Keratella* (F); malleoramate trophi of *Filinia* (G), incudate trophi of *Asplanchna* (H), malleate trophi of *Brachionus* (I), the loricated rotifer *Lepadella* with arrow showing pseudosegments of the foot (J), the illoricated rotifer *Hexarthra* showing the arms and setae needed for identification (K), and the loricated rotifer *Keratella* with arrows pointing to the facets of the lorica and anterior and posterior spines needed for identification (L).

2.3. Sexual behavior and morphology of germ line of cells in rotifers

The variations in the taxonomic characteristics, as well as the peculiar way in which mating behavior occurs in the different genera, have aroused great interest about the knowledge of sexual reproductive behavior in rotifers. Usually class Monogononta reproduces mostly via

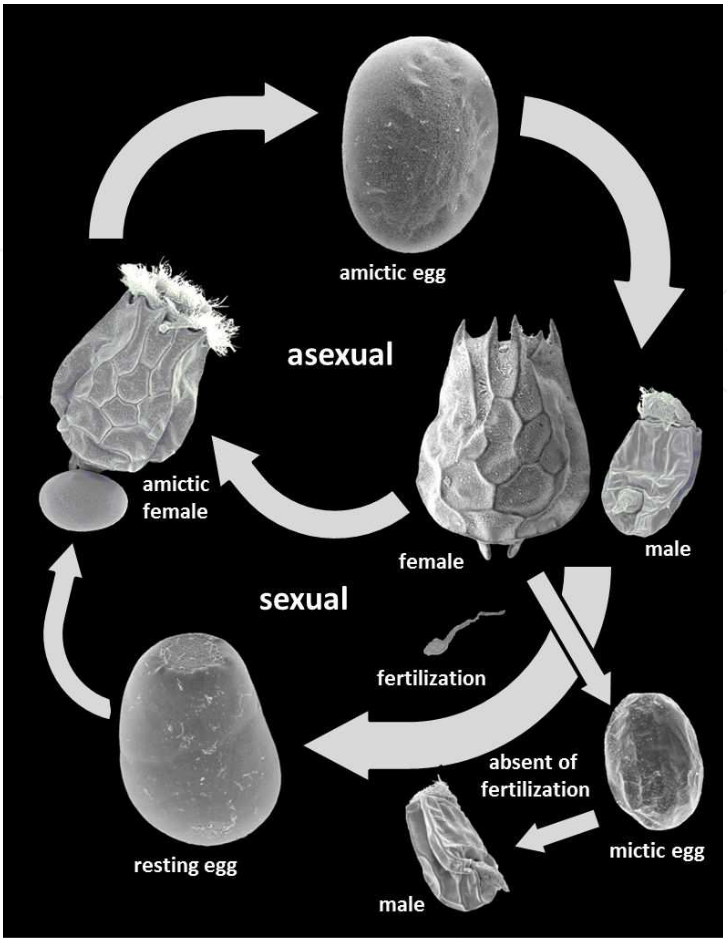

Figure 2. Monogononta life cycle: When the female produces an amictic egg, then an amictic (parthenogenetic) female is born and this asexual reproduction goes on until some environmental factors trigger sexual reproduction. However, when a mictic (sexual) female is born, it produces a mictic egg that can be fertilized to produce a resting egg (cyst), or if the egg is not fertilized, this unfertilized mictic egg produces a male. The presence of males in the environment allows cross-mating that results in production of sexual eggs or cysts (that are still known as resting eggs). Cysts represent a strategy to overcome harsh environmental conditions.

parthenogenesis, but during environmental conditions that still remain controversial, males appear in the environment and sexual reproduction takes place (Figure 2). However, the class Bdelloidea lacks sex completely [12].

The study of sexual reproductive behavior of rotifers has helped taxonomy to clarify the position of some species thought to be cryptic as is the case of *B. araceliae* which some authors suggested that it might belong to *B. bidentatus* [13]. However, it was recognized as a new species after cultivation in the laboratory for several generations showed that it was not a morphotype of *B. bidentatus*, but rather a new species [14]. Later, significant differences between the male of both species were noted [15].

Similar studies on cross-mating tests of species within the same family but belonging to different genera have been reported [11]. Unusual sexual reproductive behaviors and the peculiar life of male rotifers led to the study of the two types of sexual cells present in males (spermatozoa and rods) (Figure 3).

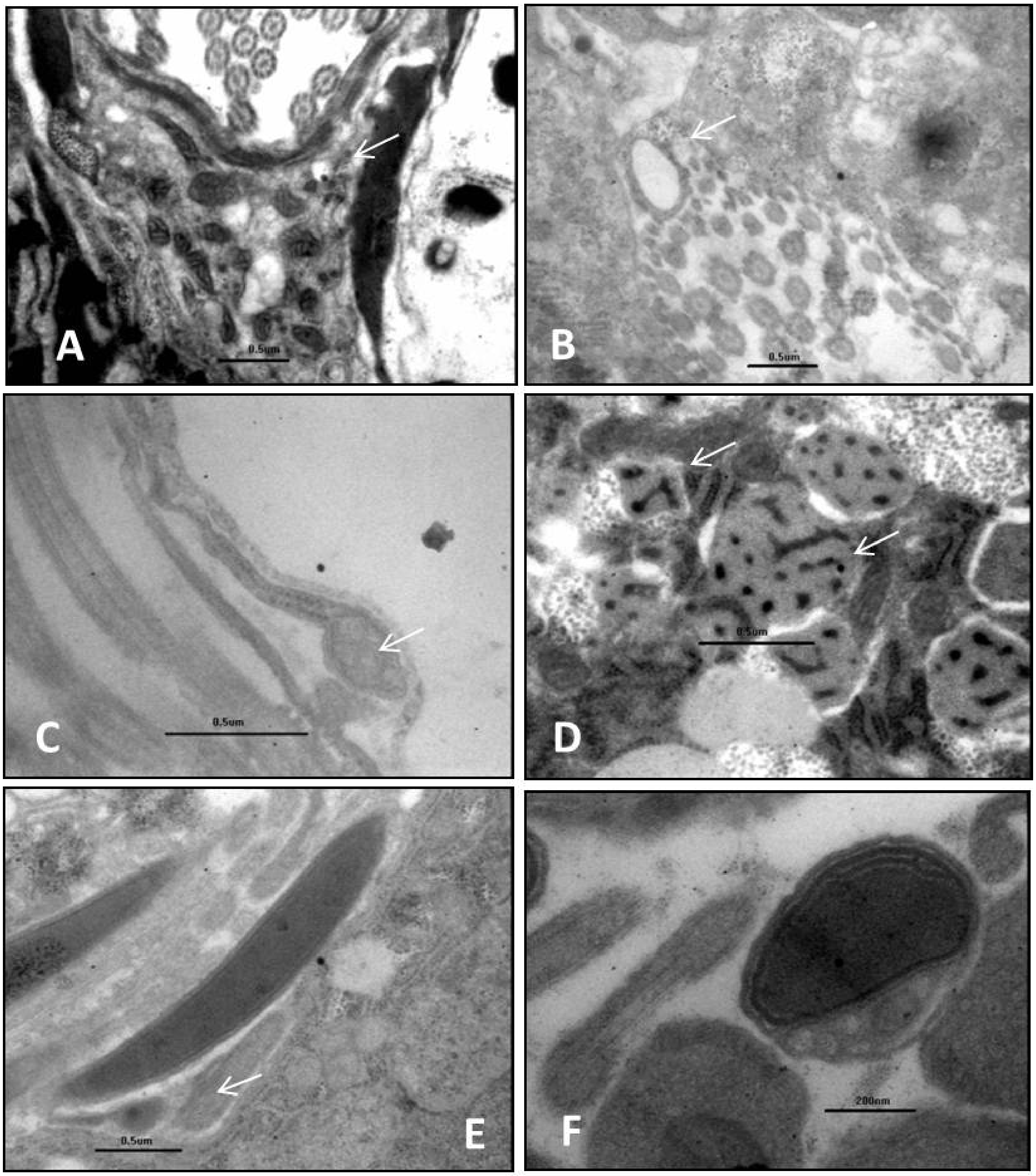

Figure 3. Cavity of the cilia in the male of *B. bidentatus* surrounded by cytoplasmatic material produced by rods (A) and (B), spermatozoon (C), different views of transversal cuts of spermatozoa (D), rod segregating cytoplasmatic material (E), and transversal cut of a rod (F).

It has been observed that these two cells are intimately related with the fertilization process. Some photographs have evidenced that the cytoplasmic substance that secretes the rods adheres to the necks of the spermatozoa. These results are still not clear, but apparently the chamber in which the spermatozoa are found is surrounded by rods waiting for the spermatozoa to appear. According to [16], rods assist in the sperm delivery during fecundation and are the first element that is introduced in the female. We are working to determine the role of rods in the reproductive mechanism of rotifers using ultrastructural studies. However, we do not have a very conclusive result yet, but it would be an important contribution to the knowledge of sexual reproductive embryology for invertebrates.

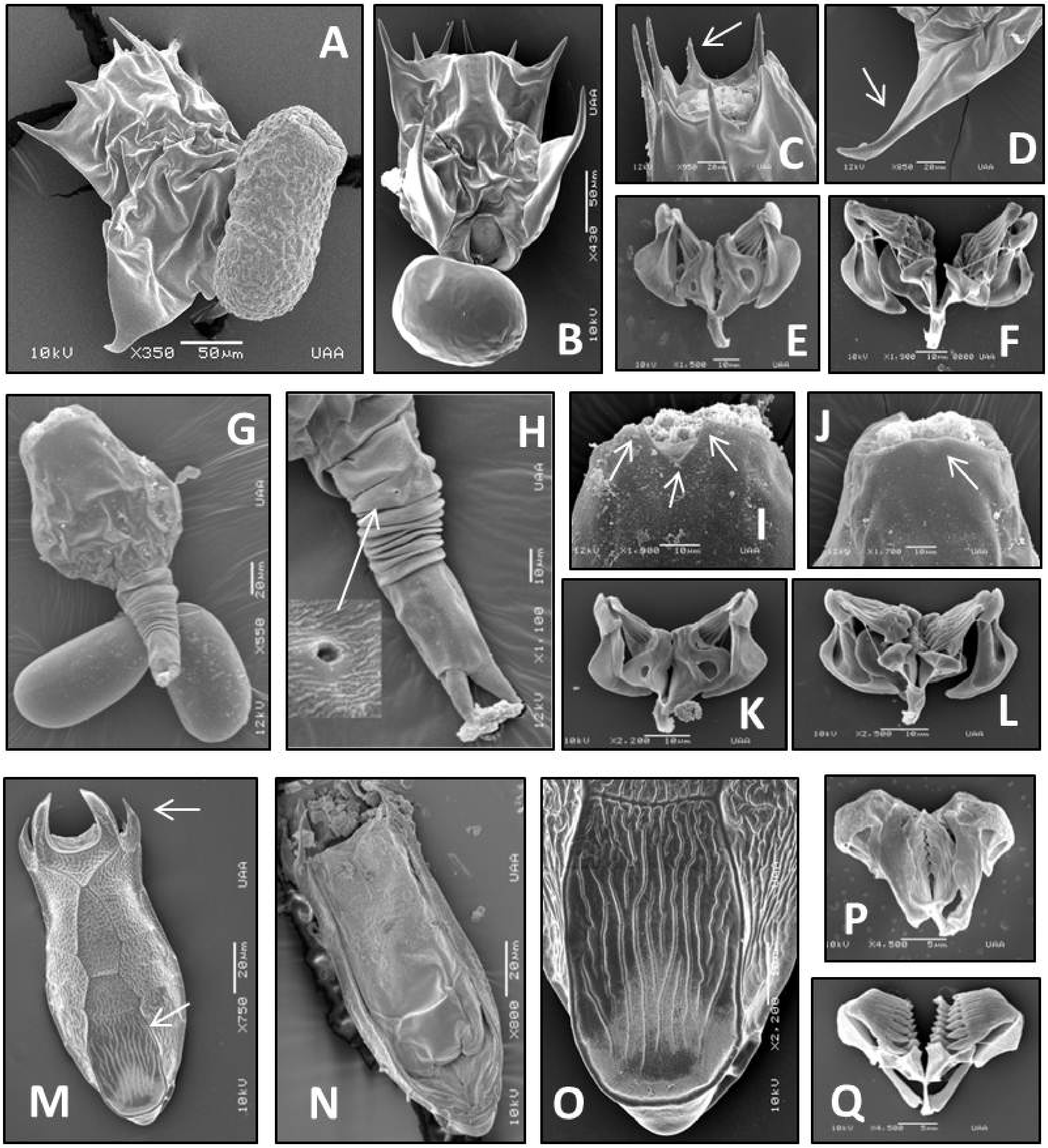

Figure 4. Three new species of rotifers recorded in Aguascalientes, Mexico. *Brachionus araceliae* dorsal view A, ventral view B, anterior spines C, posterior wing D, trophi dorsal view E, ventral view F [14]); *B. josefinae* ventral view G, foot H, dorsal spines I, ventral spines J, trophi dorsal view K, ventral view L [18, 19]; *Keratella mexicana* dorsal view M, ventral view N, plaque O, trophi dorsal view P, and ventral view Q [20, 21].

2.4. New species of rotifers from Aguascalientes, Mexico

The study of morphology and ultrastructure of rotifers along with studies of sexual reproductive behavior has led to the description of males new to science as in *Platyias quadricornis* [17] and more importantly, to the description of three species new to science. That is the case of *Brachionus araceliae* [14], *Brachionus josefinae* [18, 19], and *Keratella mexicana* [20, 21] (Figure 4).

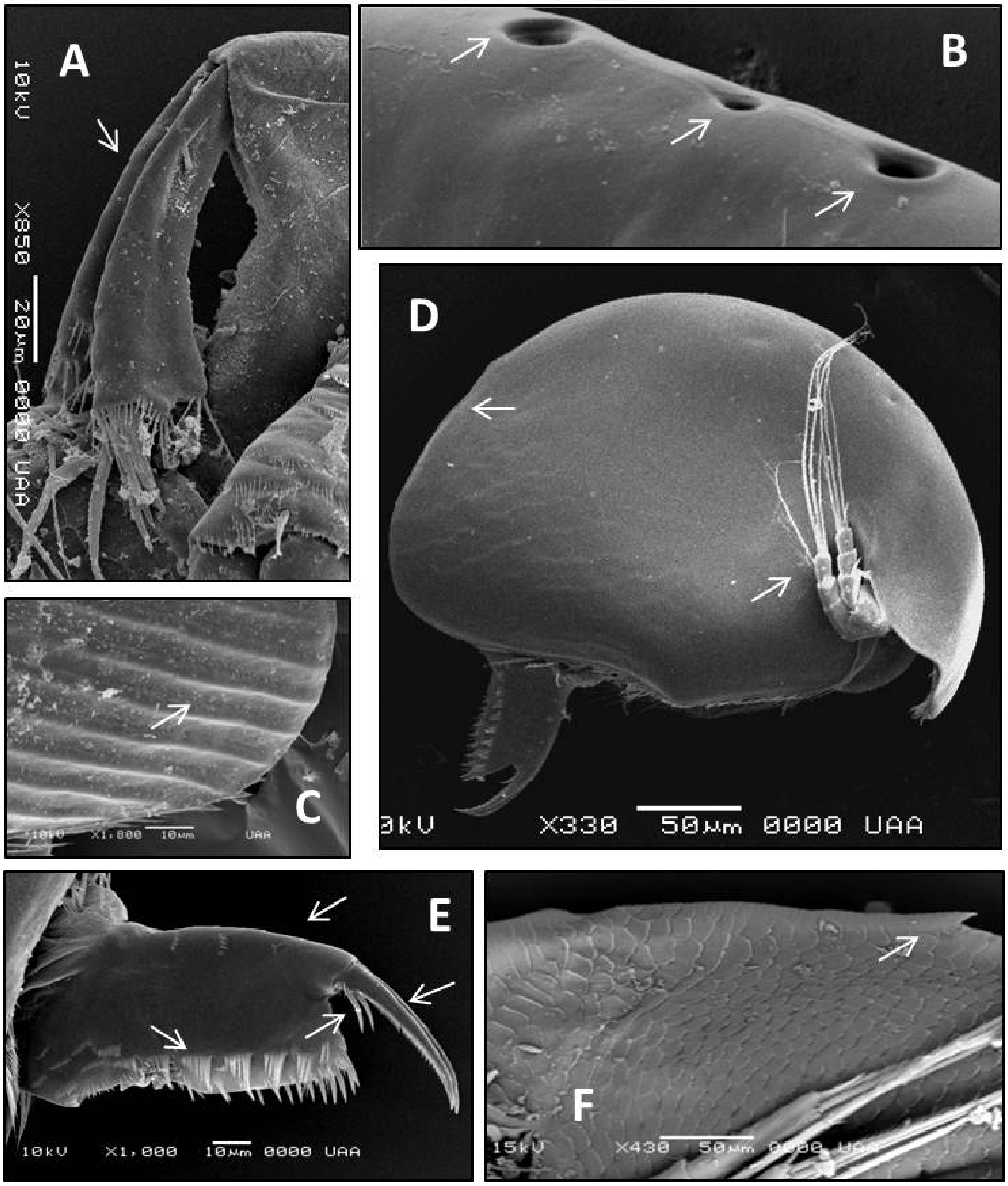

Figure 5. Several characteristics important for cladoceran identification. The first pair of antennas (A); cephalic pores (B); caudal ornamentation of carapace (C); second pair of antennas and shape of carapace (D); postabdomen, endclaw, basal spine, and lateral fasciculla of setae (E); and dorsal keel (F).

3. Cladocera

3.1. General information

Cladocerans are small-size crustaceans ranging from 0.2 to 18.0 mm in length. Most species are characterized for having a carapace that covers the entire body except for the head [22]. A clearer and more general description of cladocerans is achieved by taking the water flea (genus *Daphnia*) as a model: an organism of this genus has a body not clearly segmented; it has a head and trunk, but the latest one is covered by the carapace in the anterior part. The first part of antennas is small and the second part is big and visible; in the head, there is a compound eye. Cladocerans have 5 to 6 pairs of swimming appendages; the postabdomen is quite characteristic of these crustaceans; in the dorsal part, there is an incubation chamber where eggs are deposited.

There are 600 species of cladocerans worldwide [23]. In Mexico, some authors have estimated 150 species [24], and for the state of Aguascalientes, there are 45 species recorded which are distributed in six families [9]. Cladocerans can be found in lakes, ponds, small rivers, and streams, among others. However, some genera are found in musks, lichens, soil, and saline reservoirs.

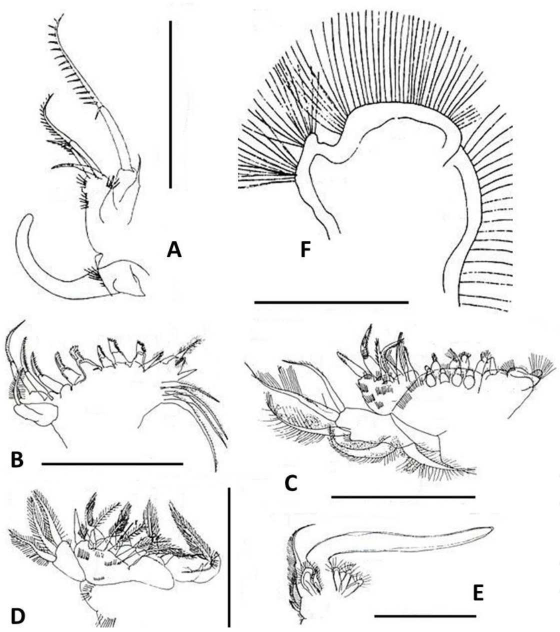

Figure 6. Typical swimming appendages of a cladoceran. First pair (A), second pair (B), third pair (C), fourth pair (D), fifth pair (E), and sixth pair (F). Scale bar equals 100 μm.

3.2. Representative characteristics for identification

The basic characteristics used for cladoceran identification are a) head with or without a compound eye, b) ocellus, c) cephalic pores, d) the first and second pair of antennas, e) shape and ornamentation of the carapace, f) postabdomen including the endclaw and a number of natatory setae, and g) the five or six pairs of swimming appendages. There are some extra characteristics that might be important tools to help with species identification, but sometimes to observe these peculiar features, it is necessary to dissect the organism. These peculiar features are many times crucial to achieve the species level. Such are the cases of a) the cephalic pores of *Alona anamariae* (Figure 5B), b) the absent or very rudimentary eyes of the genus *Spinoalona* [25], and c) the keel in the dorsal part of the carapace of *Macrothrix mexicanus* (Figure 5 F), just to mention a few cases. However, it is always necessary to support the identification with the analysis of all structures including the five or six swimming appendages (see Figure 6).

3.3. New species of cladocera from Aguascalientes, Mexico

Nearly 500 water reservoirs have been examined in the state of Aguascalientes, mainly to determine the biodiversity of zooplanktonic groups with special emphasis in rotifers, cladocerans, and copepods. The study of cladocerans in the state of Aguascalientes has led to the discovery of seven species new to science. The genus *Macrothrix* is especially well represented with four species new to science: *Macrothrix agsensis* [26], *M. mexicanus* [27], *M. sierrafriatensis* [28], and *M. smirnovi* [29] (Figure 7A, D, E, F, & G). The genus *Alona* contributed with two species new to science: *Alona aguascalentensis* (Figure 7C) and *A. anamariae* [30] (Figure 7B). The genus *Karualona* contributed with one species: *K. penuelasi* [13] (Figure 7C), which is truly endemic and only found in a small part of the state. Besides the description of new species, these studies have provided information about the distribution, endemism, and morphological variation of the different morphotypes that a particular species might show. These ultrastructure studies with SEM and TEM have strengthened the identification of certain taxa and provided criteria that can be used in the future to resolve taxonomic disputes within the Cladocera group.

4. Copepoda

4.1. General information

Copepods have several characteristics by which they can be recognized from other invertebrates. They have a cylindrical shape and a segmented body. In the anterior part, there is a pair of antennules and in the ventral part of the body, there are five pairs of swimming appendages; in the posterior part of the body, there is an abdomen or urosome containing caudal branches. These organisms are very diverse, and there are more than 11,500 species worldwide [31]. In Mexico, 100 species have been described in epicontinental waters from the three main orders: Cyclopoidea, Calanoidea, and Harpaticoidea [24]. In the state of Aguascalientes, 47 species have been recorded; however, only the orders Calanoidea and Cyclopoida have been studied (Dodson & Silva Briano 1996; [33]; CONABIO-IMAE-UAA, 2008; [35]. Copepods are located in oceans, lakes, and ponds, but they have also been found in musks, lichens, dry leaves, and bromeliads [39]; a few species have been recognized as parasites [22]. In Mexico, the species of Calanoidea and in a lesser proportion Cyclopoida have shown high endemic rates [36]. Therefore, ultrastructural studies can be important to describe new species, separate cryptic species within a species complex, and elucidate restricted distribution patterns.

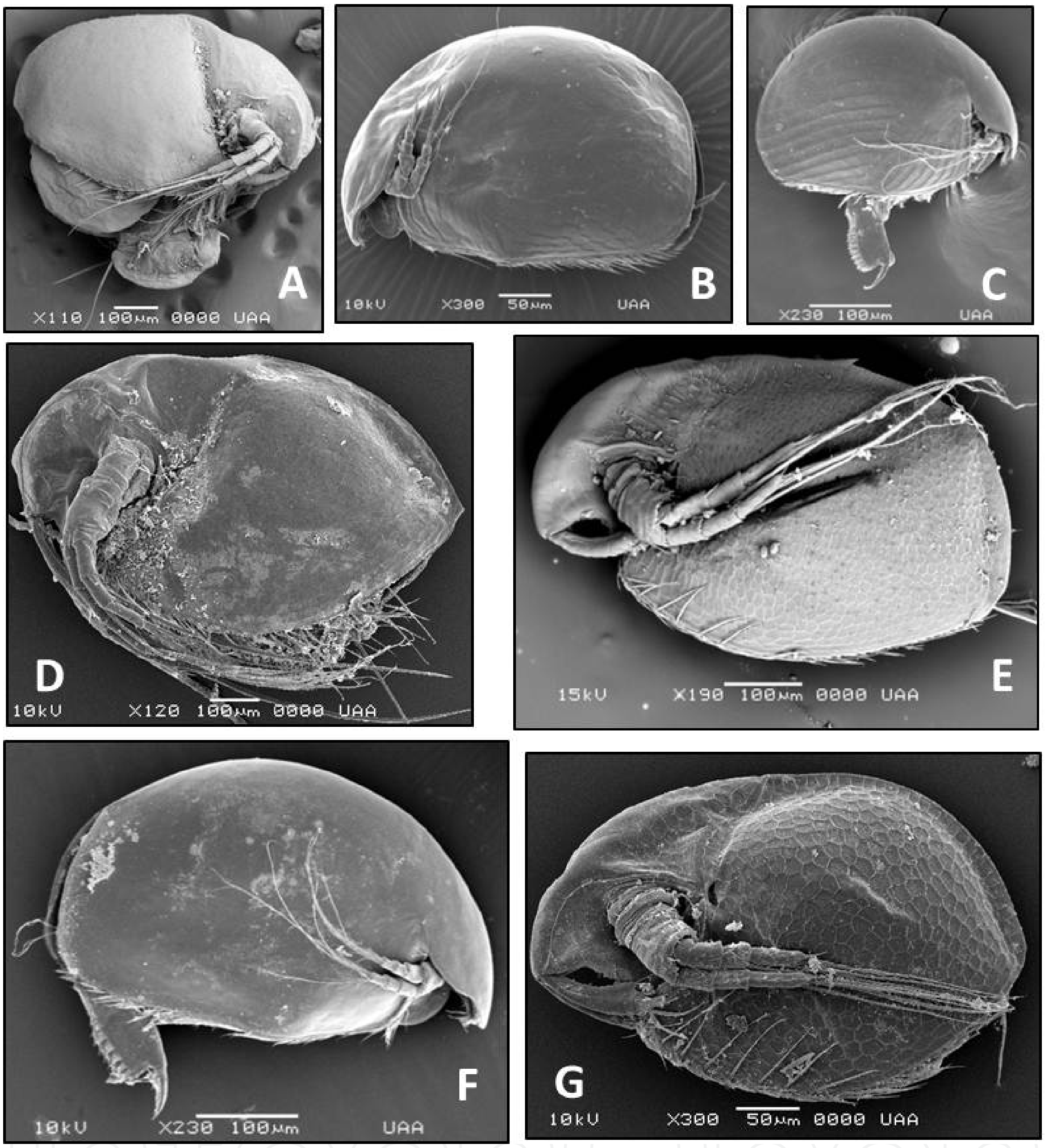

Figure 7. Seven species new to science of cladocerans found in Aguascalientes, Mexico. *Macrothrix agsensis* (A) [26], *Alona anamariae* (B) [30], *Karualona penuelasi* (C) [13], *M. smirnovi* (D) [29], *Macrothrix mexicanus* (E) [27], *A. aguascalentensis* (F) [30], and *M. sierrafriatensis* (G) [28].

4.2. Representative characteristics for identification

Identifying a copepod requires closed examination of the fifth swimming appendages of the female for cyclopoids and of the male for calanoids. For harpacticoids, it is required to closely examine the maxillipeda for the initial identification. However, the fifth pair of swimming appendages and the maxillipeda are just two of the many features that have to be analyzed. For the populations of calanoids and cyclopoids, we require to analyze a) the first two pairs of antennas, b) the buccal apparatus (mandible, maxilula, maxillae, and the maxillipeda), and

http://dx.doi.org/10.5772/60659

c) the urosome (Figure 8). For calanoids, it is necessary to observe the growth in some appendages in the last segments of the thorax. Other morphological variations that might be important for taxonomic identification include a) pores in the swimming appendages, b) different patterns of setae, and c) modifications in the second pair of antennas in some parasite copepods.

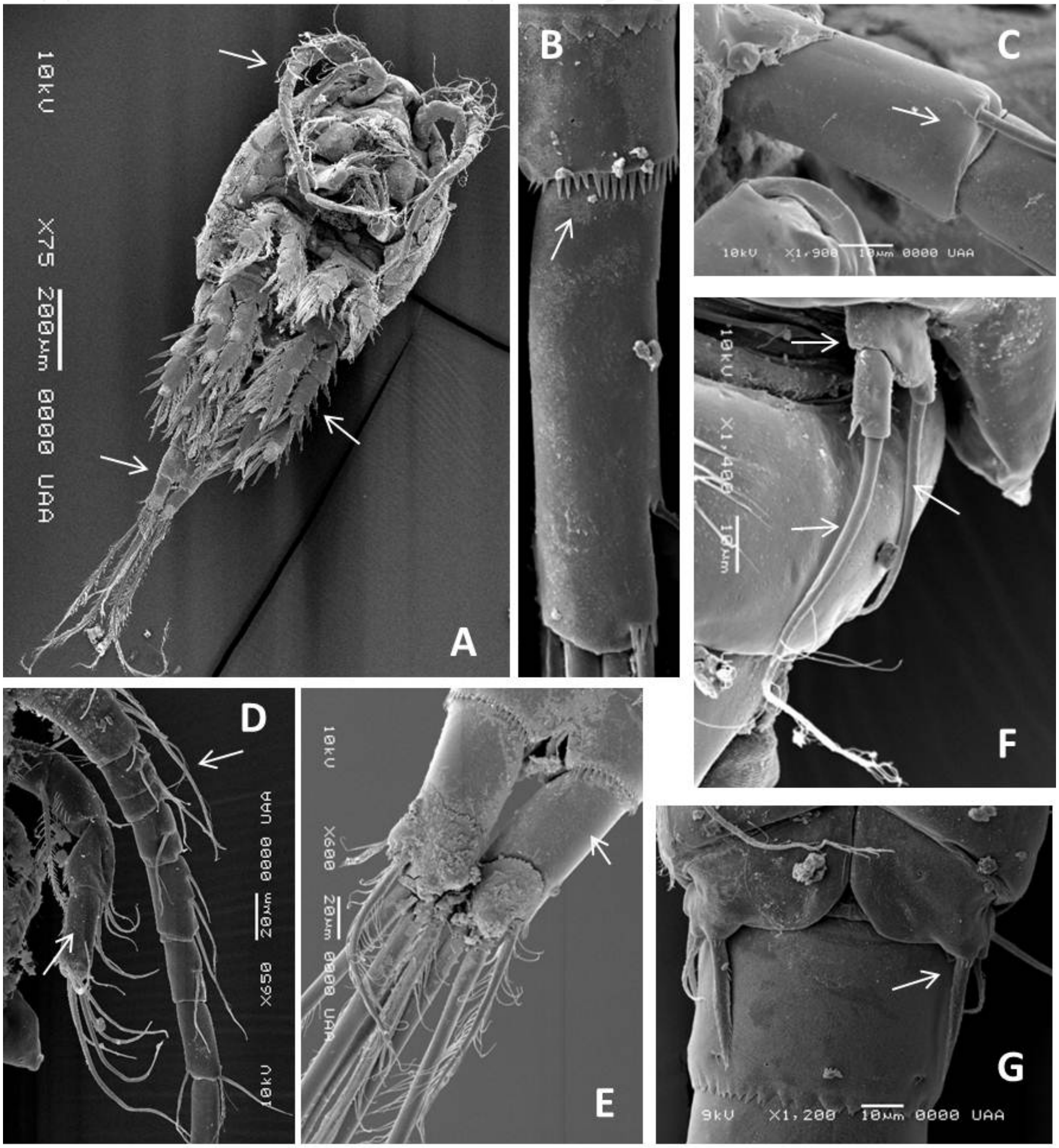

Figure 8. Habitus with arrows pointing to the second pair of antennas, a pair of swimming appendages, and urosome (A), caudal part of the urosome (B), segment of the second pair of antennas (C), the first and second pair of antennas (D), caudal part of the urosome (E), fifth pair of swimming appendages (F), and urosome showing the sixth pair of swimming appendages (G).

4.3. New species of copepods from Aguascalientes, Mexico

The study of copepods in the state of Aguascalientes has been less intense than that of cladocerans and rotifers. This is because the state lacks an established researcher with a permanent position in the main academic and research facilities, a condition that exists for the other two taxonomic groups. In spite of that, four species new to science have been found in Aguascalientes. That is the case of *Acanthocyclops dodsoni* [33] (Figure 9), *A. marceloi* [38] (Figure 9), *A. caesariatus* [37] (Figure 9), and *Paracyclops hirsutus* [38].

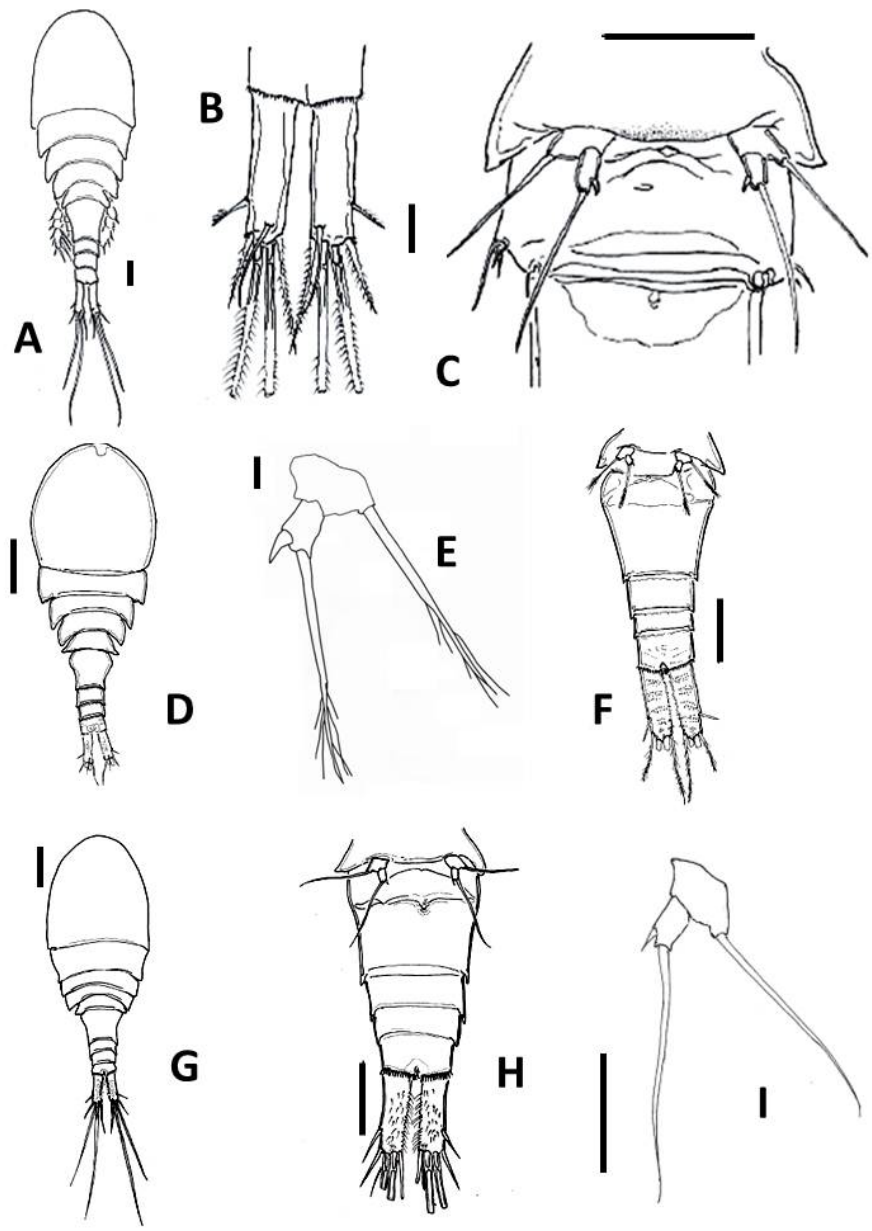

Figure 9. Three new species of copepod recorded in Aguascalientes, Mexico. *Acanthocyclops dodsoni*, dorsal view (A); caudal rami, dorsal view (B); fifth pedigerous and genital doble-somites, ventral view (C) (Mercado- Salas et al. 2006); *A. marceloi*, dorsal view (D); fifth pedigerous, ventral view (E), and urosome, ventral view (F) [37]; *A. cesariatus*, dorsal view (G); fifth pedigerous, ventral view (H); and urosome, ventral view (I) [38]. Scale bar: A, C=250 μm; G=200 μm; B, D, F, H=100 μm; I=50 μm; and E=10 μm.

http://dx.doi.org/10.5772/60659

5. Importance of the taxonomic study of rotifers, cladocerans, and copepods

The primary goal of the taxonomical study of rotifers, cladocerans, and copepods is to know the morphology and to carry on an inventory of all species located in a particular geographic area. The second goal is achieved once species are compared regarding morphological similarities and differences which allows for a better system to identify and classify them correctly. However, all these comparisons at the end allow clarification of questions related to evolution and adaptation when we combine ultrastructural morphological, physiological, and genetic studies.

The observation of small modifications among species of these three taxonomic groups only evidences the specificity that exists between the external environment and the internal function of the organism. The perfect design of each species defines the specific niche of each species in an ecosystem. Each slight modification like apparition of small setae in the fifth leg of a copepod, the changes in the structure of a trophi in rotifers, or the presence of a slightly more elongated endclaw in cladocerans is of the greatest importance since such tiny change can be the difference between a species able to perpetuate itself and the others that go extinct. These observations only corroborate the philosophy started by Darwin in his famous book *On the Origin of the Species*. With this philosophy in mind, then we can estimate the socioeconomic importance that each species implies. The precise knowledge of the species distribution, the kind of ecosystems and niches where we found each species, and the way in which each species interact are a necessity to the economical and sustainable use of each species. For example, if one wishes to use copepods as food for fishes, it would be convenient to know which species grow easily in the region. This knowledge would help to curve maintenance costs and to attain a maximal production of the cultured organisms, in the meantime avoiding producing an ecological misbalance. Another clear example is the use of the cladoceran *Daphnia magna* as model organism for acute toxicological test according to the Mexican Norm NMX-AA087-SCFI-2010, in which sometimes we exposed neonates of this organism in situ to determine the survival rate. However, this species is native of Europe and it is adapted to reservoirs with conditions quite different to the Europeans (like Mexico). The use of nonnative species to evaluate the health of Mexican ecosystems only introduces a bias into the experimental results. If instead we are able to identify and culture native species for each region and use it as a model organisms, we would obtain high-quality results and be more prepared to preserve the environmental health of our reservoirs.

Some other questions of even greater transcendence, as well as improvement in experimental and industrial designs, are the results of basic research as it is the case of the taxonomy aided by ultrastructural studies.

Acknowledgements

We are thankful to M. Sc. Reyna Lara Martinez who helped us in the management of the TEM JEOL 1010 at the Faculty of Sciences of the Universidad Nacional Autónoma de México.

Author details

Marcelo Silva-Briano[1*], Araceli Adabache-Ortiz[1], Gerardo Guerrero-Jiménez[1], Roberto Rico-Martínez[2] and Guadalupe Zavala-Padilla[3]

*Address all correspondence to: msilva@correo.uaa.mx

1 Universidad Autónoma de Aguascalientes Laboratorio de Ecología, Depto. Biología, Avenida Universidad, Aguascalientes,

2 Universidad Autónoma de Aguascalientes, Laboratorio de Toxicología Acuática, Depto. Química, Avenida Universidad, Aguascalientes, México

3 Unidad de Microscopía Electrónica. Instituto de Biotecnología, Universidad Nacional Autónoma de México, Av. Universidad, Cuernavaca, México

References

[1] Vázquez-Nin G. & Echeverría O. (2000) Introducción a la Microscopía Electrónica Aplicada a las Ciencias Biológicas. Fondo de Cultura Económica. México, 168 pp.

[2] Jiménez-García L. F., Elizundia J. M., López-Zamorano B., Maciel A., Zavala G., Echeverría O. M. & Vázquez-Nin G. H. (1989)Implications for evolution of nuclear structures of animals, plants, fungi and protoctists. *Biosystems*, 22: 103-16.

[3] Segers H., Murugan G. & Dumont H. (1993) On the taxonomy of the Brachionidae: description of *Plationus* n. gen. (Rotifera, Monogononta). *Hydrobiology*, 268, 1- 8.

[4] Wallace R., Snell T., Ricci C. & Nogrady T. (2006) Rotifera Part 1: Biology, Ecology and Systematics. 2da Ed. Kenobi Production. 229 pp.

[5] Klusemann J., Kleinow W. & Peters W. (1990). The hard parts (trophi) of the rotifer mastax to contain chitin: evidence from studies on *Brachionus plicatilis*. *Histochem*, 94, 277-283.

[6] Segers H. (2007) Global diversity of rotifers (Rotifera) in freshwater. *Hydrobiology*, 595, 49-59.

[7] Sarma S. S. S. (1999) Checklist of Rotifera (rotifers) from Mexico. *Environment and Ecology*, 17, 978-983.

[8] García-Morales A. & Elías-Gutiérrez M. (2004) Rotifera from southeastern Mexico, new records and comments on zoogeography. *Anales del Instituto de Biología*, 75, 99-120.

[9] La Biodiversidad en Aguascalientes: Estudio de Estado. (2008) Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), Instituto del Medio

Ambiente del Estado de Aguascalientes (IMAE), Universidad Autónoma de Aguascalientes (UAA). México. 26-27 pp.

[10] Rico-Martínez R., Snell T.W. & Shearer T. (2013) Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A® to the *Brachionus plicatilis* species complex (Rotifera). *Environmental Pollution*, 173, 5-10.

[11] Rico-Martínez R. & Snell T. W. (1998). Mating behavior in eight rotifer species: Using cross-mating tests to study species boundaries. *Hydrobiologia*, 356, 165-173.

[12] Melone G. & Ferraguti M. (1993). The Spermatozoon of *Brachionus plicatilis* (Rotifera, Monogononta), with some notes on sperm ultraestructure in Rotifera. *Acta Zoologica, 75*(2), 81-88.

[13] Silva-Briano M. & Adabache-Ortiz A. (2000) *Brachionus* species in Aguascalientes State, México. *Aquatic Ecosystems of México*, 203- 211.

[14] Silva-Briano M., Galván-de la Rosa R., Pérez-Legaspi A. & Rico-Martínez R. (2007) On the description of *Brachionus araceliae* sp.nov. A new species of freshwater rotifer from Mexico. *Hidrobiológica, 17* (2), 179-183.

[15] https://youtu.be/iV5VKdcQOJE

[16] Koehler J. K. (1965) An electron microscope study of the dimorphic spermatozoa of *Asplanchna* (Rotifera). I. *The adult tesis. Z. Zellforsh,* 67, 57-76.

[17] Velázquez-Rojas C. A., Santos-Medrano, G. E. & Rico-Martínez R. (2002) Sexual reproductive biology of *Platyias quadricornis* (Rotifera: Monogononta). *International Review of Hydrobiology* 87(1), 97-105.

[18] Silva–Briano M. & Segers H. (1992) *Brachionus josefinae* n. esp. *Hydrobiology,* 25, 283-285.

[19] https://youtu.be/iV5VKdcQOJE

[20] Kutikova L. A. & Silva-Briano M. (1995) *Keratella mexicana* sp. nov., a new planktonic rotifer from Aguascalientes, Mexico. *Hydrobiology*, 310, 119-122.

[21] https://www.youtube.com/watch?v=iV5VKdcQOJE&feature=youtu.be

[22] Thorp J. H. & Covich A. (2010) Ecology and Classification of North American Freshwater Invertebrates. E.U.A. 3rd Ed. Academic Press. 967 pp.

[23] Forro L., Korovchinsky N. M., Kotov A. A. & Petrusek A. (2008) Global diversity of cladocerans (Cladocera; Crustacea) in freshwater. *Hydrobiology,* 595, 177-184.

[24] Elías-Gutiérrez M., Suárez-Morales E., Gutiérrez-Aguirre M. A., Silva-Briano M., Granados-Ramírez J. G. & Garfias-Espejo T. (2008) Cladocera y Copepoda de las Aguas Continentales de Mexico. Guía ilustrada. México, 322 pp.

[25] Kotov A. A. & Elías-Gutiérrez M. (2002) Analysis of the morphology of *Spinalona anophtalma* Ciros-Pérez & Elías-Gutiérrez, 1997 (Aloninae, Anomopoda, Cladocera). *Hydrobiology*, 468, 185-192.

[26] Dumont H. J., Silva-Briano M. & Subhash-Babu J. J. (2002). A re-evaluation of the *Macrothrix rosea-triserialis* group, with the description of two new species (Crustacea Anomopoda: Macrothricidae). *Hydrobiology*, 467, 1-44.

[27] Ciros-Pérez J., Silva-Briano M. & Elías Gutiérrez M. (1996) A new species of *Macrothrix* (Anomopoda: Macrothricidae) from Central Mexico. *Hydrobiology*, 319, 159-166.

[28] Silva-Briano M., Quang Dieu N. & Dumont H. J. (1999) Redescription of *Macrothrix laticornis* (Jurine, 1820), and description of two new species of the *M. laticornis*-group. *Hydrobiology*, 403, 39-61.

[29] Ciros-Pérez J. & Elías-Gutierrez M. (1997) *Macrothrix smirnovi,* a new species (Crustacea: Anomopoda: Macrothricidae) from Mexico, a member of the *M. triseriales*-group. *Proccedings of the Biological Society of Washington*, 110 (1), 115-127.

[30] https://www.youtube.com/watch?v=iV5VKdcQOJE&feature=youtu.be

[31] Humes A. G. (1994) How many copepods? *Hydrobiology*, 292/293, 1-7.

[32] Dumont H. J. & Silva-Briano M. (2000) *Karualona* n.gen. (Anomopoda: Chydoridae), with a description of two new species, and a key to all known species. *Hydrobiology,* 435, 61-82.

[33] https://www.youtube.com/watch?v=iV5VKdcQOJE&feature=youtu.be

[34] Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO), Instituto del Medio Ambiente del Estado de Aguascalientes (IMAE), Universidad Autónoma de Aguascalientes (UAA). La Biodiversidad en Aguascalientes: Estudio de Estado. 2008. México.

[35] Mercado-Salas N. F., Suárez-Morales E. (2012) Morfología, diversidad y distribución de los Cyclopoida (Copepoda) de zonas áridas del centro-norte de México. II. Eucyclopinae y análisis biogeográfico. *Hidrobiológica* 22 (2), 99-124.

[36] Elías-Gutiérrez M., Suárez-Morales E. &Romano-Márquez B. (1999) A new species of *Leptodiaptomus* (Copepoda, Diaptomidae) from Northwestern Mexico with comments on the distribution of the genus. *Journal of Plankton Research*, 21, 603-614.

[37] Mercado-Salas N. F., Suárez-Morales E. & Silva-Briano M. (2009) Two new species of *Acanthocyclops* Kiefer, 1927 (Copepoda: Cyclopoida: Cyclopinae) with Pilose Caudal Rami from semiarid areas of Mexico. *Zological Studies*, 48, 380-393.

[38] Mercado-Salas N. F. & Suárez-Morales E. (2009) A new species and illustrated records of *Paracyclops* Claus, 1893 (Copepoda: Cyclopoida: Cyclopinae) from Mexico. *Journal of Natural History*. 43 (45–46), 2789-2808.

http://dx.doi.org/10.5772/60659

[39] Suárez-Morales E. (2000) Copépodos, seres ubicuos y poco conocidos. CONABIO. *Biodiversitas,* 29, 7-11.

[40] NMX-AA-087-SCFI. (1995) Norma Mexicana que establece el método de prueba de toxicidad aguda con *Daphnia magna* Straus (Crustacea-Cladocera).

IntechOpen