FOLIA PARASITOLOGICA 47: 241-256, 2000

The hyperparasitic microsporidium *Amphiacantha longa* Caullery et Mesnil, 1914 (Microspora: Metchnikovellidae) – description of the cytology, redescription of the species, emended diagnosis of the genus *Amphiacantha* and establishment of the new family Amphiacanthidae

J.I. Ronny Larsson

Department of Zoology, University of Lund, Helgonav. 3, S-22362 Sweden

Key words: Microspora, *Amphiacantha longa*, cytology, ultrastructure, sporogony, Amphiacanthidae

Abstract. The ultrastructural cytology and reproduction of *Amphiacantha longa* Caullery et Mesnil, 1914 is described. Merogonial reproduction was not observed. The sporogony follows two lines: free disporoblastic and enveloped, polysporoblastic, involving sporoblast mother cells. The enveloped sporogony is endogenous in spore sacs of sporont origin, daughter cells are formed by vacuolation. Probably all stages have coupled nuclei. Both free and enveloped spores are equipped with an extrusion apparatus composed of a flat polar sac, a straight polar filament of manubrium type, and a posterior globular appendix. Manubrium and appendix are enclosed in a membraneous coat. Circular elements of coat material occur in the proximity of the extrusion apparatus. The membraneous coat and the surface layer of the manubrium penetrate the polar sac. The extrusion apparatus is located at the wide pole of the spore, the nuclei at the narrower pole. Hosts are gregarines of the genus *Lecudina* Mingazzini, living in the gut of the polychaete *Lumbrinereis fragilis* (O. F. Müller). The cytology and reproduction are discussed and compared to other genera of metchnikovellideans, to the chytridiopsid genera, and to microsporidia expressing the typical cytology for the group. Metchnikovellideans and chytridiopsids exhibit cytological and reproductive similarities. The species is redescribed, the diagnosis of the genus *Amphiacantha* Caullery et Mesnil, 1914 is emended, and the new family Amphiacanthidae, comprising the genera *Amphiacantha* and *Amphiamblys* Caullery et Mesnil, 1914, is established.

Amphiacantha longa Caullery et Mesnil, 1914 belongs to a group of aberrant microsporidia which were described as haplosporidia about 100 years ago (Caullery and Mesnil 1897, 1914). The microsporidian nature was revealed by Vivier (1965a). They are all hyperparasites, and practically all use marine hosts. Spores are formed in characteristic sacs, well visible using light microscopy, and the shape and size of spore sacs and spores are used for the discrimination of the genera. They are classified in one family, Metchnikovellidae Caullery et Mesnil, 1914, with four genera: *Metchnikovella* Caullery et Mesnil, 1897, *Amphiacantha* Caullery et Mesnil, 1914, *Amphiamblys* Caullery et Mesnil, 1914, and *Desportesia* Issi et Voronin, 1986.

The ultrastructural investigations of *Metchnikovella hovassei* Vivier, 1965 revealed a cytology that in several respects differed from the typical construction of microsporidia, for example exhibited by the *Nosema* and *Pleistophora* species (Vivier 1965a, b, Vivier and Schrével 1973). Even if few more species have been studied at the ultrastructural level the isolated position of Metchnikovellidae is apparent. The ultrastructure of *Metchnikovella wohlfarthi* Hildebrand et Vivier, 1971 was treated by Hildebrand and Vivier (1971) and Hildebrand (1974). *Amphiamblys bhatiellae* Ormières, Loubès et Maurand, 1981 was described using a combination of light and electron microscopic characters (Ormières et al. 1981). The description of *Desportesia laubieri* (Desportes et Théodoridès, 1979) was entirely based on electron microscopy (Desportes and Théodoridès 1979). Despite the aberrant shape of the spores the species was originally placed in *Amphiamblys*. So far there is no ultrastructural study of an *Amphiacantha* species.

The genus *Amphiacantha* comprises three species: the type species *A. longa* and two species described by Stubblefield (1955), *A. ovalis* and *A. attenuata*. All published illustrations are line drawings and the distinction between the *Amphiacantha* species is not obvious. An *Amphiacantha* species, indistinguishable from *A. longa* and considered identical to this species, has been studied from material collected off the southern part of the Swedish west coast. Even if the results did not explain all details of the life cycle, new information on the cytology and development was obtained. The ultrastructural cytology is described herein, the results are used for a redescription of the genus *Amphiacantha* and the type species *A. longa*, and the new family Amphiacanthidae is established.

Address for correspondence: R. Larsson, Department of Zoology, University of Lund, Helgonav. 3, S-223 62 Lund, Sweden. Phone: ++46 46 222 7802; Fax: ++46 46 222 4541; E-mail: Ronny.Larsson@zool.lu.se

MATERIALS AND METHODS

Four specimens of the polychaete *Lumbrinereis fragilis* (O. F. Müller, 1766) (Annelida: Eunicidae) from Öresund, south of Helsingborg, were available for study: two specimens collected by Ragnar Hall on April 16, 1997, and two specimens collected by the author on October 9, 1998. Gregarines were dissected out in seawater. For study using phase contrast microscopy whole gregarines were mounted in Berlese's medium after fixation in 4% formaldehyde. Permanent squash preparations of gregarines were lightly air-dried, fixed in methanol for 15 min and stained by Giemsa solution, or fixed in Bouin-Duboscq-Brasil (BDB) solution for at least one hour and stained by Heidenhain's iron haematoxylin.

For paraffin sectioning entire segments of *Lumbrinereis* were fixed in BDB for several days. After washing and dehydration in a graded series of ethanols, tissue pieces were cleared in butanol and embedded in Paraplast (Lancer, St. Louis, MO, USA). Sections were cut transversely at 10 µm and stained using a modification of the polychromatic staining by Vetterling and Thompson (1972), where the nuclear staining was substituted by Heidenhain's haematoxylin. For details on the histological techniques used see the manual by Romeis (1968). All permanent preparations were mounted in D.P.X. (BDH Chemicals Ltd., Poole, England). An image analysis program (Micro Macro AB, Gothenburg, Sweden) was used for measurements.

For transmission electron microscopy infected gregarines were selected. They were fixed in 2.5% (v/v) glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4°C overnight. After washing in cacodylate buffer and post fixing in 2% (w/v) osmium tetroxide in cacodylate buffer for 1 h at 4°C, the gregarines were washed and dehydrated in an ascending series of buffer-acetone solutions to absolute acetone and embedded in Epon. Sections were stained using uranyl acetate and lead citrate (Reynolds 1963).

ABBREVIATIONS: A – appendix, C – circular element, E – endospore, ER – endoplasmic reticulum, EX – exospore, F – polar filament (manubrium), G – granular material, M – unit membrane, MC – membraneous coat, N – nucleus, NU – nucleolus, P – plasma membrane, PS – polar sac, V – vacuole.

RESULTS

Gross morphology and life cycle

The host was gregarines of the genus *Lecudina* Mingazzini, 1891 (not identified to species) (Apicomplexa: Lecudinidae) from the gut of the polychaete *Lumbrinereis fragilis*. Both *Lumbrinereis* specimens in the sample from spring 1997 contained gregarines infected by *Amphiacantha longa*, while the gregarines of the two polychaetes collected in the autumn of 1998 lacked microsporidia.

Infected and uninfected gregarines had the same shape and size. The most obvious sign of infection was the presence of fusiform sacs (Figs. 1-3). The cytoplasm of heavily infected gregarines could be almost completely filled with sacs in the region posteriorly to the nucleus (Figs. 1, 2). Sacs were rarely seen anteriorly to the nucleus, and the nucleus was not invaded by the parasites. Up to 30 sacs could be seen in a focal plane. The sacs were arranged more or less longitudinally along the longitudinal axis of the gregarines.

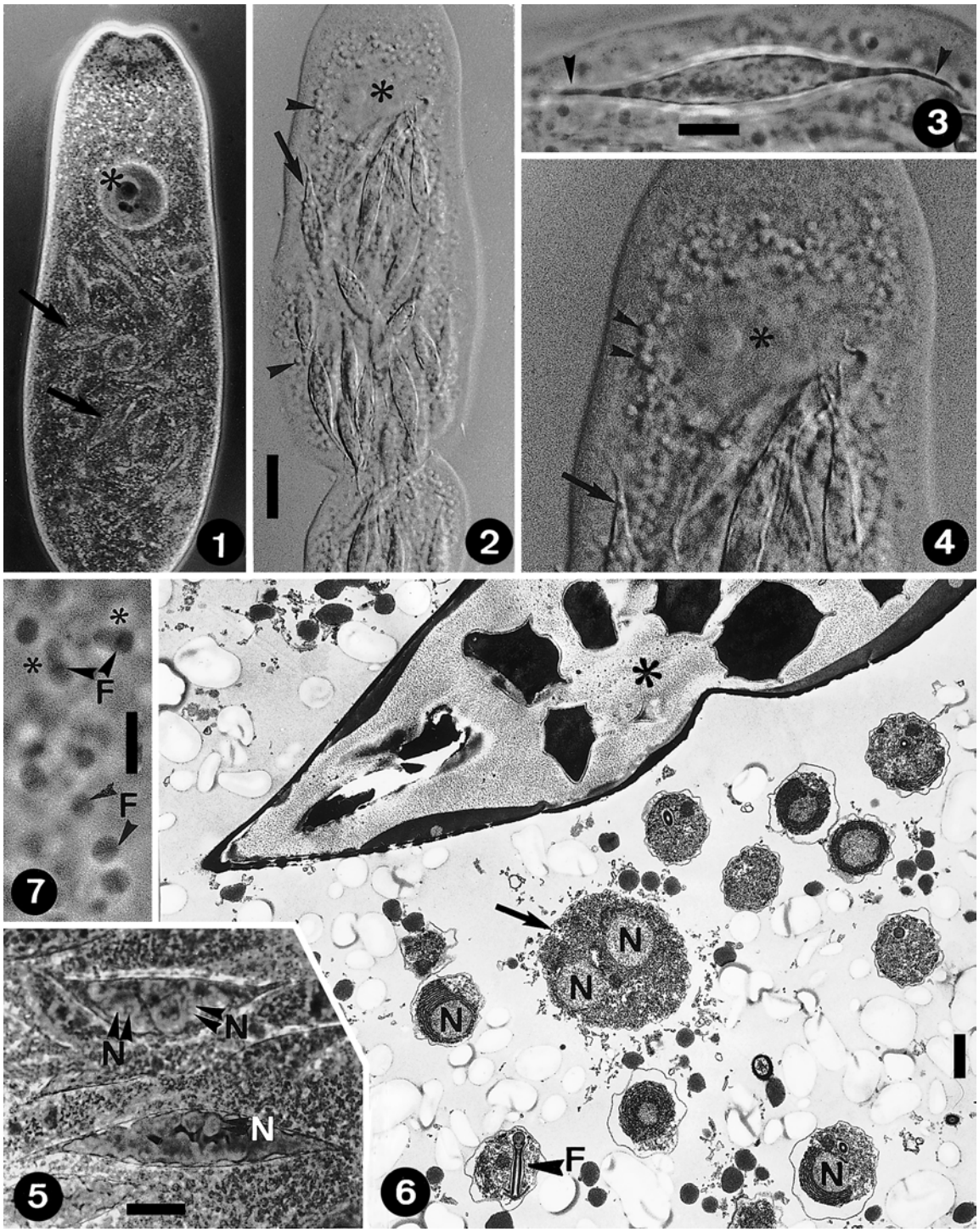
Both ends of the sac terminated in a long, often lightly curved, thread-like projection (Fig. 3). Sacs of specimens fixed in formaldehyde and embedded in Berlese's medium measured up to 72 µm long (straight distance between terminal points of projections) and were up to 9.5 µm wide. The real length was greater for the cellular component of the sacs measured up to 40 µm long, and up to 30 µm long terminal threads were measured (nearly 1/3 of the total length of the sac). It is obvious that sacs in permanent Berlese preparations were compressed and flattened out by the cover slip. Sectioned sacs were never wider than 5.1 µm. Mature sacs were filled with spores, which looked irregularly rounded when viewed inside the sacs (Fig. 5). The maximum number of spores per sac is unknown, but up to 50 spores could be seen in the focal plane or in one ultrathin section. The greatest spores measured inside sacs were 6.4 µm wide.

The cytoplasm of the gregarine, also anteriorly to the nucleus, contained numerous rounded to elongated bodies in addition to the sacs (Figs. 2, 4, 6). These cells were not released from sacs but spores produced by a free sporogony sequence. Spores viewed from the poles appeared rounded while spores viewed from the side had unequally wide ends, and appeared almost pyriform with blunt poles (Fig. 7). Free spores in Berlese preparations measured up to 4.5 µm long and up to 2.4 µm wide at the widest pole. Rounded sporonts, measuring up to 16.8 µm wide in Berlese preparations, with coupled, up to 6.0 µm wide, nuclei were seen (Fig. 6). Stages prior to the sporogony were not seen and are presumed to be absent.

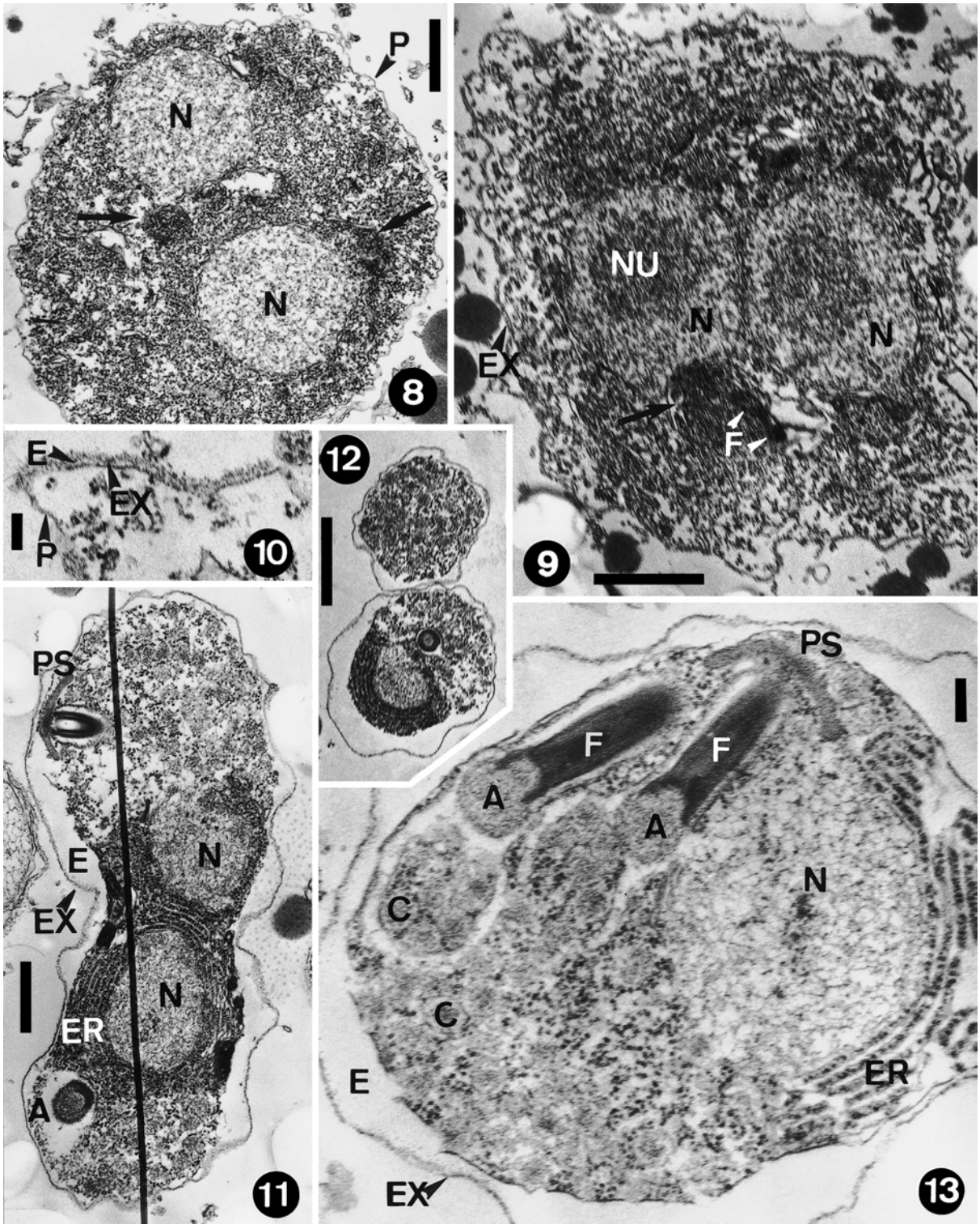
Two kinds of sporal organelles could be identified using light microscopy. In the free spores the polar filament was visible at the wide end of the spore, appearing as a dark strand perpendicular to the long axis of the spore (Fig. 7). In the sac-bound spores coupled nuclei were visible as two rounded, closely associated bodies (Fig. 5).

Free sporogony

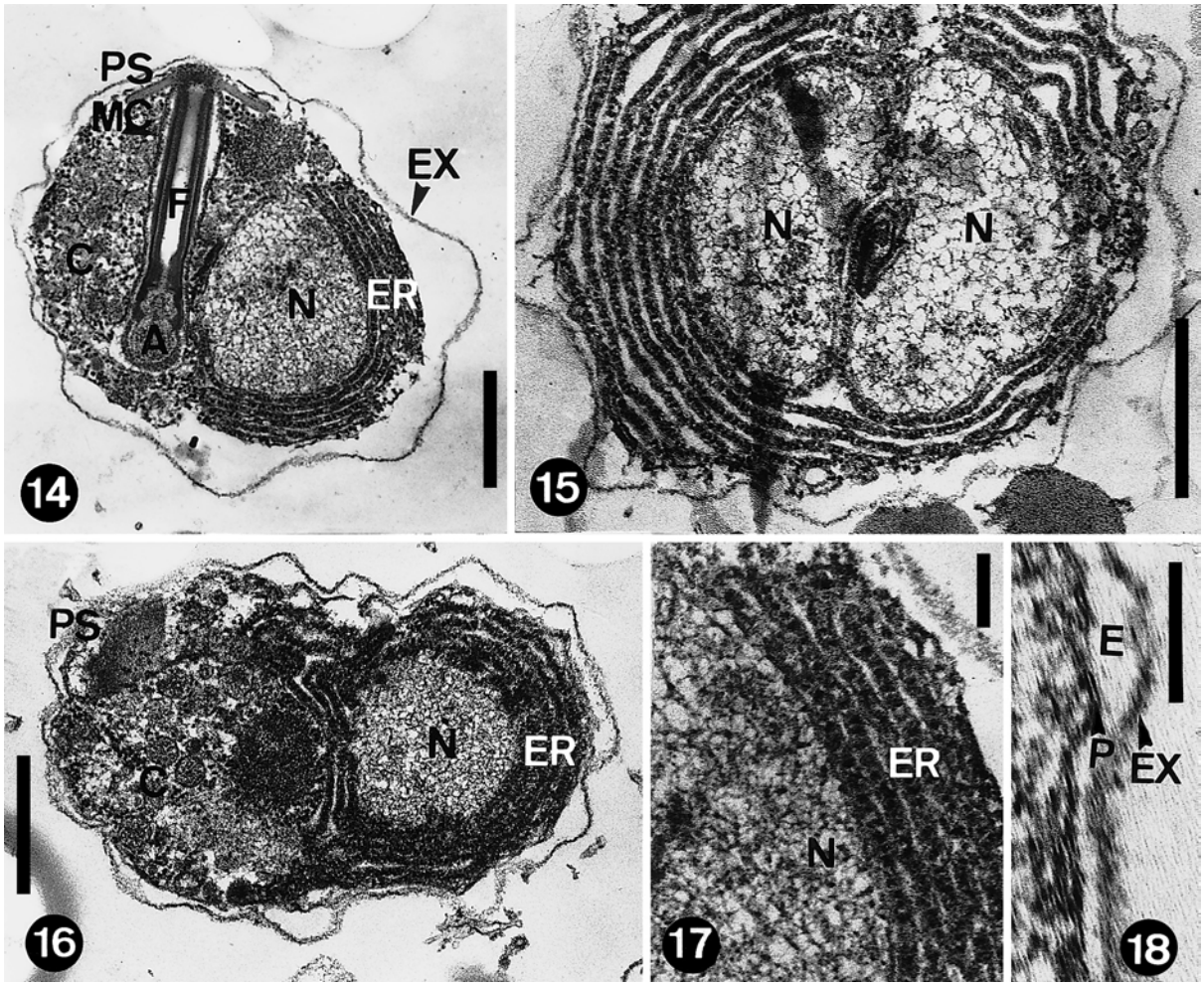
The free sporogony was disporoblastic. Early sporonts were spherical (Figs. 6, 8, 9). The most immature sporonts (Fig. 8) were delimited by an about 8 nm thick plasma membrane without external reinforcements. The cytoplasm was rather dense, containing numerous free ribosomes. In the proximity of the nuclei were rounded aggregates of closely packed ribosomes, from which the extrusion apparatus originated (Figs. 8, 9). Nuclei were probably always coupled, even if the coupled state was not always visible in sections. Nuclei were of the normal eukaryotic type with a nuclear envelope where two unit membranes were separated by a perinuclear space, and wide nucleoli were also seen (Fig. 9).



Figs. 1-7. *Amphiacantha longa* – gross morphology of spore sacs and spores. **Figs. 1, 2.** Gregarines with spore sacs (arrows) posteriorly to the nucleus (*), free spores (arrowheads) in the entire cytoplasm. **Fig. 3.** Spore sac with terminal projections (arrowheads), the complete length of the projections is not visible in the focal plane. **Fig. 4.** Anterior pole of a gregarine exhibiting free spores (arrowheads; arrow indicates spore sac; * nucleus of the gregarine). **Fig. 5.** Mature spore sacs containing rounded spores with coupled nuclei. **Fig. 6.** Ultrathin section of a gregarine showing a part of a spore sac (*), numerous free spores and one sporont (arrow) belonging to the free sporogony. **Fig. 7.** Free spores in the cytoplasm of the gregarine viewed from the pole and from the side (*). Figs. 1, 3, 5, 7 – phase contrast; Figs. 2, 4 – interference phase contrast; Fig. 6 – electron microscopy. Abbreviations for Figs. 1-38 are explained in Materials and Methods. Scale bars: Figs. 1, 2 (with common bar in Fig. 2) = 25 μm; Figs. 3, 4 (with common bar in Fig. 3), Fig. 5 = 10 μm; Fig. 6 = 1 μm; Fig. 7 = 5 μm.



Figs. 8-13. The free sporogony of *Amphiacantha longa* (electron microscopy). **Fig. 8.** Young sporont with areas of densely packed ribosomes (arrows) in the proximity of the nuclei. **Fig. 9.** Older sporont with central nuclei and with polar filament growing from the ribosome aggregates (arrow); beginning production of exospore material. **Fig. 10.** Detail of the sporont wall showing initiation of exospore and endospore. **Fig. 11.** Sporont prior to division into two sporoblasts; polar filaments (directed differently) are formed at opposite poles; nuclei are partially enveloped in the endoplasmic reticulum (dark vertical line is an artifact). **Fig. 12.** Two still connected spores. **Fig. 13.** Nearly mature anomalous spore with duplicated extrusion apparatuses; vesicular aggregates (circular elements) occur in the proximity of the filaments; nuclei (one visible) are partially enveloped in endoplasmic reticulum. Scale bars: Figs. 8, 11 = 0.5 μm; Figs. 9, 12 = 1 μm; Figs. 10, 13 = 100 nm.



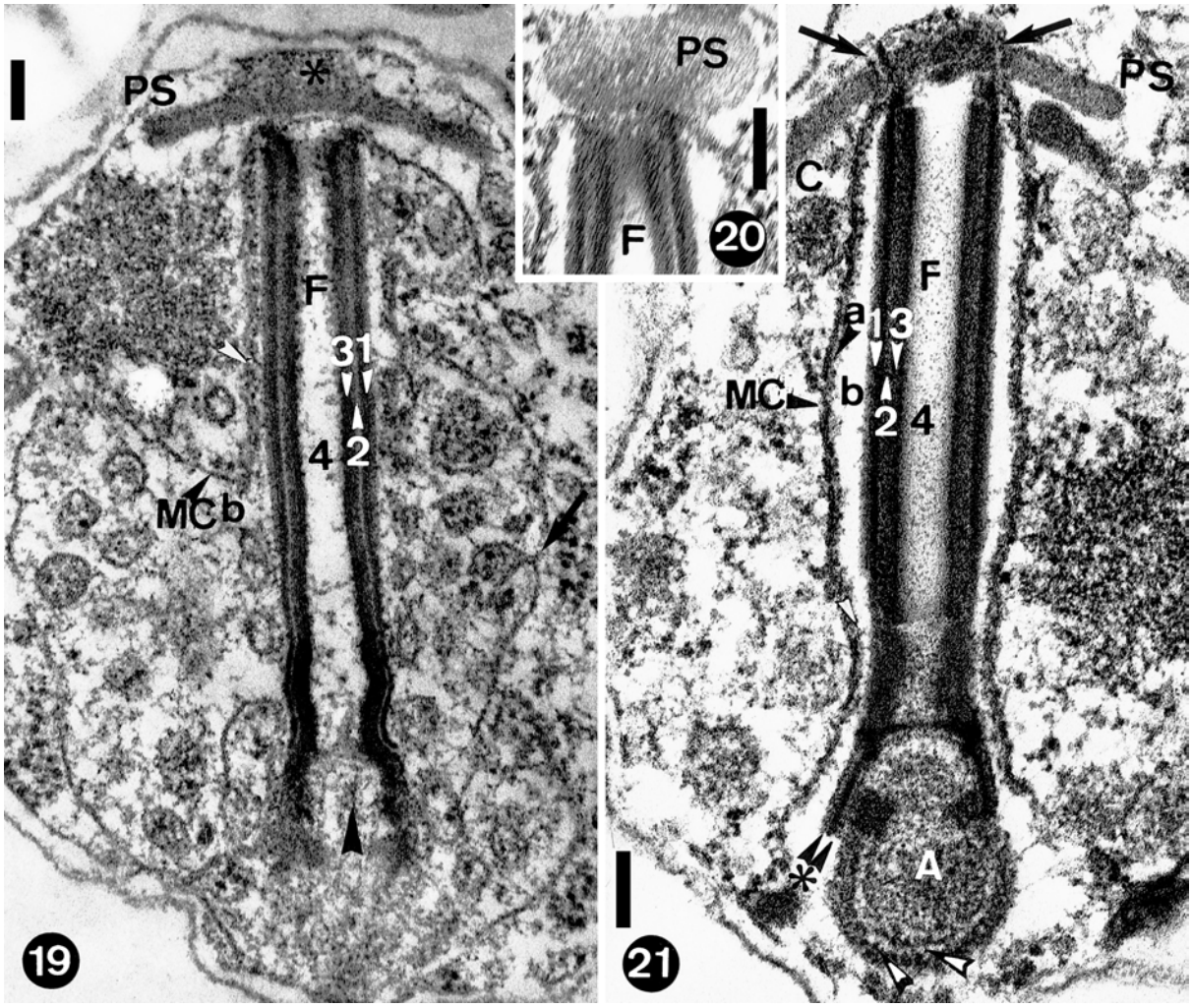
Figs. 14-18. The free spore of *Amphiacantha longa* (electron microscopy). **Fig. 14.** Longitudinal section through the longest axis of a mature spore, taken through the polar filament. **Fig. 15.** The double nuclei are visible in sections taken perpendicularly to the longitudinal axis. **Fig. 16.** Oblique section revealing circular elements close to the polar filament (manubrium). **Fig. 17.** Detail of a nucleus with folds of endoplasmic reticulum. **Fig. 18.** Detail of the spore wall. Scale bars: Figs. 14-16 = 0.5 μm; Figs. 17, 18 = 100 nm.

When the sporont matured granular material accumulated on the external side of the plasma membrane forming the future exospore layer (Figs. 9, 10). A lucent zone, obviously corresponding to an endospore, appeared between the exospore and the plasma membrane. The exo- and endospore primordia formed together an almost uniformly thick layer (about 35 nm).

In elongate sporonts, ready to divide into sporoblasts, the nuclei were localised to the centre and two extrusion apparatuses were present at opposite poles (Fig. 11). The terminology extrusion apparatus is applied herein even if it has not been proven that the structures have the same extrusion function as in typical microsporidia. The nuclei were partly enveloped in distinct folds of endoplasmic reticulum, leaving the nuclear side adjacent to the extrusion apparatus free. The two extrusion apparatuses were often directed with opposite polarity (Fig. 11). The exospore material was released as irregular exospore folds (Fig. 11). The newly formed spores occurred pair-wise (Fig. 12). Occasionally the reproduction failed resulting in spores with duplicated extrusion apparatuses (Fig. 13).

The almost pyriform spore shape (Fig. 7) was not so obvious in sections. Sections through spores appeared more rounded (Figs. 6, 14). The extrusion apparatus was found in the widest part of the spore, the almost oval nuclei at the narrower pole. The widest sectioned nucleus measured 1.1 μm. The nuclei were arranged with their longitudinal axes directed in the same way as the extrusion apparatus. Probably all spores had double nuclei, but the double condition was rarely seen (Fig. 15). Like in late sporonts, the side of the nuclei opposite the extrusion apparatus was enveloped in folds of endoplasmic reticulum (Figs. 14-16).

The external, about 15 nm thick, uniformly electron-dense exospore layer of the spore wall was distinct (Figs. 14-16, 18). A lucent zone separated the exospore

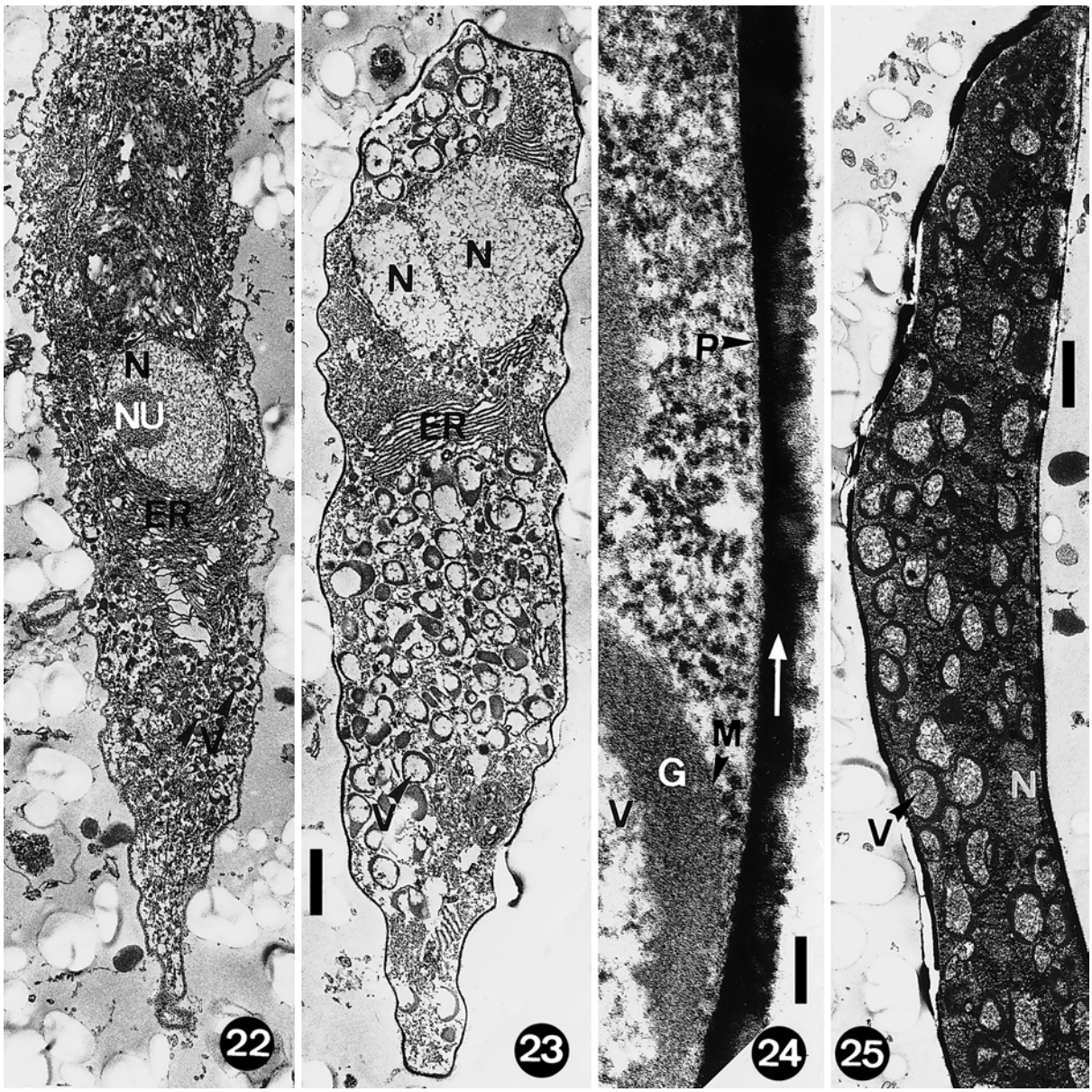


Figs. 19-21. *Amphiacantha longa* – the extrusion apparatus of the free spore (electron microscopy). **Fig. 19.** Longitudinal section through the immature extrusion apparatus; * indicates granular material of the polar sac; b denotes the cavity delimited by the membrane coat. Arrow points at a membrane-fold. White arrowhead points at fibrous connection. The filament (manubrium), with layers numbered 1-4, is open posteriorly (black arrowhead). **Fig. 20.** Anterior end of an immature spore exhibiting the primordial polar sac. **Fig. 21.** Longitudinal section through the mature extrusion apparatus. The filament, with layers numbered 1-4, ends with a posterior globular appendix (white arrowheads point at surface layers); * indicates the three layers visible in the collar. Arrows show points of penetration. Granular material (a) has accumulated below the membrane coat, the cavity (b) is practically void of structures; white arrowhead points at fibrous connection. Scale bars = 100 nm.

from the about 8 nm thick plasma membrane. This zone varied considerably in thickness, measuring from 16 nm to about 200 nm. It was completely devoid of structures and could either be an empty space or an endospore layer. It is obviously an endospore for it was initiated like an endospore layer (Fig. 10), and like a typical endospore layer, it was most narrow at the anterior pole of the spore (outside the front end of the extrusion apparatus) (Figs. 6, 14). Whether this layer varies in thickness in living spores or the irregularity was caused by the preparation is unknown.

The extrusion apparatus exhibited four parts: an anterior polar sac, a straight polar filament, a posterior oval to globular appendix, and a membraneous cover (Figs. 19-21). The most immature polar sac appeared as an agglomeration of granular material collected by an approximately 5 nm thick unit membrane (Fig. 20). During the maturation the sac became flat and the granular material successively condensed to form a disc-like structure, beginning at the posterior side of the sac (Fig. 19). In the mature extrusion apparatus the polar sac had uniform texture and thickness, appearing as an approximately 40 nm thick and up to 595 nm wide (straight distance), lightly convex disc, covered by the thin unit membrane (Fig. 21).

The polar filament was a straight rod, or using the terminology of Metchnikovellidae, a manubrium (Vivier 1965a, Desportes and Théodoridès 1979). In the few developmental steps that could be followed the shape became more regular, the electron density of the layers



Figs. 22-25. The early enveloped sporogony of *Amphiacantha longa* (electron microscopy). **Fig. 22.** The earliest stage, only delimited by a plasma membrane and with few small vacuoles. **Fig. 23.** Later stage with beginning thickening of the cell wall and with a greater number of larger vacuoles in the cytoplasm. **Fig. 24.** Detail of the cell border exhibiting the electron-dense cover of the plasma membrane (arrow) and the granular material inside the vacuole membrane. **Fig. 25.** Later stage with almost complete dense wall, large vacuoles, denser cytoplasm and smaller nuclei. Scale bars: Figs. 22, 23 (with common bar in Fig. 23), 25 = 1 μm; Fig. 24 = 100 nm.

increased, and the posterior end closed. The manubrium appeared as a thick-walled cylinder, in the mature condition measuring up to 180 nm wide, up to 1.1 μm long (Fig. 21). Four layers in the manubrium were distinct (Figs. 19, 21, numbered 1-4). Layer 1 and 3 were identical, about 25 nm wide, electron-dense with a central double-layered structure. Layer 2 was more lucent, about 6 nm wide. The 65-78 nm wide centre was finely granular (Figs. 19-21: 4).

The posterior end of the developing manubrium was open (Fig. 19). The mature manubrium terminated as a collar-like structure, enclosing the anterior third of an attached oval-globular appendix, a sac-like body measuring up to 260 nm wide, up to 340 nm long (Fig. 21). The manubrium was constricted anteriorly to the collar, in that the external zone and the granular centre were reduced. The collar was formed by three layers of approximately the same thickness (10 nm): two mod-

erately dense layers surrounding a very dense material (Fig. 21). The appendix was composed of granular material. A more lucent zone was distinct in the border to the collar, and the posterior end was enclosed by two, approximately 18 nm wide, layers with more dense internal borders: an external granular and an internal lucent material.

The immature manubrium was surrounded by a wide unit membrane-lined coat (Figs. 19, 21). The immature coat was traversed by fibrous strands, and it appeared to enclose irregularly rounded membrane-lined bodies filled with granular material (Fig. 19). The rounded bodies were apparently membrane folds enclosing ribosomes (Fig. 19). The mature manubrium was enclosed by a fairly regularly shaped coat, measuring 270-340 nm wide, which followed the outline of the manubrium (Fig. 21). The thickness of the coat increased to up to 18 nm in the mature spore by accumulation of fibrous material inside the membrane. The space between the sac and the manubrium was almost structureless. The membrane folds remained outside the coat as rounded, up to 117 nm wide, membrane-lined bodies, or circular elements using the terminology by Vivier (1965a) (Fig. 21).

The polar cap of the mature extrusion apparatus was penetrated by the membrane coat, a narrow zone of the lucent sac-material, and the surface layer of the manubrium (Fig. 21). The centre in the anterior part of the manubrium appeared to be open (Fig. 21).

The spore was devoid of an anchoring disc inside the polar sac, and neither polaroplast nor posterior vacuole were formed.

Enveloped sporogony

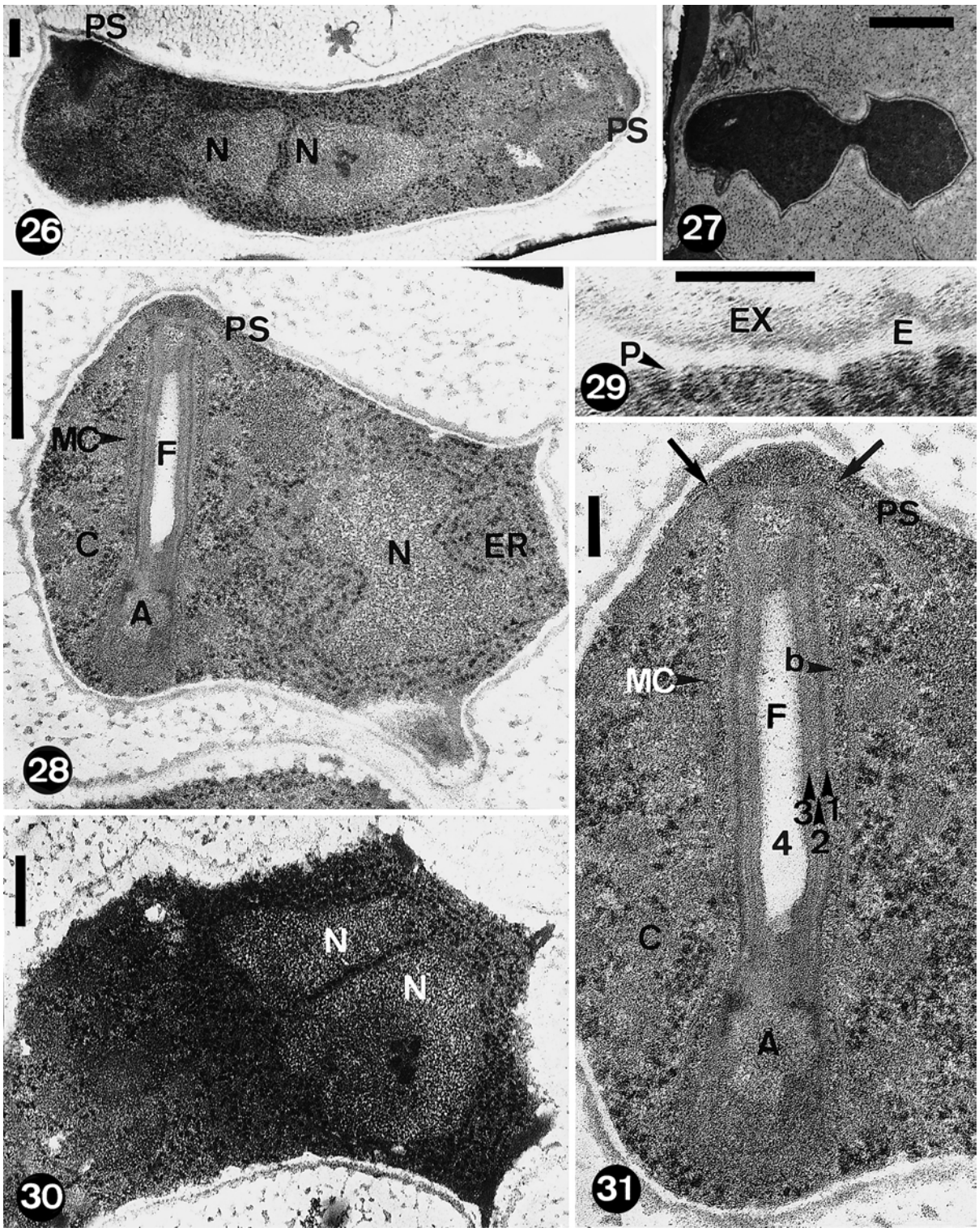
The most immature stages belonging to the enveloped sporogony appeared as fusiform cells (Figs. 22, 23). The earliest stages observed were only covered by a plasma membrane (Fig. 22). Nuclei were surrounded by an endoplasmic reticulum, nucleoli were present, and the cytoplasm was practically devoid of vacuoles. During the maturation a progressive accumulation of electron-dense material on the external side of the plasma membrane built a thick wall (Figs. 23, 24). Simultaneously vacuoles became more prominent in the cytoplasm. Vacuoles had an external unit membrane and an internal layer of granular material (Fig. 24). The cytoplasm was fairly lucent, with free ribosomes, as long as the vacuoles were small (Figs. 23, 24). With further maturation the cell wall grew thicker, and the vacuoles coalesced to form larger vacuoles with a thicker layer of granular material (Fig. 25). The cytoplasm between the vacuoles was reduced to narrow channels and as the ribosomes became densely packed the electron density increased (Fig. 25). At this stage smaller, dense and coupled nuclei were seen (Fig. 25).

The fusiform cells were the sporonts, and daughter cells were formed by vacuolation. Apparently the binucleate daughter cells produced were sporoblast mother cells which divided once, finally to yield two sporoblasts each. Elongated cells of similar type to those seen in the free sporogony (Figs. 11), with nuclei localised to the centre and with one set of the extrusion apparatus formed at each pole, were also observed in the enveloped sporogony (Fig. 26) as well as joint immature spores (Fig. 27).

The mature enveloped spores (Fig. 28) had the same organisation as the free spores (Fig. 14), but they were more electron-dense and the 30-72 nm thick spore wall (fibrous surface not included) appeared more uniform as the thickness of the endospore layer was less variable. The exospore was two-layered with an electron-dense, approximately 15 nm thick, basal layer and an up to 21 nm thick surface layer of more fibrous and lucent texture (Fig. 29). The 11-52 nm thick endospore was structureless. The plasma membrane was approximately 8 nm thick.

The extrusion apparatus, at the wide side of the spore, reached across the shorter axis of the spore (Fig. 28), while the coupled nuclei were dislocated to the other side (Fig. 30). The construction was identical to that of free spores (Figs. 28, 31). The polar sac was up to 564 nm wide and 48-65 nm thick. It was filled with uniform electron-dense material and covered by an about 5 nm thick unit membrane (Figs. 31, 32). The manubrium was up 1.1 μm long and up to 196 nm wide. A membrane-lined coat surrounded the manubrium (Fig. 31). Together with the surface layer of the manubrium the coat penetrated the polar sac (Fig. 31). The up to 81 nm wide centre of the manubrium was filled with granular material anteriorly and posteriorly but appeared almost structureless in the midsection. In the electron-dense external part of the manubrium three, partly subdivided, layers were visible (Figs. 31, 33: 1-3): about 25 nm wide and electron-dense with a central double layer (1, 3), and about 6 nm wide and lucent (2). The cavity between the surface of the manubrium and the surrounding coat was filled with dense material of two structures, appearing denser than in free spores (Fig. 33: a-b): a more fibrous and up to 13 nm wide zone close to the manubrium (b), and an almost uniform, about 9 nm wide layer below the membrane (a). The up to 340 nm long and up to 276 nm wide appendix was apparently identical to that of free spores, even if the surface layers were less distinct (Figs. 19, 31). Numerous membrane-lined, up to 127 nm wide, circular elements were present outside the membrane coat (Figs. 31, 34). The coupled nuclei were frequently observed in the enveloped spores (Figs. 5, 30, 37). The nuclei were enveloped in layers of endoplasmic reticulum, most voluminous at the pole opposite to the extrusion apparatus (Fig. 28).

Mature sporonts had a distinct thick wall where the about 8 nm thick plasma membrane was covered by an approximately 75 nm thick, very electron-dense, basal layer and an up to 13 nm thick surface layer (Figs. 23-25). The cover remained, with the same structure and thickness, as the envelope of the spore sacs. In addition



Figs. 26-31. The enveloped sporogony of *Amphiacantha longa* (electron microscopy). **Fig. 26.** Elongate cell, interpreted as sporoblast mother cell. **Fig. 27.** Two still associated spores formed by division of a sporoblast mother cell. **Fig. 28.** Mature spore sectioned longitudinally through the extrusion apparatus and following the longest axis. **Fig. 29.** Detail of the spore wall. **Fig. 30.** The coupled nuclei of a mature spore. **Fig. 31.** Longitudinally sectioned extrusion apparatus. Layers of the filament (manubrium) are numbered 1-4; the cavity below the membrane coat (b) is filled with granular material. Arrows indicate penetration. Scale bars: Figs. 26, 29, 31 = 100 nm; Fig. 27 = 1 µm; Figs. 28, 30 = 0.5 µm.

electron-dense vacuole material aggregated on the internal side of the plasma membrane and formed an irregularly thick zone (Figs. 6, 35-37). The vacuolation process by which the sporoblast mother cells were formed was visible as initiation of vacuoles (Fig. 22), vacuoles of increasing size (Figs. 23, 25), and finally as coalescence of vacuoles. The external vacuole membrane became the plasma membrane of the sporoblast mother cell. A part of the dense material inside the vacuoles (Fig. 24) remained as the surface cover of the exospore (Figs. 29, 38), while most of the material accumulated below the plasma membrane of the spore sacs. The cover on the exospore of spores, and the granular material below the envelope of the spore sac, remained connected by fibrous bridges, obviously for a long time (Fig. 38).

DISCUSSION

Identification of the species

The three genera *Metchnikovella*, *Amphiamblys* and *Amphiacantha* were distinguished by the shape of the spore sacs, illustrated by line drawings in the descriptions (Caullery and Mesnil 1897, 1914). The distinctive characters of species are the dimensions of the spore sacs and the spore yield.

The spore sacs of the genus *Amphiacantha* are elongated, mostly fusiform, and each end terminates in a straight or curved filamentous projection. The genus was established for the single species *A. longa*. The gregarine host was *Ophryodina elongata* (now placed in the genus *Lecudina*) or a related species, the polychaete host was *Lumbrinereis tingens* (see Caullery and Mesnil 1914). *A. longa* was found on a single occasion in 1906 (Caullery and Mesnil 1919). Stubblefield (1955) added two more species to the genus: *A. ovalis* and *A. attenuata*. They were found in *Lecudina* gregarines from two *Lumbrinereis* species collected off the coast of California, U.S.A. The life cycle of *A. ovalis* was described in detail. It appears to be a life cycle of Apicomplexa-type, and it includes sexual reproduction and meiosis. This partly hypothetic life cycle differs in several respects from all life cycles so far described from microsporidia, and it will not serve any purpose to make a detailed comparison. The spore sacs of *A. ovalis* have a rather uniform width and they look like sausages with thread-like ends (Stubblefield 1955: plate I: 20). The shape of the spore sacs distinguishes easily this species from the other two *Amphiacantha* species, and from the Swedish microsporidium. The second species, *A. attenuata,* produces slender spindle-shaped spore sacs, and the spores look spherical. The length and width of the spore sacs are similar to the dimensions of *A. longa*. The number of spores produced by *A. ovalis* is 14-50, while the sporulation of *A. longa* yields a variable number of up to 100 spores (Caullery and Mesnil 1919). Whether *A. attenuata* is different from *A. longa* is uncertain.

The spore sacs of the species treated herein are indistinguishable from the spore sacs of *A. longa*. The spores appear rounded when seen in spore sacs (Fig. 4), like the spores used for the description of *A. longa* were seen, and dimensions are about the same. Gregarine and polychaete hosts are identical, or closely related, and the geographical locations at the west coast of Europe are not distant. As there are no obvious differences, the microsporidium treated herein is identified to be *A. longa,* and the results are used for a redescription of the species.

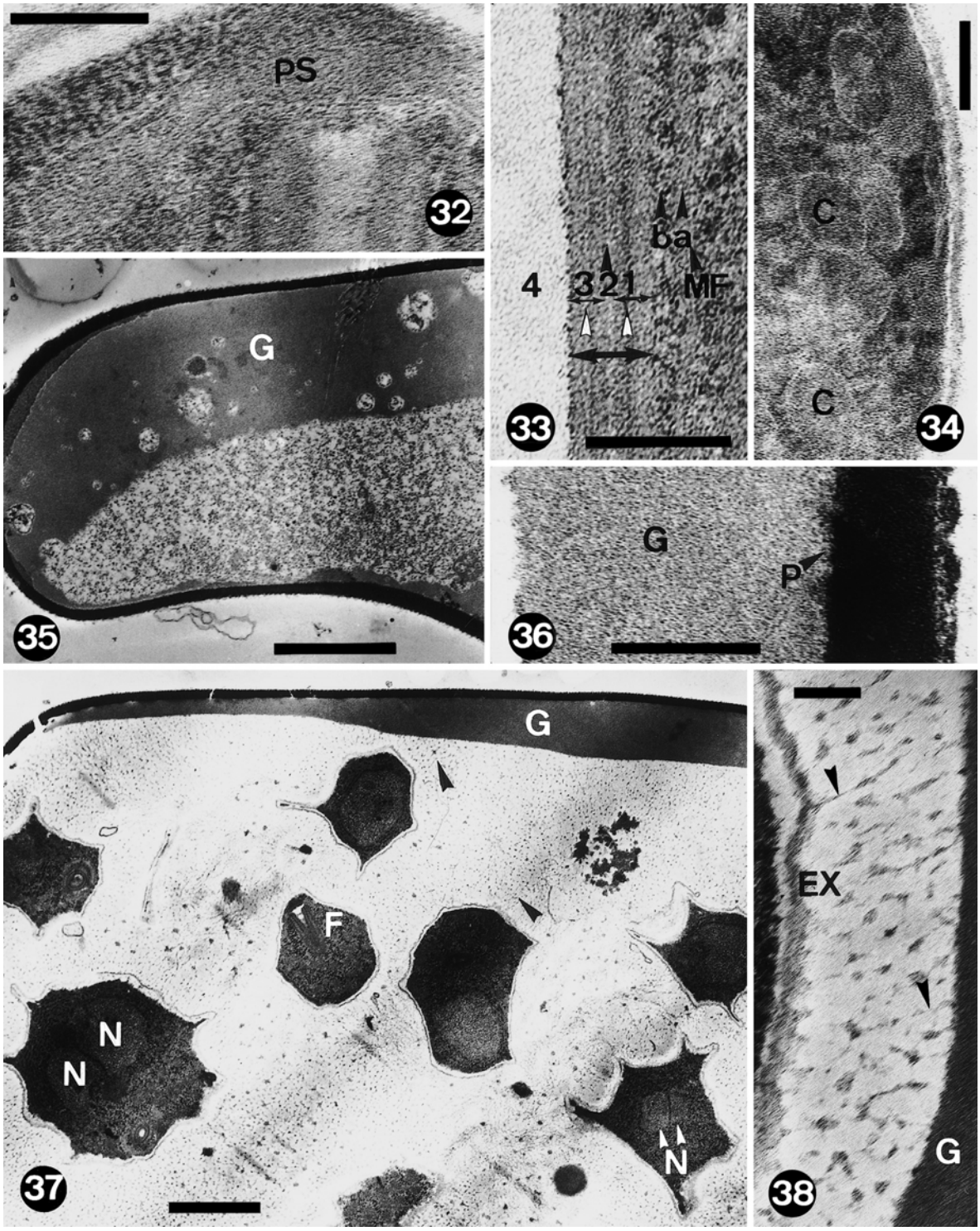
Cytology and reproduction

Vivier and his collaborators revealed the unusual or even unique traits in the cytology and life cycle of the *Metchnikovella* species (Vivier 1965a, b, Vivier and Schrével 1973, Hildebrand and Vivier 1971, Hildebrand 1974). *Desportesia* was treated by Desportes and Théodoridès (1979) and *Amphiamblys* by Ormières et al. (1981). With the evaluation of the cytology and reproduction of *Amphiacantha longa* all four genera of metchnikovellideans have been investigated ultrastructurally. This has broadened the base for an evaluation of the cytological variation among the metchnikovellideans and for a comparison with chytridiopsids and other groups of microsporidia.

Similarities between metchnikovellideans and chytridiopsids are obvious (Larsson 1993). Disregarding a few insufficiently known genera, four genera of chytridiopsids remain for comparison (references to cytological studies in parentheses): *Chytridiopsis* (Purrini and Weiser 1984, 1985, Larsson 1993), *Nolleria* (Beard et al. 1990), *Buxtehudea* (Larsson 1980) and *Intexta* (Larsson et al. 1997). The cytology of typical microsporidia, classified in the order Microsporida if the classification by Sprague (1977) is followed, has been reviewed by Vávra (1976), Larsson (1986a), Vávra and Larsson (1999), and Larsson (1999).

The enveloped sporogony of the metchnikovellideans is endogenous (Vivier and Schrével 1973). Daughter cells are formed by vacuolation and the plasma membrane of the sporont survives as the internal layer of the spore sac, not as the plasma membrane of the daughter cells like in typical microsporidia. This reproduction occurs in all four genera of metchnikovellideans and it has also been reported from chytridiopsids (Larsson 1980, Beard et al. 1990, Larsson 1993, Larsson et al. 1997). None of the typical microsporidia has been found to reproduce in this way. If typical microsporidia sporulate in envelopes, these are sporophorous vesicles secreted from the surface of the sporont, or rarely the meront.

The free sporogony has been verified from all metchnikovellideans except for *Desportesia*. It is polysporoblastic by plasmotomy in *Metchnikovella* (Vivier and Schrével 1973), disporoblastic in *Amphiacantha* (Fig. 11). Free spores are formed in *Amphiamblys*



Figs. 32-38. Details of enveloped spores and spore sacs of *Amphiacantha longa* (electron microscopy). **Fig. 32.** Polar sac with unit membrane lining. **Fig. 33.** Detail of the longitudinally sectioned polar filament (arrows indicate the electron-dense part), the layers are numbered 1-4. Finely granular material (a) has accumulated below the membrane coat, coarser material fills the cavity (b). **Fig. 34.** Circular elements in the proximity of the polar filament. **Figs. 35, 36.** Details of the envelope of the mature spore sac; the border between the granular material and the electron-dense cover is distinct, but the plasma membrane component is now hardly discernible **Fig. 37.** Spore sac with mature spores; fibrous material (arrowheads) connects the surface layer of the exospore with the granular material of the spore sac. **Fig. 38.** Detail showing nearly parallel bridges of fibrous material between the exospore and the granular material of the spore sac. Scale bars: Figs. 32-34, 36, 38 = 100 nm; Figs. 35, 37 = 1 µm.

(Ormières et al. 1981: fig. 11) but the mode of production is unknown. In the genus *Chytridiopsis* free and enveloped sporogony occur together (Larsson 1993).

The spore sacs are oval or naviculate in *Metchnikovella* and long, narrow sacs with blunt ends in *Amphiamblys* and *Desportesia*. The spore sacs of *Amphiacantha*, with thread-like terminal projections (Fig. 3), have a unique shape. Spore sacs of chytridiopsids are spherical (Purrini and Weiser 1984, Larsson 1993).

The only clearly documented reproduction of metchnikovellideans is by sporogony. Ormières et al. (1981) interpreted some of the stages of *Amphiamblys bhatiellae,* visible using light microscopy, as belonging to a merogonial reproduction. Presence of merogony was not supported by ultrastructural evidence. Merogony has not been reported from chytridiopsids. Typical microsporidia probably always have a merogonic reproduction prior to the sporogony, even if that has not been proven from all species.

The nuclei of metchnikovellideans are isolated in all stages, and in all genera except for *Amphiacantha*, and probably *Amphiamblys*. The coupled nuclei of *Amphiacantha* were frequently observed in the enveloped sporogony (Figs. 5, 30, 37), only rarely in the free sporogony (Fig. 15). In typical chytridiopsids nuclei are always isolated. *Hessea squamosa*, in the classification by Sprague (1977) treated as a chytridiopsid, has been described to have diplokaryotic stages (Ormières and Sprague 1973). The diplokaryon is a very close association between nuclei (Vávra 1976: fig. 2), and it is probably not correct to describe the coupled nuclei of *Amphiacantha* as a diplokaryon. Nuclei of typical microsporidia are either coupled, normally diplokaryotic, or isolated. The nucleus of the *Metchnikovella* spore is horseshoe-shaped (Vivier and Schrével 1973). In all microsporidia the normal shape of nuclei is rounded. However, horseshoe shape has occasionally been reported also from typical microsporidia (Vivarès 1975).

The typical spore shape of metchnikovellideans is rounded to bluntly pyriform (*Metchnikovella*, *Amphiacantha* and *Amphiamblys*). Spores of chytridiopsids are always spherical. Typical microsporidia share a great variety of spore shapes. Most commonly the spores are oval, pyriform, rod-shaped or spherical. The typical metchnikovellidean spore shape has not been reported from other microsporidia. The genus *Desportesia* is unique among the metchnikovellideans by having rod-shaped spores. However, this rod shape is not identical to the rod shape occurring in typical microsporidia, where the extrusion apparatus is polar. The elongated spore of *Desportesia* can be interpreted as a spore of the *Amphiacantha* type (Fig. 7) that has been torn out sidewards to obtain rod shape. The extrusion apparatus is central, traversing the short axis of the spore (Desportes and Théodoridès 1979: fig. 33).

All spores of microsporidia share a basic organisation where the unique component is the extrusion apparatus, composed of polar filament, polar sac, polaroplast and posterior vacuole. The spores of metchnikovellideans and chytridiopsids conform with the model, but deviate in details. It is unknown if the extrusion apparatus of the metchnikovellideans is a real extrusion apparatus functioning in the infection process.

The polar filament of the metchnikovellideans is a manubrium, a straight or lightly bent rod. The polar filament of chytridiopsids is coiled. The filament length of typical microsporidia is very variable, from shorter than the spore and uncoiled, in some species with rod-shaped spores, to a filament of great dimensions, arranged in 2-3 layers of coils. The filament of typical microsporidia has a complex sequence of layers of different electron density and thickness, and the internal organisation varies to some extent between the genera. In chytridiopsids, like in metchnikovellideans, the filament is composed of rather few layers. Aberrant spores with duplicated polar filament, like seen in *Amphiacantha* (Fig. 13), has also been observed in *Chytridiopsis* (Larsson 1993: fig. 14).

How the polar filament ends is unknown for most microsporidia and for all of the chytridiopsids. The short filament of *Cylindrospora fasciculata*, a typical microsporidium with rod-shaped spores, was described by Larsson (1986b: fig. 4 A-B). The internal organisation of the posterior pole differs from main body of the filament, but the difference can be explained as a reorganisation of the internal layers. The manubrium of the metchnikovellideans ends in a swollen structure, normally referred to as a gland, even if a glandular function has not been proven. In this paper the neutral term appendix has been used. The manubrium of *Amphiacantha* is connected to the appendix by a collar-like structure, and there is a distinct layered border between the manubrium and the appendix (Fig. 21). It is unclear how the appendix of *Metchnikovella* is connected to the manubrium. The appendix of *Desportesia* is different from the condition of *Amphiacantha*. Illustrations by Desportes and Théodoridès (1979: fig. 34) show a structure more resembling what was seen in *Cylindrospora*.

The polar sac is a more voluminous structure in typical microsporidia than in metchnikovellideans and chytridiopsids. The front end of the polar filament is connected to the polar sac in all microsporidia. In the typical microsporidia the centre of the polar sac noctains a layered structure: the anchoring disc. The polar filament enters the polar sac, and all layers of the filament attach to the disc. In chytridiopsids the posterior end of the polar sac is shaped like a socket and the anterior pole of the polar filament is collar-like (Larsson 1993, Larsson et al. 1997). Only the centre of the filament enters the polar sac and unites with the uniform material of the sac. Typical chytridiopsids lack

an anchoring disc. In *Amphiacantha* (Fig. 21) and *Amphiamblys* (Ormières et al. 1981: fig. 12) the peripheral layer of the filament and the surrounding membrane penetrate the polar sac. That type of connection is so far unique to these genera. It is unknown how the connection is constructed in *Metchnikovella* and *Desportesia*.

The typical microsporidia have a more or less prominent polaroplast, a system of membrane-lined cavities, posteriorly to the polar sac. The polaroplast surrounds the anterior part of the polar filament. The thin unit membrane components of the polar sac, polaroplast and polar filament belong to a continuous system of membranes. A polaroplast has never been observed in metchnikovellideans and chytridiopsids. However, the polar filament of chytridiopsids is ornamented with membraneous structures in the shape of a honeycomb or as narrower projections (Larsson 1980, Beard et al. 1990, Larsson 1993, Larsson et al. 1997). These constructions are probably homologous to the polaroplast of typical microsporidia (Vávra 1976). The membraneous coat of *Amphiacantha* (Fig. 21) is obviously also a polaroplast homologue. Cytoplasmic membrane folds have also been reported from the other genera of metchnikovellideans. The circular elements in the proximity of the filament are formed as folds from the same membrane system as the coat (Fig. 19).

A posterior vacuole, a prominent component of the typical microsporidian spore, has not been reported from metchnikovellideans and chytridiopsids.

To conclude, numerous cytological and reproductive characters unite metchnikovellideans with chytridiopsids and separate them from the typical microsporidia. Nevertheless, differences in the extrusion apparatus show that the two groups are distinct taxa.

Classification

There are four modern classifications of the microsporidia and all treat the group as an independent phylum. The classifications differ in several respects, also in their view of the correct phylum name and authorship. Sprague and Becnel (1998) evaluated the various names applied to the taxon microsporidia and, based on Article 29(b) (Determination of stem in names of type genera) in the International Code of Zoological Nomenclature (ICZN, 1985), concluded that the correct name of the phylum is Microsporidia Balbiani, 1882. However, Balbiani (1882) did not use the name "Microsporidia" but "Microsporidies" and the taxonomic level was not specified. The "Microsporidies" were considered as the fifth category (together with "Grégarinides", "Coccidies", "Sarcosporidies" and "Myxosporidies") in Leuckart's group "Sporozoaires" (Balbiani 1882). Further the ICZN has limitations: "Excluded from the provisions of the Code are names proposed ... (4) for taxa above the family group" (Art. 1(b), ICZN 1985). This problem was addressed by the Levine Committee and it was concluded that "the responsibility for the name of the high level taxon (suborder or above) is that of the person who established its actual level and its concept" (Levine et al. 1980). As Sprague (1977) was the first to give the microsporidia phylum status and to apply a name to the new phylum, the correct combination should be Microspora Sprague, 1977. Of interest for this discussion are differences in the way the metchnikovellideans are handled.

Sprague (1977) divided the phylum into two classes: Rudimicrosporea, with the single order Metchnikovellida (restricted to the metchnikovellideans) and Microsporea with the two orders Chytridiopsida (chytridiopsids and probably related microsporidia) and Microsporida (the typical microsporidia).

Also Weiser (1977) divided the phylum into two classes: Metchnikovellidea (with three orders: Metchnikovellida, Chytridiopsida, and Hesseida) and Microsporididea (the typical microsporidia).

Issi (1986) recognised only one class, Microsporidea, divided into four subclasses: Metchnikovellidea, Chytridiopsidea, Cylindrosporidea and Nosematidea (the last two containing the typical microsporidia).

Sprague et al. (1992) divided the phylum into two classes: Dihaplophasea (containing a part of the typical microsporidia) and Haplophasea (where typical microsporidia and chytridiopsids are united). The family Metchnikovellidae was not placed in this classification but lumped together with other problematic families as *taxa incertae sedis*.

If cytological characters are used for revealing relationships and for classifying, and so far there is no alternative to the cytology if the goal of a classification of the microsporidia is to cover all taxa, it is obvious that the metchnikovellideans are a monophyletic unit related with the chytridiopsids. Arguments are presented in the previous discussion on cytology and reproduction. Of the recent classifications only the one by Weiser (1977) recognises relationships between metchnikovellideans and chytridiopsids. The most recent classification, by Sprague et al. (1992), where the microsporidia are grouped into two classes according to the arrangement of the nuclei (Dihaplophasea with nuclei coupled at least in a part of the life cycle, Haplophasea always with isolated nuclei), cannot accommodate the metchnikovellideans. That classification would split the genera between the classes.

No microsporidian family combines genera having isolated nuclei with genera where the nuclei are coupled. A new family must be established for *Amphiacantha*. Even if all details of the cytology cannot be compared between the genera of metchnikovellideans, cytological differences as discussed above present more arguments for the establishment of a new family.

The similarities between *Amphiacantha* and *Amphiamblys* are obvious, but the genera are different – the differently shaped spore sacs tell that. When Ormières and colleagues (1981) described *Amphiamblys bhatiellae*, it was believed that the only sporogony was

sac-bound, and that the free spores seen in the cytoplasm were released from sacs. However, these spores (Ormières et al. 1981: figs. 12-14) are similar to not completely mature spores of *Amphiacantha longa* belonging to the free sporogony (Fig. 19). The spore wall is thin, the membraneous coat is wide and the manubrium is still opened posteriorly. The connection between manubrium and polar sac (penetration) is identical. The species was described to have isolated nuclei in all life cycle stages. As the spores studied belong to the free sequence, and as the free spores of *A. longa* rarely showed both nuclei, it cannot be excluded that also the nuclei of *Amphiamblys* are coupled. Sporonts of *A. bhatiellae* exhibited synaptonemal complexes, suggesting that the sporogony involved meiosis (Ormières et al. 1981). Meiosis in microsporidia seems to be almost completely restricted to diplokaryotic species (Vávra and Larsson 1999).

Descriptions and redescription

A m p h i a c a n t h i d a e fam. n.

Presporogonial development unknown, presumed absent. At least a part of the life cycle with coupled nuclei. Disporoblastic free sporogony together with polysporoblastic enveloped sporogony in elongated sacs of sporont origin. Enveloped spores produced by vacuolation. The plasma membrane of the sporont is incorporated into the envelope of the sac. Spores with an extrusion apparatus composed of polar sac, a straight manubrium, and a more or less globular appendix. Membraneous coat encloses manubrium and appendix. The coat and the surface layer of the manubrium penetrate the polar sac. Circular elements, derived from the membraneous coat, in the proximity of the extrusion apparatus. Anchoring disc, polaroplast and posterior vacuole absent. Genera: *Amphiacantha* Caullery et Mesnil, 1914, and *Amphiamblys* Caullery et Mesnil, 1914 (provided that stages with coupled nuclei are formed).

Amphiacantha Caullery et Mesnil, 1914, **emended diagnosis**

Original diagnosis: "...pour l'éspèce à kystes terminés en deux longues pointes effilées" (Caullery and Mesnil 1914).

Emended diagnosis: As for the family with the following additions: Microsporidia yielding bluntly pyriform free spores, irregularly rounded enveloped spores. Enveloped sporogony involves sporoblast mother cells. Spore sacs elongated with filamentous, slightly curved projections. Both spore types with the extrusion apparatus at the wider pole, coupled nuclei at the narrower end. Manubrium attached to the appendix by a collar-like prolongation. Parasites of gregarines.

Amphiacantha longa Caullery et Mesnil, 1914, **redescription**

Sporogony. As for the genus. Sporoblast mother cells divide by binary fission.

Spores. "Les germes ..., dans quelques cas, ... sont très nombreux, plus de 100" (Caullery and Mesnil 1919).

Both spore types: Extrusion apparatus with ≤ 1.1 µm long, ≤ 196 nm wide manubrium, flat polar sac and oval-globular appendix. Manubrium with a fairly lucent centre and a wall with 3, partly subdivided layers (c. 25 nm electron-dense, c. 6 nm lucent, c. 25 nm electron-dense). Polar sac shaped as a lightly convex disc, ≤ 595 nm wide, 40-65 nm thick. Appendix ≤ 340 nm long, ≤ 276 nm wide. Circular, up to 127 nm wide, membrane-lined bodies in the proximity of the membraneous cover.

Free spores: ≤ 2.4 × ≤ 4.5 µm (fixed). Nucleus ≤ 1.1 µm (sections). Exospore uniform, approximately 15 nm thick; endospore lucent, measuring 16-200 nm thick.

Enveloped spores: ≤ 6.4 µm in diameter (fixed). Nucleus ≤ 0.64 µm wide (sections). Exospore approximately 15 nm thick with ≤ 21 nm thick fibrous surface layer communicating with the envelope of the spore sac; endospore lucent, measuring 11-52 nm.

Spore sacs. "70-80 × 4.5 µm" (Caullery and Mesnil 1914).

Spore sacs: ≤ 72 µm long (straight distance between terminal projections, fixed), ≤ 5.1 µm wide (sections), thread-like projections about 1/3 of the total length of the sac. The wall of the spore sac three-layered: plasma membrane of the sporont (about 8 nm), electron-dense median layer (c. 75 nm) and granular surface layer (c. 13 nm). More than 50 spores produced per sac.

Host tissues involved. Both free and enveloped stages in the cytoplasm of the gregarines, spore sacs rarely anteriorly to the gregarine nucleus. Spore sacs arranged longitudinally in the gregarine.

T y p e h o s t a n d t y p e l o c a l i t y : "*Ophiodina* (=*Lecudina*) *elongata* ou espèce voisine ... Annélide *Lumbriconereis tingens*" (Caullery and Mesnil 1914); France: Anse Saint-Martin (Caullery and Mesnil 1919).

H o s t u s e d f o r t h e r e d e s c r i p t i o n : *Lecudina* sp. from *Lumbrinereis fragilis*, collected in Öresund south of Helsingborg (Sweden).

M a t e r i a l u s e d f o r t h e r e d e s c r i p t i o n : Slides, sections and blocks numbered 970416-(C-D) are in the collection of the author.

Acknowledgements. I am greatly indebted to Ragnar Hall, for collecting polychaetes, and to Lina Gefors, Inger Norling and Rita Wallén, for excellent technical assistance (all at the Department of Zoology, University of Lund). The investigation was supported by the Swedish Natural Science Research Council and the Royal Physiographic Society, Lund.

REFERENCES

BALBIANI G. 1882: Sur les microsporidies ou psorospermies des Articulés. C. R. Acad. Sci. Paris 95: 1168-1171.

BEARD C.B., BUTLER J.F., BECNEL J.J. 1990: *Nolleria pulicis* n. gen., n. sp. (Microsporida: Chytridiopsidae), a microsporidian parasite of the cat flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). J. Protozool. 37: 90-99.

CAULLERY M., MESNIL F. 1897: Sur un type nouveau (*Metchnikovella* n. g.) d'organism parasites de Grégarines. C. R. Acad. Sci. Paris 125: 787-790.

CAULLERY M., MESNIL F. 1914: Sur les Metchnikovellidae et autres Protistes parasites de Grégarines d'Annélides. C. R. Soc. Biol. 77: 527-532.

CAULLERY M., MESNIL F. 1919: Metchnikovellidae et autres Protistes parasites des Grégarines d'Annélides. Ann. Inst. Pasteur Paris 33: 209-240.

DESPORTES I., THÉODORIDÈS J. 1979: Étude ultrastructurale d'*Amphiamblys laubieri* n. sp. (Microsporidie, Metchnikovellidae) parasite d'un Grégarine (*Lecudina* sp.) d'un Echiurien abyssal. Protistologica 15: 435-457.

HILDEBRAND H.F. 1974: Observations ultrastructurales sur le stade plasmodial de *Metchnikovella wohlfarthi* Hildebrand et Vivier 1971, Microsporidie hyperparasite de la Grégarine *Lecudina tuzetae*. Protistologica 10: 5-15.

HILDEBRAND H.F., VIVIER E. 1971: Observations ultrastructurales sur le sporoblaste de *Metchnikovella wohlfarthi* n. sp. (Microsporidies), parasite de la Grégarine *Lecudina tuzetae*. Protistologica 7: 131-139.

INTERNATIONAL CODE OF ZOOLOGICAL NOMENCLATURE, Ed. 3, 1985. International Trust for Zoological Nomenclature, London, U. K., 338 pp.

ISSI I.V. 1986: Microsporidia as a phylum of parasitic protozoa. Protozoology (Leningrad) 10: 6-136. (In Russian.)

LARSSON R. 1980: Insect pathological investigations on Swedish Thysanura. II. A new microsporidian parasite of *Petrobius brevistylis* (Microcoryphia: Machilidae); description of the species and creation of two new genera and a new family. Protistologica 16: 85-101.

LARSSON R. 1986a: Ultrastructure, function and classification of microsporidia. Progr. Protistol. 1: 325-390.

LARSSON J.I.R. 1986b: Ultrastructural investigation of two microsporidia with rod-shaped spores, with descriptions of *Cylindrospora fasciculata* sp. nov. and *Resiomeria odonatae* gen. et sp. nov. (Microspora, Thelohaniidae). Protistologica 22: 379-398.

LARSSON J.I.R. 1993: Description of *Chytridiopsis trichopterae* n. sp. (Microspora, Chytridiopsidae), a microsporidian parasite of the caddis fly *Polycentropus flavomaculatus* (Trichoptera, Polycentropodidae), with comments on relationships between the families Chytridiopsidae and Metchnikovellidae. J. Euk. Microbiol. 40: 37-48.

LARSSON J.I.R. 1999: Identification of microsporidia. Acta Protozool. 38: 161-197.

LARSSON J.I.R., STEINER M.Y., BJØRNSON S. 1997: *Intexta acarivora* gen. et sp. n. (Microspora: Chytridiopsidae) – ultrastructural study and description of a new microsporidian parasite of the forage mite *Tyrophagus putrescentiae* (Acari: Acaridae). Acta Protozool. 36: 295-304.

LEVINE N.D., CORLISS J.O., COX F.E.G., DEROUX G., GRAIN J., HONIGBERG B.M., LEEDALE G.F., LOEBLICH III A.R., LOM J., LYNN D., MERINFELD E.G., PAGE F.C., POLJANSKY G., SPRAGUE V., VÁVRA J., WALLACE F.G. 1980: A newly revised classification of the protozoa. J. Protozool. 27: 37-58.

ORMIÈRES R., LOUBÈS C., MAURAND J. 1981: *Amphiamblys bhatiellae* n. sp., Microsporidie parasite de *Bhatiella marphysae* Setna, 1931, Eugrégarine d'Annélide Polychète. Protistologica 17: 273-280.

ORMIÈRES R., SPRAGUE V. 1973: A new family, new genus, and new species allied to the Microsporida. J. Invertebr. Pathol. 21: 224-240.

PURRINI K., WEISER J. 1984: Light- and electron microscopic studies on *Chytridiopsis typographi* (Weiser, 1954) Weiser, 1970 (Microspora), parasitizing the bark beetle *Hylastes cunicularius* Er. Zool. Anz. 212: 369-376.

PURRINI K., WEISER J. 1985: Ultrastructure of the microsporidian *Chytridiopsis typographi* (Chytridiopsida: Microspora) infecting the bark beetle, *Ips typographus* (Scolytidae: Coleoptera), with new data on spore dimorphism. J. Invertebr. Pathol. 45: 66-74.

REYNOLDS E.S. 1963: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208-212.

ROMEIS B. 1968: Mikroskopische Technik. Oldenbourg Verlag, München-Wien, 757 pp.

SPRAGUE V. 1977: Systematics of the Microsporidia. In: L.A. Bulla Jr. and T.C. Cheng (Eds.), Comparative Pathobiology. Vol. 2. Plenum Press, New York and London, 510 pp.

SPRAGUE V., BECNEL J.J. 1998: Note on the name-author-date combination for the taxon *Microsporidides* Balbiani, 1882, when ranked as a phylum. J. Invertebr. Pathol. 71: 91-94.

SPRAGUE V., BECNEL J.J., HAZARD E.I. 1992: Taxonomy of the phylum Microspora. Crit. Rev. Microbiol. 18: 285-395.

STUBBLEFIELD J.W. 1955: The morphology and life history of *Amphiacantha ovalis* and *A. attenuata*, two new haplosporidian parasites of gregarines. J. Parasitol. 41: 443-459.

VÁVRA J. 1976: Structure of the microsporidia. In: L.A. Bulla Jr. and T.C. Cheng (Eds.), Comparative Pathobiology. Vol. 1. Plenum Press, New York, N. Y., pp. 1-85.

VÁVRA J., LARSSON J.I.R. 1999: Structure of the microsporidia. In: M. Wittner and L.M. Weiss (Eds.), The Microsporidia and Microsporidiosis. A.S.M. Press, Washington, D. C., pp. 7-84.

VETTERLING J.M., THOMPSON S.E. 1972: A polychromatic stain for use in parasitology. Stain Technol. 47: 164-165.

VIVARÈS C.P. 1975: Étude comparative faite en microscopies photonique et électronique de trois espèces de Microsporidies appartenant au genre *Thelohania*

Hénneguy, 1892, parasites de Crustacés Décapodes marins. Ann. Sci. Nat., Zool., Paris, 12. Sér., 17: 141-178.
VIVIER E. 1965a: Étude, au microscope électronique, de la spore de *Metchnikovella hovassei* n. sp.; appartenance des Metchnikovellidae aux Microsporidies. C. R. Acad. Sci. Paris: 260: 6982-6984.
VIVIER E. 1965b: Présence de microtubules intranucléaires chez *Metchnikovella hovassei* Vivier. J. Microsc. (Paris) 4: 559-562.
VIVIER E., SCHRÉVEL J. 1973: Étude en microscopie photonique et électronique de différents stades du cycle de *Metchnikovella hovassei* et observations sur la position systématique des Metchnikovellidae. Protistologica 9: 95-118.
WEISER J. 1977: Contribution to the classification of Microsporidia. Acta Soc. Zool. Bohemoslov. 41: 308-320.

Received 20 March 2000

Accepted 25 May 2000