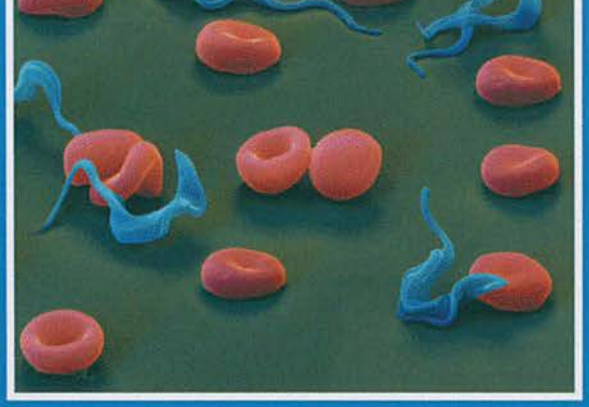

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Protistology

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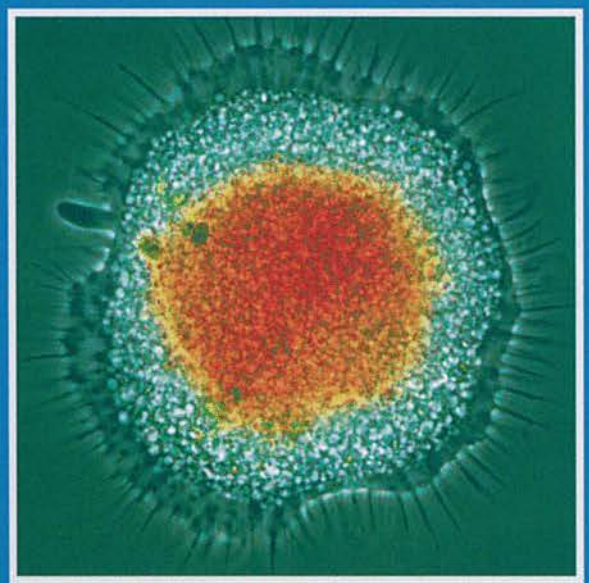

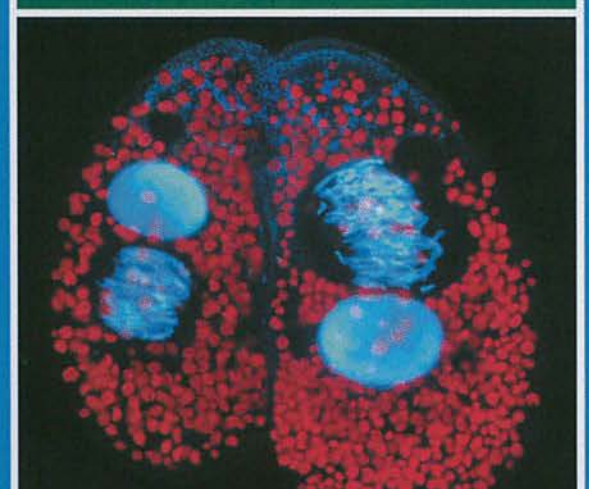

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Stuttgart

Protistology

3rd completely revised edition

Protistology

Klaus Hausmann
Norbert Hülsmann
Renate Radek

with contributions by
Hans Machemer
Maria Mulisch
Günther Steinbrück

with 384 figures
and 22 tables

3rd completely revised edition

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Upper picture: *Trypanosoma brucei* between erythrocytes (scanning electron microscopy) (micrograph: Oliver Meckes and Nicole Ottawa, eye of science, Reutlingen, Germany). Magn.: 1,450 ×.
Middle picture: Syncytium of several individuals of *Vampyrella ulothrichis* (phase contrast microscopy) (micrograph: Norbert Hülsmann, Berlin, Germany). Magn.: 450 ×.

Lower picture: *Paramecium bursaria* during conjugation with degenerating macronuclei and micronuclei during meiotic division exhibiting chromosomes (DAPI staining); the numerous red spheres represent symbiotic zoochlorellae (fluorescence microscopy) (micrograph: Arthur Hauck, Pfalzgrafenweiler, Germany). Magn.: 500 ×.

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From the Preface to the Second Edition

With the second edition of *Protozoology*, we are meeting an often-heard wish among students and teachers outside of Germany to publish this textbook in English as well, so that it can be used in protozoology courses worldwide. The first edition appeared 10 years ago in German language, and was a great success.

The book is aimed at introducing students to the amazing and bewildering world of protozoa by giving basic information on the biology of these creatures. Since the publication of the first edition in 1985, there has been tremendous progress in biological research, especially in the fields of cell and molecular biology, as well as phylogeny and ecology. These new developments have been accounted for in this second edition. This is especially evident in our treatment of such topics as phylogeny and taxonomy. We discuss – probably for the first time in a protozoological textbook – a radically different, but accessible approach to the phylogenetic relationships of the eukaryotic organisms. We are aware that some of our colleagues will passionately disagree with these new ideas, but we are absolutely sure that others will agree with them. Moreover, they may use this compilation of information as a basis for further considerations and deductions. We acknowledge that we are still a long way from a complete understanding of protozoan and eukaryotic phylogeny.

We also try to present an up-to-date synthesis of the many other facets of the biology of protozoan organisms. We have therefore made extensive use of new illustrative material.

Berlin, September 1995 Klaus Hausmann
Norbert Hülsmann

Preface to the Third Edition

With this third edition of the original textbook *Protozoologie*, published in 1985 in German language, we are again meeting an often-heard wish among students, teachers and colleagues from all over the world to update this textbook in a broader systematic and phyletic context. In consideration of the tremendous amount of knowledge gathered within the last few years on the phyletic relationships between eukaryotic organisms, we now include all the unicellular organisms, which normally are not mentioned in connection with the predominantly heterotrophic protozoans – for instance, all single-celled algae and lower fungi. Logically, we had to change the English title of the former edition of this book from *Protozoology* into *Protistology*. We do not use the word protist in the sense intended by Ernst Haeckel, for whom the term incorporated the bacteria (which at that time had not yet been recognized as prokaryotes). We consider the term to include all eukaryotic unicellular organisms, regardless of whether they are heterotrophs (protozoa), phototrophs (protophyta) or saprophytes (fungi), which live as individual organisms embodying a single-celled way of life.

This fundamental, phylogenetically based broadening of the book's scope inevitably led not only to an expansion of the text and thus to an increase in illustrative material, but also to the dramatic insight that protozoology as a discipline may soon cease to exist as a classical branch of zoology, as already stated in 1956 by Karl Gottfried Grell (Tübingen) who wrote in the preface of the first edition of his famous protozoology textbook: *Die Protozoologie ist keine besondere Wissenschaft, sondern nur die Zusammenfassung der Kenntnisse, welche wir von einer bestimmten Tiergruppe, den Protozoen, besitzen* (*Protozoology is not a particular science, but a compilation of knowledge that we possess about a special group of animals, the protozoa*). This has become more and more evident over time. In the future, protozoologists or protistologists will increasingly become experts in numerous, very divergent fields of biology. They are united by the creatures they work on and deal with: the unicellular organisms or protists.

We again thank the many colleagues who provided us with light- and electron-micrographs and diagrams that bring this book to life. We have indicated the source of the illustrations in the corresponding figure legends.

Of the numerous persons responsible for the creation of this book, a few should be mentioned individually. First, we warmly acknowledge – as we did in the second edition – the cooperation of our colleagues Hans Machemer, formerly of the University of Bochum, now retired and living in Hallenberg, Sauerland, Maria Mulisch of the University of Kiel and Günther Steinbrück of the University of Tübingen. They were kind enough to actualize the chapters *Nuclei and Sexual Reproduction* (MM), *Morphogenesis and Reproduction* (MM), *Molecular Biology* (GS), and *Behavior of Protists* (HM), contributed greatly to this book with their expertise.

Furthermore, we thank Frederic Bartlett for helping us to make our English readable and understandable. Peter Adam, the scientific illustrator of our institute, must once again be thanked for his patience and painstaking endurance during the preparation of numerous drawings and diagrams adapted or newly designed for this third edition. Hülya Tosun, Berlin, took over the greatest part of the task of digitally scanning the illustrative material, ameliorating it using computer technologies, and performing the final editing for reproduction. She did this with her special Turkish charm. Stefanie Kortfleisch, Berlin, was in the final period of computer assisted completion of the illustrative material a great help as well as Markus Schober, the technician of our working group. He was in addition extremely important when the computers decided to do things their own ways; he was always able to bring them back on track.

The first and second edition were published by Georg Thieme Verlag Stuttgart. We also wish to thank Dr. Erhard Nägele at the Schweizerbart'sche Verlagsbuchhandlung in Stuttgart for accepting the risk of publishing a third edition of this book. And as usual: last, but not least, our families should not be forgotten for being neglected to a remarkable degree for a long time in favor of our beloved protists during the preparation of this new edition.

Berlin, August 2003

Klaus Hausmann
Norbert Hülsmann
Renate Radek

Part I: Introduction and Overview

Definitions and History of Nomenclature

This book deals with the organisms we usually call protozoa or protists. We begin the book with a particularly difficult question: what are they? While the remainder of the book represents an assemblage of factual information concerning the protists, the fundamental issue of how to define the group is difficult and rather controversial. This is partly due to the rapid accumulation of knowledge concerning these organisms, and especially to the relatively recent rejection of the idea that protists (or protozoa) represent a monophyletic taxonomic unit. Thus, a phylogenetic-systematic definition of the protists cannot be given. We must consider the protists as a paraphyletic (or even polyphyletic) assemblage of small organisms of mostly microscopical dimension which do not constitute a single, natural group.

Controversial definitions of the protists have been the rule for three centuries of research. In 1818, the German zoologist and paleontologist Georg August Goldfuß (1782–1848) introduced the term protozoa. At that time, about the only thing which was definite about the protozoans was the etymology of the word itself: the Greek prefix proto- (first) and the Greek suffix -zoa (living creatures, animals), similar to the German designation Ur-Thiere. We now know that the protists were not the first creatures on earth: the pioneer role belongs to the prokaryotic-organized unicells, possibly the fermenting bacteria.

The approach of a modern understanding should take account of the term zoon. The use of zoon in terms such as zoology, zoogeography or zoophysiology led to the assumption that protozoa also are exclusively animal-like creatures, or animals that have to be carefully separated from their green, plant-like relatives, the protophytes (Ernst Haeckel, 1866). This separation has become established in tradition and has been occasionally defended with fanatical devotion. However, the modern differentiation of animalcules into plant-like/phototrophic and beast-like/heterotrophic organisms bears problems. These become obvious when monophyletic taxa such as the euglenids or dinoflagellates are grouped depending on whether or not plastids are present, i.e. as plant-like or as animal-like creatures within the corresponding zoological or botanical systems. In the middle of the last century, this unsatisfactory situation led to efforts to establish an independent system that more accurately and formally accommodated the exceptional position of the lower eukaryotes.

The frequently used circumscription of protists as single-celled eukaryotic organisms or unicells avoids such problems, but it is still inadequate. Of course, most protists present themselves as cells, but not all are fixed in their structural organization to the status of a unicell. For instance, there are some cases in which many similar conspecific individuals are able to gather together to form temporary feeding-communities (e.g. heliozoans) or to form longer-lasting syncytia (e.g. vampyrellids). In other cases the daughter cells do not separate after karyokinesis, at least not completely; they form colonies, as in the Volvocida, or they grow to form the multinucleated plasmodia of acellular slime molds, as in the Myxogastrea. In addition, alleged multicellular protists that have a complex architecture and differentiate into specialized cell types (e.g. Myxozoa) exist, but they are now unmasked as metazoans.

The problem of characterizing the protists with the necessary brevity, however, is not the result of etymological restraints, but of the fantastic diversity of organisms that we now know and must be embraced by the term. In contrast to the scientific designations for species, genera, and families, the suprafamilial categories of ranking (and therefore also the term protozoa or protista) are not subject to the restrictive rules of nomenclature. They are maintained as terms for the mostly large taxa in which organismic composition is often unknown, and neither the name of the describer nor the year are given. As in other disciplines, it is useful to give a historical overview of the conceptual framework.

In the following, the most important terms appear in chronological order. They give some information about the criteria used for distinguishing the protists from other groups of organisms.

Animalcula (Antoni van Leeuwenhoek, 1676): The term is a diminutive of animalia and came into use in the sense of water insects or small animals. The collective term embraced all microscopic creatures collectable from standing rainwaters, springs, lakes, rivers, etc., but also from body fluids of higher animals. Even sperm cells (spermatozoa) were included.

Monads (Gottfried Wilhelm Leibniz, 1714): The term was adapted from mathematical and philosophical theories of the Greek and Roman classical eras and came into fashion again after the first observations with microscopy were conducted. Primarily used in a metaphysical sense, the word served to designate indivisible and permanent smallest units thought to be the elements and sources of all creatures. Because the monads (according to Leibniz, with an origin in the unification of soul and matter) could later be demonstrated as visible realities, as in the example of a spermatozoon or a unicellular flagellate, the term was adapted by naturalists for nomenclatural purposes, mainly with reference to flagellates, e.g. Monas O. F. Müller, 1786; Cryptomonada Ehrenberg, 1838; Chrysomonadida Engler, 1898; Diplomonadida Wenyon, 1926; Trichomonadida Kirby, 1947; Proteromonadida Grassé, 1952; Prasinomonadea Christensen, 1962. At present, the flagellated phase in the life cycle of some algal groups, or the flagellate organization in general, is designated as monadal or monadoid.

Infusoria or **Animalcula Infusoria** (Martin Frobenius Ledermüller, between 1760 and 1763): *Aufgußtierchen* or infusion animals: The term primarily embraces all organisms which are able to produce desiccation-resistant stages (e.g. rotifers) and which can be reactivated by an infusion of water to hay or pepper contaminated with such resting stages. Jean Baptiste de Lamarck (1744–1829) established the zoological taxon Infusoria alongside other invertebrates. Since the acceptance of the cell theory in the 19th century, and later on until the mid-20th century (and even later in Russian literature), the term has been used exclusively as a synonym for the ciliates.

Urthiere (Lorenz von Oken, 1805): This German term served as a synonym for Infusoria, but also for the separation of single-celled organisms from higher plants and metazoans. It is the philological basis for the term protozoa (= Urtiere), but the name was not used in this taxonomic sense.

Protozoa (Georg August Goldfuß, 1818): The Protozoa embrace, besides the Infusoria in the sense of Ledermüller, also some Cnidaria, Spongia, and Bryozoa. The term protozoa was not frequently used until 1845, when Carl Theodor von Siebold formulated the definition that they represent animals that can be reduced to the status of a cell. At this point, the cell theory was incorporated into the new branch of systematic biology.

Animalia Microscopica (Jean Baptiste Bory de Saint-Vincent, 1826): Synonym for Infusoria. However, for the bell animalcule *Vorticella* and its relatives, a new kingdom (règne psychodiaire) was created (1822–1831).

Eithiere or **Oozoa** (Carl Gustav Carus, 1832): Synonym for Ledermüller's Infusoria and Goldfuß's Protozoa. However, this term did not incorporate the rather attractive idea of organisms retaining protozoan-like characters at the initial stages of their development.

Archaezoa (Maximilian Perty, 1852): This etymologically beautiful expression, meaning original creatures, was used initially as a synonym for Protozoa. This meaning has not survived. The term is presently used (spelled Archezoa) to designate original heterotrophic eukaryotes without mitochondria.

Microzoaires (Emile de Fromentel, 1874): This term was used exclusively for various microscopic unicellular creatures and has fallen out of use.

In contrast to this more or less zoologically oriented terminology (despite the compromises made by Peter Simon Pallas [1741–1881] and Felix Dujardin [1801–1860] in establishing the taxa Zoophyta and Zoophytes Infusoires, respectively), new terms developed in the middle of the last century arose from multi-kingdom concepts based on genealogical principles. As a first step, Rudolf Leuckart (1822–1898) excluded the heterotrophic single-celled organisms from the animal kingdom (1848).

Acrita (Richard Owen, 1861): This kingdom of so-called nondifferentiated cells, which contains not only the protists but also some smaller metazoans characterized by their morphologically indifferent architecture, was erected alongside the classical zoological kingdom (Animalia) and the botanical kingdom (Vegetabilia). Today the term is no longer in use.

Protoctista (John Hogg, 1861): With the Protoctista (Greek, = first creatures), a kingdom sep-

arate from the kingdoms of the animals, plants, and molds was created. Nowadays the term is used in combination with the five-kingdom concept of Robert H. Whittacker (1959). The four eukaryotic kingdoms of Plantae, Animalia, Fungi, and Protoctista are considered to be quite separate from the kingdom Prokaryota (or Monera). The term allows a precise but negative characterization: Protoctista are those microscopic and macroscopic eukaryotes that remain after exclusion of (1) all animals developing from a blastula, (2) all plants developing from embryonic stages, and (3) all higher fungi without a flagellate stage in their life cycles (Margulis et al., 1990). Thus, the Protoctista not only embrace the protozoa, but also Phaeophyta, Chytridiomycota, Oomycetes, Rhodophyta, and other taxa.

Protista (Ernst Haeckel, 1866): According to the various definitions given by Haeckel himself, the taxon Protista contains many unicellular but not exclusively eukaryotic organisms. Therefore, it differs from the unequivocally phototrophic plants (including single-celled green algae such as *Closterium*) and the clear animals (metazoans and ciliates) according to the equation: protophyta + protozoa = protista. In the modern view, the protists are eukaryotic organisms of unicellular organization. Therefore, the term embraces classical protozoa, unicellular phototrophic organisms such as diatoms, and lower unicellular fungi.

As seen by this list of terms with their short definitions, biological concepts and interpretations are involved even in the nomenclature. Of this list, only four designations have survived – Protozoa, Archaezoa, Protoctista, and Protista.

We prefer to use the terms protists and protozoa simultaneously: the former when systematic or taxonomic questions are of central interest and the latter when exclusively the obligatory heterotrophic organisms are considered. This is only partly a question of priorities; the main reason is because the other terms are not better alternatives. It should be emphasized that none of these terms represent a monophyletic or holophyletic group of organisms. This is likely to be true for the future as well, because neither the Protista nor the Protozoa represent an evolutionary lineage in the phylogenetic sense. We can, however, use both of these terms for the designation of creatures based on their body plan and the plesiomorphic character of their eukaryotic cellular organization.

Historical Overview of Protistological Research

Since the protists are usually organisms of microscopic dimension, they remained undiscovered until the development of suitable magnifying instruments. Nevertheless, the compound microscope (composed of both an objective and an ocular lens) was invented long before single-celled organisms were discovered. Around 1590, the Dutch opticians Hans and Zacharias Janssen built the first of such microscopes. Initially they were not accepted as bona fide scientific instruments, but rather as toys for upper-class adults. The real starting point for scientific protozoology came about 80 years later.

The discoverer of unicellular organisms, and hence the father of protozoology, was the Dutchman Antoni van Leeuwenhoek (1632–1723) (Figs. **1** and **2 a**). As a hobbyist, he used simple microscopes of his own design and manufacture. He cut and ground his own lenses and fabricated them into simple microscopes in a special frame (Fig. **2 b**). They were all single, high-power magnifying lenses. It was rumored that he built about 400 of these microscopes over the years and retained them for his own use.

After training to be a merchant in Amsterdam, van Leeuwenhoek moved back to his home town of Delft, where he was the local draper and also acted as a member of the town council. As he was not a professional biologist who carried out his studies because of training or occupation, he must have been considered an unusual personality who pursued his hobby with vigor, motivated by his insatiable curiosity. He described his new findings in over 100 letters which he sent to the Royal Society of London. His formal observations began in 1676, and van Leeuwenhoek was the first to observe, draw, and describe the protozoa (mainly of them ciliates). He called his little creatures animalcula. Two years later in 1678, the famous Dutch physicist Christian Huygens (1629–1695) confirmed van Leeuwenhoek's findings. This independent confirmation spurred intense research by naturalists.

Both van Leeuwenhoek and Huygens believed that the protists they observed in standing waters or in hay infusions originated from so-called air germs. The question of the origin of these protists would become the cause of animated disputes and discussion until the middle of the 19th century.

The next important date in protozoology and microscopy was 1718, when the French scientist Louis Joblot published a book on the applications of the microscope. In addition to his consideration of different types of microscopes, Joblot illustrated a variety of protists. He included some of the first descriptions of subcellular structures. Nuclei, contractile vacuoles, ciliature, and even the intestines of ciliates were described in some detail. Naturally, it was not possible for Joblot to explain the significance of these structures, although he did reflect on the question of the origin of his protists. He concluded that they would contain eggs that develop through stages resembling embryos and fetuses into identical images of their parents. Such beliefs about the ontogeny of protists are excusable, for nothing was known about the existence of cells and their ability for reduplication at that time. Nevertheless, he carried out experiments on the origin of the protists with boiled and unboiled hay infusions. As protists did not appear in boiled infusions but did appear in natural infusions or those exposed to normal air, he concluded that the eggs must be found in the air; if they drop into the water, the infusoria or animalcula develop. Thus the air-germ theory of van Leeuwenhoek and Huygens was reborn.

In 1727, an anonymous Parisian physician based a satire on these ideas. He claimed that the air is full of animalcula and homunculi that cause different diseases. He gave the creatures different names such as bellyache-ists, diarrhea-ists, pestilence-ists, faint-ists, sensual-ists, and so on. In a second publication, he went on to describe the antagonists to these creatures, calling them anti-bellyache-ists, anti-sensual-ists, and so on. He also explained how they could be used to combat disease. If we regard the animalcules and homunculi as pathogenic agents, he was right in some respects, even though he was actually trying to ridicule these ideas.

Of far greater importance to the scientific community were the doctrines of abiogenesis (generatio spontanea) introduced independently in 1749 by the French zoologist George Buffon (1707–

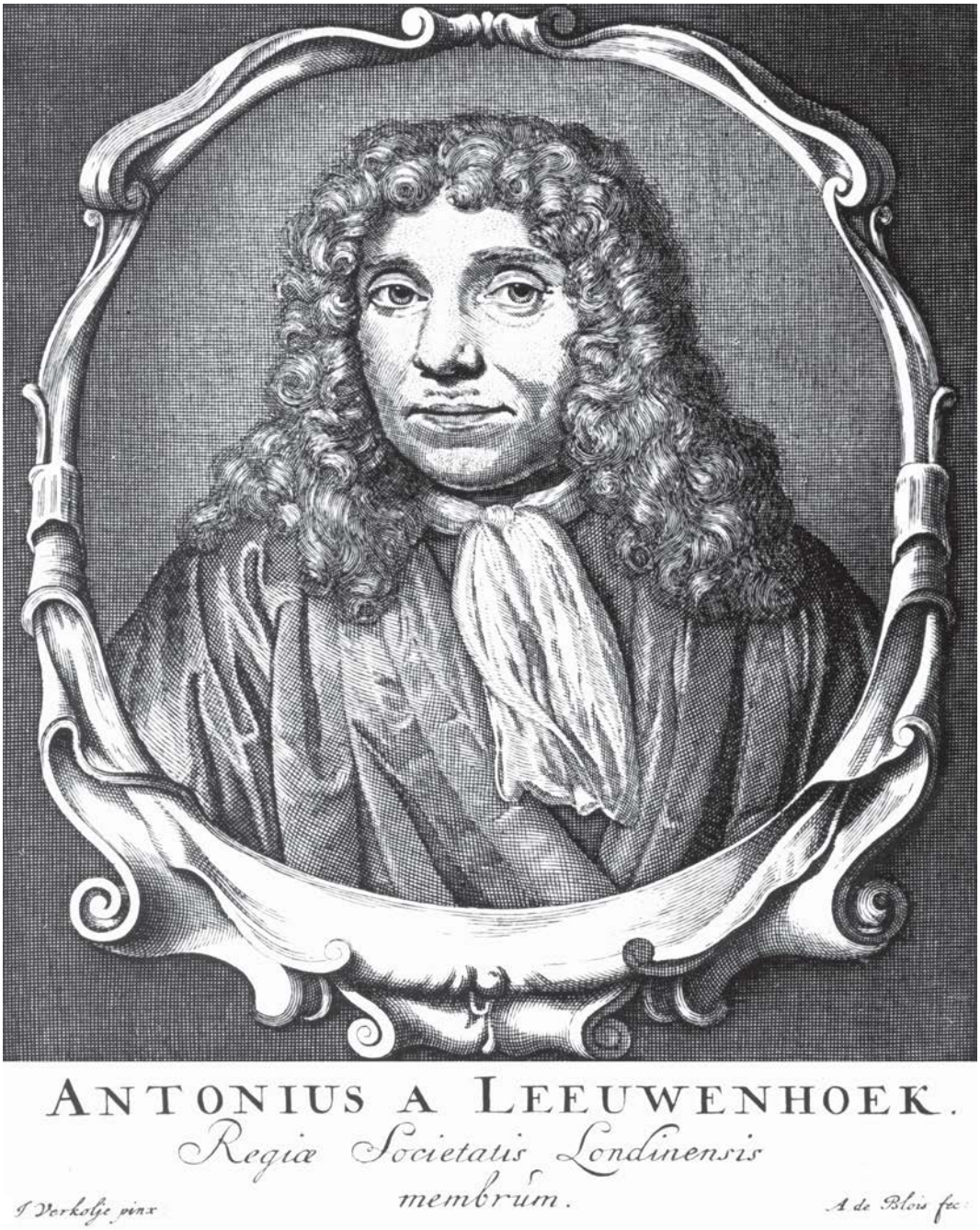

Fig. **1** Antoni van Leeuwenhoek, initiator of scientific microscopy.

1788) and by the English naturalist John Needham (1713–1781). According to Buffon's doctrine, plants and animals are composed of organic, living molecules that are ingested with food. At puberty, surplus molecules are deposited in spermatozoa. The infusoria are evidence of these living molecules, which are liberated when plants and animals die and decompose. This theory was

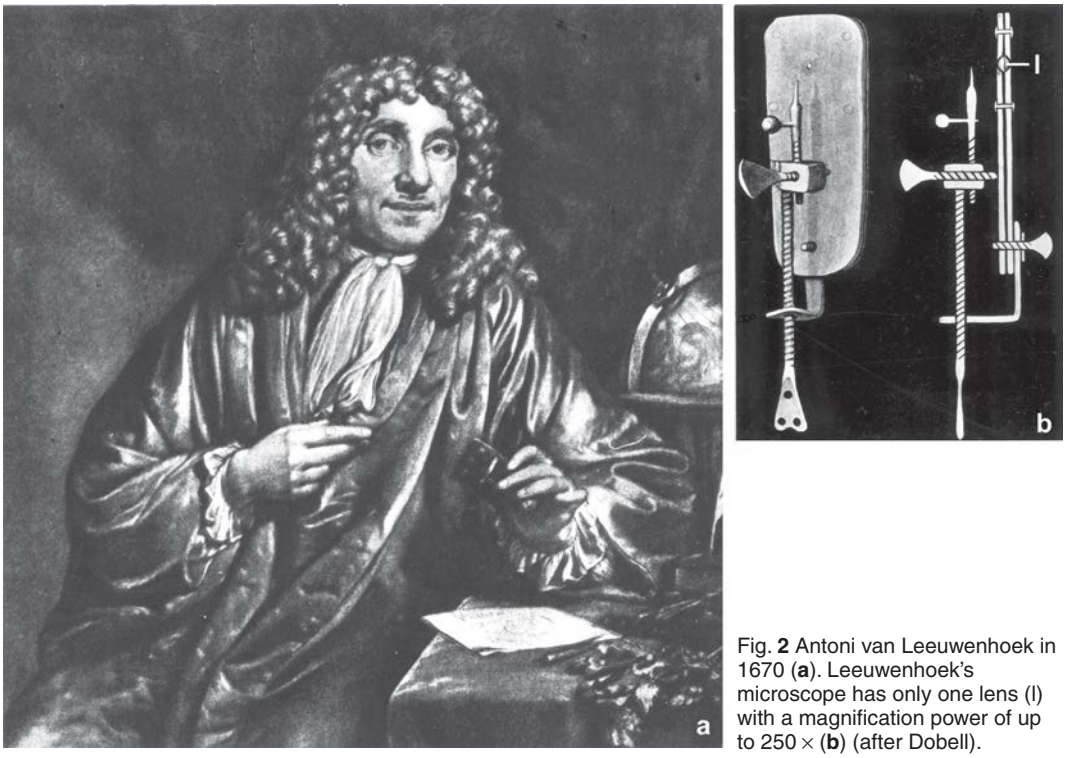

Fig. **2** Antoni van Leeuwenhoek in 1670 (**a**). Leeuwenhoek's microscope has only one lens (l) with a magnification power of up to 250 × (**b**) (after Dobell).

held until the 19th century, when the German natural philosopher Lorenz von Oken (1779–1851) wrote in 1805 that the biogenesis of spermatozoa (which he later identified as his Urthierchen) would be the result of a vital putrefaction within the testicles, a process continuing in the female after pregnancy and resulting in the formation of the fetus from decay products of blood.

Needham's doctrine of abiogenesis differed from Buffon's in that he believed that organic matter is able, through a so-called principle of expansion, to create life under favorable circumstances. In some cases, however, this process is more difficult than in others: the degree of difficulty is due to a principle of resistance that is inherent in all matter. The decay of organic matter is then the process whereby the principle of resistance is broken down. The final product of this decay is a gelatinous mass that he called zoogloea, from which new life can eventually arise.

In support of their postulates, both Buffon and Needham carried out experiments with boiled and unboiled, covered and uncovered infusions. According to their reports, infusoria appeared in all cases without exception. The doctrine of spontaneous generation was enthusiastically accepted by the scientific community.

A few years later, however, the Italian scientist Lazzaro Spallanzani (1729–1799) argued vehemently against this dogma. He built his argument on a foundation of experimental evidence and differentiated between more or less heat-resistant organisms. In spite of his investigations and insight, which were in accordance with the modern approach of science, he was not recognized for finally disproving spontaneous generation. Buffon, Needham, Oken, and Lamarck remained unconvinced of their colleagues' theories until their deaths. Not until the experimental work of Louis Pasteur (1822–1895) and Robert Koch (1843–1910) was the hypothesis finally refuted.

The pursuit of answers to general philosophical questions such as the origin of life and the finality of death was accompanied by more pragmatic attempts to collect and order the living world visible under the microscope. Following Joblot, numerous articles that dealt with the protists were published.

As examples, we might mention Henry Baker and his 1754 book *Beiträge zu nützlichem und vergnü-*

gendem Gebrauch und Verbesserung des Microscopii (*Contributions for the useful and amusing employment and improvement of the microscope*) and August Johann Rösel von Rosenhof, the discoverer of amoebae (1755). In 1769 Nicolas Théodore de Saussure was the first to observe transversal division of the infusoria (i.e. ciliates); he was also the first to establish a clonal culture, that is, a culture started with a single organism. The process of encystment was first observed around 1775, and it was at about this time that experimental research began. New observations, including the description of newly discovered organisms, have continued until the present day.

In 1768 the first systematic plan was established for the Infusoria by the Danish naturalist Otho Fridericus Müller (1730–1784; Fig. **3**). His systematics included rotifers, in addition to planktonic metazoans and those organisms we presently consider to be protists. As his book appeared after the 10th edition of Carl von Linné's *Systemae naturae* (1758) and therefore after the beginning of the scientific biological nomenclature still used today, many valid genera and species are attributed to him as author: e.g. *Monas*, *Ceratium hirundinella*, *Bursaria truncatella*, *Euplotes patella*, *Lacrymaria olor*, *Stylonychia mytilus*.

The early 19th century was a very exciting and interesting era for protozoology. Very important developments regarding terminology were made and detailed taxonomic research began in earnest. The scientific activity of the French micropaleontologist Alcide d'Orbigny (1802–1857) is often considered the starting point of this era. He is responsible for the naming of the taxon Foraminifera (organisms with shell chambers connected by holes).

In contrast to d'Orbigny, who focused his interests exclusively on the inanimate shells, his compatriot Felix Dujardin (1801–1860) also investigated the living foraminifers. He detected the living matter inside the shells and their ability to produce pseudopods and undergo active movements. This living substance, which he also described from other single-celled organisms and which he considered as having a homologous character, was named sarcode (according to the Greek term for meat-like). His 1835 thesis, the so-called sarcode doctrine, states that the living substance is a motile fluid with vacuoles and granular inclusions. However, the term sarcode, which is synonymous with protoplasm, fell into disuse and has survived only in the designations for the taxa Sarcodina or Sarcomastigophora, which are no longer in use.


ANIMALCULA INFUSORIA
FLUVIATILIA ET MARINA,
QUÆ DETEXIT, SYSTEMATICE DESCRIPSIT ET AD VIVUM
DELINEARI CURAVIT
OTHO FRIDERICUS MÜLLER,
REGI DANIÆ QUONDAM A CONSILIIS CONFERENTIÆ, PLURIUMQUE
ACADEMIARUM ET SOCIETATUM SCIENTIARUM SODALIS,
SISTIT
OPUS HOC POSTHUMUM
QUOD CUM TABULIS ÆNEIS L. IN LUCEM TRADIT
VIDUA EJUS NOBILISSIMA,
CURA
OTHONIS FABRICII.
PASTORIS ORPHANOTROPHII REGII HAUN. ET SODALIS SOCC. REG. SCIENT. HAUN.
NATURÆQUE CURIOSOR. BEROLIN.
HAUNIÆ,
TYPIS NICOLAI MÖLLERI, AULÆ REGIÆ TYPOGRAPHI.
1786.
a
Tab. XLIV.
b

Fig. **3** Title page of O. F. Müller's work (**a**) and one of the tables in the book (**b**).

After the emergence of the protoplasm theory and cell doctrine, originated in the mid-19th century by the Czech Jan Evangelista Purkinje (1787–1869) and the Germans Theodor Schwann (1810–1882) and Matthias Jacob Schleiden (1804–1881), new horizons opened for protozoologists. Could the results of work on cells of higher animals and plants also apply to protists? At first, the complicated architecture of ciliates did not seem to fit with the cell concept.

The special status of the ciliates led two Germans, Ernst Haeckel (1834–1919) and Christian Gottfried Ehrenberg (1795–1876), to neglect their unicellular nature. Haeckel hesitated to group the ciliates within his Protista until 1873, and Ehrenberg asserted that the single-celled organisms are perfect miniature animals that reflect the macroscopic, visible fauna. This is summarized in the so-called polygastric theory. In light of his point of view, it should not be surprising that he described stomach and intestines (i.e. food vacuoles), vascular systems, salivary glands (special vacuoles or probably zoochlorellae), testicles (micronuclei) with seminal vesicles (contractile vacuoles) and ovaries (probably macronuclei) in his marvelous 1838 book *Die Infusionsthierchen als vollkommene Organismen* (*The infusion animalcula as perfect organisms*) (Fig. **4**). However, even with these pardonable misinterpretations, Ehrenberg is one of the protagonists of protistology – so important are his merits in the fields of systematic zoology and micropaleontology. He showed that chalk cliffs are composed of microscopic organisms (mostly diatoms), and he described many free-living protists. The names of numerous well-known genera are a tribute to his productivity: *Actinophrys*, *Amoeba*, *Arcella*, *Bodo*, *Carchesium*, *Chlamydomonas*, *Cryptomonas*, *Dinobryon*, *Euglena*, *Euplotes*, *Loxodes*, *Nassula*, *Peridinium*, *Prorodon*, *Spirostomum*, *Synura*, and so on.

Around 1840, Ehrenberg's views were violently attacked by Dujardin and the German zoologist Carl Theodor von Siebold (1804–1885), both of

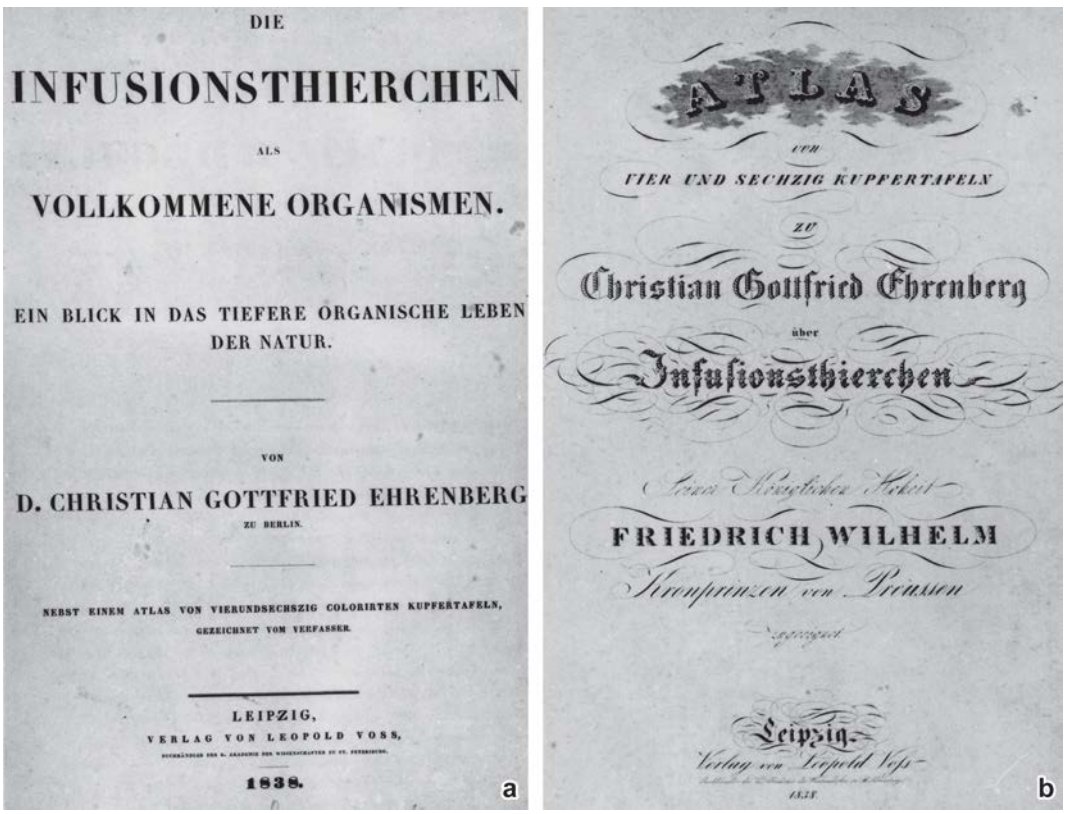

Fig. **4** Title page of Christian Gottfried Ehrenberg's most famous book (**a**) and of the accompanying pictorial atlas (**b**).

whom believed that an animal could consist of only a single cell. Siebold tried to lay the dispute to rest by redefining the protozoa as animals in which the different organ systems are not sharply defined and which consist of a single cell. In time his concept was accepted and it was he who was the first to separate the protozoa from the multicellular organisms. It is also interesting to note that the first definition of the cell was elaborated by studying living foraminifers. In 1861, the German anatomist Max Schultze (1825–1874), who introduced the use of osmium tetroxide for fixation purposes, gave the classical description of the cell as a little clump of protoplasm with a centrally located nucleus.

Although it is not our intention to review all of the protozoologists who made a mark for themselves, perhaps the following scientists should be mentioned to complete the historical perspective: the German botanist Heinrich Anton de Bary (1831–1888), who investigated slime molds; the French physician Charles Louis Alphonse Laveran (1845–1922), who discovered malarial parasites in Italy and became Nobel Laureate in 1907 in recognition of his work on the role played by protozoa in causing diseases; the Swiss zoologist Edouard Claparède (1832–1871), who, together with his coworker Johannes Lachmann, produced an impressive monograph on the infusoria and rhizopods; the British zoologist William Savill Kent (1845–1908) with his famous *Manual of the Infusoria*; the German zoologist Richard Hertwig (1850–1937), who cleared up the karyological events which occur during the conjugation of ciliates; the Russian zoologist Wladimir T. Schewiakoff (1859–1930), who studied acantharean sarcodines; the German botanist Georg Klebs (1857–1918) with his investigations of the life cycles of flagellates; the German zoologist and parasitologist Rudolf Leuckart (1822–1898), who created the taxon Sporozoa; the German heliozoan and coccidian specialist Fritz Richard Schaudinn (1871–1906); the German zoologist Franz Eilhard Schulze (1840–1917) with his investigations of rhizopods; the radiolarian expert Ernst Haeckel, who described over 4,000 species and presented them with elegant illustrations; the French zoologist Edouard Gerard Balbiani (1823–1899) as the first protistan geneticist; and the German zoologist Otto Bütschli (1848–1920), who is considered to be the *Architect of Protozoology* (according to Clifford Dobell, 1951) and was the first to write a comprehensive textbook (in three volumes) on the protozoa.

While most early protozoological studies were carried out in Europe, by the end of the 19th century protists were being studied all over the world. In 1879 Joseph Leidy and in 1888 Alfred C. Stokes wrote monographs on the protozoa of North America. Many of the protists already described from Europe were rediscovered by these pioneers, and many new species were described. By the turn of the 19th century, a solid base of knowledge had been accumulated, and many parasitic and/or pathogenic forms were beginning to be known. In some cases, significant information on the complex life cycles of some of these forms was gathered.

By 1900, a sufficient body of knowledge had been accumulated to require that new textbooks be published, and Gary Nathan Calkins (1901 and 1933) and Franz Doflein (1901, 1909, 1911, 1916, continued by Eduard Reichenow in 1929 and 1953) responded with texts that provided not only the necessary descriptive information, but also added to the knowledge of the general biology of the protista. It was then that protozoology gained sufficient respect to join other organism-oriented biological disciplines (Table **1**).

Although the description of new species continues, the protists have also been found to be ideal models for the investigation of general principles in biology. In particular, the discipline of cell biology has benefited from the special characteristics of the unicellular organisms. Notable characteristics are the ease of cultivation of these organisms, which typically have rapid rates of growth and correspondingly short generation times. Since we have learned how to establish mass cultures of protists, these single-celled organisms have become popular objects for investigations into molecular biology and biochemistry. There are, however, certain unique attributes of these organisms that have not yet been adequately explored or exploited, and we will try to point out some possibilities here as the opportunity arises.

We would like to conclude this historical overview with a short history of the most important instrument involved in the development of the field: the microscope. Van Leeuwenhoek's microscope was fairly simple; it was little more than a magnifying glass (see Fig. **2 b**). Some of the instruments of his day had a rather bizarre form (Fig. **5**) when compared to today's microscopes. By the end of the 19th century, microscopes had

Table 1: Important dates of Protistology.

EPOCH 1: Use of microscopes for detection of small invisible organisms or cells

A. VAN LEEUWENHOEK (1675)

EPOCH 2: Systematic research

G. A. GOLDFUSS (1818): Protozoa
A. D'ORBIGNY (1826): Foraminifera
CHR. G. EHRENBERG (1838): Free-living and fossil protozoans

EPOCH 3: Protists recognized as unicellular organisms

C. T. VON SIEBOLD (1845): *Animals, in which the different systems of organs are not clearly differentiated and those with irregular shape and simple organization can be reduced to only one single cell. – The protozoans can be divided into the rhizopods and infusorians.*

Sarcode doctrine and protoplasm theory:
F. DUJARDIN (1835), H. VON MOHL (1846)

Establishment of the cell doctrine:
M. SCHULTZE (1861, 1863): *A cell is a little clump of protoplasm with a nucleus in the centre*.

Studies on protozoa are combined with cell biology and embryology

EPOCH 4: Combination of protozoology with the emerging disciplines of microbiology and parasitology

Protozoa as pathogens

E. GRUBY (1843):	*Trypanosoma* (in frogs)
C. W. VON NAEGELI (1857):	pebrine (*Nosema bombycis*)
F. LÖSCH (1875):	amoebic diarrhoea (*Entamoeba histolytica*)
R. LEUCKART (1879):	Sporozoa
A. LAVERAN (1880):	Malaria and its agent (*Plasmodium*)
O. BÜTSCHLI (1881):	Myxosporidia (= Myxozoa)
G. BALBIANI (1882):	Microsporidia (= Microspora), Sarcosporidia
J. E. DUTTON (1902):	*Tryponosoma gambiense* – causative agent of sleeping sickness

EPOCH 5: Institutionalization of the discipline of protistology

Establishment of zoological institutes by protozoologists in Germany
O. BÜTSCHLI, University of Heidelberg (1878)
F. E. SCHULZE, University of Berlin (1884)
R. HERTWIG, University of Munich (1885)

F. SCHAUDINN (1902): Founder and editor of the first protozoological journal ARCHIV FÜR PROTISTENKUNDE, since 1998 continued as PROTIST.

F. SCHAUDINN (1904): Founding of protozoological laboratories at the Reichsgesundheitsamt in Berlin-Lichterfelde, and 1906 founding of the department for protozoological research at the Institute of Nautical and Tropical Diseases (presently: Bernhard-Nocht-Institute) in Hamburg
Establishment of a school of protozoology in St. Petersburg by F. DOGIEL (1908)

M. HARTMANN (1914): Founding of the department for protistology at the Kaiser-Wilhelm-Institute for Biology in Berlin-Dahlem

Table 1: Important dates of Protistology. (cont.)

Institutionalization of protistology at the international level
1947: Founding of the SOCIETY OF PROTOZOOLOGISTS in USA, presently an international society with several national sections; since 1954 editing the JOURNAL OF PROTOZOOLOGY (renamed 1993 JOURNAL OF EUKARYOTIC MICROBIOLOGY) 1963: Founding of the Polish journal ACTA PROTOZOOLOGICA 1968: Founding of the French journal PROTISTOLOGICA (1987 continued in Germany as the EUROPEAN JOURNAL OF PROTISTOLOGY) 1972: Founding of the INTERNATIONAL SOCIETY FOR EVOLUTIONARY PROTISTOLOGY (ISEP) 1999: Founding of the Russian journal PROTISTOLOGY
1961: Prague: FIRST INTERNATIONAL CONGRESS OF PROTOZOOLOGY, followed by congresses at four yearly intervals: 1965 in London, 1969 in Leningrad (St. Petersburg), 1973 in Clermont-Ferrand, 1977 in New York, 1981 in Warsaw, 1985 in Nairobi, 1989 in Tsukuba, 1993 in Berlin, 1997 in Sydney, 2001 in Jerusalem/Salzburg

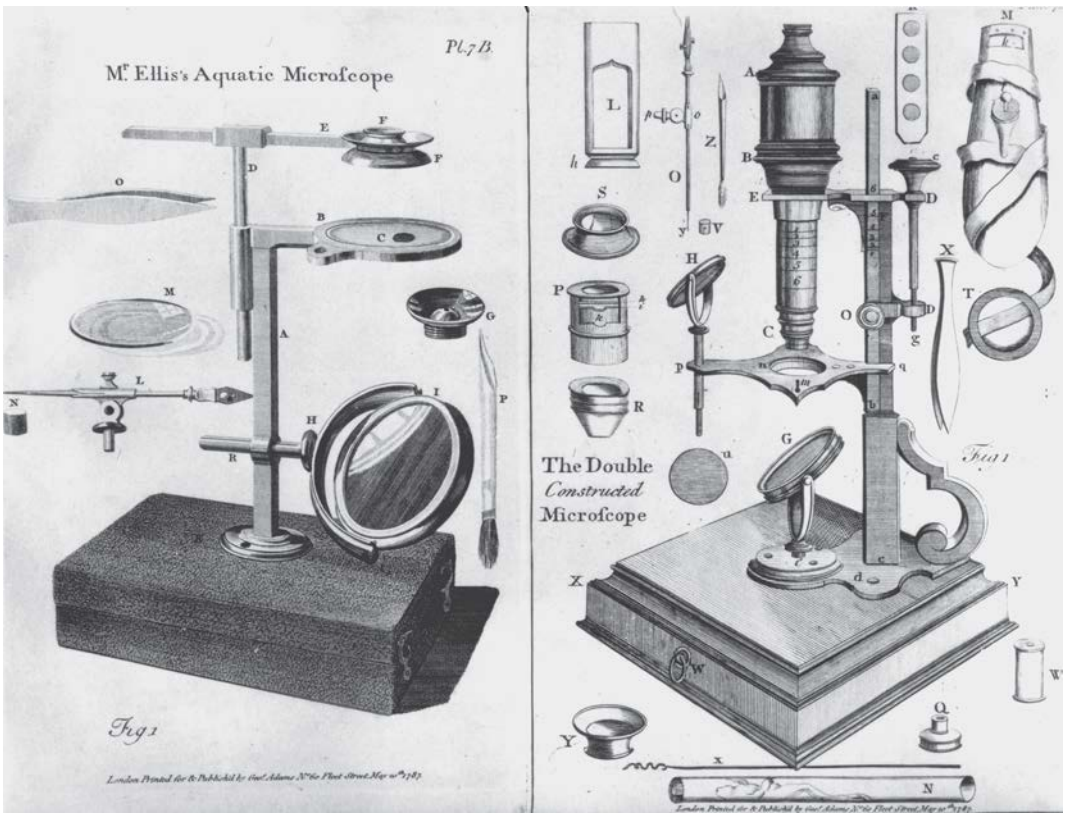

Fig. **5** Anno Domini 1787: two pages from the catalogue of a British microscope manufacturer.

evolved to a form similar to those used today. However, it is obviously not the appearance of the microscope, but its optical components that are important in the formation of the sharp and informative image it can produce. Great progress was made in the intervening years; by about 1830 optics had improved to the point where a skilled microscopist could resolve an image of approxi-

mately 1 μm. It was no longer necessary to have the skilled (and perhaps extraordinary) eye of a van Leeuwenhoek. In fact, the advances in lens design were responsible for the observation of the cell nucleus, and in Europe of the 1830s the cell and subcellular structures became known through observations made using these improved instruments. Immersion objectives came into general use by about 1880, which allowed higher magnifications at improved brightness and higher resolution. Resolution had improved to near the present standards by around 1885, following the correction for chromatic aberration through the development of apochromatic objectives with suitable compensating oculars. Later, around 1932, the first phase contrast microscopes were constructed, with which it was possible to observe faint details in living cells. This was a major breakthrough for protistologists and cell biologists alike, honored by the Nobel Prize given to the Dutch inventor Frits Zernike. Today there are diverse microscopical instruments available for our use: bright-field, dark-field, phase contrast, fluorescence, and differential interference contrast microscopy are instruments used in the routine investigation of the unicells.

The electron microscopes are of special importance in modern times. The transmission electron microscope became a useful tool for protistologists in the mid-1950s, when techniques of specimen preparation improved to the point that meaningful data could be acquired. A Nobel Prize, given to the German physicist Ernst Ruska in 1986, honored this invention too. The development of the scanning electron microscope by the German physicist Manfred von Ardenne in the mid-1960s provided graphic information on the general features of the protistan surface and overall morphology. These electron microscopes provided a new dimension to the study of protists.

Cellular Organization of Protists

Single-celled protists generally have the same cell construction as that of other eukaryotic organisms. It is possible, using electron microscopy to find some differences in the structure and number of organelles present in the different protists, but one can occasionally find such differences in the cells of multicellular eukaryotes. Few if any organelles are known to occur exclusively in protists.

However, more and more indications favor the idea that protists represent also a group of organisms in which a remarkable tendency for the secondary dismantling or even loss of organelles becomes obvious. During the course of cell evolution, mitochondria (typical for all eukaryotic organisms) and plastids (typical for phototrophic eukaryotes) may be distructed up to their complete disappearance, with exception of several genomic traits that were laterally transferred to the nuclei. It is surprising that the most important parasites belong to such protists. Examples for parasites with a secondary loss of mitochondria are *Entamoeba* and *Giardia*. Trypanosomatidea and Oomycetes have lost the plastids typical for their antecedents and in the Apicomplexa one can find an apicoplast as visible residual of a former plastid.

Membranes and Compartments

A basic characteristic of eukaryotic cells is the compartmentalization of the cytoplasm by membranes (Figs. **6**, **7**). As with all living cells, the protist is separated from its environment by a cell membrane (Fig. **8**). As seen in the electron microscope, this membrane has the typical trilamellar appearance of other biological membranes. Aggregates of regularly arranged intramembranous particles have been detected in the plasma membrane of some ciliates, for instance (Fig. **8 f**). The function of these aggregates is still unknown.

A mucoid layer, the glycocalyx, usually covers the exterior of the plasma membrane (Fig. **8**). This surface coat can contain – in a species-specific manner – longer filaments called glycostyles (Fig. **8 c**–**e**). This layer is composed of the oligosaccharide chains of the membrane glycoproteins (intramembranous particles, a.k.a. IMP) and glycolipids, plus glycoproteins and proteoglycans consisting of glycosaminoglycans attached to a protein core. The glycocalyx may have a regular structure, but when such structures occur, they do not necessarily follow the pattern of the IMP. The glycocalyx is involved in the information system of the cell, and it is in this layer that receptor molecules reside. Furthermore, the glycocalyx allows the cell to selectively absorb solutes from the surrounding medium. Absorbed molecules can be transported into the cell by a variety of mechanisms which are presently under investigation.

The perilemma is a membrane-like structure which is known from some hypotrich and tintinnid ciliates (comp. Fig. **24 f**). While this structure has the appearance of the plasma membrane, it is actually an outer, extracellular envelope-like structure of unknown function. Membranous remnants surrounding the frustules are known from diatoms (comp. Fig. **71**).

In addition to the glycocalyx, various protists possess extracellular scales (Fig. **9**), fibrillar systems, or even cell wall-like structures. Extracellular loricae may be produced and can become elaborate structures in some cases.

Intracellular membrane systems such as the endoplasmic reticulum, lysosomes, peroxisomes (microbodies) (Fig. **10**), Golgi apparatus (Fig. **11**), mitochondria (Fig. **12**) and plastids (Figs. **13**, **14**) appear similar to those in metaphytan or metazoan cells. However, it is interesting to note that members of the protists contain both the fewest and the largest number of Golgi cisternae known (one or two in *Tetrahymena* during vegetative growth, and up to as many as 30 or more in *Trichonympha*).

Three special types of organelles are represented by the glycosomes, the hydrogenosomes, and the mitosomes. Glycosomes are vesicles that are found as 0.2–0.3 µm large spheres or ovoids exclusively in trypanosomatids (Fig. **15**). About 30 of these organelles, which do not show any special morphological features, are present in a single cell of *Trypanosoma brucei*. The bloodstream forms of the parasitic trypanosomatids take up glucose constantly and degrade it into pyruvate using seven glycolytic enzymes, which are con-

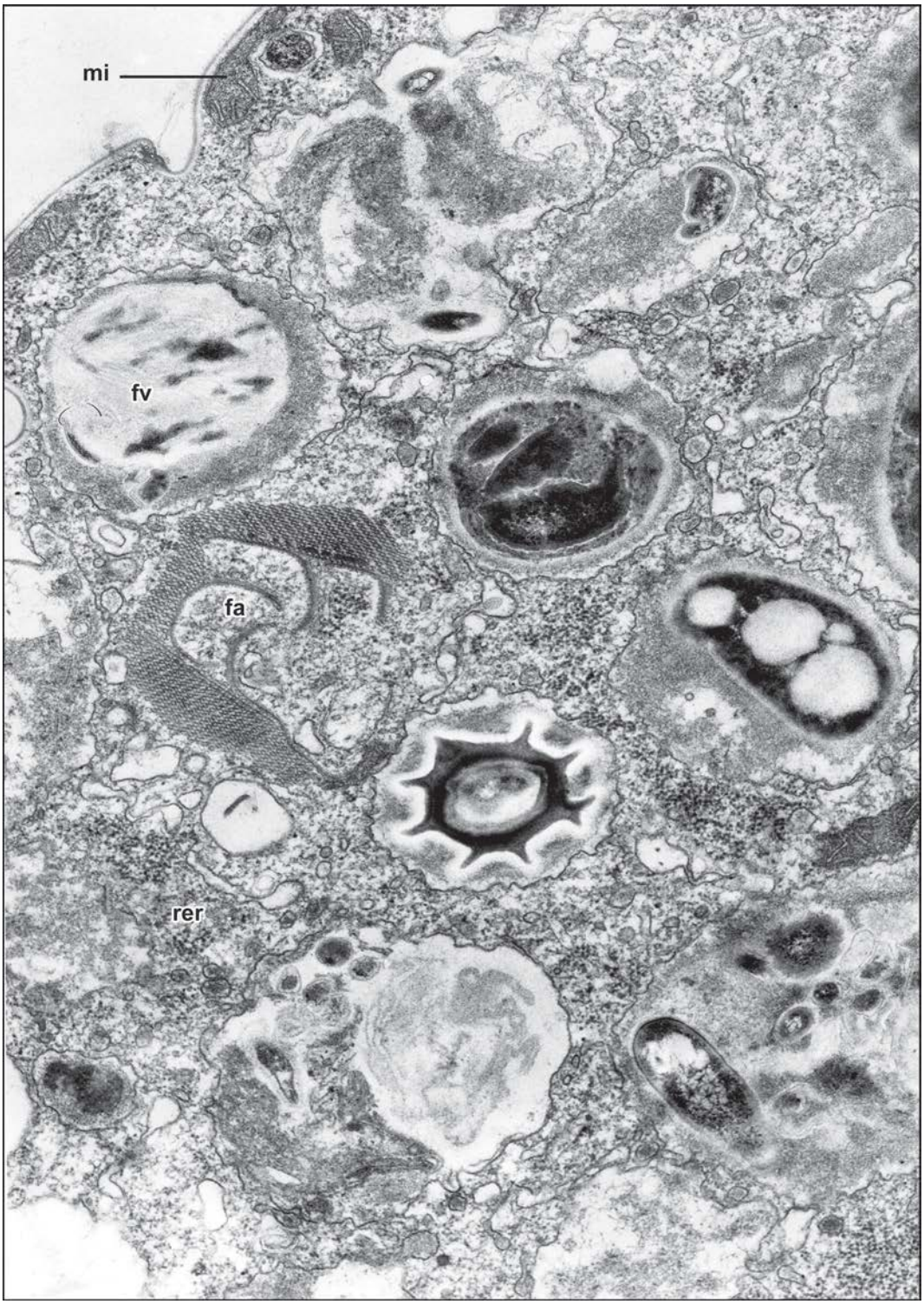

Fig. **6** Ultrastructural organization of the euglenid *Entosiphon sulcatum*: fa = feeding apparatus, fv = food vacuole, mi = mitochondrion, rer = rough endoplasmic reticulum. Magn.: 25,000 ×.

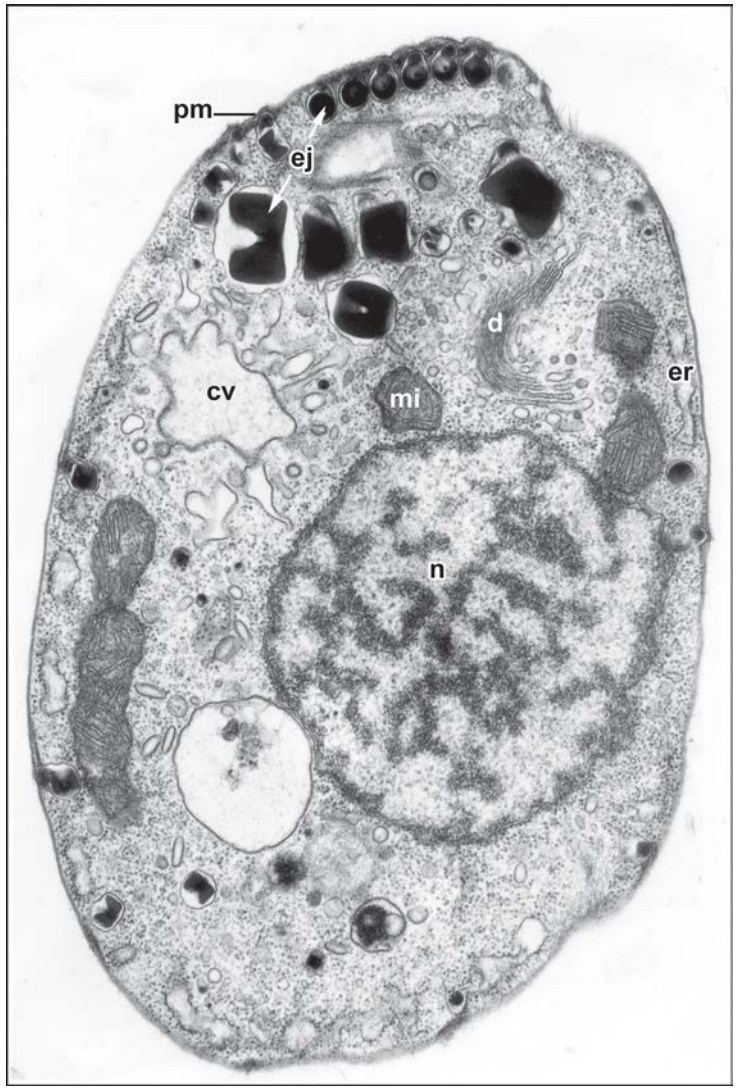

Fig. **7** Section through the cryptomonad *Cyathomonas*. The essential elements of a protistan cell are present (apart from the flagellum): cv = contractile vacuole, d = dictyosome, ej = ejectisome, er = endoplasmic reticulum, mi = mitochondrion, n = nucleus, pm = plasma membrane. Magn.: 10,000 ×.

tained in the glycosomes. Glycolysis is more efficient when it takes place in the glycosomes rather than in the cytosol, where it takes place in probably every other eukaryotic organism. It is possible that glycosomes are evolutionary related to peroxisomes, which are organelles lacking in trypanosomatids. According to the latest findings, glycosomes contain proteins encoded by genes transferred from an ancient and no longer demonstrable algal endosymbiont to the host genome.

Hydrogenosomes (Fig. **15**) are involved in carbohydrate metabolism and occur in a broad range of phylogenetically distant, microaerophilic protists including trichomonads and hypermastigote flagellates, amoeboflagellates, rumen-dwelling and free-living ciliates and chytrid fungi. They metabolize pyruvate derived from glycolysis into acetate, CO_2 and H_2. They use a fermentative pathway for this pyruvate metabolism utilizing the enzymes pyruvate:ferredoxin oxidoreductase and hydrogenase. However, their contribution to ATP production appears to be substantially smaller than that of mitochondria, and they may have other functions as well. Hydrogenosomes are surrounded by a double membrane, may contain paracrystalline structures, and multiply by binary fission; DNA is not generally contained. Hydrogenosomes and mitochondria may have a common origin.

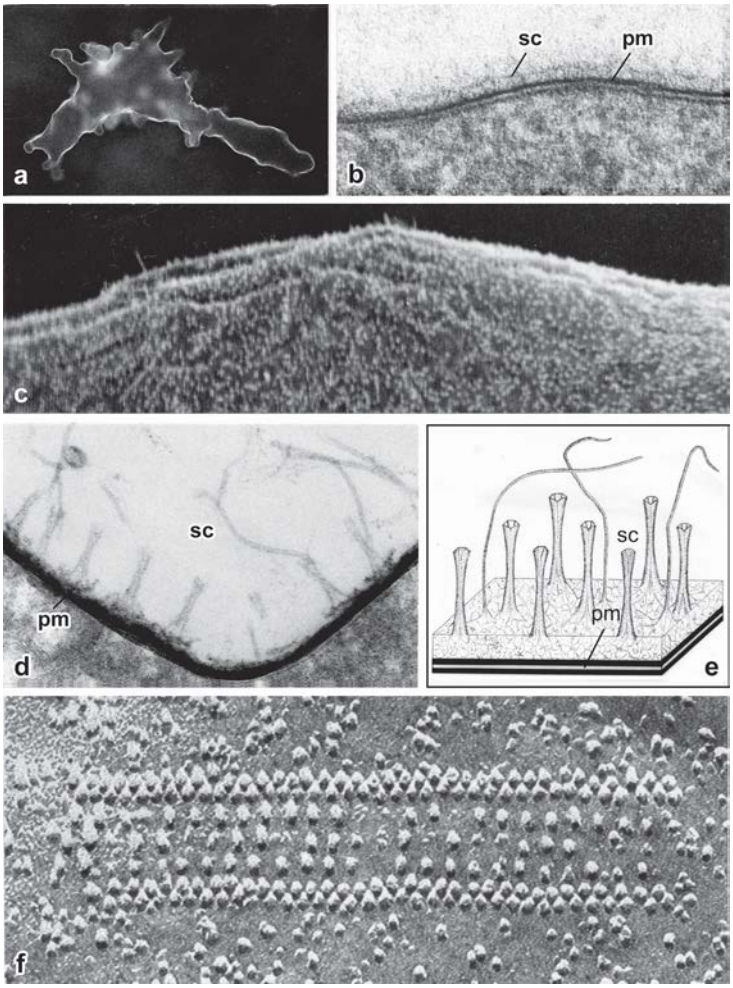

Fig. **8** Plasma membranes (pm) of various protists. Glycocalyx of *Amoeba proteus* stained with FITC-Con A (**a**); the amoeba *Vampyrella lateritia* with surface coat (sc) (**b**); surface coat (sc) of *Vannella simplex* in scanning (**c**) and transmission electron microscopy (**d**) and with schematic presentation of the highly structured glycocalyx with glycostyles (**e**). Regular arrangement of intramembranous particles in the scuticociliate *Cyclidium* (Ciliophora) (**f**). (d and e courtesy of E. Hausmann, Berlin; f courtesy of C. F. Bardele, Tübingen). Magn.: a 150 ×, b 185,000 ×, c 7,500 ×, d 150,000 ×, f 100,000 ×.

The mitosome (= cryptosome) is an organelle related to mitochondria and occurs for example in the parasitic amoeba *Entamoeba histolytica*. It is likely that *Entamoeba* once harbored mitochondria or an endosymbiont related to the progenitor of mitochondria and that the organelle lost many functions during its adaptation to an anaerobic way of life. It apparently lost its entire genome during the reduction process, as no evidence for the presence of extranuclear DNA has been obtained. The idea that a mitochondrially derived compartment originally existed was suggested by the detection of nuclear genes which encode mitochondrial proteins, and by antibodies against a recombinant protein (mitochondrial chaperonin) which label the organelle. The physiological functions of the mitosome are unknown, but it is unlikely that energy metabolism takes place here.

A special organelle derived from a plastid, the apicoplast (apicomplexan plastid), was recently recognized in several apicomplexan species. The apicoplast appears to be the result of a secondary endosymbiosis with an alga, possibly a red alga. Because up to four membranes surround the organelle (Fig. **16**), it was called a thick-walled organelle or vacuole limited by thick membrane before its plastid-like nature was clear. It is usually situated anterior to the nucleus and close to the Golgi apparatus. There is evidence for a single origin of the plastid. Each apicoplast contains a nucleoid with a relatively large amount of DNA in the form of a 35-kb, circular, extrachromosomal DNA that is clearly described. The apicoplast di-

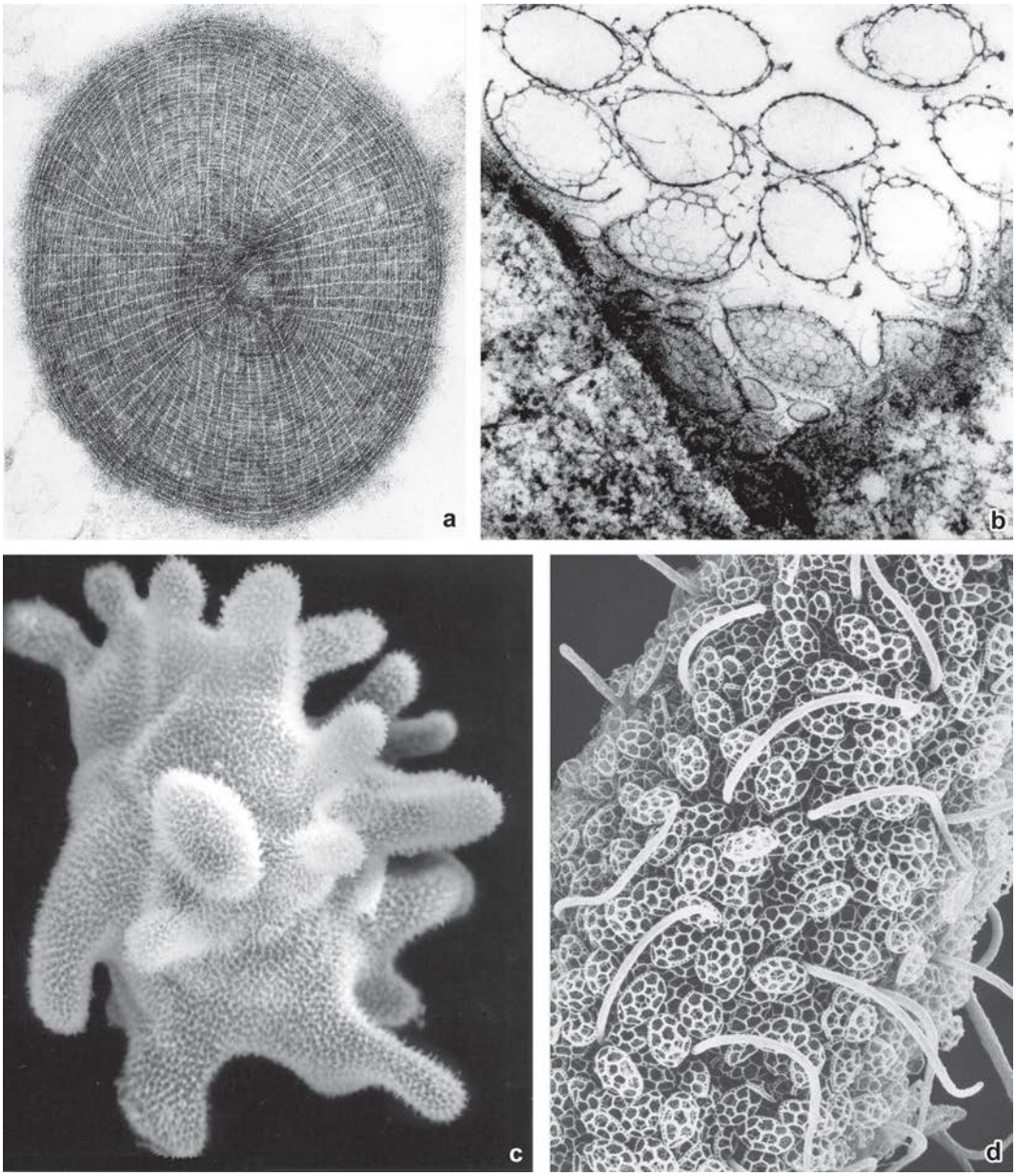

Fig. **9** Scales of unicellular organisms. Scale of the prymnesiomonad *Pleurochrysis* (**a**) and of the amoeba *Cochliopodium* (**b**); *Dactylamoeba* completely covered with scales (**c**); scales of the ciliate *Lepidotrachelophyllum* (**d**) (a courtesy of W. Herth, Heidelberg; c courtesy of W. Foissner, Salzburg; d courtesy of C. F. Bardele, Tübingen). Magn.: a 77,000 ×, b 28,000 ×, c 1,800 ×, d 4,200 ×.

vides by binary fission and is introduced into developing daughter cells very early during replication. Structures such as caps at the termini of dividing apicoplasts, a ring and a microbody-like granule may be involved in apicoplast division. The apicoplast is probably incapable of photosynthesis, but it is essential for parasite survival. Although its function is still uncertain, experiments indicate that it may be responsible for fatty acid biosynthesis. Since many apicomplexans

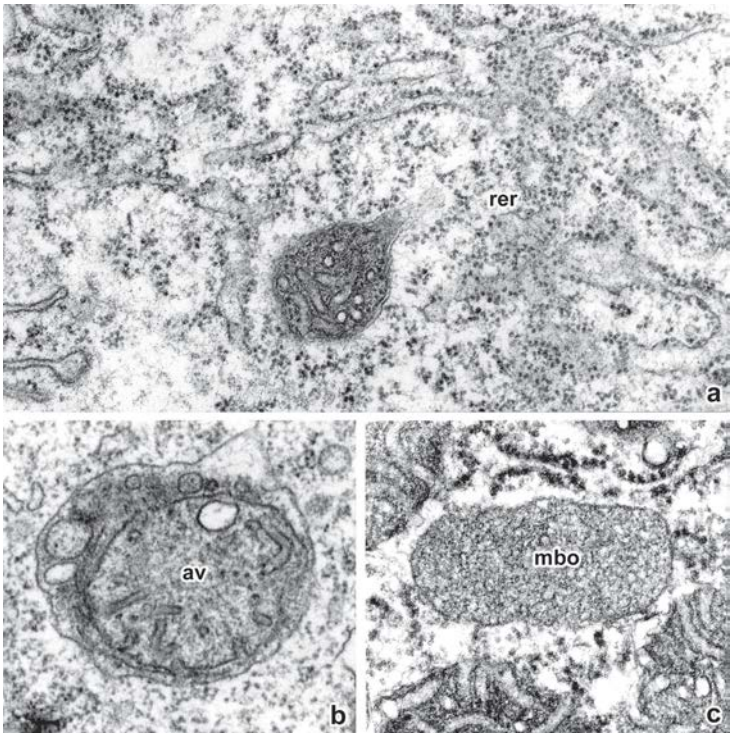

Fig. **10** Membrane systems in the ciliate *Paramecium*. Rough endoplasmic reticulum (rer) (**a**); autophagic vacuole (av) with mitochondrion during degradation (**b**); microbody (mbo) (**c**). Magn.: a 25,000 ×, b 35,000 ×, c 50,000 ×.

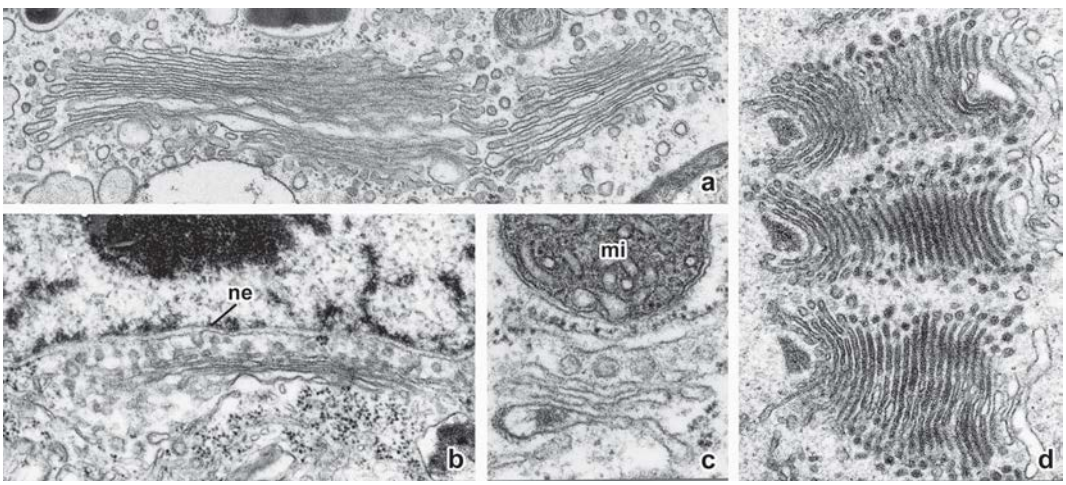

Fig. **11** Organizational types of dictyosomes in the cryptomonad *Rhodomonas* (**a**), in an unidentified amoeba (**b**), in the ciliate *Pseudomicrothorax* (**c**), and in the hypermastigid flagellate *Joenia* (**d**). mi = mitochondrion, ne = nuclear envelope. Magn.: a 25,000 ×, b 25,000 ×, c 50,000 ×, d 30,000 ×.

are important human and veterinary pathogens, it might be possible to use the newly detected organelle as a drug target for controlling the parasite. For example, it seems probable that the apicoplast is the target for macrolid antibiotics in *Toxoplasma* and for rifampicin in *Plasmodium*.

Extrusomes are basically exocytotic vesicles. In their complexity, extrusive organelles are largely specific to protists, although related structures may exist in some lower metazoans, e.g. rhabdites in flatworms. Extrusomes are membrane-bound organelles usually located in the cortical cytoplasm of these cells, although immature forms arise in the cytoplasm. While they are known to have varying functions depending on their type, they all exhibit one general characteristic: they are readily discharged when subjected to a wide range of stimuli, i.e., mechanical, electrical, and

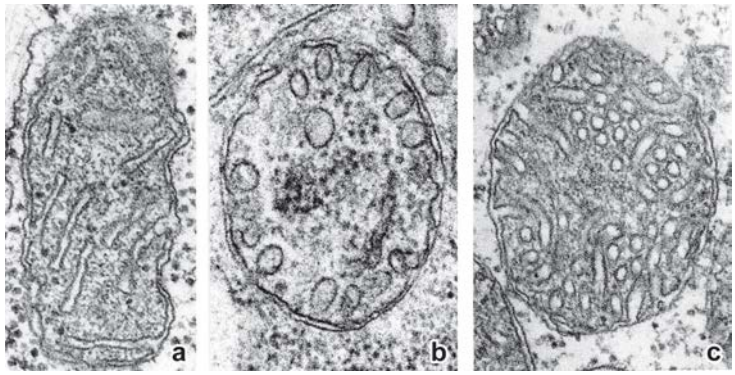

Fig. **12** Organizational types of mitochondria in the cryptomonad flagellate *Cyathomonas* (**a**), in the filopodial amoeba *Vampyrella lateritia* (**b**), and in the ciliate *Paramecium caudatum* (**c**). Magn.: a 55,000 ×, b 40,000 ×, c 60,000 ×.

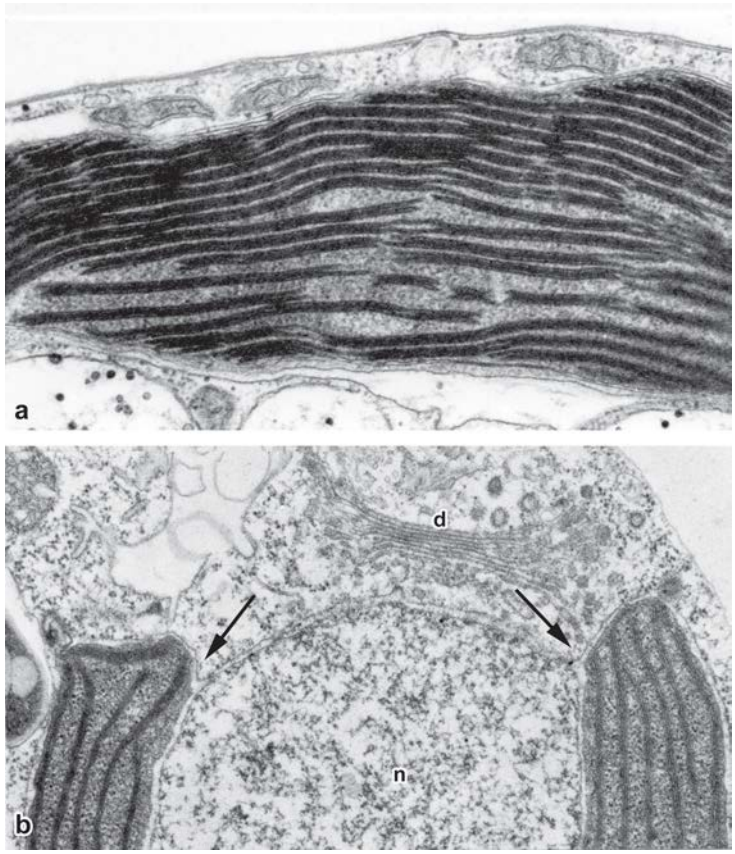

Fig. **13** Partial view of the plastid of the cryptomonad *Rhodomonas* with double stacks of thylakoids (**a**). Continuity of nuclear envelope and exterior plastidial envelope (arrows) in the chrysomonad *Ochromonas* (**b**). d = dictyosome, n = nucleus. Magn.: a 20,000 ×, b 18,000 ×.

chemical. During the transition from the resting state to the ejected form, the organelles undergo characteristic morphological changes. The best-known type of extrusome is the trichocyst of *Paramecium* (Fig. **17**). At present about fifteen different types of extrusomes are known.

The contractile vacuole is a characteristic membranous structure found in protists lacking a cell wall. This organelle, which can be seen with the light microscope, is associated with other structural elements visible only using electron microscopy. Therefore, the term contractile vacuole complex is presently used to identify those structures responsible for osmoregulation.

Some organelles, e.g. mitochondria and plastids, exhibit morphological variations which may be