PART 1

There are included here many pictures published by the kindness of the authors, to which I offer my most sincere thanks. Especially to M. Verolet, whose generosity has provided many of the most interesting pictures shown here. I have also used drawings sourced from some Internet websites for which I give the required notice. The remaining illustrations were taken (in these times of digital cameras with 5 to 10 Mpx) with a camera of 0.4 Mpx; certainly they have considerable work-up using PhotoPaint, NetImage Demo, and ACDSee.

I hope that, at least, they will be clear and convey the information that they are intended to give.

INTRODUCTION

In traditional taxonomy rotifers are considered a phylum which embraces three classes: the "Seisonacea", the "Monogononta", and the "Bdelloidea" (see Taxonomy footnote). Seisonacea are solely marine epizoics on *Nebalia*, (a genus of crustaceans considered very primitive, benthic, with some litoral species), and are represented by only one genus, *Seison*, with three species, the last one described in 2007, which have a morphology very distant from that normally assigned to a rotifer. *Monogononta*, thus called because male and female have only one genital gland, were extensively discussed by Michele Verolet, who has presented in the French Magazine *Microscopies* a very complete and heavily illustrated description of their morphology, and a splendid key to identify all the genera that the amateur has the possibility of finding with some frequency in their explorations.

Bdelloidea are all parthenogenetic females, with two genital glands. (For a long time they were called *Digononta*).

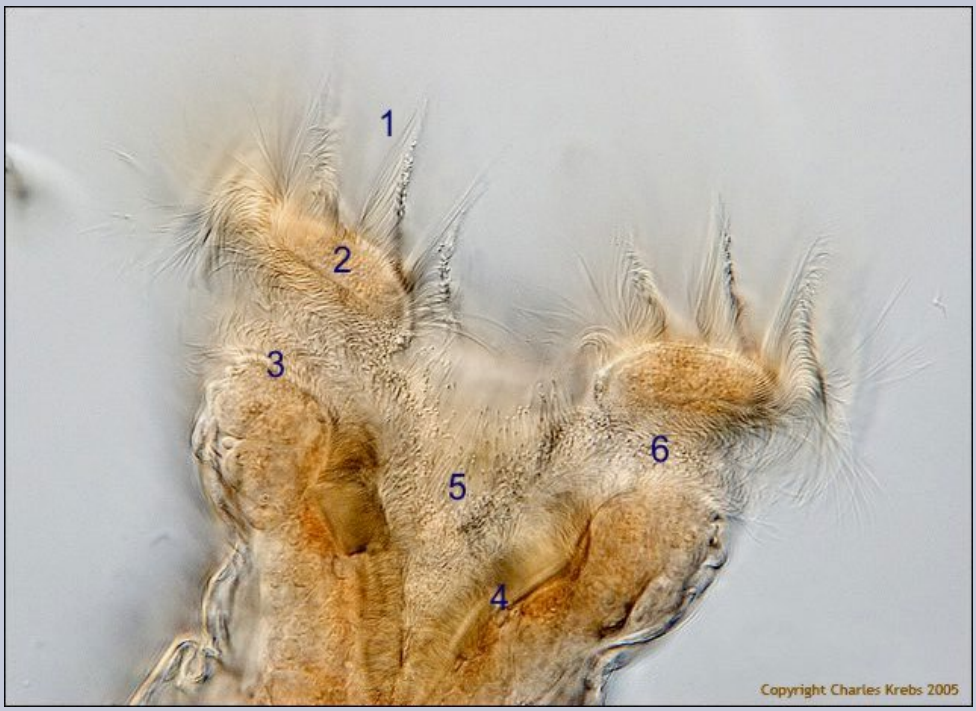
Monogononta and Seisonacea have males but Bdelloidea haven't had them ..... probably for 80 million years

(http://news.bbc.co.uk/1/hi/sci/tech/7039478.htm)

Their reproduction is exclusively parthenogenetic, and the geneticists struggle to discover the mechanisms which made it possible for these animals to maintain their genetic diversity and to avoid being banned from the list of living species. Some recent papers show that they steal genes from other species, to modify their own genome.

Of all the rotifers the Bdelloidea are the best known by the non-specialized worker. The anterior end has a "corona" divided into two retractile "trochal discs", with ciliated margins. The cilia beat rythmically, and seem to rotate, which gave the name to these animals. In fact the trochal discs are restricted to 15 of the 19 genus of this Class, but the ciliated corona are so showy that the feature gives its name to the Phylum Rotifera.

2 - Magnificent frontal view of the "head" of a Philodina (courtesy of Charles Krebs).



2a - Annotations by the author: 1 - lines of cilia implanted in the trochal disc border (2), 3 - inferior lips or cingulum, 4 - lip of the buccal funnel, 5 - buccal funnel, 6 - sulcus (a furrow between trocha and cingulum). These ciliated features help to send the food to the mouth.

They are multicellular organisms with a fairly complex organization, but are similar in size to that of the large protozoans, (between 150 and 700 microns). Only one species, *Rotaria neptunia,* is excessively long and thin, reaching a size of 1600 microns. (Ricci and Melone, 2000.)

Practically all the species can endure drying and can revive when they happen to be resubmerged in water.

Jacobs published in 1909, for the first time, the description of the amazing capacity of these rotifers, which gives them the possibility of invading really difficult habitats, like desiccable mosses, the cracks in the bark of trees, the soil, and

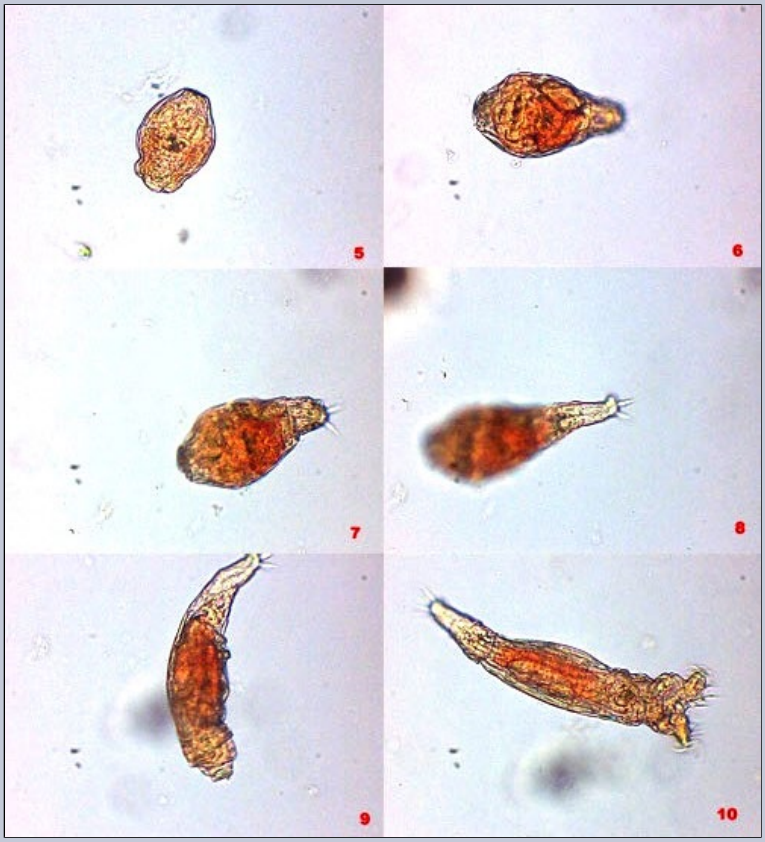
so on.

The rotiferologist Aydin Örstan was able to find bdelloids in the wall of the tubes of termites, adhered to a tree in Puerto Rico, and even described a new species from the dusty soil of the Sonoran desert in Mexico (*Macrotrachela sonorensis*).

Christian Colin recorded the images of the drying process and the revival of a bdelloid. The article that describes his technique, process, and results can be read at:

Originally this box contained an articlle on the microscpies website in France

sadly, now gone.



3 - Revival, after 46 days of anhydrobiosis. Recorded by Christian Colin.

In the state of anhydrobiosis (or anabiosis) they resemble small dust particles and, like them, they can be dispersed easily by the wind, or the waters that bathe the surfaces where they live. Notwithstanding, there are in fact few recorded studies on the real (and surely multiple) dispersal methods that the rotifers use, and which have given to many species the ability for them to become cosmopolitan.

The anydrobiosis is not a simple adaptation to colonize difficult habitats. As Ricci *et al* states (August 2007):

> "Bdelloids, although aquatic animals, are not only efficient in tolerating desiccation, but seem somehow dependent on anhydrobiosis, a circumstance that might represent a key event in their life cycle. If this is true, life in unpredictable habitats should not be seen as the result of competitive exclusion from 'easier' habitats, but a requirement for long-term survival of these parthenogenetic animals."

The name "bdelloids" comes from the Greek and indicates their characteristic way of crawling in a leech-like manner, fixing alternately the terminal toes, extending, fixing the rostrum, and retracting the previously fixed end.

Although all the bdelloids do not always show the same behavior. For example a rotifer of the genus *Adineta* moves normally by gliding over surfaces, using the cilia that cover the ventral side of their cephalic end.

Even the cephalic end, which gives the name to the class <QUERY clarify>, does not have a homogeneous structure, and there exist at least 3 different structures, which allow division of the class into 3 Orders: Adinetida, Philodinida and Phylodinavida, and will be treated in detail when characterizing them.

As in the Monogononta the body is composed of 3 basic portions: the head, the trunk and the foot. The epidermis that covers it is a sincitial layer (that is to say, a layer of cytoplasm in which separated cells do not exist, although these are represented by large dispersed nuclei) and is marked by cross-sectional furrows that separate superficial rings (pseudosegments, since they do not divide the interior of the body), and that gives to the rotifer the capacity to be contracted in a "telescopic" way. This is possible thanks to the existence of a pliable layer of intra-cytoplasmic cuticle, called the "lorica".

In many Monogononta this lorica is hard and indeformable, with important specific characteristics. (See Verolet's article.) But in the Bdelloidea it is almost always thin and folding, although in some species they can have characteristic thickening called "cuticular sculptures" (plates, furrows, spines, warts etc).

The "corona", when it exists, can be enclosed inside the head, and this, and the foot at the other end, are contracted within the trunk, acquiring a compact and rounded form. This form (named "tun" in English) is the one that they adopt as a strategy of defense in any case of a sudden threat, or even as a previous step to drying. (See the above experiment by Christian Colin.)

4 - The immediate total retraction of a Philodina as a reaction to an abrupt contact, like a stroke over the coverslip.

It is common that the head joint to the trunk has a narrowed portion called the "neck". Normally a sensitive antenna, thin, and more or less long, lodges dorsally in the "neck".

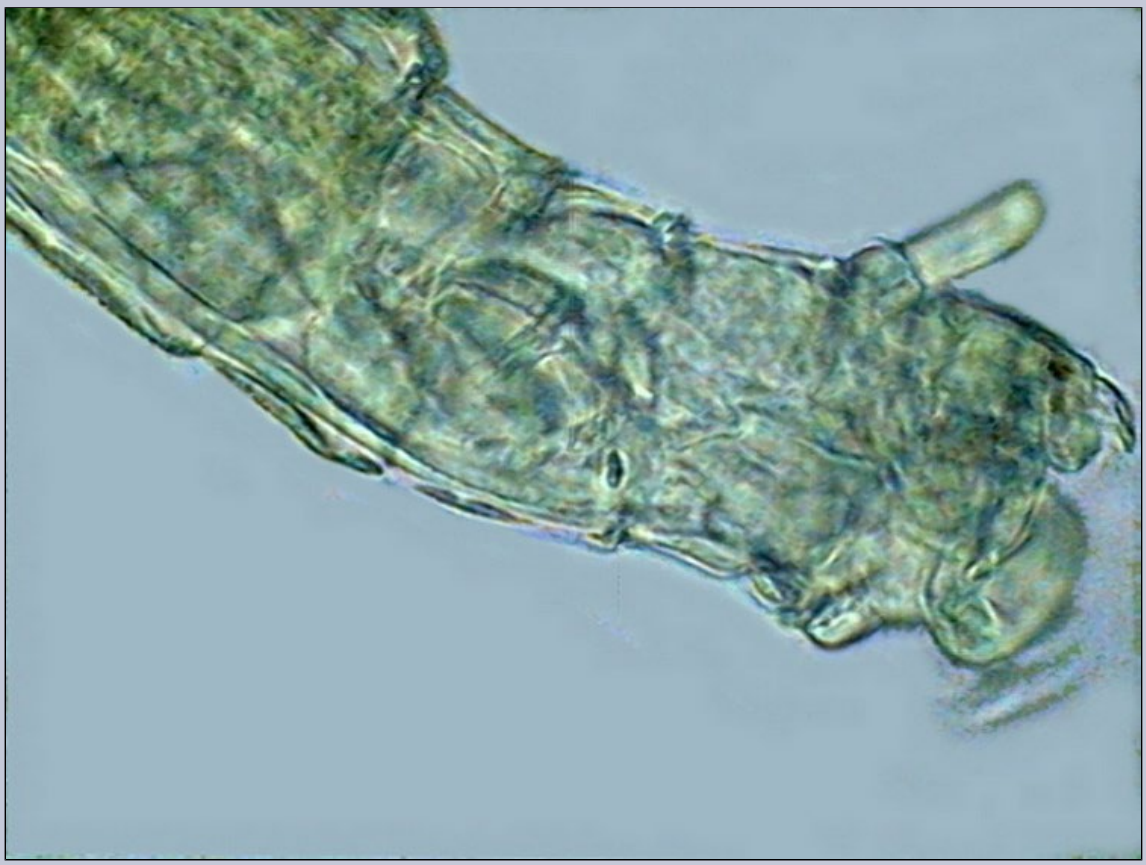
5 - Lateral view of a Philodina sp., showing the dorsal antenna. Below the antenna can be seen the red eyes. The pseudo-segmentation and the ridged trunk cuticle is also shown.

In addition, between the trochal discs it can be seen dorsally the "rostrum", one generally robust structure provided at its end with cilia, sticky cells and sensitive *lamellae*. It is retractable in most of the species.

Some species have eyes, which lodge sometimes in the anterior end of the rostrum (*Rotaria*), and other times dorsally in the neck, over the brain (*Philodina*). In *Adineta oculata* they are described as rostral eyes composed of a red spot surmounted by a concave lens.

6 - Dorsal eyes over the brain in Mniobia. They are pigmented red.

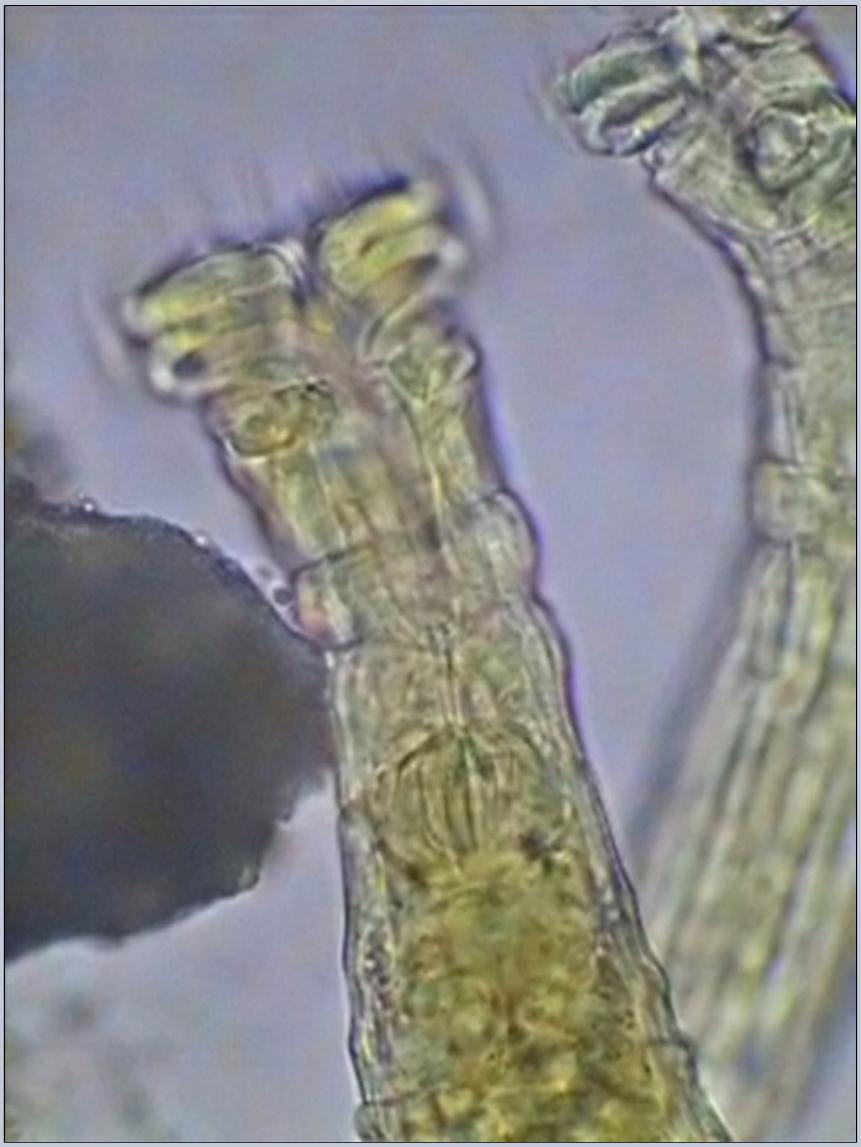
7 - Rotaria sp., dorsal view of the head, with dark eyes in the rostrum.



8 - Relation between the antenna and the rostrum. Trochal discs semi-retracted. One specimen with the "corona" totally retracted is seen in picture 27 (later article part).

The mouth opens centrally between the coronae (see fig 2) at the end of a buccal funnel, which could be followed by a buccal tube that communicates with a masticatory organ called the *mastax.*

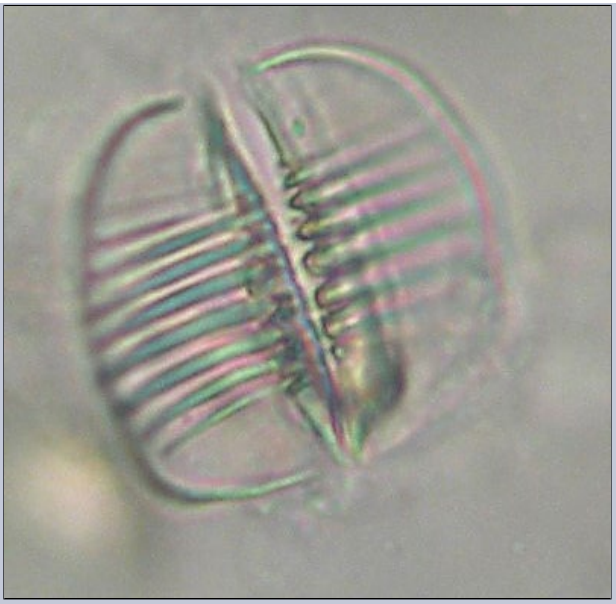
The length of the buccal funnel and the buccal tube, and the depth of the mastax is a character that depends on the style of alimentation.



9 - The deep mastax of a Habrotrochidae.

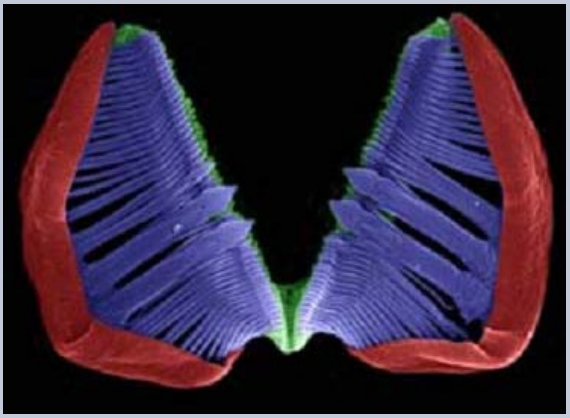
You can see a mastax very near the mouth at fig. 30 (*Henoceros*), later article part.

The mastax is a complex organ formed by strong muscles armed with several hard, cuticularized pieces, named "trophi". Its structure is characteristic of all the class and it really has little variety. The type of trophi of the Bdelloidea is called "ramate". The ramate trophi lacks the fulcrum, one unpaired piece characteristic of the remaining types of trophi of the phylum. (See Verolet's article.). The trophi consist of 6 pieces, 2 unci (sing. uncus), 2 manubria (sing. manubrium) and 2 rami (sing. ramus). The plate-like unci are armed with teeth, differentiated into 3 groups. The anterior and posterior ones, are formed by thin teeth. There is a group (generally median) that has much more heavy teeth. According to the genus and the species, these heavy teeth can number from 2 to 10.

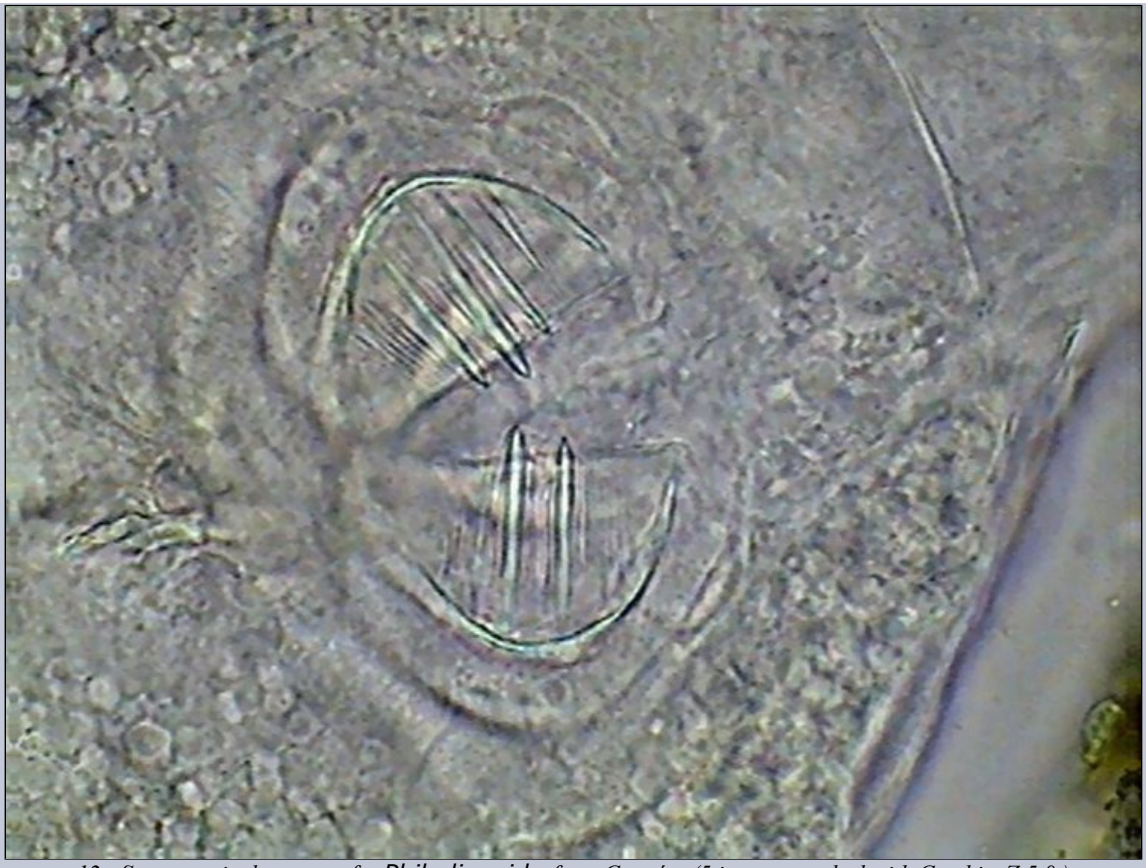


10 - Typical image of the trophi of the order Philodinida, courtesy of M. Verolet.

It can be compared with this image taken with an electron microscope, and colored to differentiate the different pieces. In red the manubria, in blue the unci and in green the rami, underneath the indented edge of the unci.

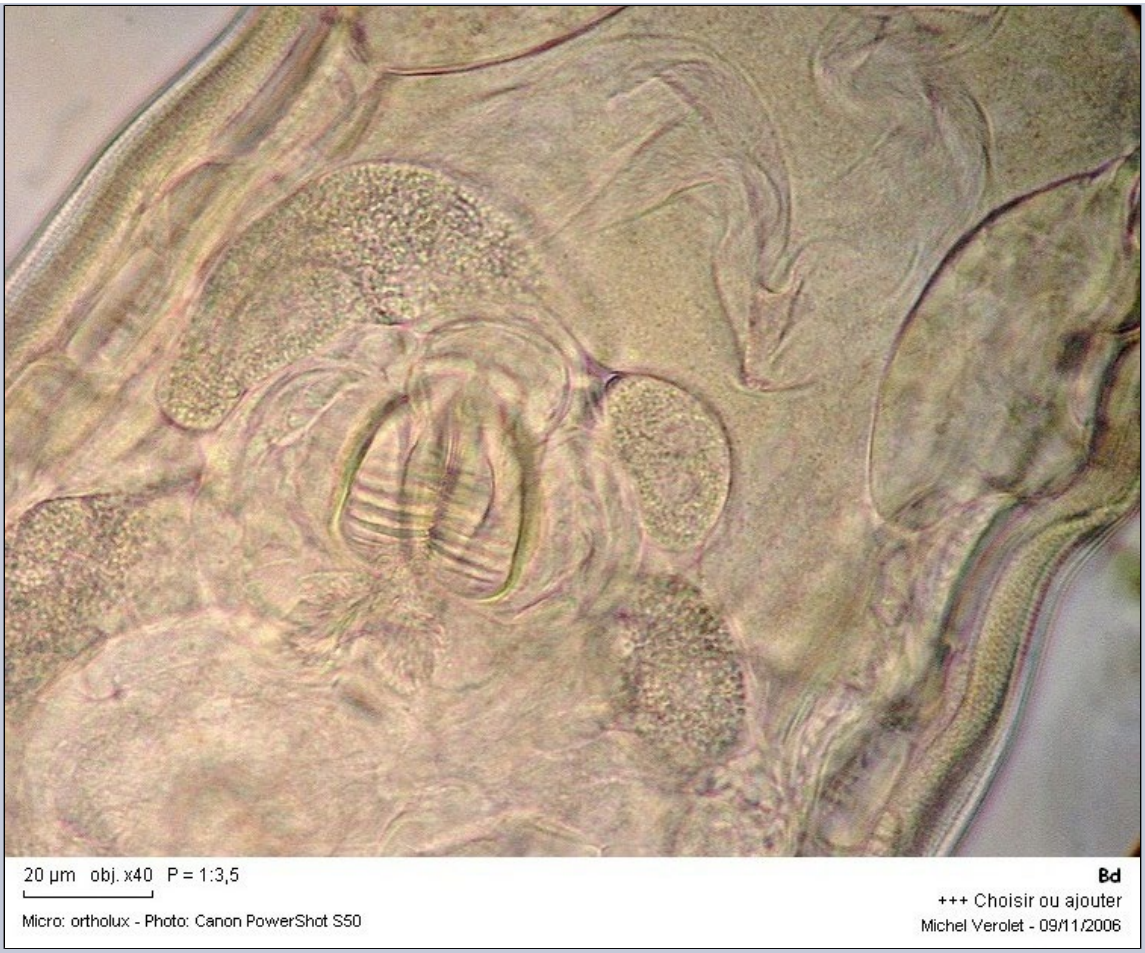


11 - SEM image of the ramate trophi electronically coloured, by G.Melone.



12 - Some atypical mastax of a Philodinavida from Cancún. (5 images stacked with CombineZ 5.0.)

Moreover the trunk contains the remaining organs in its interior: brain, sub-cerebral and retro-cerebral organs, mastax, salivary glands, digestive and excretory systems, gonads, and muscles.



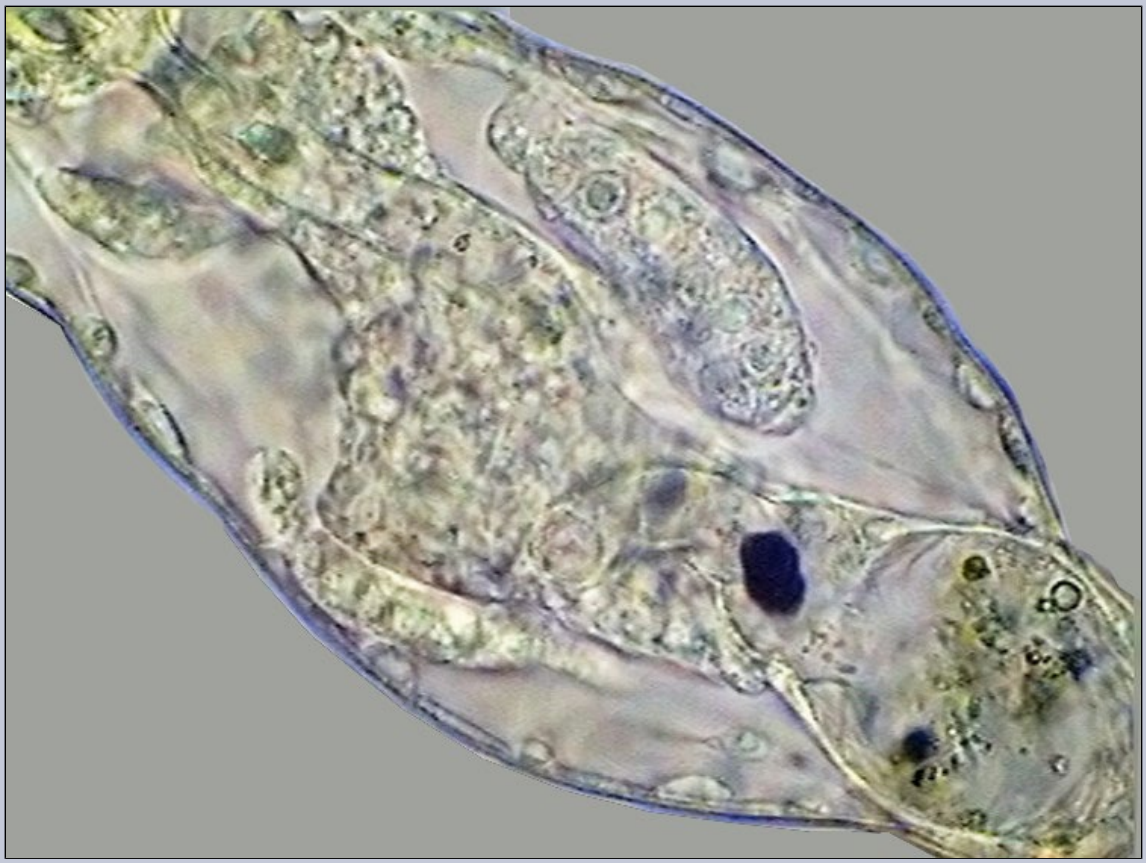
13a - Mastax, salivary, glands and intestine with heavily ciliated lumen in a bdelloid rotifer. Courtesy of M. Verolet.

The mastax is followed by a normally very short esophagus, in most of the cases difficult to see, which opens into the sac-like, stretched, stomach. Normally the stomach is syncitial, with a ciliate cavity (mostly a tube) which is followed by a short, mostly bulbous, intestine (some times called "rectum"). But in the Habrotrochidae the stomach has no lumen at all and food is formed into little balls, and included in vacuoles that give the stomach a foamy appearance.

13b - The stomach of an Habrotrochidae.

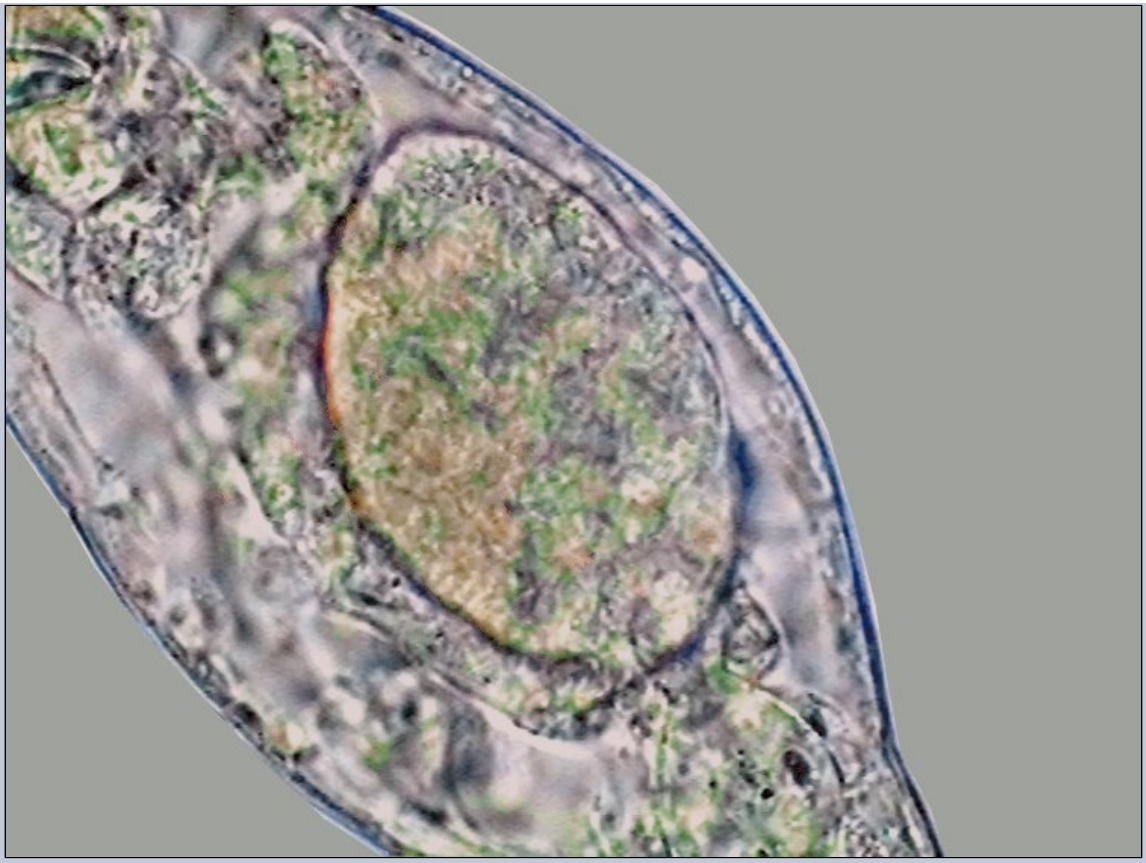
Behind the rectum, or underneath, we can see the urinary bladder, which gathers the liquid expelled from the pseudocoel by two long protonephridia culminating near the neck by some flame bulbs. Bladder and rectum both finish in a common "cloaca" that opens to the outside dorsally in the base of the trunk.

The reproductive apparatus is represented by two germovitellaria, organs that produce eggs, and at the same time provide it with the vitelo (nutritive material) necessary for its development.



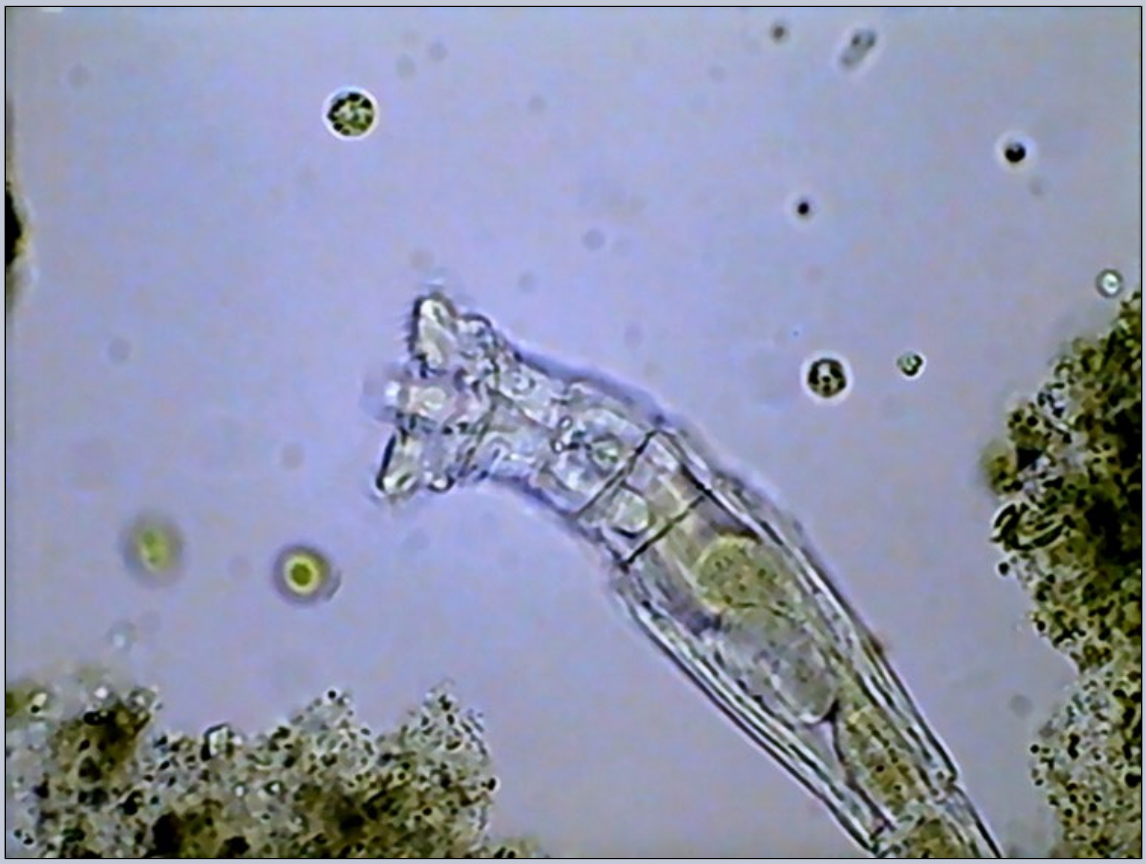
14 - Germovitellaria at both sides of the intestine, in the trunk of Adineta *sp.*

Most of the Bdelloidea are oviparous.



15 - A fully formed egg, inside a bdelloid rotifer.

But there exists some cases of viviparity. And sometimes the already formed embryos, even mobile ones, can be seen within the mother. Even if the development of the embryo is not advanced, viviparity can be denounced <confirmed? QUERY> by the fact that the egg is seen as pluricellular.

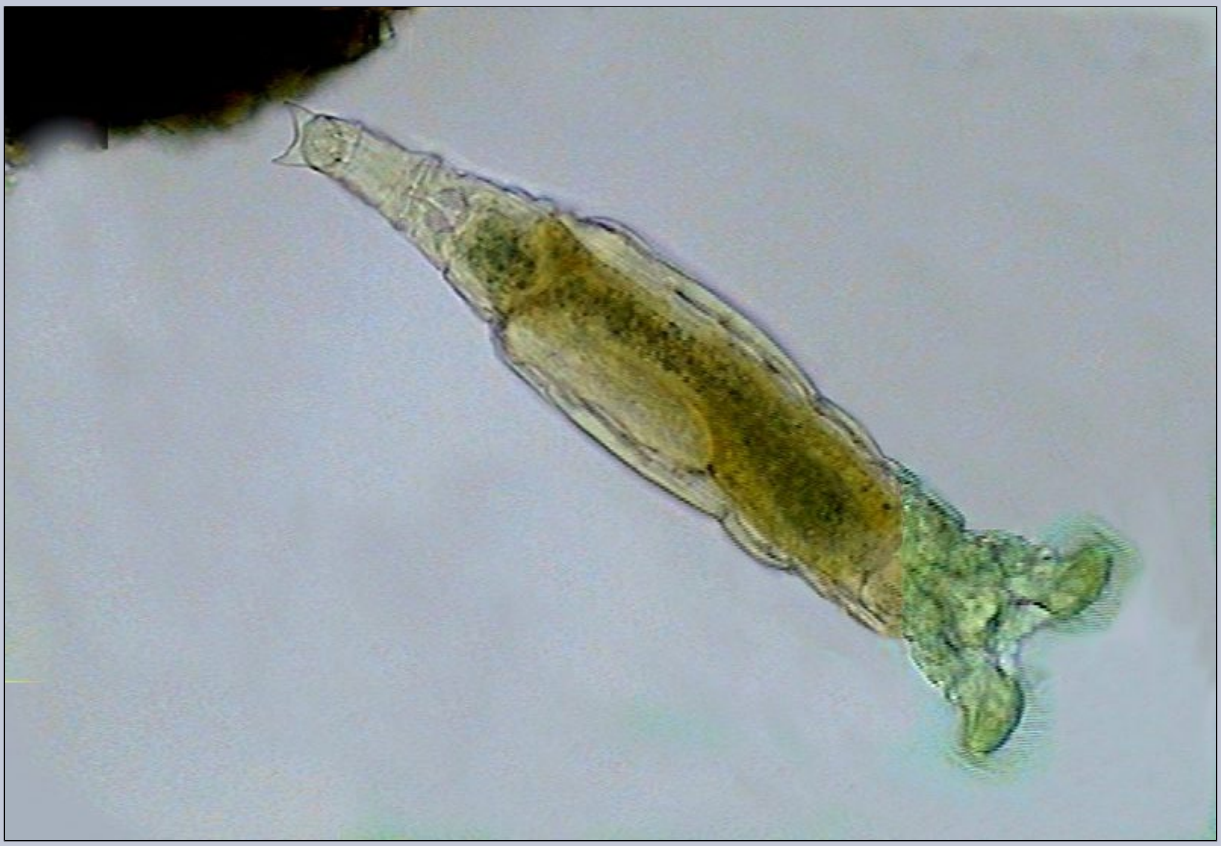


16a - Segmented egg (embryo) inside Rotaria *sp. Note the absence of a shell and the irregular surface.*

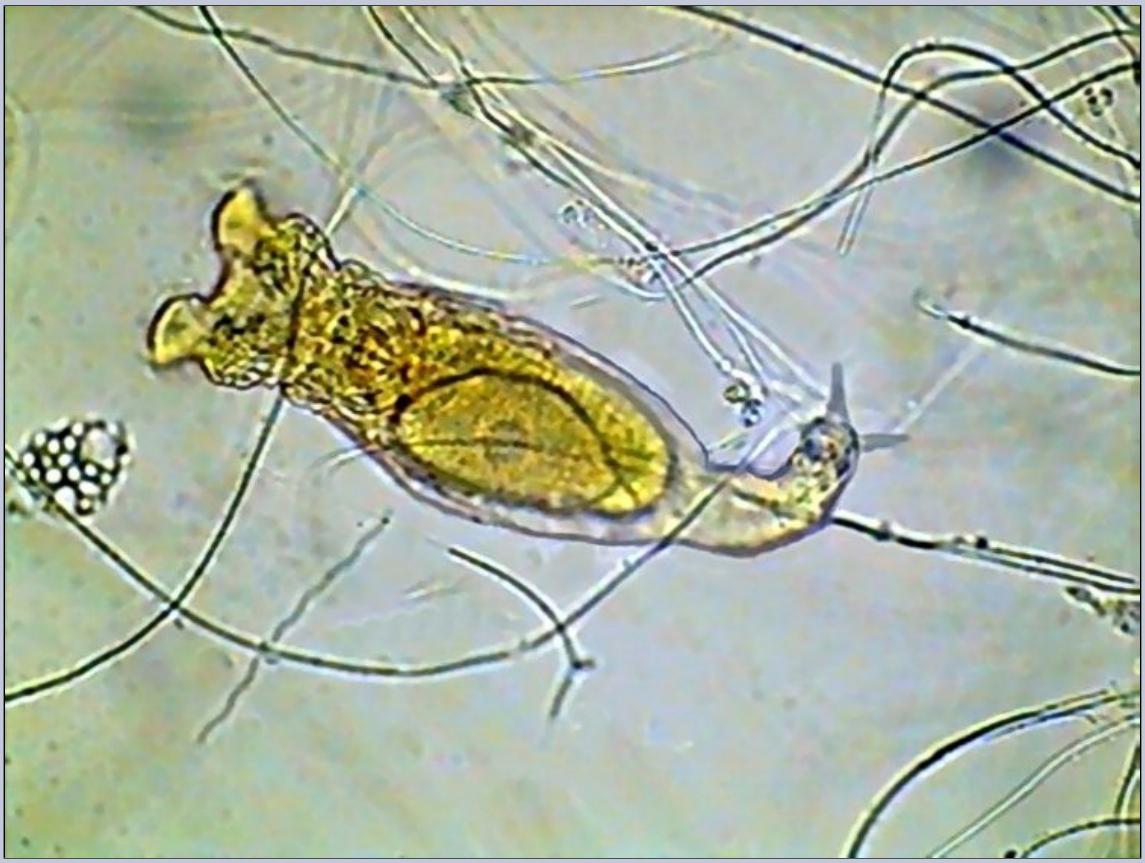
16b - Embryo and another segmented egg in Rotaria *sp.*

The segments after the anus forms the retractable foot, at whose end 2 spurs can be normally seen, and behind these, a short structure lodge the toes, connected to glands, visible in the last portion of the foot, which produce sticky substances, allowing the rotifer to adhere to the substrate.

In a few cases (*Bradiscella*, *Mniobia*, and some epizoics) these toes are replaced by a sticky disc.



17a - Adhesive disc at the end of the foot in Mniobia.



17b - Spurs in the foot of Mniobia*, note also the large egg.*

17c - Toes at the end of the foot of Rotaria (courtesy of Bertrand Parres).

I add here 3 tables with the list of the known genera, ordered under three different criteria. This can be redundant, but I think that it is an easy form to convey very interesting information.

LIST OF GENERA IN ALPHABETICAL ORDER

(in blue, epizoic genera)

Genus, author, publication date, approximate species numbers

Abrochtha	Bryce	1910	2
Adineta	Hudson & Gosse	1886	12
Anomopus	Piovanelli	1903	2
Bradyscela	Bryce	1910	2
Ceratotrocha	Bryce	1910	4
Didymodactylos	Milne	1916	1
Dissotrocha	Bryce	1910	7
Embata	Bryce	1910	5
Habrotrocha	Bryce	1910	100
Henoceros	Milne	1916	2
Macrotrachela	Milne	1886	100
Mniobia	Bryce	1910	50
Otostephanos	Milne	1916	9
Philodina	Ehrenberg	1830	40
Philodinavus	Harring	1913	1
Pleuretra	Bryce	1910	14
Rotaria	Scopoli	1777	24
Scepanotrocha	Bryce	1910	9
Zelinkiella	Harring	1913	1

A genus *Callidina* still mentioned (especially in textbooks) is not valid now; it was very heterogenous and the described species have been distributed, between many of the now accepted genera.

2) Genera ranked by number of species

Habrotrocha	Bryce	1910	100
Macrotrachela	Milne	1886	100
Mniobia	Bryce	1910	50
Philodina	Ehrenberg	1830	40
Rotaria	Scopoli	1777	24
Pleuretra	Bryce	1910	14
Adineta	Hudson & Gosse	1886	12
Otostephanos	Milne	1916	9
Scepanotrocha	Bryce	1910	9
Dissotrocha	Bryce	1910	7
Embata	Bryce	1910	5
Ceratotrocha	Bryce	1910	4
Abrochtha	Bryce	1910	2

Anomopus	Piovanelli	1903	2
Bradyscela	Bryce	1910	2
Henoceros	Milne	1916	2
Didymodactylos	Milne	1916	1
Philodinavus	Harring	1913	2
Zelinkiella	Harring	1913	1

3) Genera ordered by the publication year

Rotaria	Scopoli	1777	24
Philodina	Ehrenberg	1830	40
Macrotrachela	Milne	1886	100
Adineta	Hudson & Gosse	1886	12
Anomopus	Piovanelli	1903	2
Habrotrocha	Bryce	1910	100
Mniobia	Bryce	1910	50
Pleuretra	Bryce	1910	14
Scepanotrocha	Bryce	1910	9
Dissotrocha	Bryce	1910	7
Embata	Bryce	1910	5
Ceratotrocha	Bryce	1910	4
Abrochtha	Bryce	1910	2
Bradyscela	Bryce	1910	2
Philodinavus	Harring	1913	1
Zelinkiella	Harring	1913	1
Otostephanos	Milne	1916	9
Henoceros	Milne	1916	2
Didymodactylos	Milne	1916	1

No new genera has been described in the last 92 years

Comments to the author, Walter Dioni, are welcomed.

Taxonomy footnote: As always happens when a group of species undergoes taxonomic scrutiny with new tools (numerical taxonomy, cladistics, DNA, SEM, MET), the criteria of different investigators can vary widely.

In this article I adopt the classification and denomination of the taxonomic categories used by the most active specialists in these groups; but absolute agreement cannot be expected.

Welch and Meselson, in Science 2000, apply an ample criterion and include in the Phylum Rotatoria four groups that they consider monophyletic and to which they give category of Class: Monogononta, Bdelloidea, Seisonida (sic), and Acantocephala.

Although the frequency whereupon the Acantocephala are accepted between the rotiferologists is increasing, almost all the modern students of this group reduce the phylum to three Classes: Monogononta, Bdelloidea and Seisonacea, the latter sometimes written as Seisonidae. And the tendency at the moment exists to regroup these Classes under two orders: Eurotatoria (with the Classes Monogononta and Bdelloidea) and Pararotatoria (with only a Class, Seisonacea, a family Seisonidae and with two genera recognized at the moment Seison and Paraseison with 2 and 1 species respectively).

Again, leaving aside both mentioned orders (that seem to me very reasonable on the other hand), almost all the active specialists of the Phylum (Claudia Ricci, Georgio Melone, Roger Pourriot, and Hendrick Segers) accept the Classes Monogononta, Bdelloidea and Seisonacea.

The readers who are interested in an ample revision of the bibliography available on the Internet must be therefore prepared to find that the Classes Monogononta and Bdelloidea are widely accepted, but that the genus Seison will be attributed (sometimes by the same author) to different named categories Seisonacea, Seisonidea or Seisonida, located in different and variable hierarchies of the taxonomic classification.

Editor's Note: This three part article by the author was first published in French on the *Microscopies* Magazine website.

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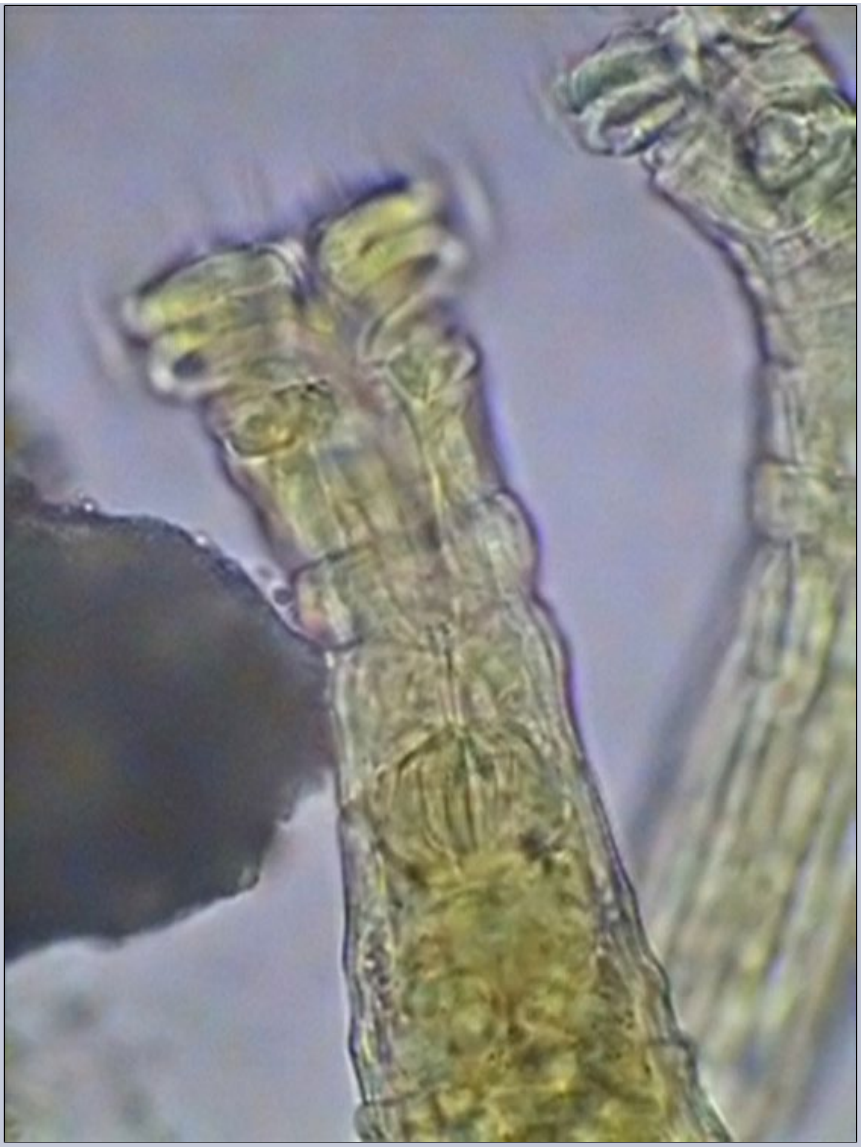
A KEY TO THE GENERA OF ROTIFERA BDELLOIDEA

by Walter Dioni

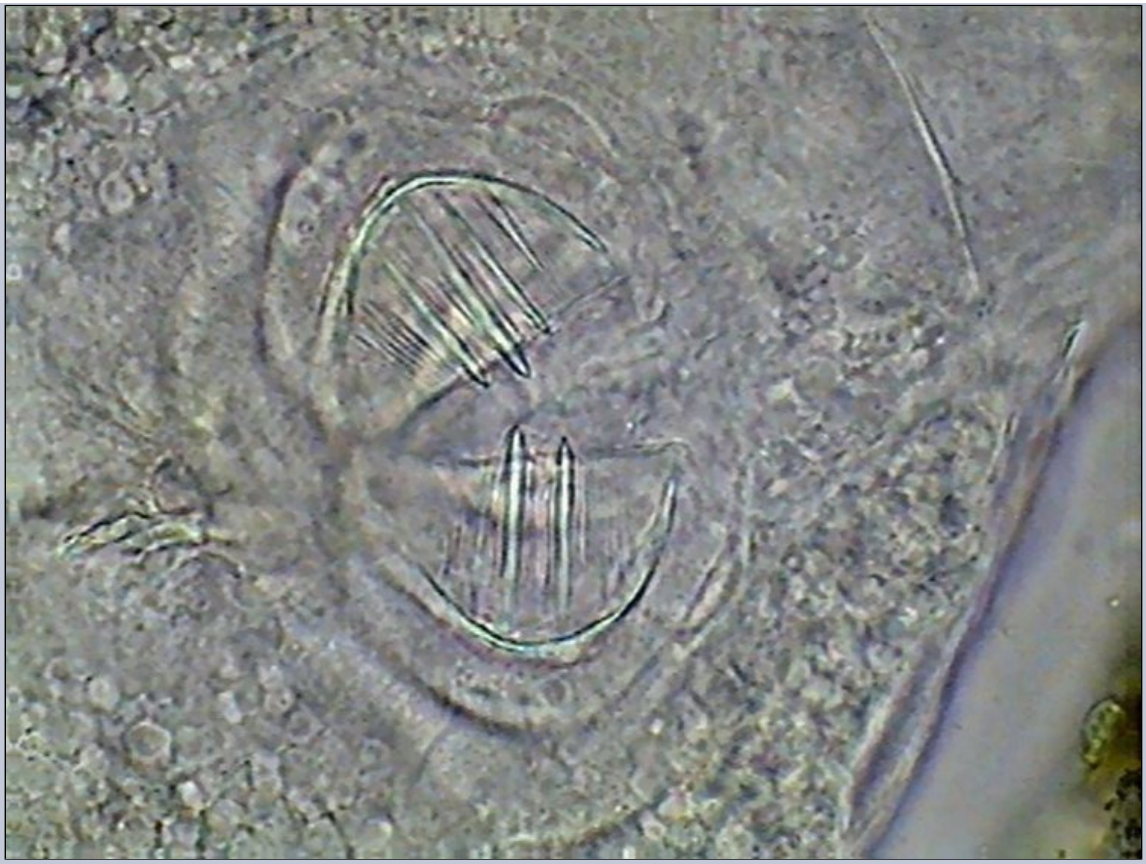
IMAGES FOR THE KEY

2 - Magnificent frontal view of the "head" of a Philodina (courtesy of Charles Krebs).

5 - Lateral view of a Philodina sp., showing the dorsal antenna. Below the antenna can be seen the red eyes. The pseudo-segmentation and the ridged trunk cuticle is also shown.



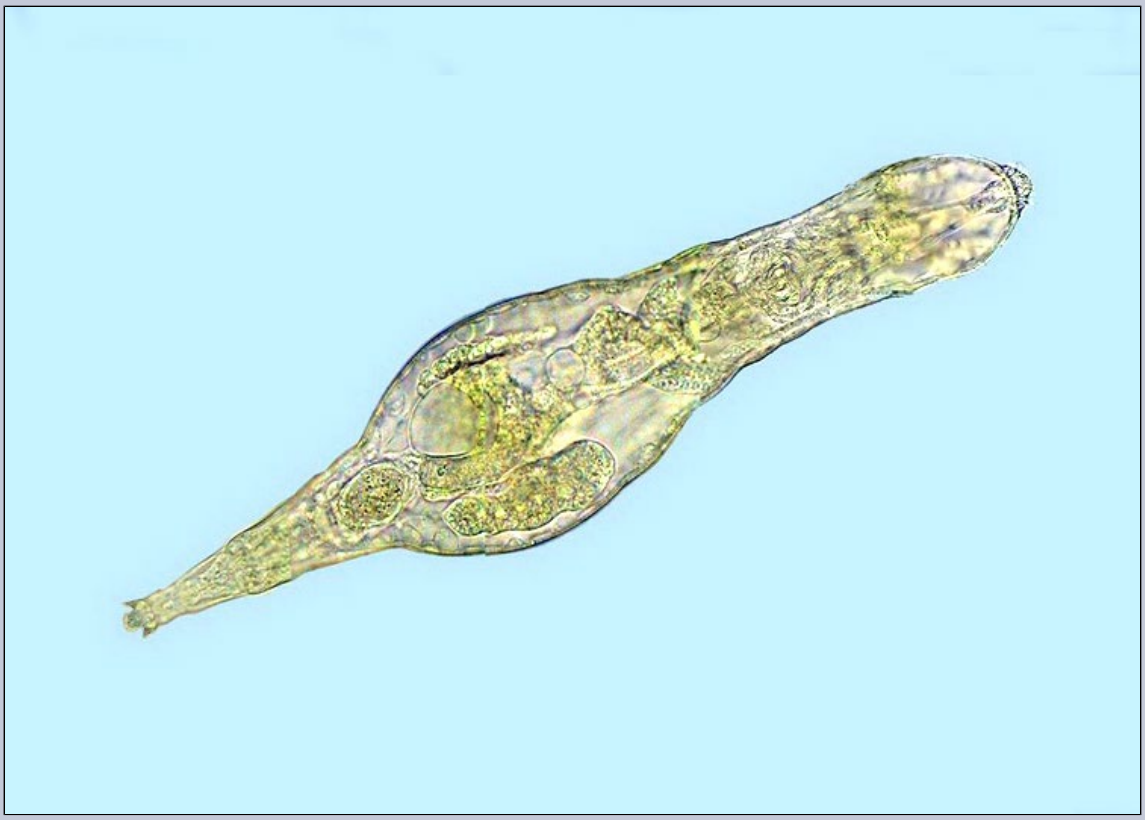
9 - The deep mastax of a Habrotrochidae.



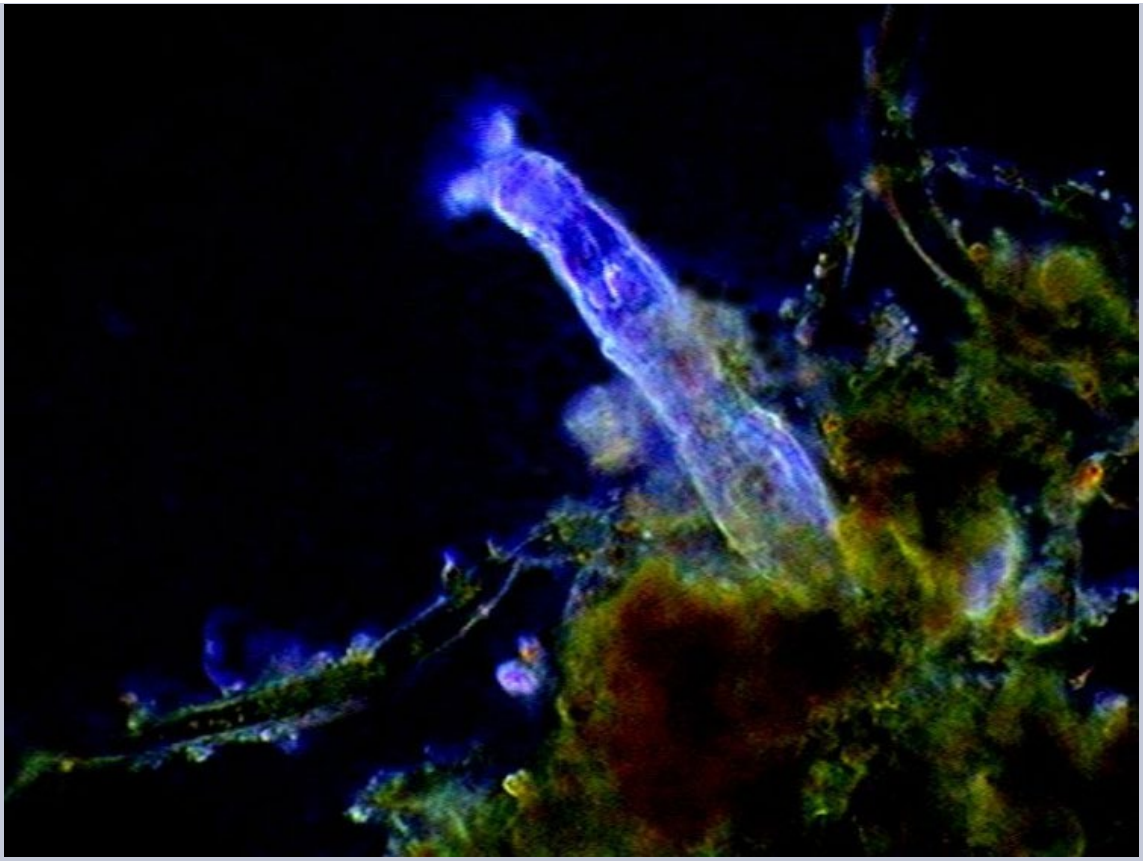
12 - Some atypical mastax of a Philodinavida from Cancún. (5 images stacked with CombineZ 5.0.)

13b - The stomach of an Habrotrochidae.

18 - Frontal view of the "head" of a species of Adineta.



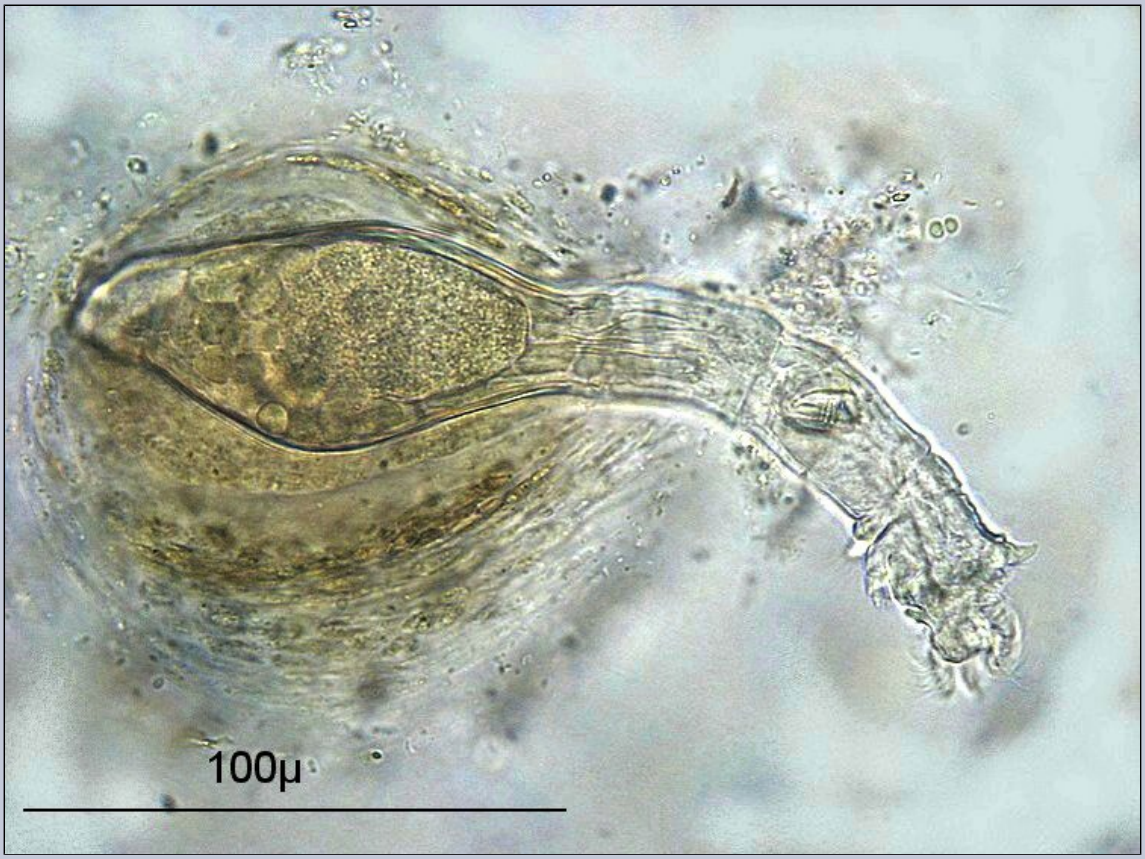
19 - Typical aspect of an Adineta.



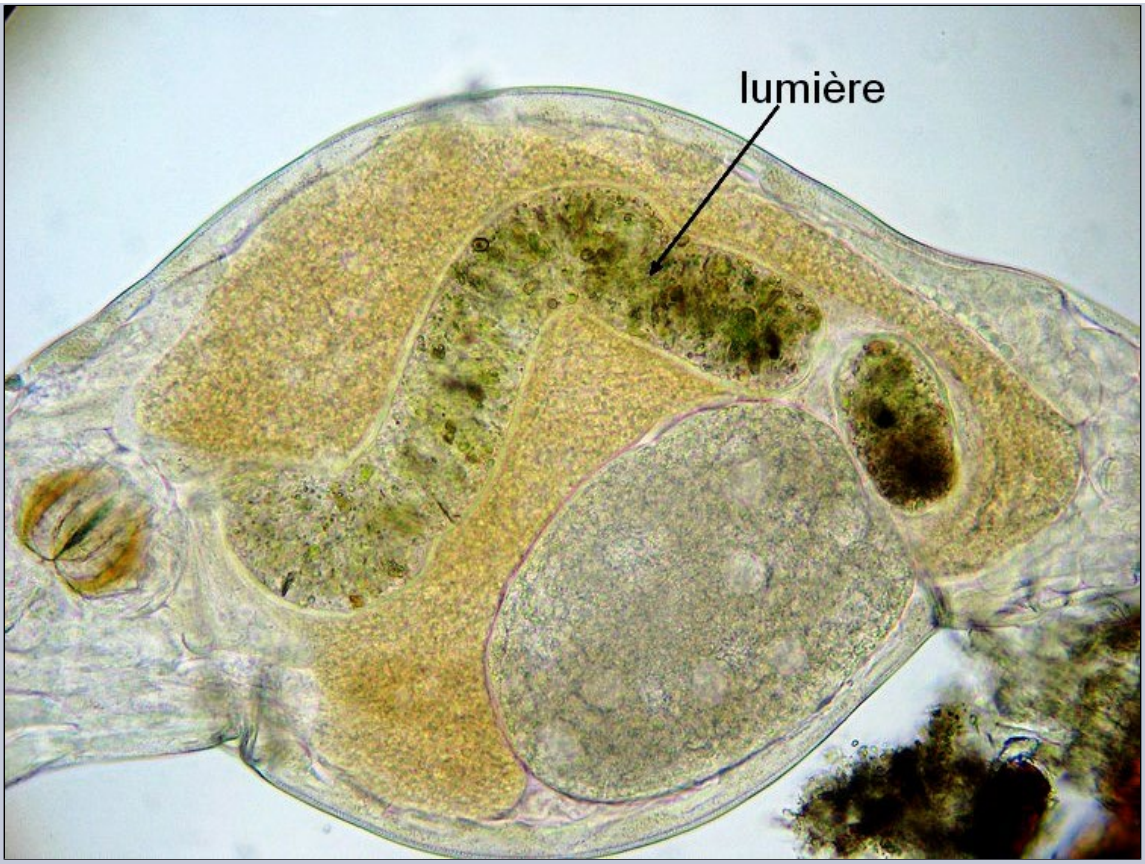
20 – A Habrotrochidae. This and fig 9 are probably Otostephanos sp. I thank Dr. Cl. Ricci for her kind suggestion.

21 – Habrotrocha swimming, courtesy of M. Verolet.

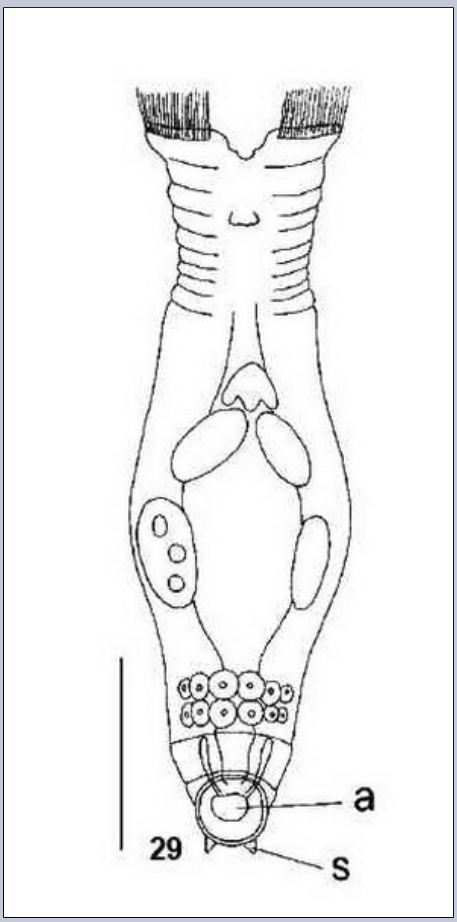
22 - Habrotrocha inhabiting a textured test probably the theca of a rhizopod. Image courtesy of Andre Advocat.



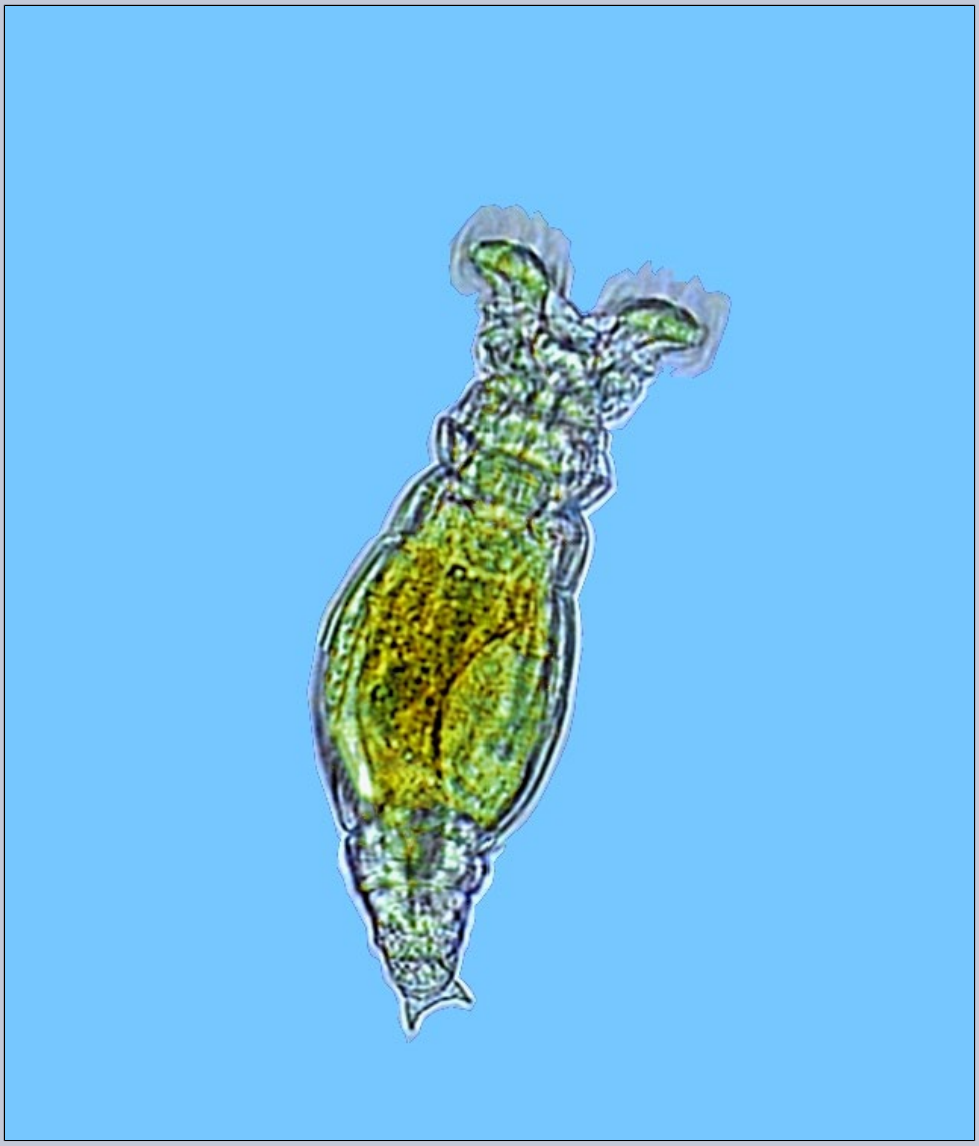
23. Habrothocha inside a mucous "house" made of many secreted layers. Image courtesy of Michel Verolet.



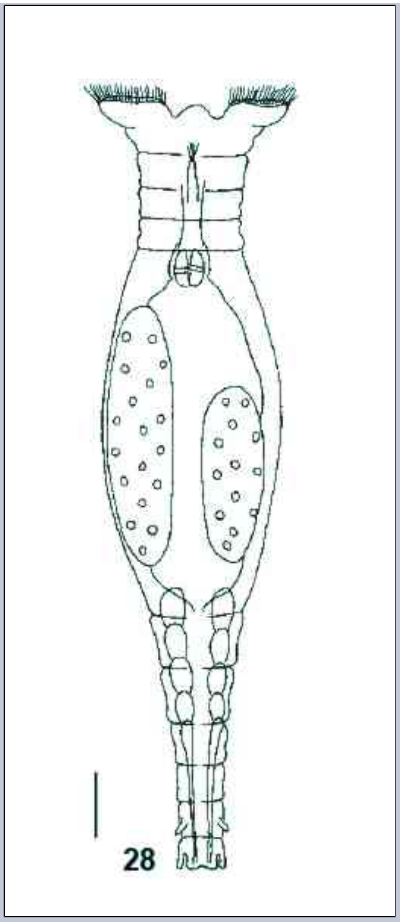
24 - The lumen in the stomach, another picture by Michel Verolet.



25 – Zelinkiella. (Drawing taken from Fontaneto et al. 2008.)



26 – Mniobia (and see also picture 17, First Part)

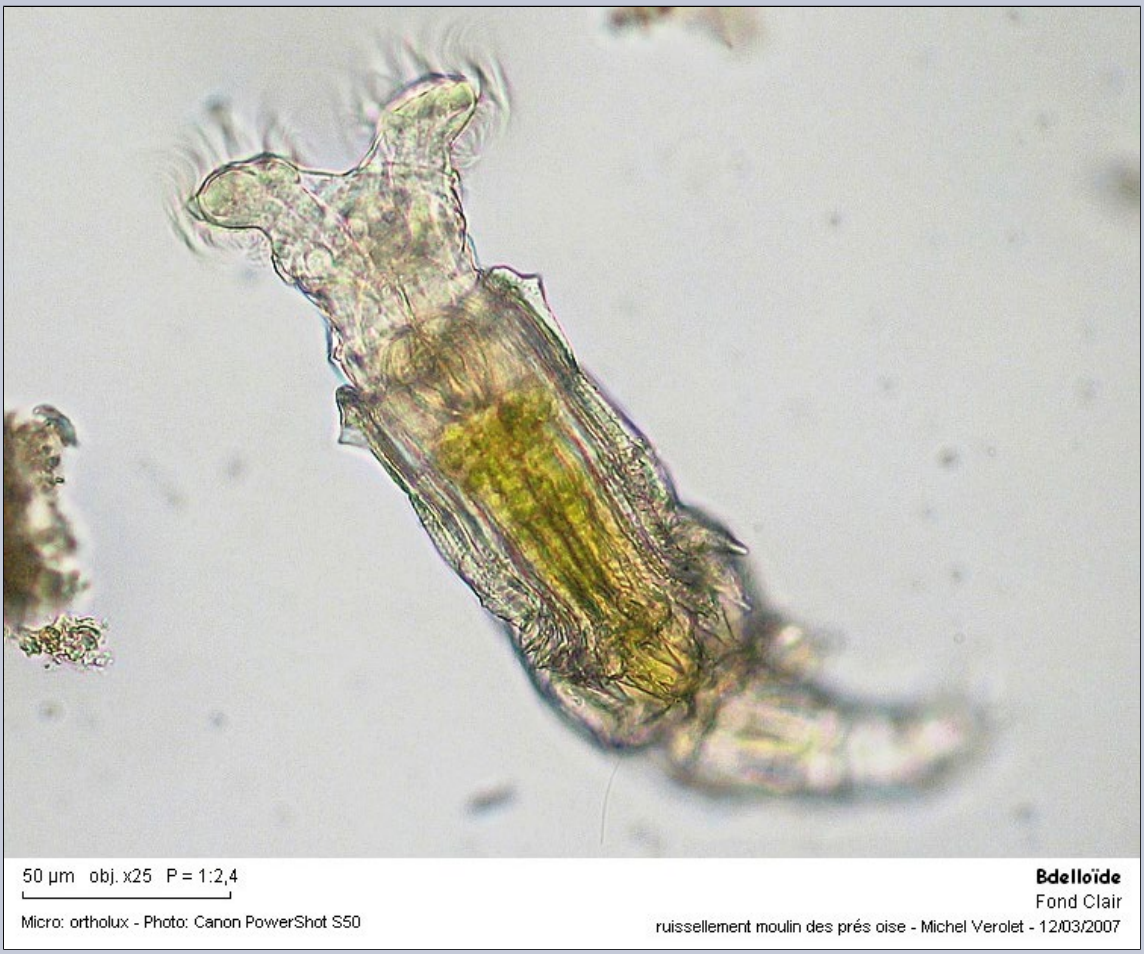


27 – Anomopus (Drawing taken from Fontaneto et all. 2008.)

28 – Rotaria with retracted "corona" See other pictures in the First part, Introduction, 7, 16b.

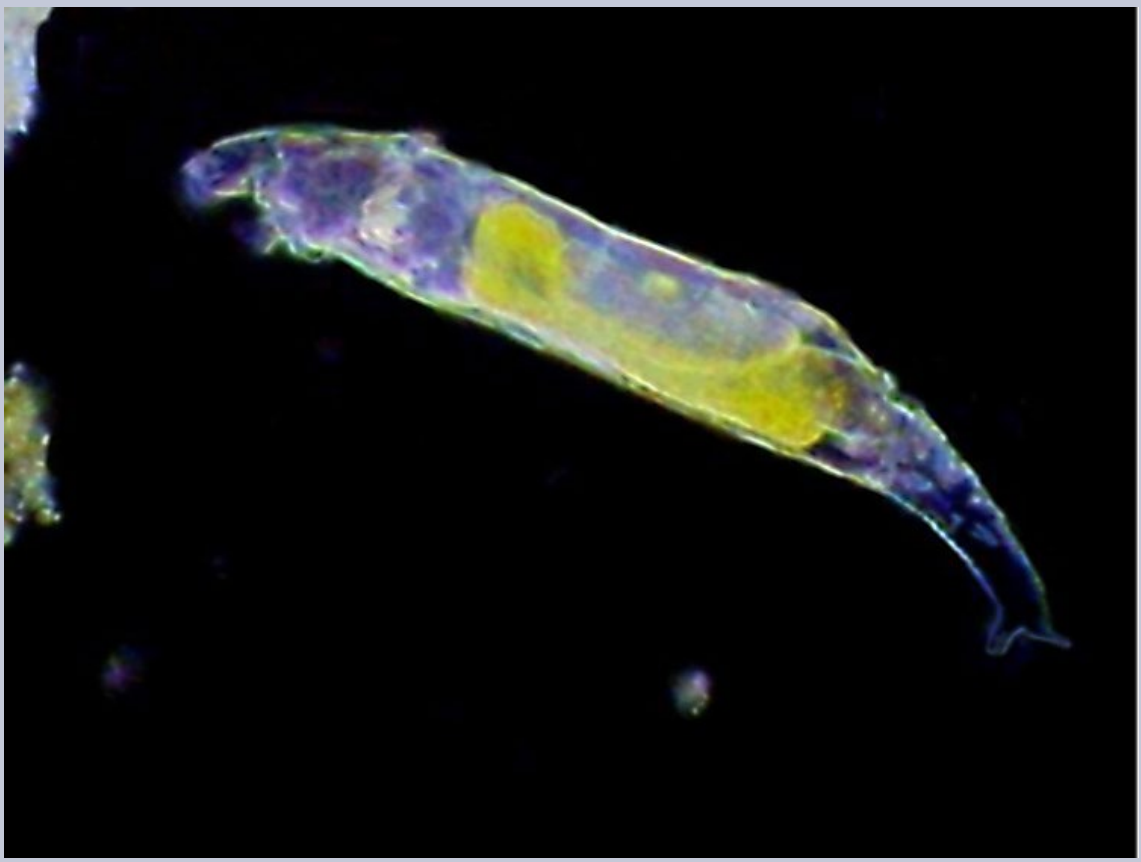
29 – Dissotrocha (SEM)

*Source: www.**zmuc.dk**/InverWeb/Dyr/Limnognathia/phylogeny/Rotifera_UK.htm*

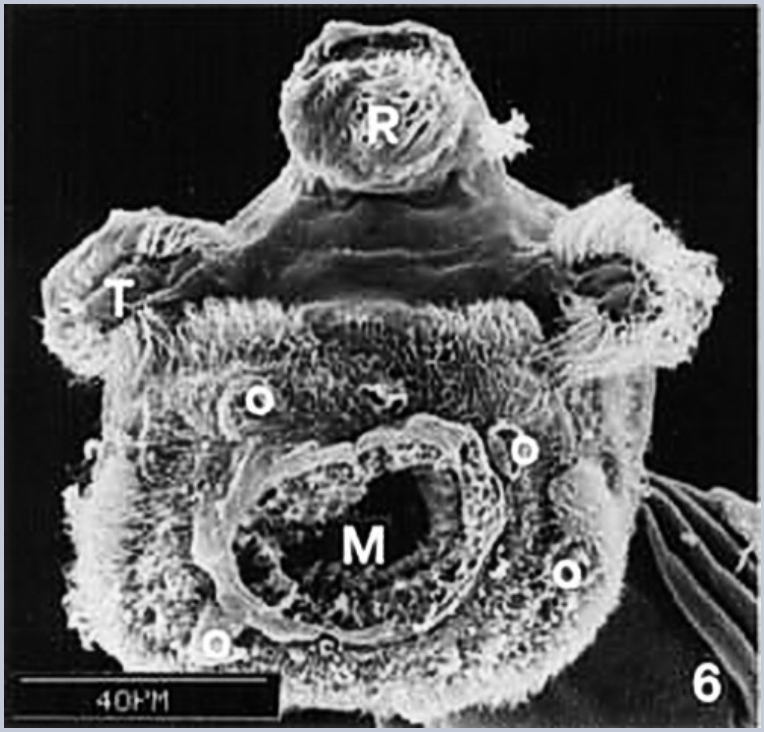


30 - Pleuretra cf. bricey courtesy of M Verolet.
The pictures shown are assigned by present author, W. Dioni to Pleuretra with some uncertainty.

31 – The same species contracted as a "tun".

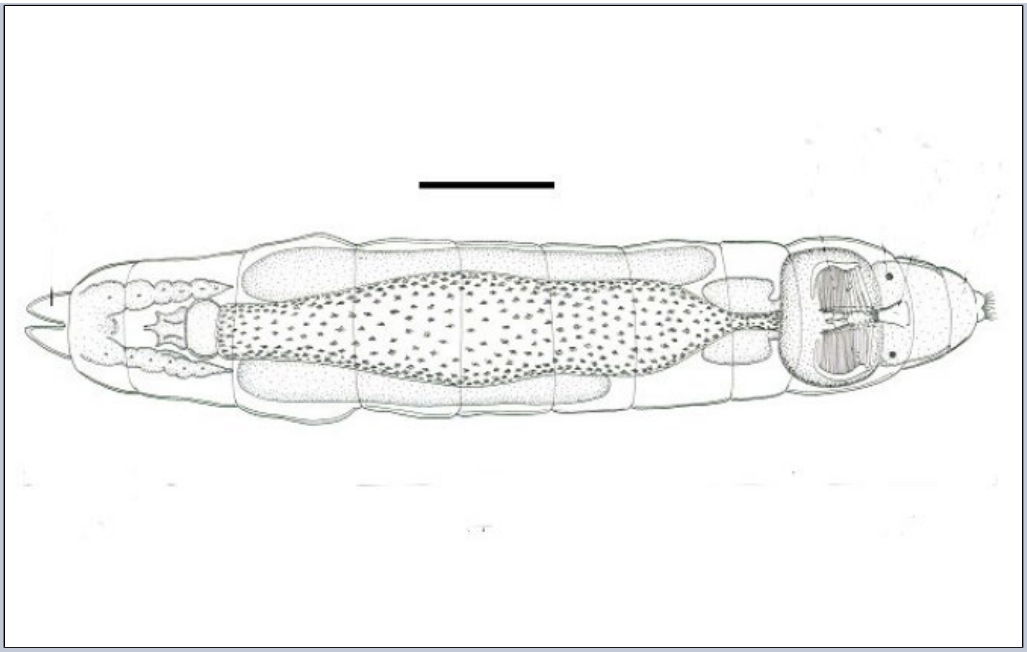


32 – A Philodinavidae from Cancun, prob. Abrochtha.

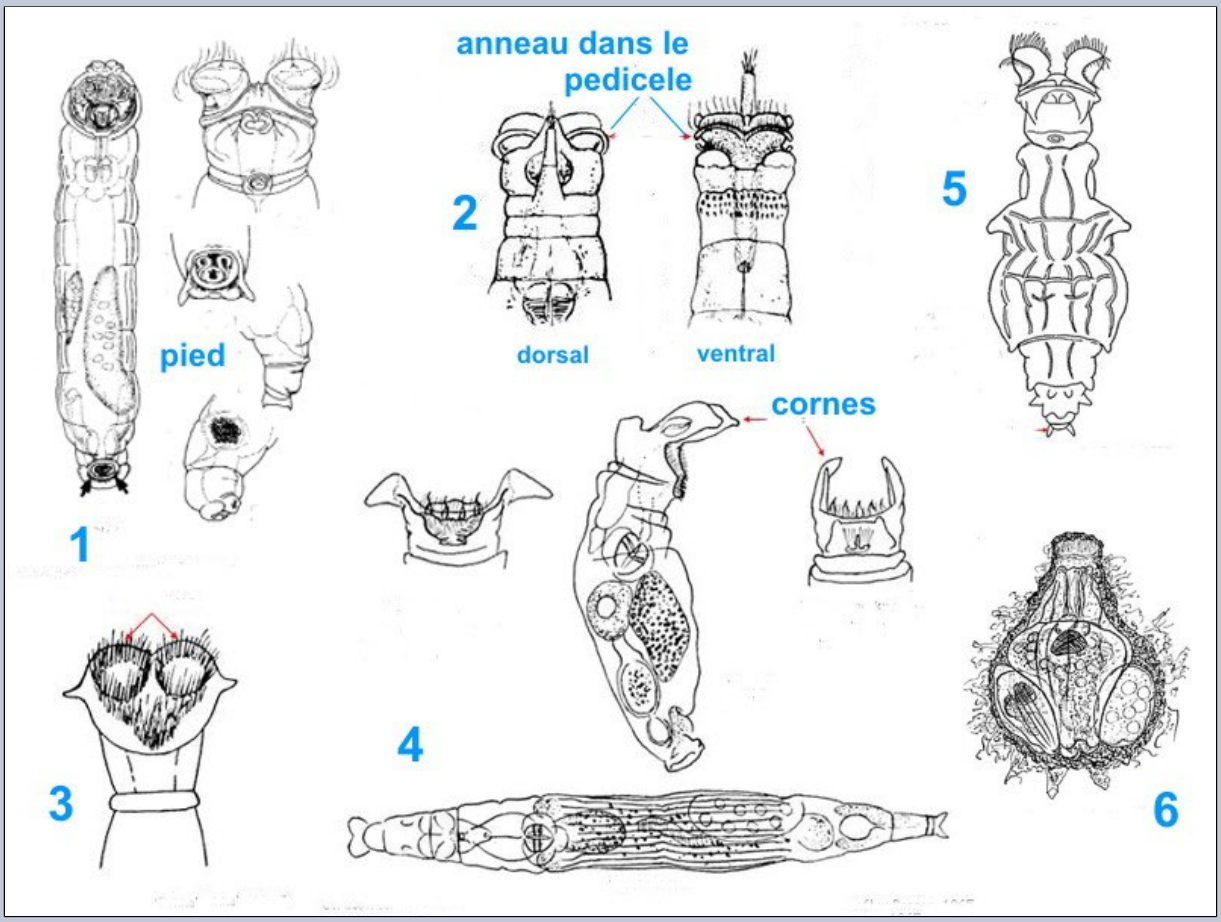


33 – Abrochtha carnivore SEM by Dr. G. Melone.

34 – Henoceros - Courtesy of M. Verolet.



35 – Philodinavus aussiensis, Ricci & Melone.



36
1 - Bradyscela after Koste (20...)
2 - Otostephanus after Donner 1965,
3 - Scephanotrocha, after Pennak 1953,
4 - Ceratotrocha, after Donner, 1965,
5 - Macrotrachela, after Pennak, 1953,
6 - Habrotrocha, within a shell secreted by the female, stuck to a moss leaf, Donner, 1965

Comments to the author, Walter Dioni, are welcomed.

All pictures by Michel Verolet had been posted previously at the French Forum Microscopies.

Editor's Note: This three part article by the author was first published in French on the *Microscopies* Magazine website.

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A KEY TO THE GENERA OF ROTIFERA BDELLOIDEA
by Walter Dioni, Cancún, México

Print version. Prints on ca. 5 pages of A4 or letter size. Web version.

(18 from fresh water, brackish water, soil or mosses, and an exclusively marine one - distributed in 3 Orders and 5 Families)

I include here this table prepared by M. Verolet, as a useful summary of the taxonomy of the Bdelloidea. In bold are the three epizoic genera.

ORDERS	FAMILIES	GENERA
Philodinida	Habrotrochidae	*Otostephanos*
		Scepanotrocha
		Habrotrocha
	Philodinidae	***Embata***
		Anomopus
		Zelinkiella
		Philodina
		Dissotrocha
		Pleuretra
		Didymodactylos
		Rotaria
		Macrotrachela

		Ceratotrocha
		Mniobia
Adinetida	Adinetidae	*Adineta*
		Bradyscela
Philodinavida	Philodinavidae	*Abrochta*
		Henoceros
		Philodinavus

The Key (Follow option in brackets if feature doesn't apply.)
(18 from fresh water, brackish water, soil or mosses, and an exclusively marine one - distributed in 3 Orders and 5 Families)

1 (32) ***Long buccal tube. (Deep Mastax)*** **2**

2 (3) **With complete "corona" (Trochal discs and Cingulum)** or at least with reduced trochal discs. **6**

3 (2) **Without a "corona"**. With a large and flat cephalic ciliated area. **4**

Order Adinetida (only one family)

Family Adinetidae

4 (5) Ribbon-like body, dorso-ventrally compressed. The head, ovoid in dorsal view, is wider than the neck. The ventral zone of the head is oblong with large ciliated cells. The anterior end generally thinner, finishes in a short but complex "rostrum". A single species with eyes (really ocelli) in the rostrum (*A. oculata*). But an Australian species (*Adineta ricciae*) with eyes at the dorsal base of the rostrum has been recently described. The ventral and posterior end of the "head" has, on its wider part, two lateral cross-sectional laminae (rakes) armed with "teeth" directed towards the front, that scrape the substrate to harvest the food and direct it towards the mouth located among them. *Adineta* seldom use the creeping movement characteristic of the bdelloids, instead they move extended, gliding very fast on the ciliated cephalic feature. They do not swim. Long pharynx. Evident small foot, with 3 fingers, and 2 spurs. Fourteen

species, litoral, or between humid mosses. Uncus with 2 teeth.

Adineta

5 (4) Cylindrical or caterpillar shaped. Very short foot, with tiny spurs and papilae (without toes). They don't swim, crawl. Uncus with 2 teeth. The head has the same diameter as the neck. Two species, that live in soil and mosses.

Bradyscela

Order Philodinida

6 (11) **Stomach without ciliated lumen**, syncitial. Plenty of digestive vacuoles, which give it a "frothy aspect". According to Burger, 1948, the food is formed like pellets in the esophagus, and then included in the vacuoles, which digest them. Feces are pelleted. Narrow "corona", but separated into 2 distinct trochal discs. **7**

Family Habrotrochidae

7 (8) The pedicels of the "corona" show, at half length, a membranous ring, incomplete, described as flat, and shelf-like, which could be difficult to see and interpret because they rarely extend the trochal discs. Uncus with 6 to 10 teeth. Short foot, without toes, with two small spurs. Seven species, in mosses that dry up, and in the soil, and very few species in the water, between aquatic plants.

Otostephanos

8 (7) Without the membranous ring in the trochal pedicel. **9**

9 (10) Corona semi-covered by a transparent expansion or lobe of the cuticle, shaped as a pointed hood, and derived from the superior lip. Rarely visible because only expands it when eating. Eyes are not reported. Uncus with 3 to 9 teeth. Short foot, without toes, two small spurs. 9 species that live in mosses or soil.

Scepanotrocha

10 (9) Without the pointed hood. More than 100 species, in soil, sediments and submerged mosses (but also in mosses that dry up). Long buccal funnel and tube. Very short foot, 2 spurs, 3 fingers. Corona often narrower than the neck. Uncus with up to 10 teeth (always more than 2) of different sizes. Very few species (perhaps only one) have eyes. Many live inside a transparent capsule in bottle form. Some species invade cells of *Sphagnum* mosses, where they inhabit and deposit their eggs. Others form mucus envelopes with adherences of vegetal detritus. In all the cases the body is differentiated in one anterior part somewhat thinner and with a smoother cuticle and a posterior one with more rigid and slightly carved cuticle.

Habrotrocha

11 (6) **Glandular stomach, with ciliated lumen,** more or less wide. The food doesn't form pellets, neither in the stomach nor being defecated. Normally the trophi have few teeth. **12**

Family Philodinidae

12 (17) **Without toes in the foot, with an adhesive disc.** **13**

13 (16) Short foot (smaller or equal to half of the length of the trunk). **14**

14 (15) Only one species, **marine**. Still now the only *strictly marine* genus of Bdelloidea. *Epizoic***,**especially on holothuroids, but also on annelids and molluscs. Foot very short, with small spurs and a sticky disc. Each uncus has 2 teeth.

Zelinkiella

15 (14) Free swimming freshwater species. Oviparous. Short foot without toes. It has a sticky disc, that is sometimes difficult to see. Two small spurs. Eyes are rare. Uncus with sometimes 2, mostly 3, and up to 10 teeth. More or less 50 species. They live on soil and mosses.

Mniobia

16 (13) Long foot (longer than half the length of the trunk), finishing in 2 short spurs, and a sticky disc, without toes. Uncus with 2 teeth. Without eyes, with lamellae in the rostrum. Two species, *epizoic* on freshwater crabs. One of them occurs in Italy on a freshwater crab, and the other in Uruguay, on a brackish water crab on the shores of the "Río de la Plata" (La Plata River).

Anomopus

17 (12) Foot with more or less visible toes. **18**

18 (23) Viviparous Species. (But see *Embata.*) Even though they do not have an advanced developed embryo, it is possible to detect the viviparity because the egg in uterus shows more than one nucleus, or is segmented into many small cells; both indications of embryonic development. **19**

19 (20) "Corona" almost always displayed. "Rostrum" (proboscis) and dorsal antenna evident. They feed generally with the rostrum extended. With or without eyes. When they have them, they are located at the end of the proboscis ("rostrum"). Uncus with 2 or 3 teeth. Fine and long foot with 2 long spurs and 3 toes generally very visible (one dorsal and two posterior ones). More or less 20 species, generally litoral, although some are epizoic, or they live in the soil.

Rotaria

20 (19) The antenna can be seen easily, but they do not have rostrum (proboscis) or this is hardly evident). **21**

21 (22) Smooth epidermis. Long, flat and wide spurs, 4 toes, very long foot (half or full the total length), 5 *epizoic* species, they are *oviparous and ovoviviparous* species, One benthic species, *E. laticeps* frequently inhabits running waters with gravel bottom. Wide trochal disks. Only the viviparous species have eyes. Uncus with 2 teeth.

Embata

22 (21) Typically ornate cuticle. They can have rigid, high and long thorns, or appendices, in the trunk. With spurs several times longer than the width of the base. Four toes, foot with four segments. Trochal disks rarely visible. Eyes over the brain like in *Philodina*. Uncus with 2-3 teeth. 7 species, mainly in coastal sediments and submerged moss.

Dissotrocha

23 (18) Oviparous species. **24**

24 (25) Large, more than 400 µm when creeping. Short foot with just *two small blunt toes*. Uncus with 4 or 5 teeth. Without eyes. Oviparous, found in mosses and soil. Only one species known.

Didymodactylos

NOTE: This is the original description of the genus as per Milne. (Didymodactilos carnosus Milne, 1916) but some authors spell it as Didymodactylus.

25 (24) With more than two toes. **26**

26 (29) With three toes. **27**

27 (28) The exterior angles of the cingulum stretch and curve forming 2 long membranous horns or wings. They are extended when eating. Specimens are more or less long, but with a short foot. Uncus with 2-3 teeth. Four species, many times cited as living in acidic water mosses. Ricci and Melone state that they all live in the soil.

Ceratotrocha

28 (27) Over 100 species. Generally in mosses that dry frequently. Without cuticular expansions in the head. Without eyes. Uncus with 2 teeth, some times 3, rarely 5. Short foot and fingers. Robust aspect. Very marked cuticular segments. Some species with the cuticle of the trunk "carved", or with thorns. Ample "corona", generally extended. Some species can be confused at first sight with *Rotaria*, but they have relatively small spurs and fingers, and they do not have eyes in the proboscis.

Macrotrachela

29 (26) With four toes. **30**

30(31) They seldom has the trochal discs open. Without eyes. Short foot with quite short spurs (character of fast differentiation with *Dissotrocha* when they have thorns) and 4 toes. Somewhat rigid cuticle, like a type of armor, sometimes ornamented with thorns. Uncus with 2 teeth. Only one species has 5 teeth. 14 species, all found in submerged mosses, sometimes in acid waters.

Pleuretra

31 (30) Body generally extended, although it can be fusiform and even robust. Long foot. Two spurs and 4 toes often visible. Typical "corona" generally extended. Uncus generally with 2 teeth (some species with 5). There is a viviparous species of *Philodina*, but is easily distinguished from *Rotaria* because it does not have the eyes in the rostrum. The majority with reddish eyes, placed under the antenna, on the brain and behind the mastax. Most of the species live in water, although there are some in mosses, or in the soil. Approximately 40 species.

Philodina

Although Macrotrachela and Habrotrocha have more than double the species, their habitat makes them less frequent in the samplings of the amateur microscopists. Philodina is probably the more mentioned genus of Bdelloids and its structure (or the one of Rotaria) is the generally chosen one to illustrate the Bdelloidea in handbooks, or articles.

Order Philodinavida

Family Philodinavidae

32 (1) **Mastax superficial, near the mouth**. **Short buccal tube.** The big mastax is protrusible and this allows them to directly scrape the food off the substrate. The inferior lip of the poorly developed corona, forms a 'V' that skirts the buccal opening, which is bilaterally bordered by bent cuticular structures (cheeks), (Ricci and Melone). Stout rostrum, very evident, with a strong ciliated end. Short dorsal antenna. **33**

33 (34) With "corona", but very reduced and atypical. The upper lip and the lower lip completely lacking. One frontal field, with two areas. The upper one, near the rostrum, only surrounded by cilia (the circumapical waist, De Beauchamp), the lower, and more ventrally oriented (the buccal field, De Beauchamp) covered with much smaller cilia. If the rotifer is seen in ventral or dorsal view the circumapical waist can be distinguished at both sides of the rostrum as small "pseudo-trochas". They have a stout rostrum, but shorter than in the other two genera. In some pictures by Pourriot (1974) and Ricci et all. (2001) the rostrum is difficult to see between the pseudotrochas. Short foot, Philodinidae style, (approx. 25% of total length) with two spurs and 4 short toes. Two species, one in Europe and North America, that eats cyanobacteria (de Beauchamp, 1909, Pourriot, 1974) and one (carnivore) in the Barbados (Ricci, Melone and Walsh, 2001).

Abrochtha

34 (33) Without "corona". Strong, and always extended ciliated proboscis. **35**

35 (36) Long proboscis. Short antenna. A very small pre-oral ciliated area protected by oval cuticular flaps. A very short (3 segmented) foot, with only one, immobile, large spur and four strong toes with form and sizes very similar to the spur. Two species in running water on submerged vegetation.

Henoceros

36 (35) Cilia are reduced to a small field around the mouth, and those at the end of the always extended short but strong rostrum. Evident short antenna. One of the species (*P. aussiensis*) has two eyes on the brain. Short foot with 4 strong, often visible toes. Two short, parallel, conical spurs. The existing illustrations show the species with the aspect of caterpillars, due to

their cylindrical body, and its short head and very short foot. Two litoral species.

Philodinavus

Comments to the author, Walter Dioni, email: < wdioni2002d AT yahoo DOT com > are welcomed.

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Web address www.microscopy-uk.org.uk/mag/artoct08/wd-rotifer2.html

Part 1 Introduced the taxonomy and biology of the Class Bdelloidea.
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The three part article by the author was first published in French on the *Microscopies* Magazine website.
http://www.microscopies.com/DOSSIERS/Magazine/Articles/WD-CLE BDELLOIDEA/BD ARTICLE 1.htm

PART 3:
TECHNICAL NOTES FOR THE COLLECTION

AND STUDY METHODS FOR

BDELLOID ROTIFERS

(AND OTHER AQUATIC MICROINVERTEBRATES)

Part 1: Introduced the taxonomy and biology of the Class Bdelloidea.

Part 2: The key to the 15 genera of the Class Bdelloidea.

Introduction

This article forms the third part (*Technical Notes*) of the

Key to the Genera of the Bdelloid Rotifers

already published. Once written I realized that most descriptions of the micro-habitats, and also the techniques to be used for each of them, could even be applied almost without modification not only to Bdelloidea but to many other groups of micro invertebrates (gastrotrichs, nematodes, rotifers Monogononta, the inhabitants of the meiobenthos, including the kinorhyncha, and with a few modifications that surely the imagination of the amateurs will discover quickly, to the entomostracans and the hydracarina). Consequently I modified the title to make it more accessible to the indexing for the Internet, although I maintain the text without change. My primary objective is still to help stimulate more amateurs' work on bdelloids.

I use here some terms to designate “biocenosis” that probably are not supported by the European or even American bibliography (they were coined in Institutes of Limnology from the southern hemisphere, where the researchers must deal with a much diversified biological world). I believe that it is very useful to separate which most of the time are different and identifiable species groups, which could be otherwise confused if you don’t make a careful selection of the microhabitats. I make a short definition of the used terms.

Habitat

Leaving alone ***Zelinkiella***, an exclusively marine genus, which lives as a commensal on **holothurians**, the bdelloid rotifers are found in practically all ecological habitats, although most of the time in freshwaters (or systems that could dry completely, being periodically dampened by the

rains). Only 21 species in 8 genera have representatives which have been found in continental waters, salty or brackish. (Fontaneto *et al*, 2008) and the majority are haloxenic species, that is to say, species that are suspected to have only arrived and survived by chance in those habitats.

In fresh waters they can, and they must, be looked for in zooplankton of the larger water bodies (fishing with a fine mesh of about 70 microns at the most) but there will be found only a few species mixed with the frequent Monogononta. The most fruitful habitats are described next.

The classification of the biocenosis is not capricious. Each one has its own physical, chemical and structural characteristics, which determine which fauna can live there. One must learn how to recognize them.

a) **littoral** forms: those that swim **free** near the edges of the water bodies, (sometimes called **heleoplankton**) or crawl on the littoral plants. Between these must be distinguished those related fundamentally to **pleuston**,(floating plants, like *Salvinia, Pistia* or *Eicchornia*) and those that are ***periphytic*** (growing on and around a support) over the **bafon**,(submerged part of the rooted plants). Or the ones bound to **plocon** (floating filamentous algae, fixed to the shore, stones, or objects) and to **heteroplocon** (free floating filamentous algae). The relationships of these faunas, between them and with those free swimming, are debatable, but in most cases they are distinct species.

b) inhabitants of other ***bioderms*** (also called *biofilms),* thin layer of bacteria, micro-algae and micro-invertebrates that occur on permanently or temporarily submerged substrates, like stones, wood, the submerged areas of the floating or emergent plants, submerged roots of trees, walls, wood, etc. (**periphyton**).

c) those that live between the grains of littoral sands (**psammon**) or in the thin layer of the superficial benthos (**ooze**).

d) **sapropelic**, those that live in ponds and small pools contaminated by organic matter in decomposition (**in any one of the previously described situations**).

e) those that live in waters that are collected periodically in cavities of trees (tree-holes), or in the "pitchers" (pitfalls at the end of leaves) of the carnivorous plants like *Nepenthes*, or in the "cup" or "vase" reservoir, that forms in the center of many bromeliads, because of a rosette of overlapping leaves (**phytothelmics**).

f) those that live in periodically dried mosses or lichens (**bryophytes**), in and under pine needles and litter (**edaphic**) or in cavities, be they always humid, or flooded periodically, on rocks, walls, buildings, pavements, etc. (**lithothelmic**).

g) in addition, there are 3 **epizoic** genera (*Anomopus, Embata, Zelinkiella*), commensals on freshwater animals; (one of them, *Anomopus* in brackish water).

It is becoming increasingly common to call the species that thrive in the **e** and **f** situations **limnoterrestrial,** because evidently they are in terrestrial environments, but thriving only when they are wet or flooded.

Sampling and conservation

All trips in search of **qualitative** samples of bdelloid rotifers must be specific, directed to a certain habitat (i.e: periphyton, bafon, etc), and very limited in the sampled volume. A single live sample can provide sometimes many species, with few individuals each, which will force a slow and

individualized study. Three or four samples would be the ideal. An ample investigation of a selected habitat, must be divided into many small samplings throughout a certain time. This will allow not only a better idea of the specific richness (the number of species present at the site) but to obtain a first sight of the possible successive substitution of the species, driven by physical events such as the seasons of the year, or the state of humidity of the materials. The live samples must be protected from abrupt changes of temperature, they may be best transported in a thermally isolated box.

Of course the sampling methods are as varied as the habitats that must be investigated, and in addition not only provide rotifers but a very varied microfauna, although here I have concentrated on the methods best adapted for bdelloids.

The **epizoic** rotifers can only be collected by examining the animals that lodge them and washing the open surfaces (which include, by example, the cavities where gills of the crustaceans are sited, or the mantle cavity of mollusks) to gather the **agile** or fixed organisms that inhabits them. (Vagile: not fixed, moving animals.)

The coastal forms raise a true challenge to the talent of the collector. It is easy to gather, with small plankton nets, the forms that swim free near the shore, or between the plants. But there also exists a varied microfauna that adheres more intensely to the surfaces, or that inhabits more intricate habitats.

For **pleuston**, **plocon** and **heteroplocon** there is no other remedy than to gather portions of the corresponding flora, carefully sliding them with its water and fauna within bags or bottles.

Periphytic fauna. This is most difficult to gather. For the inhabitants of the bioderms which cover the submerged part of diverse rooted plants, stones, wood, etc., one generally resorts to lift off, with care and skill, with the help of a brush or spatula, the material adhered, directing it (with luck) towards the harvesting containers. For rooted emergent plants (e.g. rushes) a somewhat safer method is to cut (whenever you can) the aerial part, inverting a bottle or plastic bag to surround the submerged stem with its adhered microflora and microfauna, and cutting the plant below the mouth of the container, closing this and removing from the water. This even allows advanced researchers a quite exact quantitative sampling.

For plankton the conventional plankton nets with 70 or 45 micron mesh are used. They can be towed behind a boat driven at a low speed. Or the fauna can be fished-out of a "water column", lowering the net to the bottom and recovering it vertically with a slow and steady ascent.

For the rotifers of **mosses, lichens, litter, sands and soil,** samples will be taken which must be investigated later at the laboratory.

The sands must be collected with abundant water from the sampling site. Shaking them, to suspend them, in a great volume of water, can help to loosen and suspend the interstitial fauna. Let it rest some seconds so that the sand settles, and pour off the supernatant water. Concentrate it by straining through a fabric of 40 to 70 microns mesh. As many of the **psammobionts** (animals which live in the interstices of the sand, at the shore of the water bodies) adhere strongly to the sand particles, researchers generally try to anesthetize the microinvertebrates using magnesium sulphate, or bupivacaine, (see "anesthetics") which facilitate its removal. It must be investigated with care the effectiveness and the form of administration of the anesthetics when collecting bdelloids. The times and suitable concentrations vary according to the species present in the samples.

Some investigators propose to leave the sample alone for two days. The oxygen at the bottom will be consumed and the animals will creep towards the surface. It will be possible to gather them with a pipette at the interphase between the sandy substrate and the water. The best site to

search is the angle between the sand and the glass walls of the container. Exploration can be continued over one week or more.

The lichens and mosses must be wetted with distilled water or, better, rain water, and they will be let to rest for two days. They can be squeezed to separate the water from its microfauna, and the liquids will be investigated during several days with the stereoscopic microscope or under the low power objective of the binocular to separate the detected fauna.

The rotifers of **ground** and **litter** are collected by treating the samples in the laboratory, using a **Baermann** funnel. This is a funnel with a sieve applied to its mouth, where the sample is placed. The tip of the funnel is closed with a rubber tube and a Mohr clamp. The funnel is filled with water up to the level of the sample. The vagile animals can cross the mesh of the sieve and accumulate in the rubber tube, from where they will be collected periodically.

Phytothelmic fauna is better studied if the plants are carried to the laboratory, if it is possible. Otherwise, the liquids in the vegetable container must be siphoned to a sampling tube or bottle and must be transported as any other liquid sample.

Lithothelmic habitats will be sampled, absorbing the water filling the cavities, and scraping the sediment, generally rich in algae, and especially in cyanobacteria.

Anesthesia

In the following technical discussion I refer sometimes to the late H. Taylor. He was a specialized technician that worked all his life with rotifers, and started his career with the great rotiferologist F.J. Myers. He published a series of technical articles that are now bound in a book. Although aimed at the professional researcher, many of his suggestions are worth trying by the amateur microscopist.

Those individual fauna selected for a later more detailed study, or to be mounted as " vouchers", (duplicate specimens, which are filed for future reference and comparison) will have to be anesthetized, if it is possible.

The best ever known anesthetic for rotifers, including bdelloids, is the "Liqueur de Rousselet", for which we have two formulations, the original, and a modification due to De Beauchamp. The Italian limnologist Mario F. Canella says (1954) that even traces of this reagent were so effective that they stretched immediately the individuals of *Rotaria neptunia*.

Even if all the species were not so collaborative, it is worth the trouble to try the Rousselet **if its basic ingredient can be obtained**: **Cocaine Chlorhydrate**.

ROUSSELET original (0.006%)

Cocaine chlorhydrate 2% solution……..3 ml

Methyl alcohol (full strength)……..1 ml

Distilled water…….....6 ml

De BEAUCHAMP, modification (0.05%)

Cocaine chlorhydrate……..0.5 g

Methyl alcohol (full strength)…….5 ml

Distilled water……....5 ml

With either of the two formulae it is recommended to add 1 drop of anesthetic to each mililiter of sample, every 5 minutes, until obtaining the narcosis.

In addition to the Rousselet formulae, many other anesthetics have been tried.

Most used now is Bupivacaine (also known as Marcaine).

Bupivacaine (Marcaine) was recommended by H. Taylor as a 0,5% mother solution. It is an anesthetic used by dentists, which I obtained in presentation of 30 milliliter with 150 mg. of active substance. Dilute it and use carefully drop by drop, or infiltrate the diluted solution under the coverslip. It is a proven narcotic with Monogononta, I tried it with bdelloids, but results were successful only a few times. Segers has successfully anesthetized *Adineta ricciae* with this substance.

1% Magnesium chloride or sulphate, was also recommended by Mario F. Canella (1954). Also use it drop by drop. There is some note of its successful use in some species of bdelloids, but most of his pictures are from Monogononta. An 8% solution is regularly used by meiobenthologists to loosen the microfauna clinging to the sand from marine habitats.

Neosynephrine (Phenylephrine) ophthalmic drops are used by ophthalmologists to dilate the pupil of the eye. They are also sold as nasal sprays. You must take care with these, because the drug is very addictive. Doctors recommend not to use it as a medicine for more than five days! I had tried it a long time ago, and it was very good (as always) with Monogononta. It was deceptive applied to bdelloids.

Homatropine can be obtained in drug stores as a medication for liver diseases, and also as ophthalmic drops. This, and a lot of other drugs as **atropine**, **benzamine hydrochloride**, **xylocaine** (lidocaine), **tetracaine**, **metoprolol**, etc. were recommended to be tried. But no one reports today an always successful anesthetic for bdelloids. But all of them merit a trial.

The anesthesia must be judged by the lack of response to the contact stimulation, because cilia do not anesthetize generally, and the rotifers continue moving, even if they are well anesthetized.

Fixatives

Sometime ago the anesthetized rotifers were fixed adding to the containing drop a similar volume of 8% neutral formol, or 6% glutaraldehyde (H. Taylor used it as a 2% solution). Formol is now banned as **carcinogenic**, but glutaradehyde is not. (See footnote, added March 13th). Although bibliography shows that professionals continue to use both products, even if many laboratories have changed to less toxic fixatives.

GALA 60 (Dioni) was used in Italy by Dra. Ulrique Uehliger at 10% concentration, in **planktonic rotifers samples**, and they reported a good behavior in samples conserved in the fixative for two months, (of course most of the species were Monogononta). Nevertheless it is highly advisable to discard the liquid of the sample, and wash it with 70% alcohol after no more than a week, and preserve it afterwards in 70% alcohol plus a 5% of glycerin. This has three advantages: it prevents the possible corrosion of organisms by the strong acids, provides a permanent preservation medium, and facilitates the later work and the faunistic counts eliminating the irritating fumes of acetic acid.

As the specimens (fixed or alive) are selected from the sample being searched, they will be withdrawn with a micropipette and accumulated in a small watchglass or any small capsule, to conserve them for future study.

Although it is always advisable to bring a live sample to the laboratory, at least in the beginning of a project, most of the time the samples will be generally fixed in the field. If bdelloids are the objective, the traditional methods of fixation will show only unidentifiable contracted units.

So, I propose to divide the sample in three:

A subsample will be anesthetized with Bupivacaine (or the anesthetic that would be finally selected by experimental trials) until it only shows ciliar activity, and the affected animals do not contract. H. Taylor suggested, as a standard technique, to split the concentrated sample in 5 or 6 subunits of 4 to 5 ml each, and to apply to each one an increasing number of drops of the anesthetic, expecting that one turns to be optimal. *He also says that a uniform time (9 min. max.) would be used, to fix the samples. It seems that more time allows for the specimens to contract even if they were already well expanded*. At this moment one will add to each subsample, 10 to 20% GALA, with or without any *in toto* stain. Before, I used 0.2% Rose Bengal, but it is now considered strongly carcinogenic. Try the use of much diluted Allura Red, or Tartrazine (respectively red and yellow food colorants), or, even better, 0.5% to 1% aqueous Eosin.

The second can be treated with CO2 (mixing with the sample some gasified commercial water) or by asphyxia (small flask, concentrated collection, very tight sealing), which, in this material, can rarely produce some anesthetized bdelloids. If the rotifers do not die stretched by the boiling water, there would be certainly many other invertebrates that do, which could also be very interesting for the microscopist which for this reason could be prone to do a more complete inventory of the sample.

Third unit - The samples gathered by filtration of the sediment detached from pleuston or bafon, or gathered as benthonic ooze, are generally too voluminous for an individualized treatment in the field. In this case the best strategy (apparently designed by Frank Myers) is to place the sample in a container of a volume 6 times greater, located as a safety precaution, within another one 2 to 3 times bigger. Time is allowed, so that individuals stretch out and retake their normal rate of activity, at which moment, 3 to 5 volumes of almost boiling water is poured into the first container. Sediment is allowed to settle for a long time and upper liquid is poured out to the maximum possible. A volume of fixative similar to the sediment volume is added to the sample. As in the second suggested treatment only a few species of bdelloids respond to this treatment, which is normally very useful with Monogononta.

Alternatively, the filtrates or sediments can be placed in a "detachable-neck flask", with the base painted in opaque black (Dioni). After a time, the geotrophic negative, and phototrophic positive, swimming animals, plus those that suffer with the oxygen rarefaction, will meet in the detachable neck. This one is taken apart, its content is poured in the definitive container, and the fixative is added; or the microfauna is first massively anesthetized before to fix it, or it is treated with almost boiling water or almost boiling fixative.

Methods of study

The sample is first studied with a stereoscopic microscope, or the low power objectives of your microscope. Preferably over a dark background. And the individuals whose morphology must be specially studied, or which must be identified taxonomically, will be separated, using micropipettes with buccal or mechanical control…… (if you try your pipette under the low power, remember that

the movements has its directions inverted. If your microscope allows it, use the "Dioni's poor man stereoscope". Some investigators consider that pipettes allow the adhesion of the animal to the glass, preventing them to be unloaded to the slides, and suggest to learn to manipulate the rotifers, even live, exclusively with micro-loops, micro-spatulas or micro-needles. They are saved in *watchglasses*, or suitable capsules of any type, where they are accumulated, and are later transferred to slides in a drop of water, and first studied uncovered, to verify its behavior in an unrestricted medium, style of swimming, etc., or they are covered with a coverslip. As water under the coverslip evaporates the weight of the coverslip can apply pressure on the specimens. If this is not prevented the rotifer could be squeezed. To avoid this

1. For voluminous species some support must be included (paper, cellophane, small coverslip parts, hairs, etc).

2. A more technical solution, but a not so simple one, is to create a petroleum jelly compressor.

   Place a small drop of water, with the specimens to be studied, on one slide. Spread a very thin layer of petroleum jelly on the palm of your hand, and slide over it two opposite edges of a coverslip, to gather a thin line of jelly on them. Invert the coverslip and, carefully, lower it VERTICALLY over the drop, watching to keep it centered. With the aid of two thin and blunt needles, (or even toothpicks), adjust the height of the coverslip, looking at the preparation with the stereoscopic microscope, or with the low power objective of the binocular, until a delicate compression of the rotifers is reached, that will be controlled with great care, to avoid destroying them.

Individualized processing:

a) The selected living individuals, can be treated with some anesthetic, if any one is useful, fixed with GALA, to be stained and mounted in permanent preparations, as described below.

b) The individuals of the species already fixed in the field, which would be needed for further study, must be separated and collected in a small capsule. If they have been massively stained **with Tartrazine, or Allura Red or Eosin,** they go directly to the glycerin as it is explained below. But, if not, 0.5% Eosin will be added to them letting it act for the necessary time. By means of micropipettes, or working with microloops or microspatulas or even microneedles, the animals must be worked out of the staining solution, and they will be washed in water, to be mounted in glycerin.

Glycerin will be applied through several graduated steps of 10, 25, 50, 75 and 100%, a few minutes in each one, or they can be placed in a 5% solution that are left to concentrate under a dust cover, over several hours. The goal is to avoid the specimens wrinkling, but to concentrate and mount them in pure glycerin (H.Taylor used glycerin with a "touch" of phenol, as a bactericide and an aid in clearing). Apparently the fragile species that wrinkle during the mounting process can become suitably hardened if they are previously treated with 10% acetic acid.

The mastax is indispensable to identify the species, even if its general structure is very similar throughout the class. A solution of 0.3% of commercial sodium hypochlorite (supermarket cleaner bleach) is recommended by H.Taylor. The commercial solution is normally a 5% solution. Dilute 1 drop of commercial solution with 8 drops of water (9 final drops). This gives a 0,6% solution approx (5%/9 = 0,6%) Add 1 drop to another water drop with the rotifer, the working solution is now 0,3%, and it starts dissolving the cells and leaving only the hard structures. It is better to work with a well slide, and with small drops to prevent losing the tiny structure.

It is also possible to work under the coverslip, adding a 0.5-1% solution drop to the cover margin and absorbing with great care the water at the opposite margin with a thin but long strip of filter paper. It is also possible to use the same technique to replace the water by glycerin which has a

better refractive index.

ELEMENTS FOR THE DESCRIPTION OF THE SPECIES OF

BDELLOIDEA

Put your sample in a drop of water on a slide. Ricci and Melone (2000) suggest that, at first, you study your material without applying a coverslip. This is easy with the low power stereoscopic microscopes, but only for the 4x and 10x objectives with the compound microscopes, and with more or less quiet animals, but could give you a good appraisal of the general morphology and activity of the rotifer.

The general morphology will be described from observations with a 10x eyepiece and the objectives 4x and 10x, with complementary details with a 40x, and some details (the trophi as an example) will even be recorded with the 100x Oil Immersion objective, if you have one. The law here is to "document all that is possible, in the best way which will be possible".

Digital pictures are a rapid recording technique. Now you can resort to one of a wide range of digital cameras, from the "big names" valued at many thousands of dollars, to the most modest webcams. In Europe Philips is the preferred trade mark, replaced by Logitech's in America. Be cautious and do not acquire for your microscope any camera with less than 1.3 megapixels. Study with care the many articles on this theme published in Micscape. If you can read French, a detailed view of the posts in the Forum Microscopies (see the link in Micscape front page), could be of your interest, long theoretical discussions and detailed camera presentations has been published. You can work without a photomicrography camera, even if one of them can make things more easy for you. But if no camera at all, you can draw, as hundreds of rotiferologists did fruitfully in the past. They study and document with drawings and descriptions almost all the actually known species.

If the rotifer is more or less immobile, take the needed images to later compose a whole body mosaic. In all the cases it is highly useful to make z-arranged stacks, open the pictures in the desired order, and using the Screen Grid function of your image processor, make detailed drawings, even if some of them are only sketchy ones. When you have a clear concept of the relationship of the organs, you can select and arrange the images of the stack to apply CombineZ (in any of its later versions) or to use instead Helicon-Focus.

When studying a new specimen it could be useful to adhere to the following list, (It is not exhaustive, **even if in many cases could be excessive**).

Write and draw all that you can, you will verify that this is a very useful approach. Don't forget that, with every note, drawing, or picture, you must record the date and, if it could be important, the hour. Record also the microscope, objective and illumination technique, the technique used to record the data, any other instrument involved. Put a scale on the picture or drawing **now**. Not a general statement about a group of images, but an individual scale. You don't know when, or what kind of phenomena can hit tomorrow, and separate you from your materials and notebooks. Record all you can do for your own benefit, or to make your laboratory work comprehensible to other scientists. And don't worry about the word. If you work seriously along this proposal, what you are doing is SCIENTIFIC WORK (yes, let stand the capitals) even if you don't have the ultimate tools to do the publications, or you don't work on "The Big Problems" of Physiology, or Evolution, your contribution to taxonomy and zoogeography will be recognized and stimulated. These two areas are those which need more of the push up that the amateurs can give. Many, many years ago, Huxley states that "Taxonomy is in the hearth of Biology".

General data

First of all register the origin of your sample, describe the habitat in your notebook, record, if you can, latitude and longitude (NO, now you don't need complicated apparatus, nor even expensive topographical maps. You need to use your FREE "Google Earth"?) In some cases and in many countries you can even include in your files one or more screen captures to record the locality. Record any data you can obtain about the source (this includes of course physical and chemical data if you can) …But, surely you have your computer, your word processor, your Photoprocessor, your digital camera and your Google Earth. But a kit of chemical tests, even for aquarists, is not in the standard equipment of the amateur: search in your wallet if you can afford the expenses, this would be a great addition to your lab. Use your digital camera to file some pictures of the site. They are invaluable aids today, and especially month or years in your future.

External anatomy

It is necessary to describe their appearance and activities when eating, crawling and swimming.

Body structure - Verify the shape, in dorsal and lateral view, and the relationship between the pseudo-segments. Measure the length of the foot, trunk, neck, and the segments of the head.

C*uticle* - Write down the appearance and thickness of the cuticle, and the presence of folds, longitudinal furrows, or reliefs. If they exist, note the number, disposition, form and size, of the cuticle thorns, or any projection, warts, or papillae they have.

Head and corona - Verify the form of the head, its width, and the shape of the "corona", its size with respect to the head when they are displayed, the order in which the trochas opens, pedicel length, and its mobility.

Superior lip - While the specimen eats, verify in detail the shape of the superior lip, **especially in dorsal view.**

Antenna - Verify the position, form, and length of the antenna (in lateral view).

Eyes - Verify the presence or absence of eyes, color and position (on the brain or at the end of the rostrum or proboscis, or at any other situation).

Foot: number and shape of segments, form and details of union with the trunk.

Spurs: Shape and length, insertion on the foot, and width at their base, separation among them, and any mark that they have.

Toes: number, and disposition. Absolute and relative lengths, among them and with the spurs. (It is better to study the bdelloid in a hanging drop, over an o-ring or well slide. It sticks to the coverslip and one can see much better its foot and toes, or any other adhesive system it has).

Adhesive disc: (if present) position, orientation, structure.

The study of the foot could take a long time, and a lot of effort, most of the time it is hidden between algae or detritus. A very fine pipette to isolate some clean specimens is a must.

Internal anatomy

Position, form and size of the *brain*

Form and disposition of the *sub-cerebral glands* and the *retro-cerebral sacs*

Mastax: position, form and size

Trophi: form and size of each piece. Teeth, dental formula (100x)

Gastric and salivary glands: aspect, size, position.

Stomach (Intestine): form, size disposition presence or absence of a lumen, (it can be necessary to verify it at 400x in some compressed individuals). Only in the *Habrotrochidae* there is no lumen

Intestine (rectum): location, size.

Feces: if the opportunity allows their observation. In Habrotrochidae the feces are pelleted, similar to the gastric content, in the rest of the bdelloids they are a loose material

Protonephridia: 40x and 100x; compressed; they could be easily overlooked, look for the flame cells.

Bladder: shape, position, size, relationships. Time for repletion and voiding?

Foot glands: also called *cement glands* (number and disposition) 10 and 40x with extended foot.

Gonads (germovitellarium): made from two syncitia:

Vitelogen, The big nuclei that are normally seen even at moderate powers.

Ovaries (Small nuclei between the nuclei of the vitellaria. They are only well seen in compressed individuals.)

Oviduct: one, common to both germovitellaria, difficult to see or to identify.

Developed eg:, presence, size (in oviduct).

Uterus (in the viviparous species): mostly indistinguishable.

Position of the opening of the cloacae: Shape of the near segments if they have any interesting characteristic.

Egg (layed) - Form, size, type of covering and any sculpture on the surface.

If it can be recorded: time to eclosion. You must separate some females into a solution innoculated with bacteria, with very few gross particles, in a capsule protected from evaporation, and record the laying time, the egg structure and the time to eclosion.

As far as is possible all the studied details would have to be photographed.

Probably the specimens that are used for the complete study do not survive the treatment.

If pictures are not completely satisfactory (as rarely they are) one can make drawings based on them, using as a guide the screen grid which can be displayed over the picture in almost all the image processors. Complete your drawings with details from live specimens

Some times ago I published in Micscape an article on the utility of the drawing for the microscopists http://www.microscopy-uk.org.uk/mag/artsep02/wddraw.html

If a specialist can appraise most of the pictures, drawings, notes and measurements here advised, (and it would be a lot of good information) they will have very little difficulty to classify the material, even if this could be new to science.

THIS IS WORK, HARD WORK, BUT WE ARE SEARCHING ALL THE DAY FOR A GOOD SUBJECT TO BE SEEN AND RECORDED WITH OUR EQUIPMENT. HERE IS A GOOD OPPORTUNITY TO BE HAPPY AND REALLY USEFUL.

Present situation

Claudia Ricci and Giorgio Melone in their work of 8 years ago (2000) hoped that their paper would wake up the interest of amateurs and professionals on Bdelloidea.

The reasons that prevented a fast concretion of this desire are also explained by the authors: small size, excessive mobility, lack of useful media to slow or anesthetize them, incredible speed for total contraction, difficulty to stop them by compression without badly deforming them, necessity to study the individuals for hours, alive, to be able to decipher their organization.

The books with suitable keys and descriptions are mostly out-of-print editions, and it even influences negatively the fact that outside Europe few students have a good knowledge of German, because the fundamental work on Bdelloids is

Donner. J. 1965. Ordnung Bdelloidea. Bestimmungsbücher zur Bodenfauna Europas, 6, 297 pages.

This last difficulty could be partially resolved if this book is translated to English and or French.

This illustrated Key, which we include today in the Micscape Magazine tries to make available to students, in direct visual form the morphological basis that allows separation of the Bdelloidea genera.

It is a pity that I cannot include a picture of each genus, When I found them I took some drawings from Internet. But even then there are some for which I could not find images (I live far from the scientific libraries, and on the Internet there are not many images).

But it will continue being true that their does not exist a sure medium to anesthetize the Bdelloids. Apart from Rousselet's, any tried technique gives as a most probable result the contraction of the rotifers so fast and complete that it disqualifies any further study, except for the recovery of the trophi by dissolution of soft parts with hypochlorite. In this Class the trophi are not generally a very important specific character, mostly confirming, better than defining, the determination. But the Italian team at the University of Milano is assembling an important library of trophi images captured with the SEM and this could change things in the future.

Nevertheless there is a tool that surely will facilitate the investigation of the species of Rotifera. The professionals of course, but now even many advanced amateurs, have incorporated to their equipment not only the powerful Nomarski DIC microscopes, but also the photography with electronic flash.

Figure 2, of the first part, which we reproduce below by courtesy of its author, Charles Krebs (who with it inaugurated a new approach that surely will attract the efforts of many other amateurs) illustrates the magnificent results that augur this technique. We add other pictures by Krebs and Abel Lear, and others shown below, which confirm that.

The availability of flash, digital photography in hi-res, and the now popular programs for three-dimensional reconstruction (CombineZ5, CombineZM, Combine ZP, and Helicon Focus (a and b methods)), augur an important generalization of the capacity to investigate and to document this difficult group of microinvertebrates.

Amateurs have an important opportunity here to collaborate with the professionals (who have the suitable bibliography, and the scanning electronic microscopes) sending to them high quality descriptions, measurements, and detailed photos of the species they observe. And this can be made on a world-wide scale thanks to the aid of Internet overcoming the geographic barrier.

I hope that the insatiable curiosity of present and future members of the forums of microscopists, will produce an ample harvest of documents to make them available to the specialists.

Nevertheless, it is clear that, due to the exigencies of bibliographical availability, and access to electron microscopes, the specialists in the Universities and Research Institutes, will continue to be the ones who can identify with certainty the species of bdelloids, and while the number of those does not increase substantially, and the photomicrographic documents harvested by amateurs don't run fluently: "The biogeography of the Bdelloidea, (*also in general for all Rotifers – author's note)* will continue to be, really, the biogeography of the students of the group." (Ricci and Melone, 2002)"

To facilitate the contribution of amateurs to this discipline I add at the end a list of the more active specialists I know. I think that most of them would be glad to receive taxonomic novelties from all over the world.

SENDING SAMPLES - If the amateur does not feel himself able to accomplish the more difficult tasks, he or she can make a huge contribution by sending samples to the specialists. Remember you are working with species that (almost all) can survive after desiccation. Using a piece of laboratory filter paper (or coffee-makers filter paper) you can prepare a sample that could withstand the hazards of even the surface mail, and can be included in any mail envelope. THIS IS SIMPLE AND VALUABLE.

But remember that a researcher is a very busy person. Take care to contact first the specialist of your election to ask for their permission to send your materials, pictures and dehydrated samples.

If after seeing your notes, pictures and drawings, the scientist tells you that your material is of real interest, prepare a desiccated sample to send her or him some specimens.

How to prepare the sample

Concentrate as many as you can of the individuals in a little volume of water. Prepare a Petri dish, or a similar flat dish with a cover, with a piece of filter paper on the bottom. Lightly wet (not flood) the paper with some drops of distilled water. Deposit the sample with the living rotifers in the center, and cover, letting a thin open gap to allow evaporation. Dryness must be attained in 4 or 5 hours. Cut the dry filter paper to recover the area with the sample and put it inside a folded paper. Include this with the letter you send with data. Don't use plastic for the holding paper or the envelope. There is a good chance that the individuals in your sample can be easily recovered in the destination laboratory.

Even if you don't result being the happy parent of a new species, your sample could have zoogeographical and ecological interest. If you are doing that you are a very careful amateur, and of course you know that a full series of data must be sent with the sample.

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Ricci Claudia y Giulio Melone – **1998** – The Philodinavidae (Rotifera Bdelloide): A special family. *Hydrobiología* 385:77-85

Ricci Claudia, Russell Shiel, Diego Fontaneto & Giulio Melone– 2002 - Bdelloid rotifers recorded from australia with description of *Philodinavus aussiensis* n.sp**.**

Bdelloidea specialists: *In view of the possibility of annoyances to the specialists by mechanically sent spam, finding their e-mails addresses is left to the initiative of the advanced amateurs. Communication methods are mentioned in their bibliography, and most titles are published in Internet in PDF format.*

Caprioli, Manuela (Italy)

De Smeth, Willem (Belgium)

Fontaneto, Diego (Italy, UK)

Melone, Giulio (Italy)

Ricci, Claudia (Italy)

Schmid-Araya, J.M. (UK)

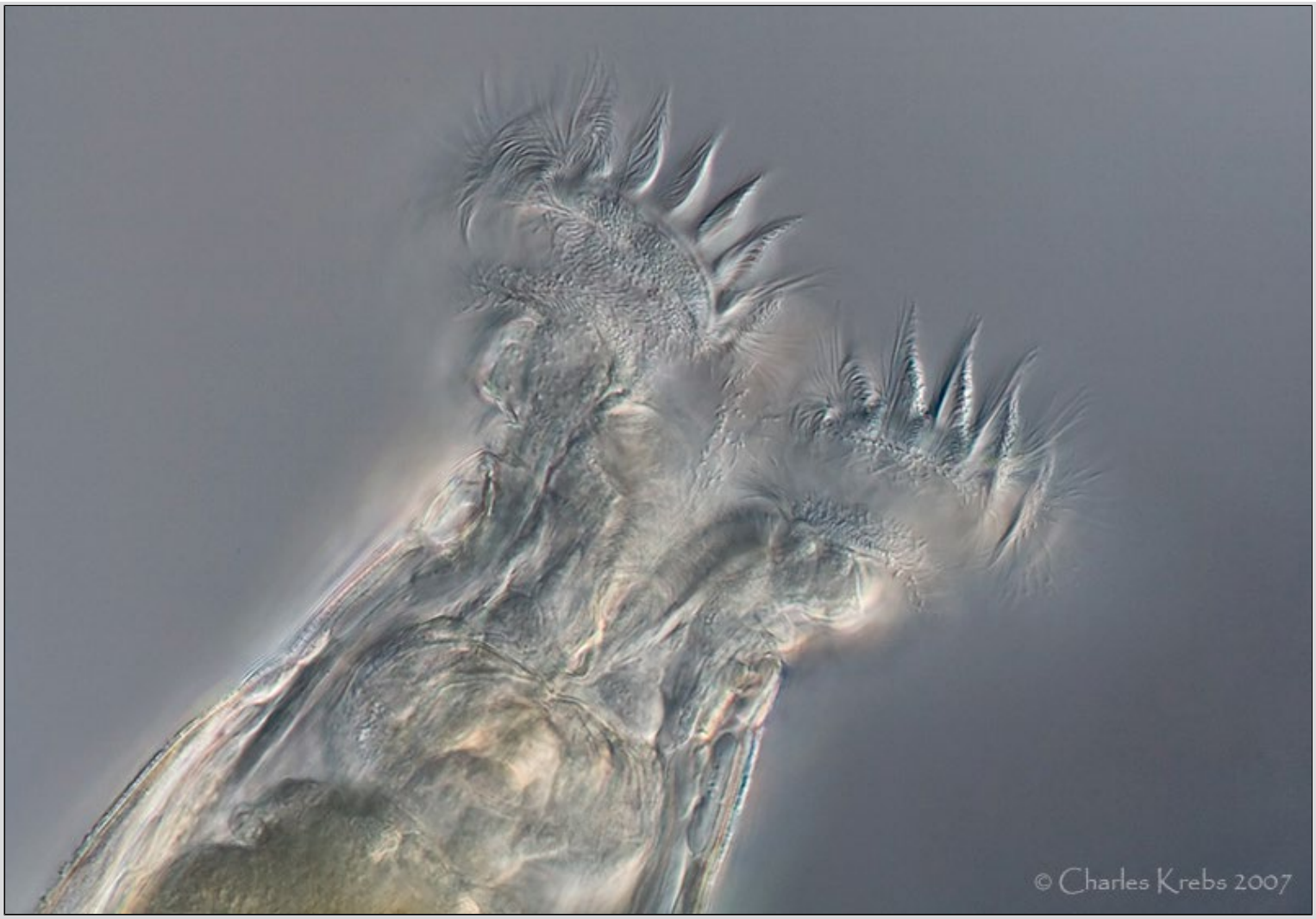
Segers, Hendrick (Belgium)

Song, M. O. (S. Korea)

Sarma, S.S.S. (Mexico)

Images

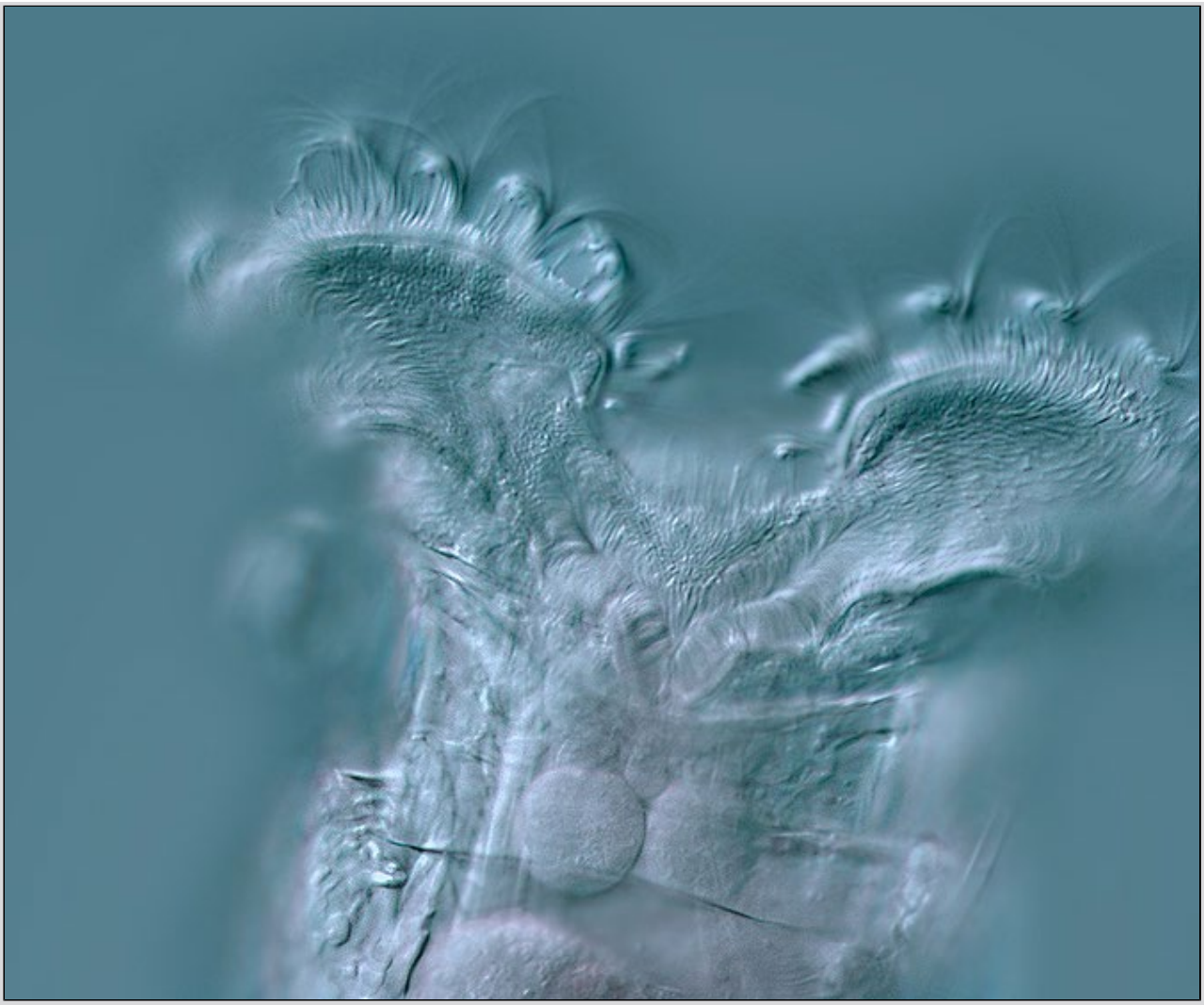
1 – eyes at the end of the rostrum in Rotaria, bright field – Oliver Barth



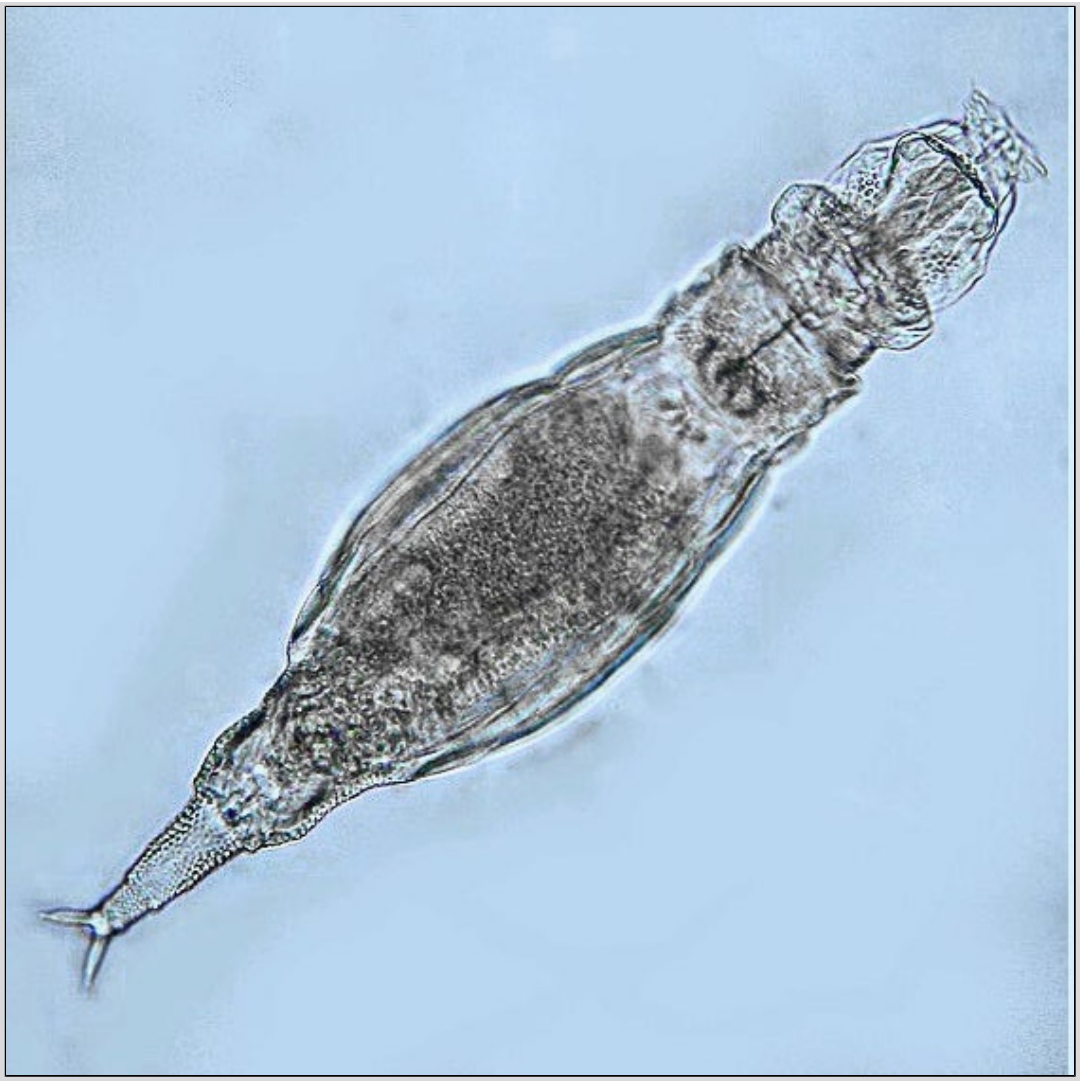
2 – head of Philodina – electronic flash – Charles Krebs

3 - another head of Philodina – bright field, electronic flash, by Charles Krebs, showing the red eyes

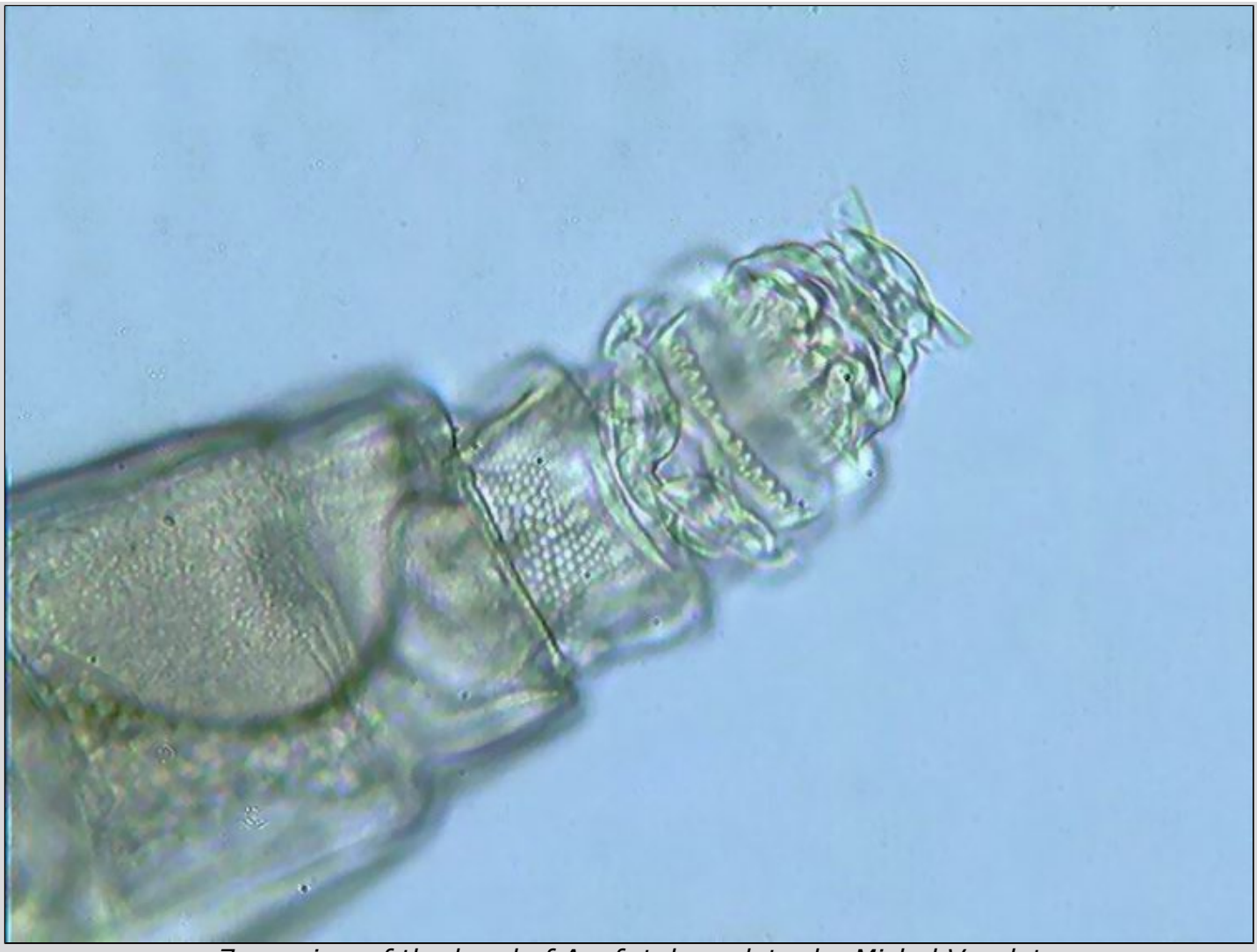
4 – Philodina megalotrocha – DIC – a picture by Abel Lear



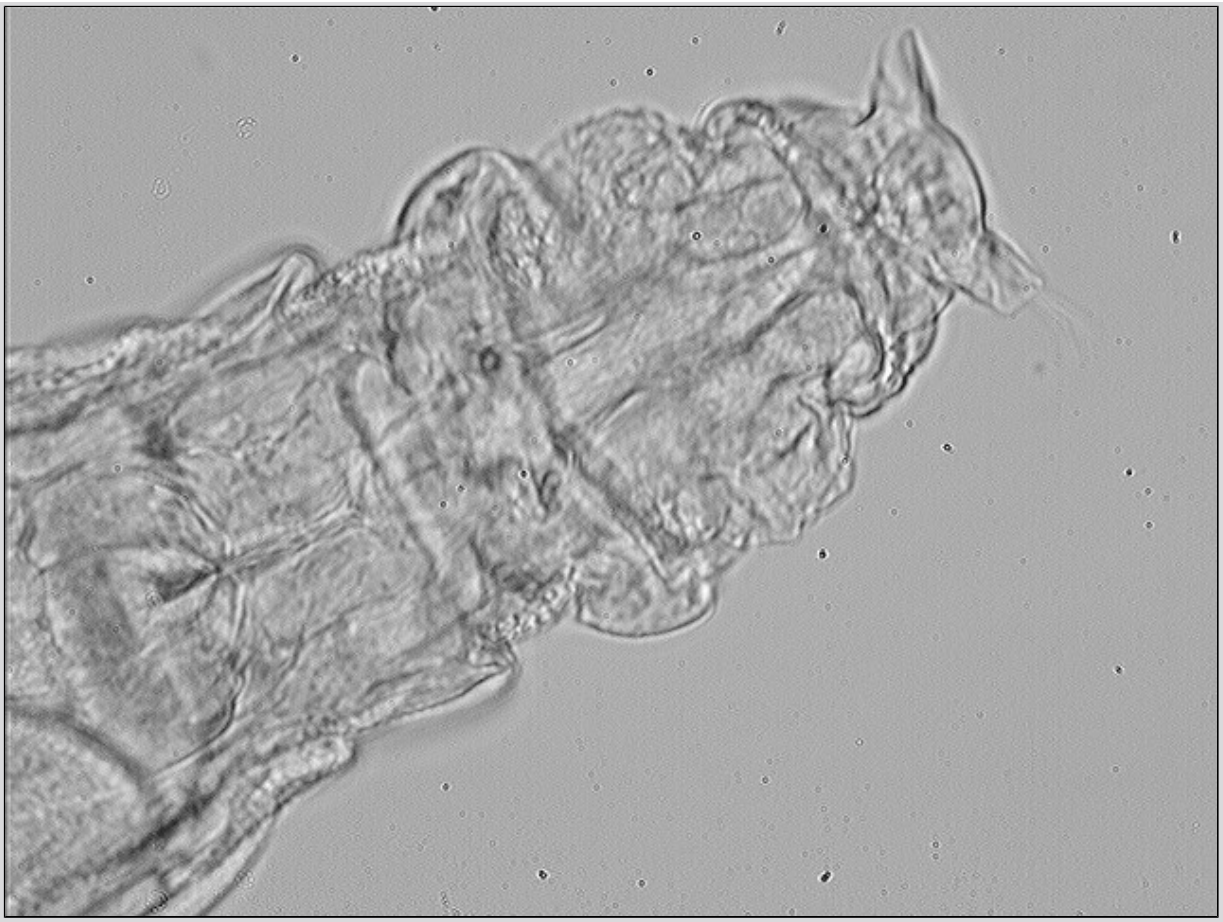
5 – Another DIC image of the head of a probable Philodina, by Abel Lear



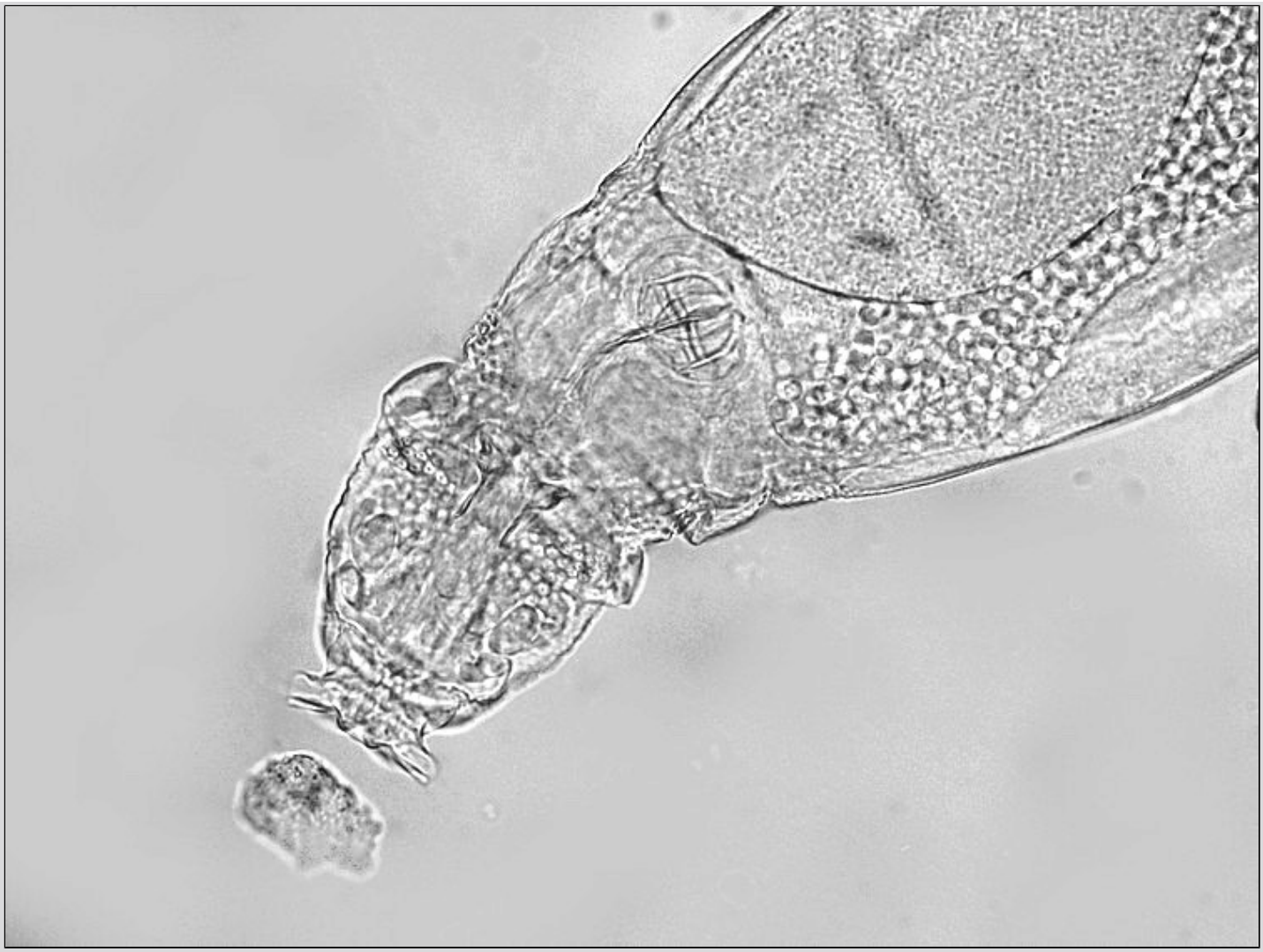
6 – Adineta cf. tuberculata, bright field, Michel Verolet



7 – a view of the head of A. cf. tuberculata, by Michel Verolet



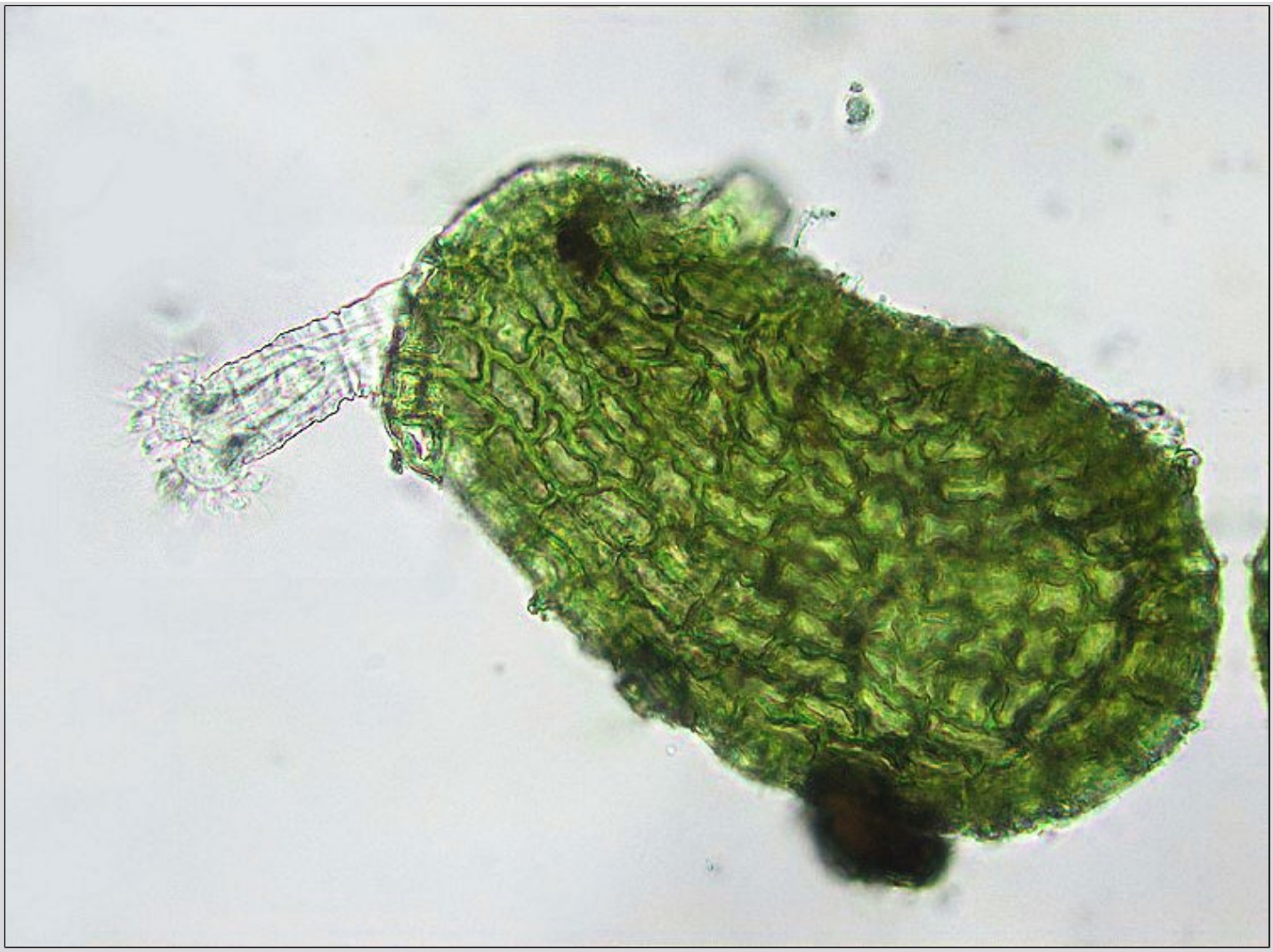
8 – dorsal view of the head of the same, bright field, Michel Verolet



9 – bright field at its best, detailed anatomy of the head of Adineta, showing the complex rostrum
Michel Verolet

10 – The new megapixel digital cameras allow detailed pictures of active animals, and even movies at a good resolution. Adineta, by M. Verolet

11 – Habrotrocha attached to a bryophyte, picture by M. Verolet



12 – one of the attached examples - a stretched Habrotrocha, M. Verolet

13 – Rotifer's eggs glued to an algae filament, M. Verolet

14 – One of the eggs, the ramate trophi confirm it is from a bdelloid

The author, Walter Dioni

Editor's Note: This three part article by the author was first published in French on the *Microscopies* Magazine website.

Footnote added March 13th 2009: Glutaraldehyde is not carcinogenic but considered an irritating and sensitising agent. In short expositions and even at low doses it produces irritation of the mucous and of the high respiratory tract, although in this respect its low vapour pressure must be considered (it must be remembered that its boiling point is near 200ºC). Skin irritation is only reported at concentrations above 0.05%, and sensitisation only above 0.1%. Long, or repeated, contact with the skin at higher concentrations, produce dermatitis and sensitisation. There is no agreement about whether prolonged and repeated inhalation can produce asthma.

Studies on genetoxicity, carcinogenicity and reproductive toxicity haven't shown positive results, neither in experimental toxicological nor epidemiological studies made in hospital workers.

With thanks to Aydin Örstan.

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Please report any Web problems or offer general comments to the Micscape Editor.

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