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Ralph O. Schill *Editor*

Water Bears: The Biology of Tardigrades

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Preface

Some of the most delightful hours of my scientific career have been spent studying tardigrades. And even after many years, I observe these little animals enthusiastically under my microscope. They are true survival artists that occur in the most diverse habitats on earth. I discovered them in dry desert regions, on glaciers, in rivers and lakes, in cold and tropical seas, and at my front door in the German forest.

Everything started with Johann August Ephraim Goeze, a protestant pastor in Quedlinburg, Germany. He noted that in his hours of rest he preferably refreshed himself by the microscopic examination of the boundless wealth of nature in the water. And that led to the first discovery and description of a small animal which looked similar to a little bear. Therefore, he gave him the name "kleiner Wasserbär" (small water bear) in 1773.

A continuously growing number of scientists became interested over centuries in these fascinating animals, but there are no comprehensive summaries of their history, morphology, phylogeny and taxonomy, biogeography, paleontology, cytology, ecology, and adaptive strategies. The idea for this comprehensive tardigrade book was born during the 11th International Symposium on Tardigrada in 2009 at the University of Tübingen, Germany. This symposium series goes back to the inaugural meeting in Pallanza, Italy, in 1974. Subsequent meetings were held every three years in various locations around the world.

I'm extremely grateful to my friends and colleagues who immediately agreed to work on this important task, to share ideas, provide references, and review chapters. Therefore, I would like to take this opportunity to thank all the authors named below in the order of the chapters: Hartmut Greven, Nadja Møbjerg, Aslak Jørgensen, Reinhardt Møbjerg Kristensen, Ricardo C. Neves, Sandra J. McInnes, P. J. A. Pugh, Roberto Guidetti, Roberto Bertolani, Lorena Rebecchi, Diane R. Nelson, Paul J. Bartels, Noemi Guil, Tiziana Altiero, Atsushi C. Suzuki, Steffen Hengherr, K. Ingemar Jönsson, Eliana B. Levine, Andrzej Wojcik, Thomas C. Boothby, and Peter Degma. I also wish to thank our project coordinator at Springer, Andrea Schlitzberger, for her support, encouragement, and patience. It has been a pleasure to work with her.

Lastly, my deepest thanks go to the families of the authors, for their support, and especially for their help and understanding during the time this comprehensive and at the moment unique book about tardigrades evolved. I hope this work will serve as a useful guide for everyone interested in these fascinating animals.

Tübingen, Germany Ralph O. Schill
2018

Contents

Chapter 1
From Johann August Ephraim Goeze to Ernst Marcus: A Ramble Through the History of Early Tardigrade Research (1773 Until 1929)

Hartmut Greven

There is a time for everything…
Ecclesiastes 3:1

Abstract A survey is presented about the early history of tardigrade research spanning the time from 1773, when the first description of a tardigrade was published by Goeze, until 1929, when the most comprehensive monographic approach by E. Marcus, unsurpassed today, was published. Almost from the beginning, two topics dominated "tardigradology", i.e. phylogeny and systematics as well as cryptobiosis, especially anhydrobiosis, but also other issues (e.g. morphology, development and life history) have followed successfully with ongoing technical and preparatory improvements.

1.1 Introduction

Occasionally it seems useful to remember the origins and pioneers of scientific disciplines, not to look amused from our present point of view on their curious views or their ignorance (this does happen not only to ancient authors like Aristotle[1]

[1]**Aristotle** (Gr. Aristotélēs; 384–322 BC), Greek philosopher, who joined Plato's Academy in Athens. His writings cover physics, biology, zoology, metaphysics, logic, ethics, aesthetics, poetry, etc. He was the teacher of Alexander the Great (356–323 BC). Aristotle is the earliest natural historian, whose work has survived, among others his writings on natural science such as (translated

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or Pliny the Elder[2]), but to acknowledge their work, their forward-looking ideas, often completely forgotten today, sometimes consciously ignored (however, nobody will and can go back to Aristotle with his citations) or distributed by succeeding researchers as their own ideas, and last but not least to understand their errors caused by a general poor knowledge of the matter at the time, by insufficient techniques or by ideologically narrow-minded views.

Tardigrada, which belong to the so-called minor phyla, were known for decades only to a small group of insiders commonly called tardigradologists.[3] Nevertheless, the early studies in tardigradology[4] have quite a few surprises in store. From the few surveys on the history of tardigrade research, the summarizing chapter in Ernst Marcus's[5] remarkable monograph on Tardigrada published in 1929 is still the most concise, critical and informative overview (see Marcus 1929b). Thereafter limited historical information can be found in specific monographs (e.g. Cuénot[6] 1924; Greven 1980; Maucci 1982; Ramazzotti and Maucci 1983; Kinchin 1994) and more recently on various websites. Due to general interest, early studies on tardigrades have also been reviewed repeatedly in the literature on anabiosis,[7] in particular detail by Keilin (1959) and less detailed by Wright et al. (1992) and Rebecchi et al. (2007).

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into Latin) the "Historia animalium" [History of animals], "De generatione animalium" [On the generation of animals] and "De partibus animalium" [On the parts of animals].

[2]Gaius **Plinius Secundus** (Pliny the Elder) (ca. 23–79 AD), Roman naval and army commander, as well as author and naturalist, wrote an encyclopaedia entitled "Naturalis Historiae" [On Natural History] that comprises 37 books, into which he collected much of the knowledge of his time.

[3]Commonly assigned to people predominantly concerned with research on tardigrades. However, at all times knowledge of tardigrades has been promoted by people with very different backgrounds and various provenances including "heroes" of natural sciences such as L. Spallanzani, laymen and even people, who never have seen a living tardigrade (especially in modern times, in which sophisticated methods need the experience of specialists). Most researchers mentioned in the present essay were engaged in studying tardigrades only for a certain period of their life, often only during their doctoral dissertation and sometimes only along the way.

[4]Composed of the Latin words "tardus" (= slow) and "gradi" (= to walk) and the ancient Greek lógo*s* (= word, study, research). The term Tardigrada comes from Spallanzani's "il tardigrado" (= sluggard). See footnote 15.

[5]Ernst Gustav Gotthelf **Marcus** (1893–1968), German zoologist, worked closely with his wife Eveline Du Bois-Reymond (1901–1990), granddaughter of the famous physiologist Emil Heinrich **Du Bois-Reymond** (1818–1896). Due to his Jewish origin, Ernst M. moved in 1936 from Berlin to Brazil and became professor at the University of São Paulo, where he taught and researched for 37 years (until 1963). Eveline M. did all the drawings for the publications of her husband and her own work. Ernst Marcus's research covered a very wide spectrum of topics and organisms. In his doctoral thesis, he dealt with lamellicorn beetles.

[6]Lucien Claude Jules Marie **Cuénot** (1866–1951), French biologist, zoologist and geneticist. In 1898 he became chair of zoology in Nancy (France).

[7]The term anabiosis (aná, Gr. = upwards; bíos, Gr. = life) was introduced by Preyer (1880, 1891) (see note 89). Keilin (1959) replaced this term with cryptobiosis (kryptós, Gr. = hidden) to describe a specific type of hypobiosis (hypó, Gr. = under), viz. "the state of an organism when it shows no visible signs of life and when its metabolic activity becomes hardly measurable, or comes reversibly to a standstill" (p. 166). He distinguished cryobiosis (krýos, Gr. = ice) induced by cooling, anhydrobiosis (a(n), Gr. = a denial prefix; hýdor, Gr. = water) induced by loss of water, osmobiosis

Initially, I planned to give an overview of the history of tardigradology at least until the First International Symposium on the Tardigrada. This Symposium, held June 17–19, 1974, at the Istituto Italiano di Idrobiologia in Pallanza (Italy) in honour of Giuseppe Ramazzotti[8] on the occasion of his 75th birthday, marked a turning point in tardigradology. After that tardigradologists began to organize themselves and to arrange further symposia (see Bertolani and Nelson 2011). However, a presentation even of this period would result in a simple listing of what has been done. I therefore dismissed this idea in favour of the present essay, which attempts to delineate the history of tardigrade research from the first published description of a water bear by Goeze (see Figs. 1.1 and 1.2 top left) to the above-mentioned monograph by Marcus (see Figs. 1.1, bottom right, and 1.23) by means of examples (in part subjectively selected) from the literature. It was also my intention to familiarize the reader with a period in which tardigradology was primarily a European matter and in which results were published in various languages, with which many contemporaries may be unfamiliar. Early studies outside of Europe were restricted to finding, describing and naming of species (α-taxonomy) and were performed largely by European tardigradologists. However, the first mention of tardigrades written by a US scientist was previously published in 1850 (Bailey 1850).[9]

Further, I included several "historical" images of tardigrades and their organization to give the reader an impression of how differently and subjectively these animals were visualized, whether or not authors had an appropriate microscopy equipment. I excluded those articles with pure α-taxonomy and local faunas, which only have increased the number of species of already known taxa, as well as the treatment of tardigrades in text books of zoology and popular books. These subjects may be worth investigating separately.

Undoubtedly, Marcus's monograph is a milestone in tardigradology that, apart from the valuable and critical overview of previous literature, includes observations not published elsewhere and provides important ideas for future research in nearly all fields of tardigradology. Strictly speaking, the present essay follows closely the chapter "Geschichte der Tardigradenforschung" [History of Tardigrade Research] in this monograph. However, some parts I have shortened and others I have

(osmós, Gr. = impulse) induced by high levels of concentration and anoxybiosis (oxýs, Gr. = acidic) induced by absence of oxygen or any combination of these factors. Wright et al. (1992) suggested not to restrict the term cryptobiosis to organisms surviving in an ametabolic state (as Keilin did), but "to define cryptobiosis as a collective term for those quiescent states in which metabolism may be reversibly arrested" (p. 22) and to exclude anoxybiosis and encystation, since both "qualify for quiescence but not cryptobiosis" (p. 23).

[8]Giuseppe **Ramazzotti** (1898–1986), Italian engineer, enthusiastic collector of pipes, began research of tardigrades in 1938 and presented in 1962 a comprehensive review of the literature focussing on the description and determination keys of the known species. The second edition was published in 1972 and the third (with W. Maucci as coauthor) in 1983.

[9]Jacob Whitman **Bailey** (1811–1857), US American naturalist, professor of chemistry, mineralogy and geology at West Point, pioneer in microscopic research in America. He corresponded with Ehrenberg (see footnote 46). Bailey noted in 1850 "The waters in which I detected the species above recorded, also abounded in many other forms of microscopic life; as, Entomostraca, Tardigradi, Anguilluli, &c., &c. Of these I have made no record, as I did not possess sufficient knowledge concerning them." (p. 44).

Fig. 1.1 Portraits of influential scientists studying tardigrades. Top left: Johann August Ephraim Goeze (1731–1793), painting attributed to Fiedrich Schlüter from around 1780. With kind permission of Gleimhaus Halberstadt—Museum of German Enlightenment. Top right: Lazzaro Spallanzani (1729–1799). http://ihm.nlm.nih.gov/images/B24049. Bottom left: Karl August Sigismund Schultze (1795–1877), painting by Wilhelm Titel from 1837. From Schmitt and Schultze (1931). Bottom right: Ernst Marcus (1893–1968), drawing by JB Santos in 1981 according to an earlier photo. With kind permission of the Department of Zoology, Institute of Biosciences, University of São Paulo

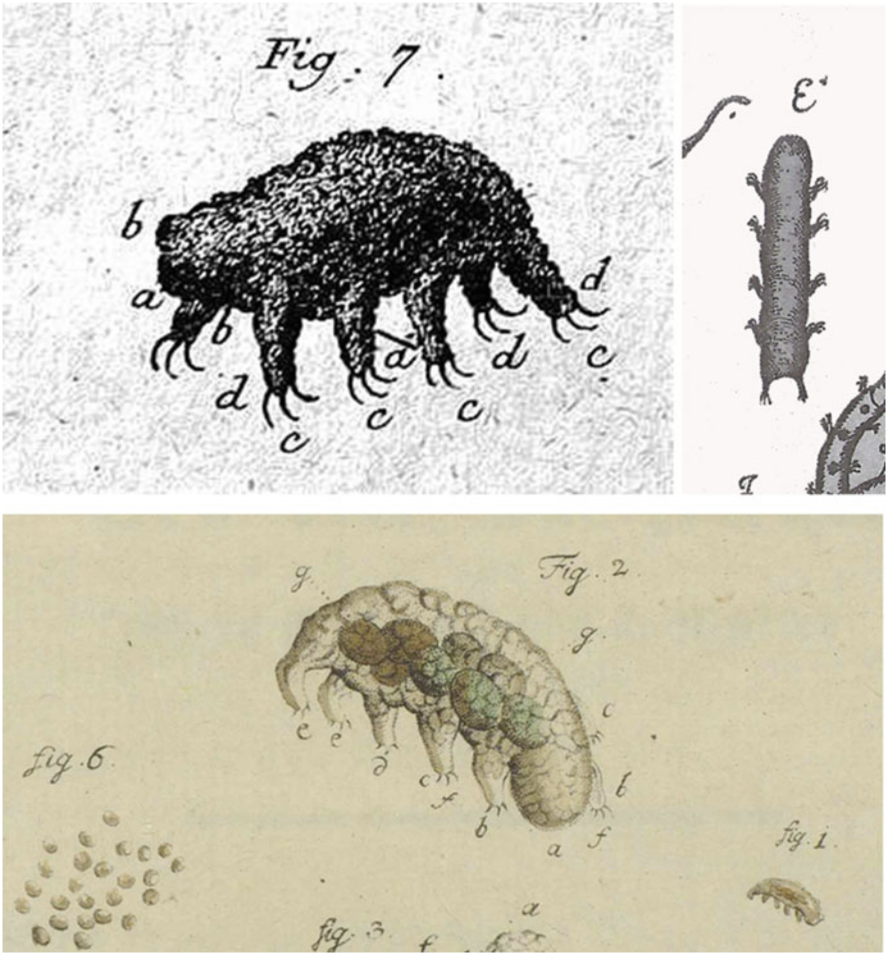

Fig. 1.2 Early pictorial presentations of tardigrades. Top left: The little water bear. From Goeze (1773), section from Plate 4 in the appendix of the second part. Top right: The water bear. From Eichhorn (1775), Plate 7, fig. E. Bottom: The bear animalcule. From Müller (1785), section from Plate 36 (with kind permission of the Zentralbibliothek Zürich Scan NNN827)

extended. For that I consulted again the original sources, as available, and, contrary to Marcus, provided herein some literal citations from the texts of earlier authors including the English translation (mostly in endnotes), to give the reader an idea why tardigradologists of earlier times were fascinated about these microscopically small life forms and how they studied them.

To keep this essay reasonably short, I decided not to describe the current state of knowledge. Only in a few cases do I refer to articles published after 1929. Recent findings that may modify or disprove the interpretations and conclusions of the data reported herein are, at least in part, summarized in the following chapters of the present book.

The endnotes also contain some explanations (including etymological ones) and biographical data of the researchers quoted (primarily year of birth and year of death, research activity and main profession). The latter were collected by means of online encyclopaedias (not cited) and checked in some cases with other sources (cited, but some only in the endnotes). In some cases, detailed bibliographic data could not be ascertained.

Besides α-taxonomy, which started, albeit with a certain delay, soon after the first discovery of tardigrades and which continues unabatedly, tardigrade research was dominated almost from the beginning by two topics, i.e. (1) anabiosis or abiosis or, the more modern term, cryptobiosis and here especially anhydrobiosis (the ability many tardigrades share with other organisms such as the metazoan taxa rotifers and nematodes, in which this phenomenon was detected earlier than in tardigrades[10]) and (2) possible relationships to other organisms; almost every author has discussed relationships broadly recapping previous opinions either to endorse or to modify them or to work out new proposals. These two subjects occasionally appeared to overshadow other findings such as functional morphology, development and life history data, which have suffered from the lack of adequate equipment and techniques for a long time.

In the following text, individual sections deal with arbitrarily specified periods. It would certainly be useful to further subdivide these sections into specific subject matters, but I preferred to present information in chronological order, so far as possible, to avoid too much repetition, as earlier authors often covered more than one subject in their articles. I have retained the species names originally used by the authors, although most have changed in the course of time. Moreover, many could not be assigned to a specific species by later researchers.

1.2 The Early Findings (Eighteenth Century)

The first mention and descriptions of tardigrades (only eutardigrades) were published in quick succession within a period of 9 years (Goeze 1773; Corti 1774; Eichhorn 1775; Spallanzani 1776; Müller 1785; the latter note was published posthumously). Considering (1) that publications needed a certain lead time, (2) that the authors had different backgrounds—Eichhorn[11] was an amateur scientist

[10]This phenomenon was known in rotifers observed by the Dutch tradesman, amateur scientist and "father of microbiology" Antonie Philips **van Leeuwenhoek** (1632–1723) (see Leeuwenhoek 1702) and in nematodes observed by the English biologist and Roman Catholic priest John Turberville **Needham** (1713–1781) (see Needham 1743).

[11]Johann Conrad **Eichhorn** (1728–1790), German protestant pastor and amateur researcher, who described microorganisms including a tardigrade in the moat and other water bodies near his home city Danzig, now Gdansk (Poland). In 1775 he published the booklet "Beyträge zur Natur-Geschichte der kleinsten Wasser-Thiere" [Contributions to the natural history of the smallest aquatic animals], primarily for lovers of natural history, which was reprinted in 1781.

throughout his life and Goeze[12] was just at the start of his career as a respected zoologist[13] (see Greven 2015), whereas Corti,[14] Spallanzani[15] and Müller[16] were trained scientists—(3) that authors probably did not know each other (only Müller cites Spallanzani and Eichhorn) and (4) that both Eichhorn and Müller claimed that they had seen a water bear long before they published their observations (see Greven 2015; unpublished manuscript), it is most likely that tardigrades have been independently discovered several times. However, formally Goeze is the first describer of a tardigrade, probably a *Hypsibius* species (Goeze 1773; see Fig. 1.2 top left). This description was published under the heading "Von einigen merkwürdigen Wasserinsekten" [On some strange water insects] in an appendix of the German translation of Bonnet's[17] "Traite d'Insectologie". The appendix contains personal observations made by Goeze in the environment of his hometown Quedlinburg. Goeze underlined the rarity of this animalcule (later he qualified this statement; see Müller 1785) and compared its appearance with that of a bear, giving it the name "water bear"[18]; his illustration reflects this bearish appearance, somewhat exaggerated (see Fig. 1.2 top left). Goeze found water bears in duckweeds (especially in winter), mistook the pharynx for an "egg sac", saw the floating body cavity cells, considered the lateral folds as tracheae and was surprised at the eight short feet,

[12]Johann August Ephraim **Goeze** (1731–1793), protestant pastor in Quedlinburg (Germany). He began his career as a translator of various scientific and philosophical works and became a highly respected and productive zoologist, especially helminthologist, only in the last third of his life. The description of the little water bear is considered as one of the first steps of an interested amateur. http://www.deutsche-biographie.de/ppn116745509.html. For further details see Greven (2015).

[13]"In meinen Erholungsstunden erquicke ich mich am liebsten durch mikroskopische Betrachtungen der unermesslichen Reichtümer der Natur im Wasser" [in my hours of rest I preferably refresh myself by the microscopic examination of the boundless wealth of nature in the water] (Goeze 1773, p. 361).

[14]Bonaventura **Corti** (1729–1813), Italian botanist, studied under L. Spallanzani (see Footnote 15), detected the plasma flow and investigated stoneworts (Characeae), jelly fungi (*Tremella* sp.), rotifers, ciliates and their reviviscence after dehydration (see footnote 20).

[15]Lazzaro **Spallanzani** (1729–1799), Italian polymath, catholic priest and naturalist, studied bodily functions, animal reproduction, echolocation, biogenesis, preservation of food, generation of microbes, etc. Stages in his life were lived in Reggio (professor of logic, metaphysic, Greek), Modena and Pavia (chair of natural history and director of the museum). "No biological phenomenon of any interest ever escaped the attention of Spallanzani (...)" (Keilin 1959, p. 153).

[16]Otto Frederick **Müller** (1730–1784), Danish naturalist (botanist and zoologist), studied later primarily Infusoria (= collective term for minute aquatic organisms including Protozoa and small "invertebrates"); he used for the first time Linné's binomial classification.

[17]Charles **Bonnet** (1720–1793), Swiss naturalist and philosopher, detected as a young man parthenogenesis in aphids and wanted to name the science of insects "insectology" (s. also Goeze 1773, p. 39).

[18]"Seltsam ist dieses Thierchen, weil der ganze Bau seines Körpers ausserordentlich und seltsam ist, und weil es in seiner äusserlichen Gestalt, dem ersten Anblicke nach, die gröste Aehnlichkeit mit einem Bäre im Kleinen hat. Das hat mich auch bewogen, ihm den Namen des kleinen Wasserbärs zu geben". [Strange is this little creature, because the whole organisation of its body is extraordinary and strange and because of its external appearance. At the first glance, has the closest similarity to a little bear. This also led me to give it the name little water bear.] (Goeze 1773, p. 368).

where he noticed three sharp, curved claws. Further he saw the exuviae with developing eggs and compared the reproduction of the water bear with that of the green freshwater alga *Volvox*.[19] Eichhorn's description and figure of the water bear with ten legs (Fig. 1.2 top right) do not compare with Goeze's presentation; the drawing and description are poor and virtually worthless and, therefore, are omitted here (for the translation of Eichhorn's text, see Greven 2015).

The next researchers to be acknowledged are the Italian scientists Corti and Spallanzani. Corti observed anhydrobiosis in jelly fungi, rotifers and some other organisms and Spallanzani in rotifers and nematodes. Both researchers occasionally observed tardigrades during their studies. Although not depicted and insufficiently described, the small caterpillars ["brucolini"] Corti (1774) found in rain gutters are most probably tardigrades.[20] Thus, Corti was the first who discovered anhydrobiosis in tardigrades; he wondered whether anhydrobiotic animals were dead or not, but he fully agreed with his academic teacher Spallanzani, who believed them to be dead.[21]

Spallanzani (1776) also studied tardigrades from the sandy substrate of the gutters; he compared their movement with that of a tortoise and called this animal "il Tardigrado". He described the animals with six (!) legs (considering the posterior pair of legs to be hooked filaments), noticed the position of the midgut and the pharyngeal bulb that he called the oesophagus but failed to breed them. Curiously, he compared its overall appearance with the testicle of a cock. The figures—Spallanzani employed an illustrator—are far below the quality of his observations (see Fig. 1.5). Further, he accurately described how tardigrades enter anhydrobiosis, pointing out

[19]"Sie hatten die größte Aehnlichkeit mit der Lage und Gestalt der sogenannten jungen Kugelthiere, die auf eben die Art in den Alten eingeschlossen sind". [Concerning overall appearance, they had the greatest similarity with the so-called globular animalcules, which are encased in the old ones in just the same way] (Goeze 1773, pp. 374/375).

[20]"Intorno al risorgere, questa proprietà è singolare, ma non è delle sole Tremelle: è gia stata osservata in altri animali, e in altri piante ... Anch'io in bagnando della polvere delle grondaje ho veduto tornare a vita non solamente i *rotiferi*, ma ancora certi animaluzzi, cui ho chiamati i *brucolini* della polvere delle grondaje, a cagione di qualche somiglianza, che hanno coi bruchi;..." [With regard to reviviscence, this property is not only unique to the jelly fungi (this was considered in detail on the previous pages; translator's note), but was also observed in other animals and plants already....I also saw in the dust of the gutter I had moistened, not only reviving rotifers, but also certain creatures that I have called the caterpillars of the gutter dust, because of some similarity they had with caterpillars.] (Corti 1774, p. 97).

[21]"Il punto più rilevante si è quello di decidere se le Tremelle, e gli accennati animalucci risorgenti sieno veracemente morti, oppure soltanto in apparenza: poi supposta la morte verace, come accada il loro risorgimento. Il Sig. Abate Spallanzani è di opinione, che il *rotifero*, e gli altri animaluzzi, i quali seccati in pria ritornano agli usati movimenti per mezzo dell'acqua, sieno rigorosamente morti, e ne soggiugne prove degne di lui, che è quanto dire da bravissimo filosofo naturalista". [The most relevant point is to decide whether the jelly fungi and the resurging little creatures in question are in fact truly dead, or only seem to be dead: assuming that they are truly dead, how does it happen that they come to life again? Signor Abate Spallanzani thinks that the rotifer as well as other little animals, which have been previously desiccated and are thus definitely dead, obtain their previous ability to move through contact with water. And he adds proofs that are worthy of his reputation as an exceptional natural philosopher.] (Corti 1774, p. 97/98).

that dehydration should be gradual to survive[22] (this observation has been ignored for a long time or has been forgotten), and emphasized that these animals cannot desiccate any number of times. The secondary (summarizing and rather popular) literature of this time appeared to accept this interpretation by writing that the sloth belongs to those animals that suffer death but are able to rise from the dead several times (Senebier 1795).[23]

The well-illustrated note (Fig. 1.2 bottom) on the "Bärthierchen" [bear animalcule] by Müller (1785), obviously published posthumously, contains corrections of Goeze's folds (tracheal tubes) and Eichhorn's descriptions (number of legs) and points out the similarity with Spallanzani's "sloth". Among other things Müller identified eggs in the animal, observed egg deposition during moulting, noted that the shed cuticle may protect the developing offspring, observed that bear animalcules are herbivores feeding on disintegrated duckweeds and vividly described their clumsy movements.[24] Müller was the first who classified tardigrades and assumed

[22]"I fenomeni del morire, mancando l'acqua, e del risorgere, sosituendone della nuova, succedono nel Tardigrado al modo sesso, che nel Rotifero. Il moto in lui via via si va perdendo, le gambe ſi ritirano, e s' internano totalmente dentro del corpo, questo rimpicciolisce assaissimo, si secca affatto, ed acquista forma globosa (…). Ed il contrario del fin quì narrato accade vivificando il Tardigrado con acqua novella. E siccome il Rotifero è limitato nelle volte, che può risorgere, così interviene al Tardigrado. Sembra però questo portarsi meglio in ciò, che quantunque l'arena conferisca per gran maniera al suo risorgere, non è però sì strettamente richiesa, come veduto abbiam nel Rotifero.

Que' gradi di calore, che son fatali ai Rotiſerí risorti o da risorgere, lo sono ai Tardigradi, e lo stesso vuol dirsi degli odori, e dei liquori. Il freddo all'oppoſito, per quantunque aspro che sia, nulla può contra di essi, onde anche in ciò si accordano coi Rotiferi". (Spallanzani 1776, p. 225/226).

[The phenomena of its death, from the want of water, and of resurrection when water is supplied, are precisely the same with those of the wheel animal. Motion gradually ceases: the limbs are contracted and drawn entirely within the body, which diminishes very much, is completely dried, and assumes a globular figure, … The reverse succeeds when the sloth is revived by supplying water. As the wheel animal can only revive a certain number of times, so it is with the sloth. And, although sand is necessary for its resurrection, it does not appear as essential as for the wheel animal.

The degrees of heat, fatal to revived or dead wheel animals, are also fatal to sloths; and the same must be said of odours and liquors. Cold, however intense, does them not harm, and in this they likewise coincide with wheel animals] (Spallanzani 1803, pp. 162–163; translated by J. G. Dalyell).

[23]Jean **Senebier** (1742–1809), Swiss reformed pastor, naturalist and bibliographer, studied vegetable physiology, mainly the influence of light on vegetation. The above quoted statement is to be read in the German translation (from French) "…die, wie das Kugelthier, das Räderthier, das Faulthierchen (le Tardigrade) den Tod leiden und mehrmals wieder auferstehen können". […which, as the globular animalcule, the wheel animals, the little sloth, meet death and are able to rise again several times] (Senebier 1795, p. 41/42).

[24]"Der kleine Bär ist ein schwerfälliges, kaltblütiges und sanftes Thierchen, er lässet die Mitbewohner seines Tropfens mit gleicher Gleichgültigkeit als der Löwe das Hündgen um und an sich fahren". [The little bear is a clumsy, cold-blooded and gentle animalcule; he let himself be touched by the occupants of his drop with the same indifference as the lion the dog] (Müller 1785, p. 28).

their relationship with mites[25]; mites were considered as insects at that time, i.e. tardigrades were included into the much later created taxon Arthropoda.[26] Müller (l.c.) gave the bear animalcule the binomen *Acarus ursellus*[27] and added a short Latin diagnosis.[28] The binomen and some further remarks were included under the heading insects (mites) in the 13th edition of Linné's "Systema Naturae" (Linné 1790; Fig. 1.3).[29]

As a side note, Dalyell,[30] the translator of Spallanzani's "Opuscoli" (see Spallanzani 1803), noticed in his comments on the translation that he also observed a few sloths, similar to caterpillars. He considered them as new species and named them *Tardigradus Octopdalis* (sic!), i.e. the tardigrade with eight legs, and *Tardigradus Italicus* (see Spallanzani 1803) but qualified this statement in an added note after he had read Müller's article (see Greven 2015).

In brief, at the threshold of the nineteenth century, a few tardigrades (exclusively eutardigrades) were known. One species received a binomen that was included in the 13th edition of Linné's "Systema Naturae" (Fig. 1.3). Further, anhydrobiosis of tardigrades and some important parameters that enabled the animals to withstand dehydration were described, but generally anhydrobiotic animals were considered dead.

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[25]"...den Milben, die in die Klasse der Insecten gehören, kömmt es in der Gestalt, der Zahl und dem Gebrauch seiner Füße am nächsten" [Concerning the general appearance, and the number and use of his feet, he is most similar to mites that belong to the class of insects] (Müller 1785, p. 26).

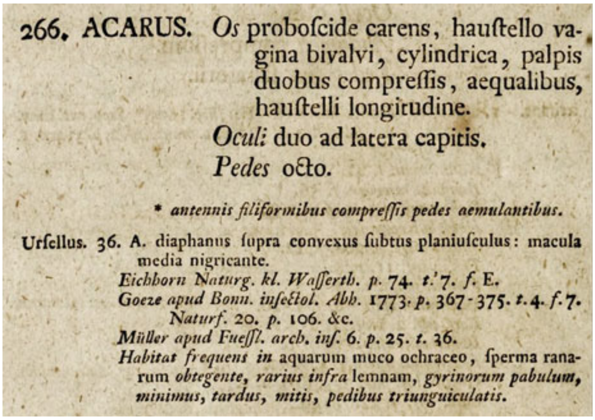
[26]The term "Arthropoda" was used for the first time in 1848 by the German physiologist and zoologist Karl (Carl) Theodor Ernst **von Siebold** (1804–1885) in his "Lehrbuch der Vergleichenden Anatomie der wirbellosen Thiere" [Textbook of Comparative Anatomy of Invertebrates]. Arthropods included the classes Crustacea, Arachnida and Insecta. Tardigrades were considered as arachnids: "(...) die Tiere sind wohl nirgends passender unterzubringen als gerade hier, nur müssen sie obenan gestellt werden, da sie den Uebergang von den Arachniden zu den Annulaten bilden" [...the animals may be placed nowhere better than just here, but they must be placed at the top, since it forms the transition from the arachnids to Annulaten more appropriate than just here, but they must be placed first as they form the transition from arachnids to annelids] (von Siebold 1848, p. 506). According to Haeckel (1896, p. 597), the full separation of the annelids and arthropods was "einer der größten Rückschritte der neueren Systematik und hat 30 Jahre hindurch das Verständniss des Articulaten-Stammes in hohem Maasse erschwert; (...)" [was one of the biggest steps backwards of the recent systematics and has extremely complicated the conception of the phylum Articulata for 30 years]. See footnote 130.

[27]ácari, Gr. = mite; ursellus is the diminutive of úrsus, Lat. = bear.

[28]"Dieses in den allgemeinen Thiergeschichten noch nicht angeführte Thierchen kann mit folgenden Namen bezeichnet werden. Acarus Ursellus corpore rugoso, pedibus conicis" [This animalcule, not yet recorded in general histories of animals, can be described with the following words. Acarus Ursellus with wrinkled body and conical feet] (Müller 1785, p. 30).

[29]Carl **Linné** (Carolus Linnaeus), after his ennoblement in 1761. Carl von Linné (1707–1778), Swedish botanist, professor of medicine and botany at the University of Uppsala (Sweden), was the inventor of the binomial system. The first edition of his "Systema Naturae" was published in 1738. Apparently there were 13 editions, of which Linnaeus wrote only 5 (Usinger 1964).

[30]Sir John Graham **Daylyell** (1775–1851), Scottish antiquary and naturalist, translated Spallanzani's "Opuscoli" from Italian to English and provided the second translated edition with comments and his own observations (see Spallanzani 1776, 1803).


266. ACARUS. *Os* proboſcide carens, hauſtello vagina bivalvi, cylindrica, palpis duobus compreſſis, aequalibus, hauſtelli longitudine.
Oculi: duo ad latera capitis.
Pedes octo.

* *antennis filiformibus compreſſis pedes aemulantibus.*

Urſellus. 36. A. diaphanus ſupra convexus ſubtus planiuſculus: macula media nigricante.
Eichhorn Naturg. kl. Waſſerth. p. 74. *t.* 7. *f.* E.
Goeze apud Bonn. inſectol. Abh. 1773. *p.* 367-375. *t.* 4. *f.* 7. *Naturf.* 20. *p.* 106. &c.
Müller apud Fueſſl. arch. inſ. 6. *p.* 25. *t.* 36.
Habitat frequens in aquarum muco ochraceo, ſperma ranarum *obtegente, rarius infra* lemnam, *gyrinorum pabulum, minimus, tardus, mitis, pedibus triunguiculatis.*

Fig. 1.3 Page 2924 from the 13th edition of Linné's "Systema Naturae" (1790) showing the entry of the water bear *Acarus ursellus* ([Ursellus 36. translucent *Acarus* on the dorsal side convex, on the ventral side slightly flattened: with a blackish spot in the middle. Habitat: Common in the yellowish slime covering the sperm of frogs in water bodies, rarer under *Lemna* (duckweed), food for the tadpoles, very small, slow, peaceful, with three-clawed feet]. This is exactly what one can read in Müller (1785) and the notes added to this article by Goeze)

1.3 Moves in Various Directions: The First Half of the Nineteenth Century

In 1803 Paula von Schrank[31] briefly introduced a new species, *Arctiscon*[32] *tardigradum*, which he described as caterpillar-like and equipped with two short antennae (cephalic papillae of *Milnesium*?). He did not provide a drawing, but criticized the figures in Spallanzani's (bad) and Eichorn's work (not better), which were the only authors he cited. Schrank belonged to those researchers who doubted reviviscence of anhydrobiotic animals including tardigrades. His fault was that he did not allow them to dry together with sand,[33] a prerequisite for the success of such an experiment as was already emphasized by Spallanzani (1776).

---

[31]Franz **Paula von Schrank** (1747–1835), German Jesuit priest, botanist and entomologist and professor at the University Ingolstadt and Landshut (Germany); first director of the botanical garden in Munich (Germany), editor of a multivolume Fauna of Bavaria (1803), one of the most important botanists of Bavaria (http://www.deutsche-biographie.de/pnd11861066X.html).

[32]árktos, Gr. = bear.

[33]"Es ist völlig falsch, was Senebier nach flüchtigen Beobachtungen behauptet. . ., dass dieses Thierchen, das Kugelthier, und das Räderthier den Tod öfter leiden, und mehrmals wieder aufleben können. Alle diese Thierchen platzen, wann der Wassertropfen abdunstet, und sind dann unwiederbringlich verloren. Die Naturgeschichte bedarf der angeblichen Wunder nicht, sie hat der wahren genug". [It is quite incorrect to assert, as Senebier does based on superficial observations... that this animalcule (tardigrade in water bodies, translator's note), the spherical sphere, and the wheel suffer death more often, and are able to revive many times. All these little creatures burst

Thereafter remarkable studies were issued in quick succession. The systematic position of tardigrades changed their place mostly within "arthropods" several times, and publications attempting classification based on superficial similarities often appeared almost simultaneously. Researchers, most of them trained scientists (arranged according to the year of their publications with a note on their classification proposal), were Paula von Schrank (1803; wingless insect between fleas (*Pulex*) and mites (*Acarus*)), Dutrochet[34] (1812, 1837; insects or larvae of mites; see Fig. 1.4 top) and Blainville[35] (1826; larvae of beetles). The works of Schultze[36] (1834a, b), Perty[37] (1834), Dujardin[38] (1838, 1851) and Doyère[39] (1840, 1842a, b, c) are considered in more detail below.

In 1834 Schultze published two relatively short studies of almost identical content, focussing on the new species and its capacity to survive dehydration with just a single relevant quotation[40] paying tribute to Spallanzani.[41] Schultze (1834a, b)

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when the water droplet evaporates, and are then lost forever. The natural history does not require the alleged miracles, it has true ones enough] (Paula von Schrank 1803, p. 195/196).

[34]René Joachim Henri **Dutrochet** (1776–1847), French physician, botanist and physiologist with a broad spectrum of scientific interests, particularly known for his studies on osmosis, respiration, embryology, and the effect of light on plants. The two volumes of the "Memoires..." (Essays...) from 1837 are a collection of his most important biological articles.

[35]Henri Marie Ducrotay de **Blainville** (1777–1850), French zoologist and anatomist at the Faculté des Sciences at the Sorbonne (Paris) and later successor of Georges Cuvier (1769–1832) to the chair for Comparative Anatomy at the Muséum National d'histoire naturelle in Paris.

[36]Carl August Sigismund **Schultze** (1795–1877), German physiologist and anatomist. He was from 1812 onwards professor at the University of Freiburg im Breisgau (Germany) and from 1931 professor at the University of Greifswald (Germany). He wrote four articles about tardigrades (Schultze 1834a, b, 1840, 1861), numerous medical reports and a textbook of comparative anatomy. The two publications from 1834 are of similar content, one of them was issued as Festschrift dedicated to his distinguished contemporary, the physician Christoph Wilhelm Hufeland (1762–1836) on the occasion of the 50th anniversary of his doctorate (Schultze 1834a).

[37]Josef Anton Maximilian **Perty** (1804–1884), German naturalist and natural philosopher, professor at the University of Bern (Switzerland) as of 1834, published among other things about rotifers, Infusoria and insects, mainly Coleoptera. http://www.deutsche-biographie.de/pnd116092386.html?anchor=adb

[38]Félix **Dujardin** (1801–1860), French biologist, professor at the University of Rennes, researched on protozoan and other "invertebrates", e.g. echinoderms, helminths and cnidarians, and wrote a "Histoire naturelle des zoophytes" (Dujardin 1841).

[39]Louis Michel François **Doyère** (1811–1863), student of H. Milne-Edwards (see footnote 56), French agriculturist, (1850–1852) professor of natural history (1842), 1850–1852 professor of Applied Zoology at the "Institute agronomique de Versailles" (see Maire 1892) and then at the "École centrale des arts et manufactures" (a well-known school of engineering founded in1829). He developed a process for conservation of grain silage. His thesis was published in three parts (Doyère 1840, 1842b, c) and is also available as a separate volume (thesis) (Doyère 1842a).

[40]This is in contrast to his later article (Schultze 1861), where he considered the literature very detailed.

[41]"Quae quidem descriptio, sicut alia diligentissimi naturae scrutatoris inventa, oblivioni omnino tradita esse videtur; certo a nemine confirmata est". [Of course, his description as well as other findings of this very diligent student of nature seems to have been entirely forgotten; certainly, nobody has confirmed them.] (Schultze 1834b, p. 5).

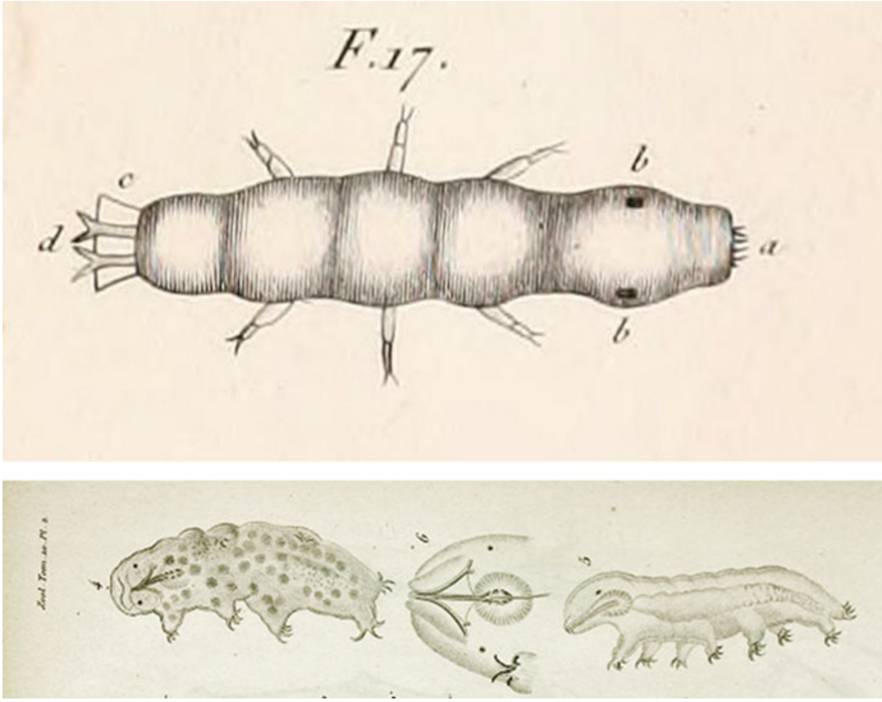

Fig. 1.4 Early pictorial presentations of tardigrades (without species name). Top: From Du Trochel (1812); detail from Plate 18. Bottom: From Dujardin (1838); detail from Plate 2

named his new species *Macrobiotus hufelandii*[42] (to my knowledge the first scientific name of a tardigrade dedicated to a person, which is still valid today). He noted the correct number of claws, the different parts of the digestive tract (but was wrong as far as the function is concerned), the position of the ovary and the sculpturing of the egg chorion (see Fig. 1.5). He classified this animal as a crustacean taxon based on its relatively strong integument divided into segments like a suit of armour, jointed (!) legs with claws and blood vessels; the absence of a heart and respiratory

[42]The genus name refers to the famous book of the German physician Christoph Wilhem **Hufeland** (1762–1836) entitled "Makrobiotik oder die Kunst das menschliche Leben zu verlängern" [Makrobiotik or the art of prolonging human life] that was reprinted several times and translated in several languages. The title is derived from the Greek word makróbios = long living. In this book Hufeland laid down the principle: " Je weniger intensiv das Leben eines Geschöpfs und je geringer seine innere und äußere Consumtion, desto dauerhafter ist es" [The less intense life of a creature and its internal and external consumption, the longer its life will last.] (Hufeland 1826, p. 82). Hufeland believed, however, that this rule does not apply to human beings due to their exceedingly great portion of spiritual power.

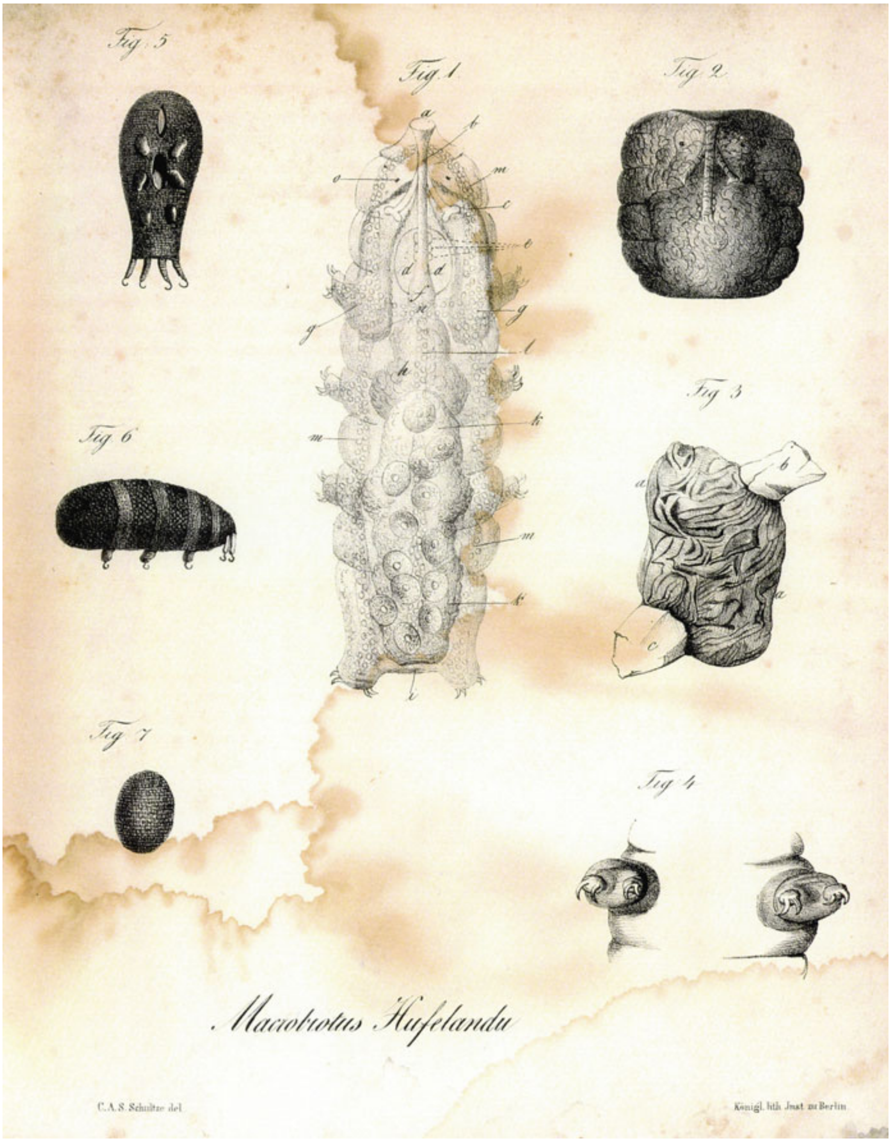

Fig. 1.5 Plate from Schultze (1834a) showing (1) the organization of *Macrobiotus hufelandii*. (2) *M. hufelandii* contracted by evaporation of water. (3) The same with adhering sand grains after complete dehydration. (4) Forelegs, from below. (5–7) Figures from Spallanzani's treatise showing the animalcule from below (5), the side (6) and desiccated (7)

organs he considered as a sign of primitiveness.[43] Concerning anhydrobiosis, Schultze (re)described the "tun" (figured by Spallanzani; see Fig. 1.5) of *M. hufelandii* and considered anhydrobiotic tardigrades as being in a state of long-lasting suspended animation ("mortis simulatione diuturna insigne", p. 2, the article is not paginated) or—even more provocative at that time—assumed that animals during evaporation of water are able to contract but then unable to pick up stimuli, being brought into a death-like state retaining the ability to restore all phenomena of life, i.e. a state that might considerably prolong their life.[44] This wording contrasted with previous views assuming a resurrection (from death). This way of looking at anhydrobiosis provoked distinguished contemporary scholars to disagree vehemently. An influential opponent, for example, was Ehrenberg.[45] Disregarding previous studies, he believed that rotifers and nematodes were not able to revive due to their complex structure, and he considered their reanimation (and that of tardigrades) an illusion, assuming that the individuals seemingly animated after years are the great-great-grandchildren of the formerly dried individuals (Ehrenberg 1834). This was the prevailing opinion of all those who denied "latent life" or "vita minima" (not only in tardigrades) in the sense of an extremely retarded or even arrested regular metabolism. Even the already disproved abiogenesis ("generatio spontanea")[46] was

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[43]"Macrobiotus simplici structurà ac parvitate Protozoiset Annulatis affinis est. Attamen duriusculo corporis tegumento, in speciem loricae e segmentis composito, pedibus articulatis ungues ferentibus, truncis vasorum sanguiferorum Crustaceorum signa offert. Qua in classe autem nonnisi infimum occupare potest, cum organa respiratoria propria et cor eum deficere videantur." [Due to its simplicity and smallness Macrobiotus is near the Protozoa and Annulata. However, with the rather tough integument, composed of segments like armour, with jointed legs that bear claws and with trunks supplied with blood they show traits of crustaceans. But in this class, it can hold only the lowest place, as it seems to lack own respiratory organs and a heart.] (Schultze 1834b, p. 5).

[44]"Maxime memorabilis est indoles animalis nostri, aqua omni evaporante sese contrahendi (...) omissa ipsa stimulos percipiendi facultate in statum morti simillimum transeundi, solamque vim restitutionis omnium vitae phaenomenorum retinendi. In hoc statu devinctarum virium vitalium (...) Macrobiotus non per breve solum tempus, sed per plures annos durare potest, multo igitur diutius, quam si vita ejus non turbata fuisset." [Most curious is the ability of our animal to contract (...), and to transform in a state very similar to the death after having lost the ability to be receptive to stimuli, and to retain only the power to restore again all phenomena of life. In this state of bound vitality (...) Macrobiotus is able to persist not only a short time, but many years, i.e. much longer than if its life would not be interrupted.] (Schultze 1834b, p. 4).

[45]Christian Gottfried **Ehrenberg** (1795–1876), a very productive and influential German zoologist, microgeologist and micropalaeontologist, professor of medicine at the University Berlin, friend of Alexander von Humboldt (1769–1859). He published on Hydrozoa, molluscs, coral polyps, rotifers, etc. but later concentrated on microscopic organisms (Protozoa, microalgae and bacteria) and founded the science of micropaleontology. He employed the term "Infusoria" for a wide range both of animal and vegetable life, i.e. he did not yet separate the multicellular organisms from the unicellular ones. One of his most famous monographs is "Die Infusionsthierchen als vollkommene Organismen" [The Infusoria as complete organisms] in 1838. He was one of the strongest opponents of the concept of resuscitation of desiccated "Infusoria".

[46]The first scientists to challenge the theory of spontaneous generation, i.e. formation of living organisms from non-living matter, were Francesco **Redi** (1626–1697) and Lazzaro Spallanzani (1729–1799; see footnote 15).

brought into play, e.g. by Purkinje.[47] Later Schultze erected the genus *Echiniscus*[48] and described two species of this genus (Schultze 1840, 1861). Interestingly, he also observed *Arctiscon* (=*Milnesium*) species, but did not describe them in detail.[49]

Perty (1834), who had at that time no other involvement with tardigrades but later published faunistic articles on Swiss tardigrades, summarized most of the existing taxonomic literature noting that Schultze (1834a) and Ehrenberg (1834; this is a letter commenting on Schultze's experiments; see below) had ignored the observations of Paula von Schrank (see above). He asserted the "right of the earlier observer" and, therefore, preferred the genus name *Arctiscon*, distinguishing five species and giving each the name of the first observer as the epithet, e.g. *Arctiscon mülleri*, *A. schrankii*, *A. hufelandii*, *A. spallanzanii* and *A. dutrochetii*.[50] Further, he included tardigrades in the newly created Xenomorphidae,[51] a family of crustaceans, which he positioned between Lernaeidae (Copepoda, Crustacea) and Annelida with similarities to Infusoria Rotatoria.[52] However, he did not provide reasons for that.

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[47]See footnote 49.

[48]échinos, Gr. = hedgehog.

[49]A letter by Schultze read aloud at the 15th Meeting of the Society of German Researchers and Physicians in Prague says under point 31 1. "Uiber den *Macrobiotus Hufelandi* und noch vier andere Species dieser merwürdigen Krusterfamilie. Zwei davon sind mit Fühlhörnern, eine mit zwei, die andere mit vier, und mit Fressspitzen versehen, welche, sowohl die Fühlhörner als Fressspitzen, der Gattung *Macrobiotus* fehlen. Das mit zwei Fühlhörnern versehene Thierchen dürfte das von Schrank beschriebene *Arctiscon tardigradum* seyn; auch legt dieses Thier seine Eier (7-11) immer in die abgelegte Haut, was der *Macrobiotus* niemals thut. Hr. Hofr. Schulze (sic!) hat ein kleines Päckchen Sand, welcher diese Thierchen enthält, eingesendet, den er seit sechs Monaten trocken aufbewahrt hat, worin gewiß einige Exemplare dieses *Arctiscon* durch Benetzung mit frischem Regenwasser oder destillirtem Wasser werden sich beleben lassen. (...). "[About *Macrobiotus Hufelandi* and four other species of this strange family of crustaceans. Two of them are equipped with antennae, one with two, the other with four, and with feeding tips (= peribuccal papillae, translator's note); both, the antennae and feeding tips, are lacking in the genus *Macrobiotus*. The animalcule with the two antennae is probably *Arctiscon tardigradum* described by Schrank; in addition, this animalcule lays its eggs (7–12) in the shed skin, which *Macrobiotus* never does. Hofrat Schulze (sic!) has sent a small parcel with sand containing these animalcules, which he has stored in a dry place for six months; certainly it will be possible to reanimate some specimens of this *Arctiscon* by wetting <the sand> with rain water or distilled water].

On the same page under point 32, Mr Purkinje, professor of physiology at the University of Breslau (at that time Germany), informs "(...) dass man die Entstehung des *Macrobiotus* und ähnlicher Thiere in seiner Gewalt habe. Man braucht nämlich Sand mit Wasser zu benetzen und stehen zu lassen, wo dann nach einigen Tagen solche Thiere sich zeigen" [that the origin of Macrobiotus and similar animals can be mastered. You have only to leave sand moistened with water, where then such animals emerge after a few days] (Sternberg and von Kromholz 1838, p. 187).

[50]Except for *Macrobiotus hufelandii*, none of these names, neither genus nor epithet, survived due to the impossibility to identify the species in question.

[51]xénos, Gr. = strange; morphé, Gr. = form, shape

[52]Friedrich Siegmund **Voigt** (1781–1850), professor of medicine and botany and director of the Botanic Garden at the University Jena (Germany), translated the second edition of "Le règne animal...." by the famous French naturalist Georges **Cuvier** (1769–1832) into German and extended the text considerably (Voigt 1843). Voigt classified tardigrades as the first order of the Infusoria, in which also Rotatoria, Polygastrica (Infusoria sensu Ehrenberg) and Spermatozoa were included.

Concerning anhydrobiosis (resurrection versus reawakening), he agreed with Schultze, using the term “latent life”.[53]

Dujardin (1838) corrected some data given by Schultze (1834a), noting that tardigrades have folds and wrinkles instead of segments, non-jointed legs, eyes like those of planarians and “blood corpuscles” that do not float in vessels, but freely in the body cavity. Further, he described the organization and the function of the buccal apparatus and added drawings from living tardigrades (see Fig. 1.4). His notes on reproduction (egg deposition, moulting) and anhydrobiosis were also correct. He considered rotifers and tardigrades as Systolides,[54] a class of organisms with a strong ability to contract, with a relatively tough outer covering of the body, a simple intestine and a “jaw apparatus” equipped with articulated elements and specific muscles. Tardigrades were called “Systolides marcheurs”, i.e. the walking Systolides, lacking cilia. They were suggested to form the transition between helminths on the one side and annelids and arachnids on the other side (Dujardin 1841).

The unanimous opinion of all later researchers is that Doyère’s dissertation “Memoire sur les Tardigrades” is an indisputable milestone in tardigradology, providing a profound basis for subsequent studies in anatomy and physiology. In 144 pages, he contributed to a wide spectrum of topics, e.g. taxonomy, morphology, reproduction and anhydrobiosis (Fig. 1.6), based on deep knowledge of the relevant literature. The thesis was published in three parts (Doyère 1840, 1842b, c), but is available, although rarely, as single-volume thesis (1842a; see Fig. 1.6). In the first part (Doyère 1840), he started with a broad historical introduction, following Dujardin’s view concerning “Systolides broyeurs” (crushing systolids, i.e. rotifers) and “Systolides suceurs” (sucking systolids, i.e. tardigrades) and their relations to crustaceans and annelids and distinguishing three genera, the genus *Emydium*[55] (three species; see above), the still valid *Milnesium*[56] (one species), both created by him, and *Macrobiotus* (four species). He detected asphyxia[57] and described in

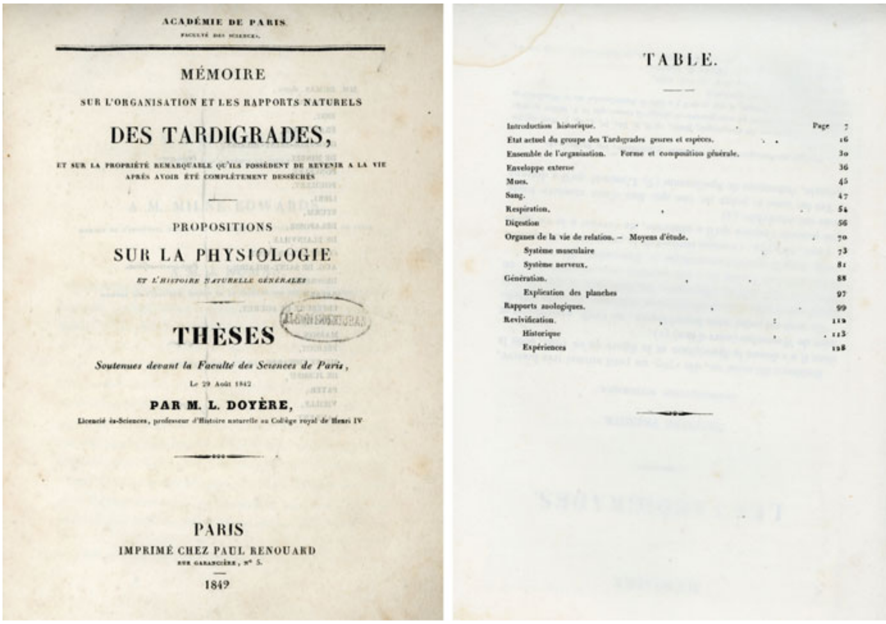
[53]The term was adopted from physics (e.g. latent heat) and introduced in 1834 for physiology by the German physician and natural philosopher Carl Gustav **Carus** (1789–1869) (see Carus 1834).

[54]systolé, Gr. = contraction.

[55]emýs, Gr. = turtle. In an annex of his thesis, Doyére noted the similarity of Schultze’s *Ech. bellermanni* with his *Em. testudo* and accepted the priority of the term *Echiniscus* (see Doyére 1842c).

[56]Dedicated to Henri Milne-**Edwards** (1800–1885), eminent French zoologist, at that time professor of entomology at the Muséum National d’Histoire Naturelle and at the faculty of Sciences at the Sorbonne in Paris and later chair of zoology

[57]asphyxía, Gr. = stopping of the pulse. “L’asphyxie est le moyen qui réussit le mieux, celui qui donne les plus beaux résultats. Je prends des Tardigrades vivans, je les place dans un tube en verre plein d’eau préalbablement privée d’air par l’ebulliton, et au-dessus de laquelle j’ai le soin de mettre une couche d’huile pour la séparer de l’atmosphère. Après vingt-quatre heures l’engourdissement est complet, il est plus complet et plus durable après deux, trois, quatre jours; ce n’est qu’après cinq à six jours que les Tardigrades perdent la faculté de revenir à la vie.” [Asphyxia is the most successful way, one that gives the best results. I take living Tardigrades, I place them in a glass tube filled with water, deoxygenated beforehand by boiling, and cover it carefully with a layer of oil to separate it from the atmosphere. After twenty-four hours, numbness is complete, it is even more complete and lasting after two, three or four days; it was only after five or six days that Tardigrades lose their ability to come back to life.] (Doyère 1840, p. 333). This technique was simplified later and was the method of choice for decades to study tardigrades (e.g. Greeff 1865; Plate 1889; Basse 1905).


ACADÉMIE DE PARIS
FACULTÉ DES SCIENCES.

MÉMOIRE
SUR L'ORGANISATION ET LES RAPPORTS NATURELS
DES TARDIGRADES,
ET SUR LA PROPRIÉTÉ REMARQUABLE QU'ILS POSSÈDENT DE REVENIR A LA VIE APRÈS AVOIR ÉTÉ COMPLÈTEMENT DESSÉCHÉS

PROPOSITIONS
SUR LA PHYSIOLOGIE
ET L'HISTOIRE NATURELLE GÉNÉRALES

THÈSES
Soutenues devant la Faculté des Sciences de Paris,
Le 29 Août 1842
PAR M. L. DOYÈRE,
Licencié ès-Sciences, professeur d'Histoire naturelle au Collège royal de Henri IV

PARIS
IMPRIMÉ CHEZ PAUL RENOUARD
RUE GARANCIÈRE, n° 5.
1842

TABLE.

Introduction historique. Page 7
Etat actuel du groupe des Tardigrades genres et espèces. 16
Ensemble de l'organisation. Forme et composition générale. 30
Enveloppe externe 36
Mues. 45
Sang. 47
Respiration. 54
Digestion 56
Organes de la vie de relation. – Moyens d'étude. 70
Système musculaire 73
Système nerveux. 81
Génération. 88
Explication des planches 97
Rapports zoologiques. 99
Revivification. 112
Historique 113
Expériences 128

Fig. 1.6 Title page and table of contents of Doyère's thesis (Doyère 1842a)

detail how to put tardigrades in this state that he found very effective to study their anatomy. Thus, he dealt with myology, neurology and reproduction (he recognized spermatids) and described some stages of oogenesis, egg deposition and much more. Despite the poor optic equipment (by today's standards), the quality of his observations is excellent (irrespective of whether his interpretations are correct), and the reader can only be amazed by the many details he saw, for example, when he described that newly hatched *Echiniscus testudo* possess only two claws per leg.

Obviously he was also the first who saw cysts, but he did not realize their true nature.[58] He also discovered "anomalies" in the buccopharyngeal apparatus (see below). His accuracy is perhaps best illustrated (without going into detail) by the plates added to the thesis, from which four are shown in Fig. 1.7.

Starting with Dujardin's Systolides, the second part (Doyère 1842b) contains a further, broad discussion about the relationships of tardigrades considering the previous existing literature and his own morphological findings. However, concerning the class Systolidae as well as its relations to other "articulated" animal classes, he did not come to a clear decision.

[58]"J'eus d'abord quelque peine à reconnaître l'animal, dans la petite masse, inert, en apparence granuleuse et amorphe que je rencontrais parfois à l'interieure de certains peaux qui me semblait abandonnés. C'était le Tardigrade lui-méme. Déjà dépouillé, mais non encore sorti de sa dépouille." [Initially I had some difficulty to recognize the animal, in the small mass, inert, granular and amorphous in appearance, which I sometimes found within a skin. It was the tardigrade itself. I thought it had already been shed. Already shed, but not yet outside of its skin.] (Doyère 1840, p. 308).

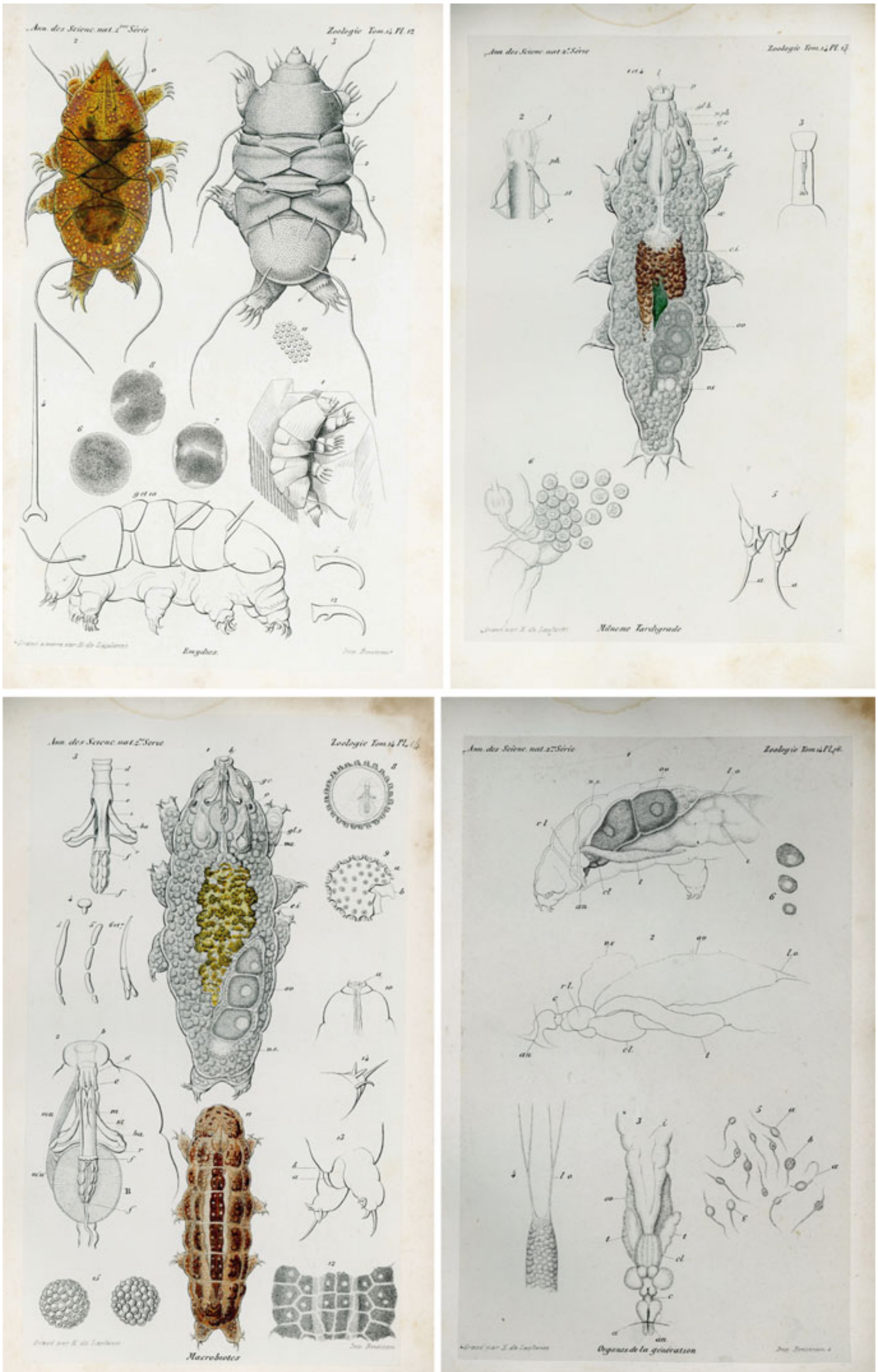


Fig. 1.7 Four plates from Doyère's thesis. Top left: Plate 12, counting of the plates starts with 12, *Emydium testudo*. Top right: Plate 13, *Milnesium tardigradum* (six squeezed buccal apparatus

The last part (Doyère 1842c) contains the dehydration experiments on tardigrades. Among other things the results of this part have led to the controversy between Doyère and Pouchet[59] (see below). In the historical review on anhydrobiosis, he highly appreciated Leeuwenhoek, Needham, Schultze and especially Spallanzani. Then he described his experiments (mainly desiccation and the effect of elevated temperature on desiccated tardigrades) using *Emydium* and *Macrobiotus* and emphasized the worthlessness of a "desiccation à nu" (means literally translated "nude desiccation", i.e. direct desiccation without an environment that releases moisture slowly) and concluded from his experiments that the "organized matter" contains life *in potentia* that passes into life *in actu* after moistening the anhydrobiotic animals, i.e. their pass from potential life into active life provided that the molecular integrity within the tissue and their connections are maintained.

The above-mentioned dispute concerning anhydrobiosis was submitted 1859 to the Société de Biologie in Paris[60] for decision; it can be presented in abbreviated form as follows (see also Lance 1896; Keilin 1959). Both parties agreed that a completely desiccated body is dead. The question was, however, whether such bodies can regain their lost life. According to Doyère a dried organism has the capability to revive, provided desiccation and moisturization are slow (e.g. in a medium releasing humidity slowly), whereas Pouchet stated that an organism, once fully dehydrated, cannot escape the final death. The 156-page report written by Paul Broca[61] on behalf of the high-ranking commission has been critically examined in detail by Keilin (1959), who also corrected some of Broca's errors. Both sides carried out experiments; the control experiments made by the commission fully

Fig. 1.7 (continued) of *Macrobiotus hufelandii*). Bottom left: Plate 14, *Macrobiotus hufelandii* (1–11) and *Macrobiotus oberhaeuser* (11–15). Bottom right: Plate 16, reproductive organs of *Macrobiotus hufelandii* (1, 4, 5, 6) and *Milnesium tardigradum* (2–4). From Doyère (1842a)

[59]Félix-Archimède **Pouchet** (1800–1872, French physician and naturalist, founder of Rouen Museum of Natural History (1828), from 1838 professor at the School of Medicine at Rouen, a leading proponent of spontaneous generation of life from non-living materials.

[60]Société de biologie, a learned society founded in Paris (France) in 1848. The members of the society held regular meetings; proceedings were published in the scientific journal "Comptes rendus de la Société de Biologie" first issued in 1849.

[61]Pierre Paul **Broca** (1824–1880), French physician and anthropologist, known for his research on a region of the frontal lobe involved with language that was named after him (= Broca's area).

confirmed Doyère's view.[62] Negative results[63] were attributed to fluctuating humidity during anhydrobiosis as alternating swelling and drying might cause mechanical damages. Animals in anhydrobiosis tolerated the highest temperature. This was not accepted by some contemporaries and "intellectual latecomers" (Marcus 1929a). However, the controversial issue was no longer the ability to undergo anhydrobiosis, but how to explain it, i.e. whether the metabolism was extremely reduced (vita minima, see above) or suspended without dying.

In several publications issued between 1848 and 1862, Ehrenberg promoted knowledge of α-taxonomy and distribution of terrestrial tardigrades especially in high altitudes (Alps, Himalaya), in which he suspected specific forms. He distinguished tardigrades from the Acaridae mainly by the absence of metamorphosis in the presence of moulting. Among others he described several *Milnesium* species, i.e. *M. alpigenum* (Ehrenberg 1853a, b; see Fig. 1.8), and even a new genus *Acrophanes* related to *Milnesium* with *A.* (*Milnesium*) *schlagintweitii* as species from the Himalaya (Ehrenberg 1859), using, among other distinguishing features (e.g. claw configuration), the number of the "tentacles" (=the laterally positioned cephalic papilla) and peribuccal papilla.[64]

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[62]The key conclusion of the commission was: "La résistance des tardigrades et des rotifères aux températures élevées paraît s'accroitre d'autant plus qu'ils ont été plus complétement desséchés d'avance. Les rotifères peuvent se ranimer après avoir séjourné quatre-vingt-deux jours dans le vide sec et subi imdiatement aprés une température de 100° pendant trente minutes. Par conséquent, des animaux desséchés successivement à froid dans le vide sec, puis à 100° sous a pression atmosphérique, c'est-à-dire amenés au degré de desiccation le plus complet qu'on puisse réaliser dans ces conditions et dans l'état actuel el a science, peuvent conserver encore la propriété de se ranimer au contact de l'eau." [The resistance of tardigrades and rotifers to high temperature appears to increase, the more they had been completely dehydrated before. Rotifers can be become alive again after having stayed for eighty-two days in a dry vacuum and immediately thereafter being exposed to a temperature of 100° for thirty minutes. Therefore, animals which had been dried first in a cold dry vacuum and then at 100° under atmospheric pressure, i.e. brought to the most complete degree of desiccation we can achieve under these conditions and in the present state of science, still retain the ability to revive in contact with water] (Broca 1860, p. 139).

[63]In this context, a short note from 1858 by the French physician Casimir **Davaine** (1812–1882), co-discoverer of *Bacillus anthracis*, should be mentioned. He showed that, in contrast to moss-dwelling tardigrades and rotifers, fully aquatic species of both are not able to withstand dehydration (Davaine 1858). Later Marcus (1928a, 1929b) thought that such differences depend to a large extent on the habitat rather than on the general inability of the species in question to tolerate desiccation. He further suggested to leave up the distinction between freshwater and terrestrial tardigrades in favour of strongly and less strongly hygrophilous (hygrós, Gr. = wet; phílos, Gr. = friend, loving) species and rejected the term xerophilous (xéros, Gr. = trocken) as all tardigrades can only be active in a medium containing humidity.

[64]*Milnesium* was considered over decades as a monospecific genus with a single cosmopolitan species, which currently, however, contains more than 30 species. No single tardigrade species described by Ehrenberg has been revalidated so far. However, more recently *Milnesium alpigenum* appears to be revitalized using animals from a parthenogenetic lab strain collected near Tübingen (Germany) (Morek et al. 2016), but Ehrenberg collected his material in the Mone Rosa massif in the Pennine Alps at approx. 3600 m above sea level (Ehrenberg 1853b). According to Ehrenberg, Doyère's *M. tardigradum* and his *M. alpigenum* differ in the claw configuration and also in the number of peribuccal papilla, i.e. three in the former and six in the latter. However, also