ORIGINAL PAPER

In the shadow of Darwin: Anton de Bary's origin of myxomycetology and a molecular phylogeny of the plasmodial slime molds

T. Hoppe · U. Kutschera

Abstract In his *Origin of Species* (John Murray, London, 1859), Charles Darwin described the theory of descent with modification by means of natural selection and postulated that all life may have evolved from one or a few simple kinds of organisms. However, Darwin's concept of evolutionary change is entirely based on observations of populations of animals and plants. He briefly mentioned 'lower algae', but ignored amoebae, bacteria and other microorganisms. In 1859, Anton de Bary, the founder of mycology and plant pathology, published a seminal paper on the biology and taxonomy of the plasmodial slime molds (myxomycetes). These heterotrophic protists are known primarily as a large composite mass, the plasmodium, in which single nuclei are suspended in a common 'naked' cytoplasm that is surrounded by a plasma membrane. Here we summarize the contents of de Bary's 1859 publication and highlight the significance of this scientific classic with respect to the establishment of the kingdom Protoctista (protists such as amoebae), the development of the protoplasmic theory of the cell, the introduction of the concept of symbiosis and the rejection of the dogma of spontaneous generation. We describe the life cycle of the myxomycetes, present new observations on the myxamoebae and propose a higher-order phylogeny based on elongation factor-1 alpha gene sequences. Our results document the congruence between the morphology-based taxonomy of the myxomycetes and molecular data. In addition, we show that free-living amoebae, common protists in the soil, are among the closest living relatives of the myxomycetes and conclude that de Bary's 'Amoeba-hypothesis' on the evolutionary origin of the plasmodial slime molds may have been correct.

Keywords Darwin · de Bary · Evolution · Myxomycetes · Phylogenetic tree · Spontaneous generation

Introduction

In his famous book *On the Origin of Species*, Charles Darwin (1809–1882) presented his 'principle of descent with modification by means of natural selection', which later evolved into one of the most important concepts of the biological sciences. However, as pointed out by Mayr (2004) and others, Darwin (1859) did not describe one coherent theory of evolution. Rather, the British naturalist deduced a set of five different hypotheses to explain the mass of empirical facts he had collected over the past 20 years which were summarized in condensed form in his monograph: Species have evolved and multiplied gradually from pre-existing varieties by means of natural selection, and all living beings originated from one primordial form. Throughout his book, Darwin (1859) referred to animals and plants, but lower organisms (protists, bacteria) are ignored by the author. Darwin's principle of common descent, which was briefly outlined in the first (1859) and described in more detail in the definitive last edition of his monograph (Darwin 1872), is based on a questionable analogy between extant 'lower algae' and his hypothetical 'primordial proto-organism' that may have lived in the distant past (Kutschera and Niklas 2008).

Darwin (1859, 1872) had very little to say on the topic of cells and their similarities to free-living protists such as amoebae, euglenids etc. This important area of research in

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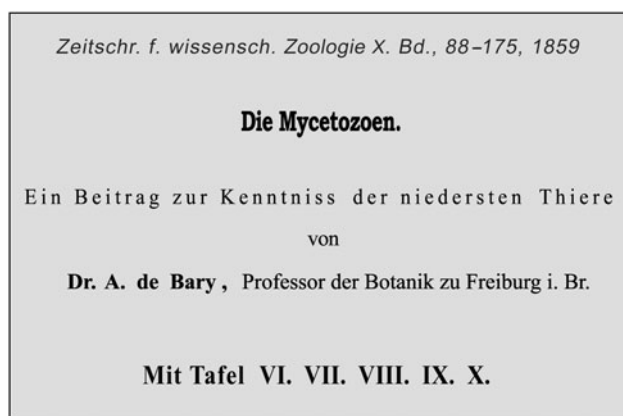e-mail: kut@uni-kassel.de

Fig. 1 Facsimile of the title page of Anton de Bary's seminal publication of 1859 on the Mycetozoa, with the sub-title, 'A contribution to our knowledgement of the lowest animals'. In the second edition (1864) of this scientific classic, the author used the terms 'Myxomycetes' and 'lowest organisms', respectively (adapted from de Bary 1859)

the emerging evolutionary sciences of the nineteenth century was pursued by naturalists that remained 'in the shadow of Charles Darwin' up to the present day. The life and scientific achievements of Darwin's most enthusiastic disciple, Ernst Haeckel (1834–1919), has been described in a recent monograph (Hossfeld 2009).

In this article, we outline the seminal studies of the less better known German botanist Anton de Bary (1831–1888) on the biology and systematics of the myxomycetes (plasmodial slime molds). This cosmopolitan group of microorganisms was ignored by Darwin (1859, 1872) and most evolutionary biologists of the twentieth century. De Bary's monograph was published in 1859 (Fig. 1), but remained unknown outside the field of slime mould research (myxomycetology). In addition, we describe the life cycle of these enigmatic organisms, provide observations on the amoebae and present a higher-order phylogeny that is based on newly acquired elongation factor-1 alpha gene sequences.

Materials and methods

Field expeditions were conducted during the summer and autumn of the years 2004–2008. Our study areas were the forests around Kassel, Germany (Habichtswald). The following species were collected in the field: *Hemitrichia clavata*, *H. calyculata*, *Trichia persimilis*, *T. verrucosa*, *T. alpina*, *T. decipiens*, *T. varia*, *Metatrichia vesparia*, *Arcyria denudata* (Trichiales); *Tubulifera arachnoidea*, *Lycogala epidendrum*, *Cribraria cancellata* var. *fusca* (Liceales); *Didymium nigripes*, *Didymium serpula*, *Physarum compressum*, *P. didermoides*, *P. viride*, *P. hongkongense*, *P. virescens*, *P. bivalve*,

P. daamsii, *P. oblatum*, *P. leucophaeum*, *Badhamia utricularis*, *B. versicolor*, *B. panicea*, *Craterium leucocephalum*, *Fuligo septica* var. *candida*, *F. septica* var. *flava* (Physarales); *Ceratiomyxa fruticulosa*, *C. fruticulosa* var. *porioides* (Ceratiomyxales); *Stemonitis fusca* (Stemonitiales).

Cultures or sporophores (mature fruiting bodies) of the following taxa were kindly provided by H. Müller (Rudolstadt, Germany): *Trichia persimilis*, *T. verrucosa*, *T. alpina*, (Trichiales); *Didymium serpula*, *Physarum compressum*, *P. didermoides*, *P. viride*, *P. hongkongense*, *P. virescens*, *P. bivalve*, *P. daamsii*, *P. oblatum*, *P. leucophaeum*, *Badhamia utricularis*, *B. panicea*, *Craterium leucocephalum*, (Physarales) (Neubert et al. 1993, 1995, 2000). All samples were air-dried and stored in cardboard boxes at room temperature. Spore germination and growth of the swarm cells/ plasmodia took place on agar plates (closed Petri dishes, diameter: 10 cm) at a temperature of 25 °C in darkness.

Scanning electron micrographs of haploid amoebae, fixed in glutaraldehyde (2% v/v), were prepared as described by Kutschera (2002).

For deoxyribonucleic acid (DNA) extractions, 0.05–0.1 g of sporophores were ground with a pestle in lysis buffer (DNeasy plant mini kit, Qiagen, Hilden, Germany). Total DNA was obtained according to the manufacturer's protocol. The eukaryotic gene elongation factor-1 alpha (ca. 1300 bp) was amplified using the following primers (Baldauf and Doolittle 1997):

Primer	Sequence (5′–3′)	Position
MYX f1	GGA TTC GTT ATT GGN CAY GTN GA	37–60
MYX f2	CTC GAG AAR AAY ATG ATN CAN GG	301–324
MYX f3	CGG AAG CTT TCA AYA ARA TGG A	477–499
MYX f4.1	GGA ATC GAT ACA GTN CCN GTN GG	804–827
MYX f4.2	GTA CGT GAG CTC CAR GAY GTN TA	771–794
MYX r1	CCN GTY TGN TCT CTT GTA CGA GAG	402–378
MYX r4	GTY CTR CAN ATG TTC TAG CCA GCC	762–738
MYX r7	CCN CTR TTR CAA CCT AGG TTG CA	924–901
MYX r10.1	GTY TGN CAN CGS CAG CCT TAA GAC	1311–1287
MYX r10.2	CGN CAN CCT CAT TAC CTA GGT CT	1320–1297
Additional primer sequences		
(A) Based on *P. polycephalum* (AF 016243)		
PhNG1f	CAC CAC CAC TGG ACA CTT GAT	13–33
PhNG1r	ATG TCA CGG ACG GCG AAA	1288–1305

Primer	Sequence (5′–3′)	Position
(B) Based on *L. epidendrum* (FJ 546677) und *A. denudata* (FJ 546659)		
TLEF 1f	GGT TCY TTC AAR TAY GCS TG	63–83
TLEF 1r	TGT TTG WCK CAT ATC AC	1136–1152
LEF 1f	CAA CGA AAT CGA AGA AAG AG	392–412
TEF 1f	GGT ATT GCT AAG AAC GGA CAG	556–576
(C) Based on *S. flavoginita* (AY 643819)		
EFSF 1f	GCT GAA ATG GGC AAG GGT	58–75
EFSF 1r	ACA CAT GGG CTT GGA AGG	1245–1228

All DNA sequences, which were obtained with an automated sequencer (ABI 310, Applied Biosystems, Foster City, USA), were aligned electronically using Bio Edit software 7.0.9.0 (Hall 1999). New sequences reported here have been deposited in the GenBank database. The accession numbers are summarized in Table 1.

Phylogenetic trees, based on our 32 myxomycete taxa and the GenBank sequences of other organisms, were constructed using maximum likelihood (ML) and Bayesian interference (BI) methods as described by Fiore-Donno et al. (2005, 2008). All DNA extractions and sequencing steps were repeated at least two times using different sporophores.

Results

Sporophores of representative myxomycetes

In his classical monograph (Fig. 1), de Bary (1859) depicted the mature fruiting bodies (sporophores) of the most common myxomycetes in such a detail that his drawings are of the same quality as modern photographs of these organisms. The first slime mold ever described in the scientific literature, *Lycogala epidendrum* (L.) Fr. 1654, is shown in Figs. 2a and 3a, together with the sporophores of two species that live in the same habitat (Figs. 2b, c, 3b, c). These common myxomycetes co-occur in temperate forests of Europe, mostly on rotten logs or on the bark of old trees (Neubert et al. 1993).

Life cycle and morphology of the amoebae

A major achievement of de Bary (1859) was the elucidation of all important stages in the life cycle of different myxomycetes. He showed that the spores enclosed in the mature fruiting bodies of all species investigated germinate essentially in the same manner and develop into one or more swarm cells. Moreover, de Bary (1859) observed and

documented that these cells divide repeatedly and thereafter fuse to form an amoeba-like microbe that enlarges into small amoeboid organism which he interpreted as plasmodia. These large composite masses, in which individual nuclei are suspended in a common cytoplasm surrounded by a single plasma membrane, should not be confused with *Plasmodium*, the genus of apicomplexan Protoctista that causes malaria (Winzeler 2008). To our knowledge, de Bary's sequence of developmental stages in the plasmodial slime molds has been confirmed by all subsequent myxomycologists who have studied this topic. However, many cytological details have been added to de Bary's original scheme (Martin and Alexopoulos 1969; Everhart and Keller 2008).

Our current view of the life cycle of myxomycetes, deduced from observations on two European species (*Fuligo septica* var. *candida* and *Didymium nigripes*) that were maintained in the laboratory, is shown in Fig. 4. The ontogeny of these microorganisms can be divided into a haploid (n) reproductive and diploid ($2n$) trophic phase. The relatively large diploid plasmodium produces sporulating organs (sporophores or another kind of fruiting bodies). Within the sporangium (sporocarp), haploid spores are produced that, after germination, develop into one to four unicellular myxamoebae or swarm cells that lack a cell wall and are therefore only surrounded by the plasma membrane. The amorphous myxamoebae divide via mitosis, whereas the swarm cells, which also divide, have two flagellae of unequal length. Two myxamoebae (Fig. 5) or swarm cells, which belong to the same morphospecies, fuse as sexual gametes to form a zygote ($2n$). This diploid ($n + n$) cell starts to feed, a process that initiates mitosis without cytokinesis. As a result, a multinucleate, amorphous mass develops, which is called the plasmodium. It should be noted that the common name 'plasmodial slime molds' is derived from this multinucleate, amoeba-like complex, which is, together with the sporophore, a diagnostic feature for species identification (Schnittler and Mitchell 2000; Clark 2000, 2004; Neubert et al. 1993, 1995, 2000).

Under unfavourable environmental conditions, the mobile, growing plasmodium, which feeds on bacteria and decaying organic material, will desiccate, harden and finally develop into a dormant stage that is called sclerotium. As soon as favourable conditions such as a supply of water, higher temperatures or food items return, the hardened sclerotium will hydrate and the re-vitalized plasmodium crawls out. After another feeding period, the mature diploid plasmodium produces fruiting bodies.

In the common plasmodial slime mold species shown in Figs. 2 and 3, the entire life cycle, from spore germination to mature sporophore development (Fig. 4), can be completed within 5–10 days when spores are placed on agar

Table 1 Species names, GenBank accession numbers, taxonomic status and length (base pairs, bp) of 32 newly acquired gene sequences (elongation factor-1 alpha) from different myxomycetes

Species	GenBank number	Order	Length (bp)
Badhamia panicea	FJ 546661	Physarales	710
Badhamia utricularis	FJ 546662	Physarales	539
Physarum leucophaeum	FJ 546685	Physarales	758
Physarum oblatum	FJ 546686	Physarales	609
Fuligo septica var. *candida*	FJ 546670	Physarales	735
Fuligo septica var. *flava*	FJ 546671	Physarales	753
Physarum hongkongense	FJ 546679	Physarales	627
Physarum virescens	FJ 546687	Physarales	666
Physarum viride	FJ 546688	Physarales	706
Physarum compressum	FJ 546682	Physarales	702
Physarum didermoides	FJ 546684	Physarales	705
Craterium leucocephalum	FJ 546664	Physarales	755
Physarum daamsii	FJ 546683	Physarales	755
Badhamia versicolor	FJ 546663	Physarales	722
Physarum bivalve	FJ 546681	Physarales	772
Didymium nigripes	FJ 546668	Physarales	632
Didymium serpula	FJ 546669	Physarales	714
Arcyria denudata	FJ 546659	Trichiales	707
Trichia decipiens	FJ 546691	Trichiales	754
Trichia varia	FJ 546673	Trichiales	736
Hemitrichia calyculata	FJ 546672	Trichiales	709
Metatrichia vesparia	FJ 546678	Trichiales	1066
Tubulifera arachnoidea	FJ 546675	Liceales	721
Trichia alpina	FJ 546690	Trichiales	735
Trichia verrucosa	FJ 546674	Trichiales	700
Hemitrichia clavata	FJ 546676	Trichiales	600
Trichia persimilis	FJ 546692	Trichiales	812
Cribraria cancellata var. *fusca*	FJ 546665	Liceales	540
Lycogala epidendrum	FJ 546677	Liceales	719
Stemonitis fusca	FJ 546689	Stemonitiales	663
Ceratiomyxa fruticulosa	FJ 558514	Ceratiomyxiales	655
Ceratiomyxa fruticulosa var. *porioides*	FJ 558513	Ceratiomyxiales	780

The organisms were collected in forests of central Europe

medium with optimum moisture, light conditions and nutrients (Fig. 5).

Taxonomy and phylogenetic relationships

Despite the fact that the plasmodial slime molds have been known to generations of naturalists, de Bary (1859) was the first to analyze the systematic relationship within the myxomycetes and to other organisms. In his monograph, he recognized the following orders: Physareen (*Physarum* spp.), Lycogaleen (*Lycogala* spp.), Trichiaceen (*Trichia* spp.) and Stemoniteen (*Stemonitis* spp.). In his first publication on these lower organisms (Fig. 1), de Bary (1859) was convinced that slime molds are animals and therefore re-named them 'Mycetozoa'. In later publications, he changed the taxonomic status of these enigmatic

microorganisms, but was not sure into which Kingdom of Life the myxomycetes belong. The implications of de Bary's conclusions concerning the protoplasmic theory of the cell and the origin of life on Earth are discussed below.

In a series of papers, a DNA-based taxonomy (molecular phylogeny) of a selection of plasmodial slime mold taxa was established (Baldauf and Doolittle 1997; Baldauf et al. 2000; Fiore-Donno et al. 2005, 2008). In the present study, we collected representative myxomycetes in the forests of Europe, determined their taxonomic status according to the classical morphospecies-concept (Clark 2000, 2004), and extracted DNA. We utilized polymerase chain reaction-amplification and sequencing to obtain relatively long (ca. 500–1000 bp) fragments of the highly conserved gene 'protein synthesis elongation factor-1 alpha' (Table 1). Our phylogenetic tree (Fig. 6), rooted with the protist *Euglena*
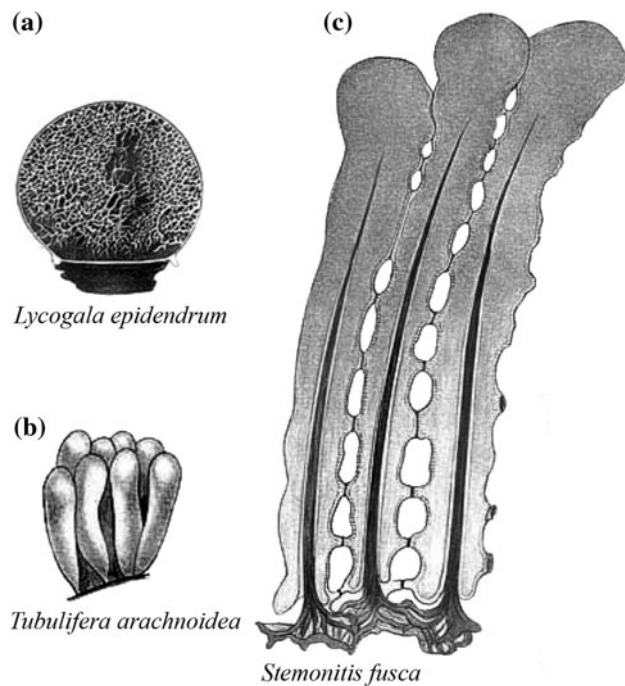
Fig. 2 Fruiting bodies (sporophores) of three common myxomycetes that occur on rotten wood and leaves on the forest floor throughout Europe. The drawings were reproduced from de Bary's monograph of 1859 (see Fig. 1)
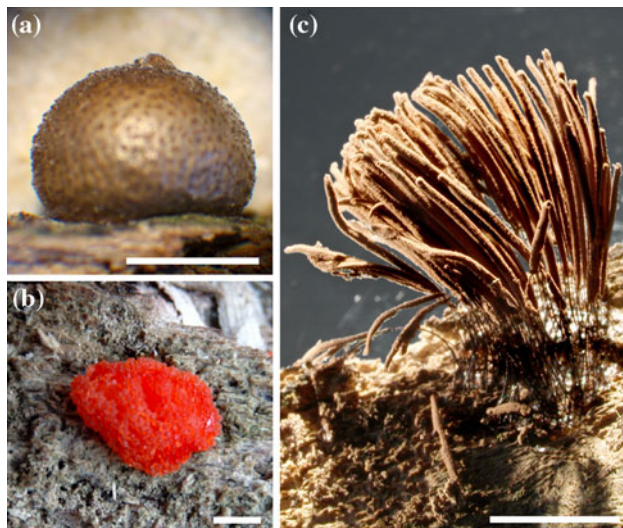

Fig. 3 Photographs of the fruiting bodies (sporophores) of three common plasmodial slime molds from Europe, collected in the field. *Lycogala epidendrum* (**a**), *Tubulifera arachnoidea* (**b**) and *Stemonitis fusca* (**c**). Bars = 1 cm

gracilis and the mammal *Homo sapiens* as outgroups, resolved five of the six 'classical' orders of the Myxomycetes: Physarales, Liceales (*L. epidendrum*, Fig. 2a, b; *Tubulifera arachnoidea*, Fig. 2a, b), Stemonitiales (*Stemonitis fusca*, Fig. 1c), Trichiales and Ceratiomyxiales.

Our molecular phylogeny differs in a number of points from those published previously (Fiore-Donno et al. 2005, 2008), which is to a large extent attributable to our selection of taxa (i.e. morphospecies). However, in general, our new molecular taxonomy of German myxomycetes is in accordance with the morphology-based systematics of the plasmodial slime molds (Neubert et al. 1993, 1995, 2000).

Discussion

One hundred and fifty years ago, Darwin (1859) and de Bary (1859) published their seminal monographs that later became the reference works for entire scientific disciplines. In contrast to Darwin's book, which is still in print today and one of the most cited monographs in the biological sciences, de Bary's work, a publication that marks the 'origin of myxomycetology', is only known to a few specialists (Martin and Alexopoulos 1969; Sparrow 1978; Horsfall and Wilhelm 1982; Drews 2001; Everhart and Keller 2008).

One reason for the unequal impact of these scientific classics is obvious. Darwin (1859) published a work of general significance with reference to animals and plants, whereas de Bary (1859) discussed one little-known group of 'lower micro-organisms', living beings that have no economical value for man. Here we argue that this superficial evaluation of de Bary's work is not appropriate for the following reasons.

First, de Bary (1859) addressed the general question whether or not the slime molds (Schleimpilze) are fungi, as generally assumed by those naturalists who had studied this group of organisms before his monograph was published. In this seminal paper, de Bary (1859) came to the conclusion that plasmodial slime molds are animals and therefore renamed them 'Mycetozoa' as more in accordance with their supposed nature. It should be noted that de Bary (1859) published his manuscript in a zoological journal. In his subsequent book, *The Mycetozoans*, which must be viewed as the second edition of his article of 1859, de Bary (1864) changed the sub-title from 'der niedersten Thiere' ('the lowest animals') (Fig. 1) to 'der niedersten Organismen' ('the lowest organisms'). In these two publications, de Bary (1859, 1864) recognized that the traditional view of all living beings as either animals or plants loses its validity when lower microorganisms, such as slime molds, amoebae or unicellular algae, are considered. Darwin (1859, 1872), however, adhered to this simplistic taxonomy and deduced, based on this premise, his questionable 'Proto-Euglena-Hypothesis' of the last common ancestor of all life on Earth (for a critical evaluation of this concept, see Kutschera and Niklas 2008). Anton de Bary, on the other hand, abandoned Darwin's 'animal–plant-dichotomy' and, based on novel

Fig. 4 The life cycle of the myxomycetes. Fruiting bodies form spores that, after germination, develop into myxamoebae or swarm cells. Trophic stages (swarm cells, plasmogamy), dormant stages when the organisms are resting (spores, microcysts, sclerotia) and developing fruiting bodies are shown. n = haploid, $2n$ = diploid cells. n = number of chromosomes of the nuclei. The plasmodium contains numerous diploid nuclei
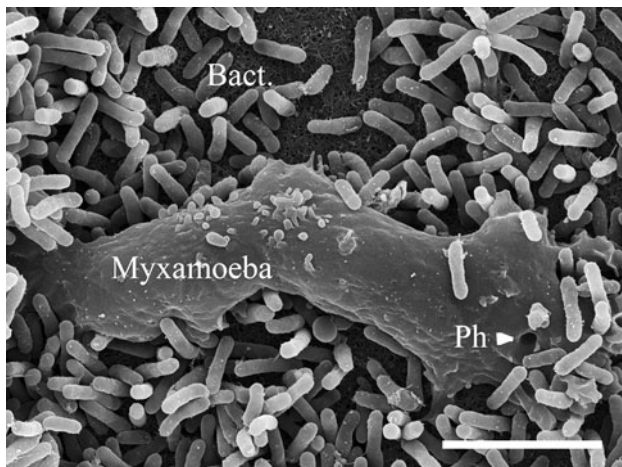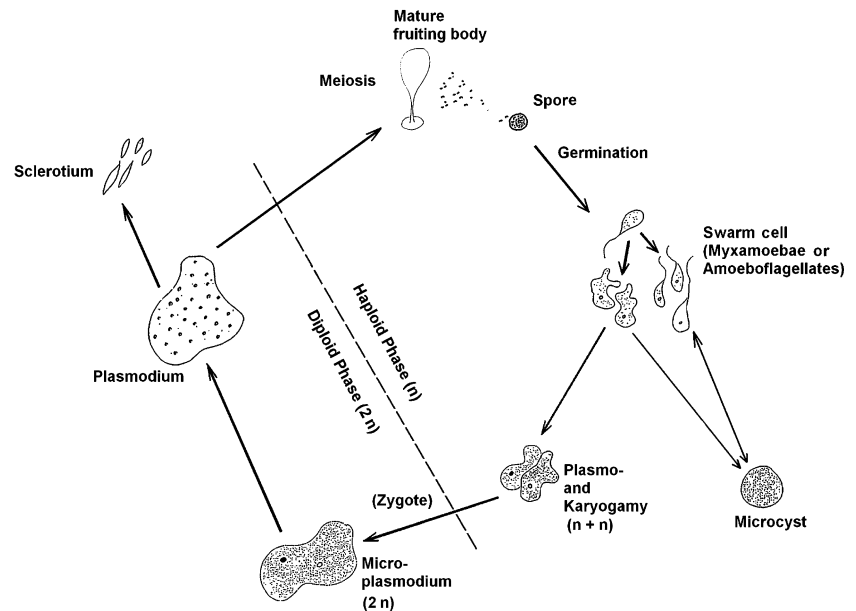
Fig. 5 Scanning electron micrograph of a haploid myxamoeba that feeds on methylotrophic bacteria (*Methylobacterium mesophilicum*). The cells were cultivated on agar plates and show membrane bodies with unknown function. *Ph* phagocytosis ('mouth pore'). The samples were fixed before microscopical examination. Bar = 10 μm

empirical data, included the myxomycetes within the fungi (de Bary 1866). In a later work of general scope, he added the bacteria (de Bary 1884), a group of microorganisms that was entirely ignored by Darwin. Today we know that bacteria and other prokaryotes dominate the protoplasmic mass of the biosphere (Whitman et al. 1998; Kutschera and Niklas 2004). Hence, with respect to the 'lower forms of life', de Bary (1859, 1864, 1866, 1884), who was the first to study the myxamoebae of slime molds in detail, held more modern views than Darwin (1859, 1872).

Second, de Bary's discovery of the naked, amoeboid mass called plasmodium, a single cell with many nuclei, provided the basis for the protoplasmic theory of cellular life. It demonstrated that living protoplasm could function without the presence of a surrounding wall. Accordingly, de Bary (1859) concluded that the cell must be viewed as 'an independent body of protoplasm separated from others'. This commentary and other observations on the same topic led Max Schultze (1825–1874) to the statement that the existence of a wall was a non-essential feature of the cell, as suggested by two of the 'fathers' of the 'cell-as-a-walled-unit'-concept, Robert Hooke (1635–1703) and Matthias Schleiden (1804–1881). In a classical monograph, Schulze (1863) reformed the cell theory and re-defined these units of life as a 'naked speck of protoplasm with a nucleus'.

In this context, it should be noted that Anton de Bary also studied the origin of lichens, and, like in his research on the myxomycetes, traced all the developmental stages through which these organisms grew and reproduced. Based on these and related observations, de Bary coined the word 'symbiosis' in 1879 and defined this term as 'the living together of unlike organisms' (Sparrow 1978). This discovery and definition later gave rise to an entire new branch of evolutionary cell research that resulted in the concept of symbiogenesis (primary and secondary endosymbiosis; see Kutschera 2009; Kutschera and Niklas 2005, 2008; Niklas 1997).

Finally, it should be noted that Darwin (1859, 1872) postulated a mechanism for the transformation and diversification of species that was based on the gradual modification of existing ones. However, he was unable to answer the question where the earliest forms of life came from. Louis Pasteur (1822–1895) is well known for his experiments that refuted the dogma of spontaneous generation. This long-held assumption states that living beings

Fig. 6 Molecular phylogeny of 32 myxomycetes based on newly acquired elongation factor-1 alpha gene sequences (orders Physarales, Liceales, Trichiales, Stemonitiales and Ceratiomyxiales) (Table 1). The freshwater flagellate *Euglena gracilis* and the mammal *Homo sapiens* were included as outgroups. The DNA tree was derived by maximum parsimony analysis. The results of 10 bootstrap replicates are depicted above or below the lines as percentages
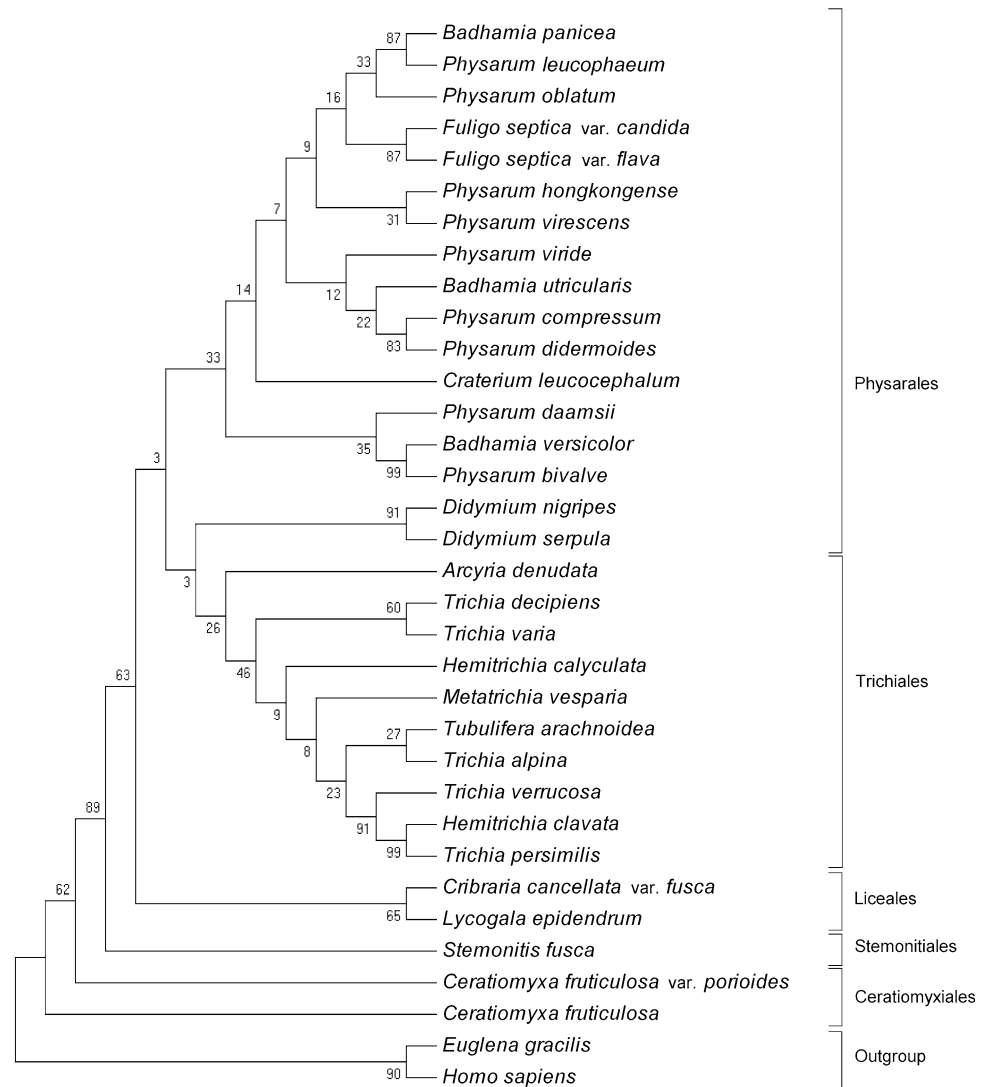
can arise today de novo from non-living material (McLaughlin 2005). Pasteur documented instead that life forms always originate from organisms. In a series of publications, de Bary (1859, 1864, 1866) documented that fungi can cause plant diseases. Based on these observations he provided independent evidence, by means of germinating spores, for Pasteur's general conclusion. In a prize-winning paper to the French Academy entitled 'Researches on the development of some plant parasites', de Bary definitively proved that organisms cannot be generated spontaneously (Sparrow 1978). In the introduction to his book on the *Comparative morphology and biology of the fungi, slime molds and bacteria*, de Bary (1884) pointed out that he was glad that he no longer feels obliged to include a chapter on spontaneous generation, since this dogma is no longer taken seriously by competent scientists.

Based on Pasteur's and de Bary's insights it was concluded that all forms of life developed in unbroken lineages of descent through time, with modifications, to produce the biological diversity we observe today. Darwin (1859, 1872) was possibly aware of this fact, but in his book *On the Origin of Species* we find no explicit statement concerning this general rule of the biological sciences.

In his last textbook, de Bary (1884) mentioned that the plasmodial slime molds are 'large amoebas' and hence have to be placed, together with the 'true amoebas', outside the animal and vegetable kingdoms. What is the current status of de Bary's classical 'amoeba-hypothesis'?

In this study, we collected 32 species of plasmodial slime molds in the field, extracted DNA and sequenced the elongation factor-1 alpha gene from all taxa. In addition, we included corresponding gene sequences from GenBank data base for the following putative phylogenetic relatives of myxomycetes: the free-living ciliate, *Stylonychia mytilus* and amoebae of the genera *Entamoeba* and *Cryptosporidium*. Our phylogenetic tree (Fig. 7) indicates that the next living relatives of the myxomycetes are the Ciliates and Amoebae. Based on our data we conclude that the

Fig. 7 Molecular phylogeny of a selection of animals, a fungus, red algae, flagellates, oomycetes, a plant, myxomycetes, a ciliate and amoebae, based on elongation factor-1 alpha gene sequences
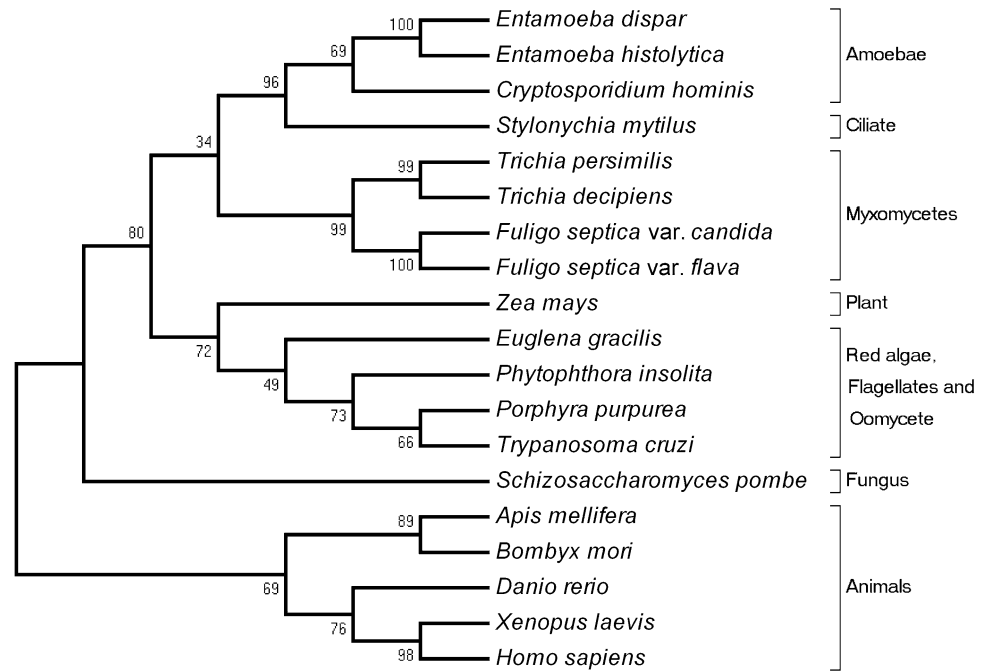
plasmodial slime molds are closely related to these 'Wechseltierchen' and presumably evolved from free-living amoebae or flagellates, as suggested by de Bary (1884).

In previous studies that were based on elongation factor-1 alpha and a number of other gene sequences the close phylogenetic relationship between the plasmodial slime molds and amoebae (*Acanthamoeba, Entamoeba, Naegleria*) has been suggested (Zettler et al. 1997; Baldauf et al. 2000; Fiore-Donno et al. 2005). Hence, protists such as *Acanthamoeba*, which have been isolated from soil sediments, compost, pond-, sea- and freshwater lakes may be one of the sister taxa to the myxomycetes. Free-living Acanthamoebae feed on microorganisms such as bacteria by phago- or pinocytosis (Kahn 2006). As in the haploid myxamoebae of plasmodial slime molds (Fig. 5), the diploid *Acanthamoeba* usually take up bacteria by phagocytosis, followed by lysis of the prey in phagolysosomes. This largely identical mode of nutrition further corroborates the close evolutionary relationship between free-living amoebas and the myxamoebae of plasmodial slime molds, as postulated by de Bary (1884).

Finally, our molecular phylogeny documents that the morphology-based (classical) taxonomy of the myxomycetes with the recognized orders Physarales, Liceales, Trichiales, Stemonitiales and Ceratiomyxiales (Neubert et al. 1993, 1995, 2000) is confirmed by a DNA-based classification (Fig. 6). Such a congruence between the morphospecies taxonomy and our molecular data lends further credence to the notion that the *Tree of Life*, as envisioned by Darwin (1859, 1872), can be reconstructed on the basis of DNA sequences, which reflect the evolutionary history of the organisms on this ever changing planet (Carroll 2006; Kutschera 2009).

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