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Ultrastructural characterization of *Acarispora falculifera* n.gen., n.sp., a new microsporidium (Opisthokonta: Chytridiopsida) from the feather mite *Falculifer rostratus* (Astigmata: Pterolichoidea)

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Abstract

Only about 20 species of microsporidia have been described from mites. All except one species produce typical spores with a long polar filament and a polaroplast. This paper is the first study of an atypical microsporidium infection in a feather mite (*Falculifer rostratus*). The infection of the pigeon feather mite is restricted to the colon epithelium where it leads to hypertrophy of the concerned cells. During sporogony, a multinucleate plasmodial aggregate is formed within a sporont (endogenous sporogony resulting in a polysporophorous vesicle). The cisterns delimiting the single sporoblasts later form the spore walls. Sporogonial stages are in direct contact to the host cell cytoplasm. Merogonial stages were not present. Spores are tiny (3.6 µm × 2.6 µm), broad oval in form and monokaryotic. The spore wall of mature spores consists of a three-layered endospore and a thin, electron-dense, wavy exospore. The polar filament is anisofilar and completely coiled in 3–4 turns. In cross-sections, it has a star-like appearance because the electron-dense core forms rounded compartments of lucent material at its surface. In superficial sections, this results in a honeycomb-like pattern. A polaroplast is missing. The polar filament arises subapically at a polar sac that lacks an internal anchoring disk. These atypical spore structures clearly classify the species from the feather mite as a member of the order Chytridiopsida. It could not be clearly affiliated to one of the known genera, so we created a new genus, *Acarispora*, with the species *A. falculifera*.

Keywords

Acarispora, arthropod, Chytridiopsida, Falculiferidae, microsporidia, mite

Introduction

With roughly 55,000 described species, mites are among the most speciose lineages of the Arachnida (Alberti and Thaler-Knoflach 2013). Mites (Acari) are no longer considered to be a monophyletic taxon and consist of the convergently similar but unrelated orders Actinotrichida and Anactinotrichida (Dabert *et al.* 2010). Despite this high number of potential arachnid hosts, only a few of their parasites are known. Since many mite species are of economic importance, more attention is paid to acaropathogens. Fungi, viruses and bacteria, for example, were shown to play important roles in the regulation of phytophagous mite populations (van der Geest *et al.* 2000). Microsporidia have also been observed in several mite species and are often found in mass rearings of beneficial arthropods (Bjørnson 2009; Schütte and Dicke 2009). Since a microsporidian infection of predatory mites (phytoseiids) used for biological control could have an impact on host fitness, their presence and effect should be controlled (Bjørnson 2009). Microsporidia do not, however, seem to have a potential for biological control of undesirable mites (van der Geest *et al.* 2000).

According to Larsson *et al.* (1997), 21 named and three unnamed microsporidia are parasites of mites, whereas van der Geest *et al.* (2000) only listed 17 named and one unnamed

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species. During fine structure analysis of the feather mite *Falculifer rostratus* (Astigmata: Pterolichoidea: Falculiferidae), two of the authors (Alberti and Dabert 2012) incidentally found one animal infected with microsporidia. *Falculifer rostratus* is a feather mite species found exclusively in the plumage of pigeons, where it is considered to live as parasite or paraphage. It is a representative of a huge assemblage of about 2,500 described species of feather mites that are widely distributed and occur on every bird species (Mironov and Proctor 2008). They comprise several evolutionary lineages which belong to different families and are closely related to sarcoptiform mammal parasites and free living dust mites (Dabert *et al.* 2010; Klimov and OConnor 2013). Since to date there has been no report on a microsporidium from a feather mite, we decided to study this pathogen in more detail.

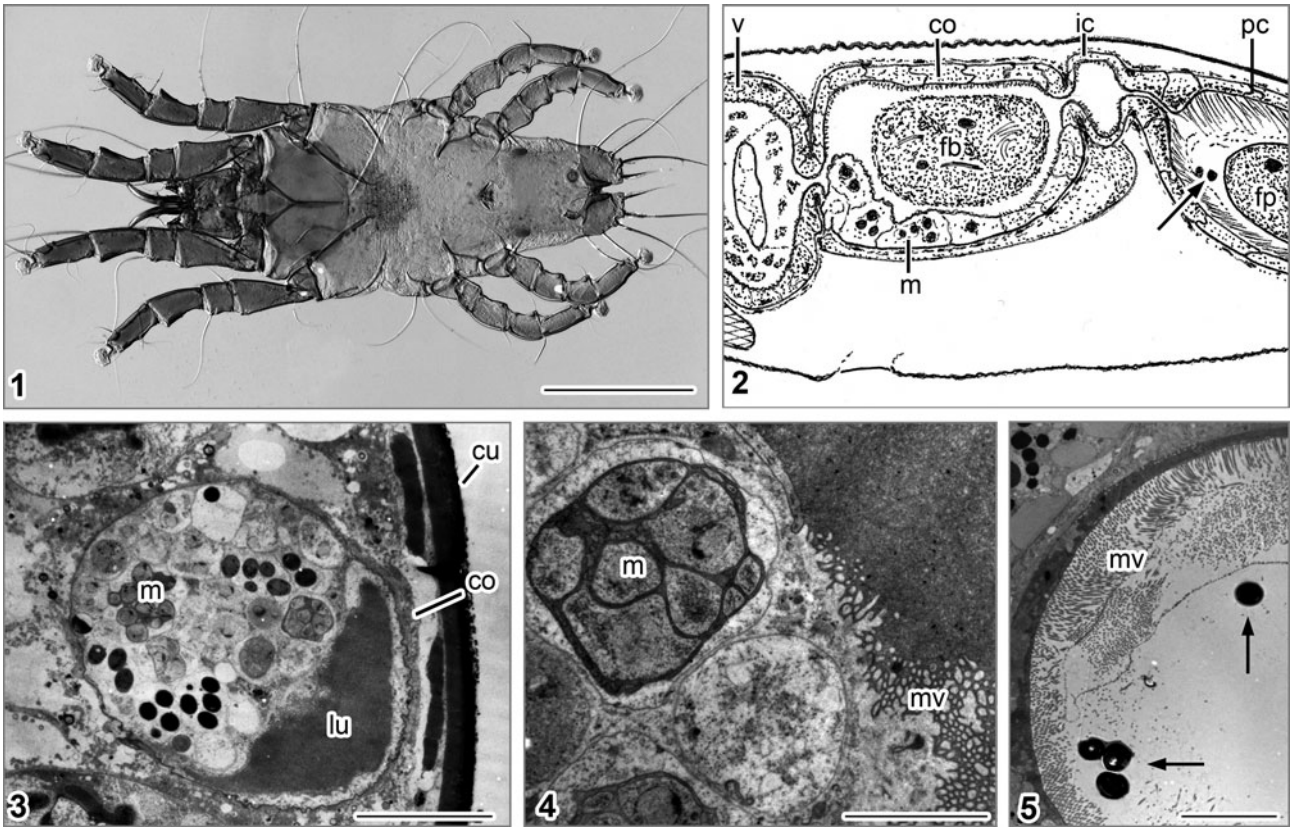
Materials and Methods

Specimens of *Falculifer rostratus* were collected in October 2007 near Greifswald, Germany from the domestic pigeon *Columba livia* f. *domestica*. For transmission electron microscopy (TEM), living specimens were placed into cold fixative (4°C, 3.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4) for two hours and transversely cut into halves with a razor blade. After rinsing with buffer solution, the specimens were post-fixed in 2% buffered OsO_4 solution for a further two hours, rinsed again, dehydrated in a graded series of ethanol and transferred into Spurr's embedding medium (Spurr 1969). Ultrathin sections (70 nm) were contrasted with a saturated solution of uranyl acetate in 70% methanol and lead citrate according to Reynolds (1963) and studied with a JEOL JEM-1011 or a Zeiss EM 208 transmission electron microscope. Semi-thin sections (300 nm) were stained according to Richardson *et al* . (1960) and studied with an Olympus BX60 light microscope (equipped with digital camera DP10).

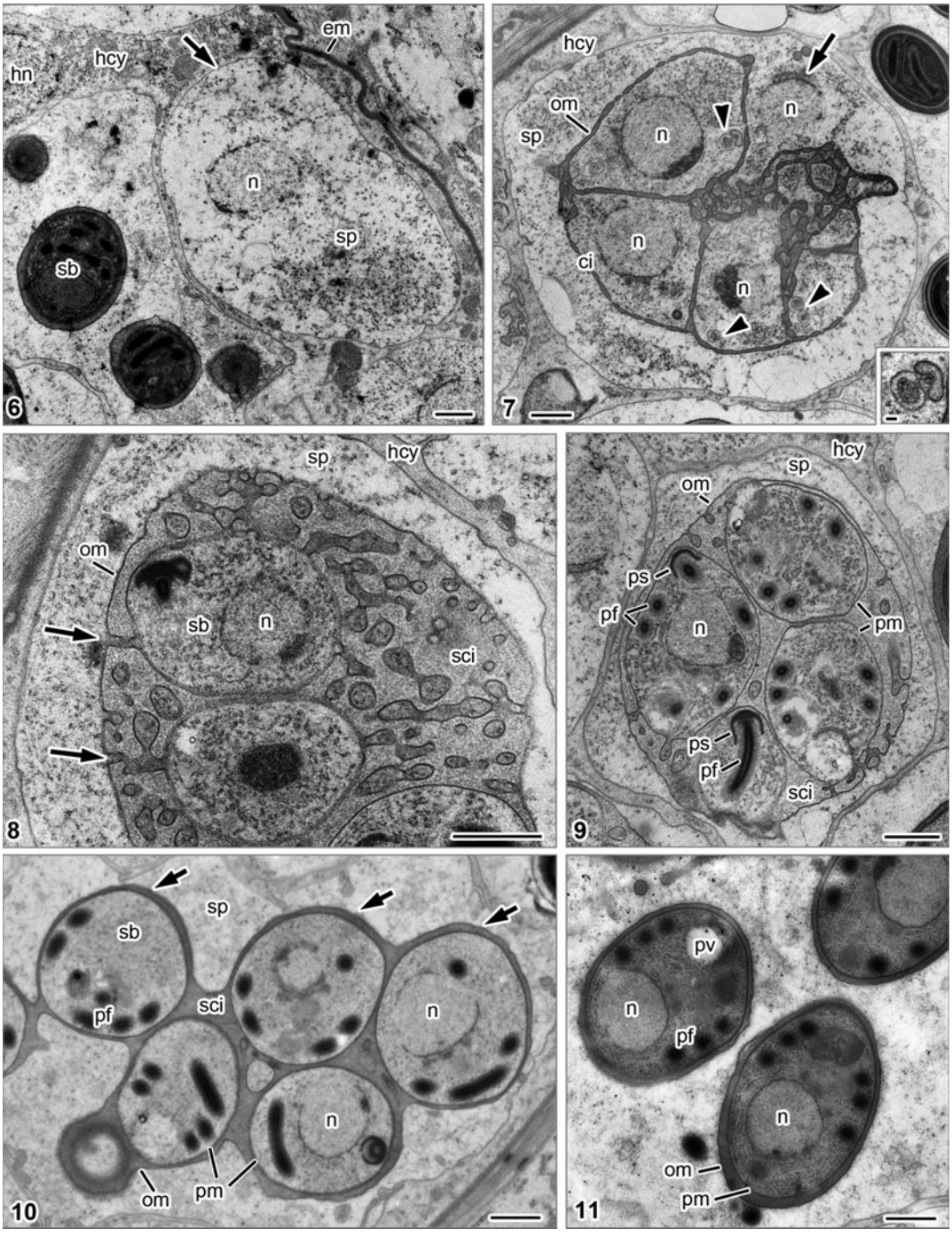
Results

Infection

During morphologic and ultrastructural investigation of the pigeon feather mite *Falculifer rostratus* (Fig. 1), an infection with



Figs 1–5. Distribution of the microsporidial infection in the host *Falculifer rostratus.* Arrows – microsporidia in lumen of postcolon, co – unaltered colon cells, cu – cuticle, fb – food bolus, fp – fecal pellet, ic – intercolon, lu – lumen of colon, m – microsporidia, mv – microvilli, pc – postcolon, v – ventriculus. **Fig. 1. –** Heteromorph male individual of the pigeon feather mite *F. rostratus*. Light microscopy. **Fig. 2. –** Scheme of a sagittal section through the digestive tract of a female *F. rostratus* depicting microsporidia in hypertrophied epithelial cells in the ventral part of the colon. **Figs 3–5. –** Ultrathin sections of infected gut regions. **Fig. 3. –** Hypertrophied colon cells heavily infected with microsporidia at ventral side (left). **Fig. 4. –** Colon cells infected with microsporidia show an irregular microvilli border. **Fig. 5. –** Released spores in the lumen of the postcolon. Scale bars Fig. 1 – 100 µm, Figs 3, 5 – 10 µm, Fig. 4 – 5 µm



Figs 6–11. Sporogony. ci – cistern, em – extracellular matrix supporting epithelium cell, hcy – host cell cytoplasm, hn – host cell nucleus, n – nucleus of microsporidium, om – outer membrane of (former) cistern, pf – polar filament, pm – plasma membrane of sporoblast, ps – polar sac, pv – posterior vacuole, sb – sporoblast, sci – swollen cistern, sp – sporont. **Fig. 6. –** Large sporont; its membrane is not thickened (arrow). Different life cycle stages occur in the same host cell. They are in direct contact to the host cell cytoplasm. **Fig. 7. –** Sporont with several nuclei. Membranous cisterns deliminate compartments around the nuclei, i.e. internal sporoblasts begin to be formed in a central aggregate. One nucleus is still free in sporont cytoplasm (arrow). The formation of polar filaments (arrowheads) begins. Inset: at left side polar filament surrounded by membrane, at right side young polar cap. **Fig. 8. –** The central aggregate produces finely granulated material in the lumen of the swollen cisterns embedding the developing sporoblasts. Cytoplasmic bridges (arrows) connect the peripheral cytoplasm with the cytoplasm of the sporoblasts. **Fig. 9. –** The sporoblasts gain an ovoid form and their polar sacs and filaments are formed. The number of cytoplasmic bridges diminishes. **Fig. 10. –** In the central aggregates the contents of the swollen cisterns condense around the sporoblasts and transform to their spore walls (arrows). **Fig. 11. –** The central aggregate disintegrates into single, uninucleate, ovoid sporoblasts which are now free in the sporont cytoplasm. A homogenously granulated spore wall is formed between the two bordering membranes of the former cisterns. Scale bars Figs 6–11 – 1 μm, Fig. 7 inset – 0.1 μm

microsporidia was discovered by chance. Only one adult female out of the investigated nine animals (3 females, 3 males, 2 nymphs and 1 larva) was infected. Pathogens were found exclusively in the epithelium of the colon (Figs 2–4). The infected epithelial cells were hypertrophied and had an irregular border of microvilli, while the uninfected gut cells were unaltered (Figs 2–4). The host cell nuclei were close-fitting to the sporogonial stages so that they had no typical rounded or elongated form but filled the gaps between the parasites (not shown). Released spores were found in the lumen of the postcolon and the anal atrium (Fig. 5), suggesting a possible horizontal route of transmission. Vegetative reproduction by merogony could not be observed. Structural features and life cycle details were deduced from the study of ultrathin sections.

Sporogony

Sporonts, sporoblasts and mature spores were occasionally found together in the same host cell (Figs 3, 6). The sporonts lay directly in the host cell cytoplasm; membranous envelopes or material layers were not formed around them. Thus thick walled cysts were not present. All stages had isolated nuclei; diplokarya did not occur.

The earliest stages in the life cycle were uninucleated sporonts, recognized in the host cell cytoplasm as relatively large inclusions (e.g. 8 × 5 µm) with loose, slightly granulated cytoplasm (Fig. 6). The granules correspond to ribosomes. The sporont plasma membrane was not supported by external material deposits as in most other microsporidia. During the process of sporogony, spores were formed endogenously. The membrane of the sporont thus developed into the membrane of a polysporophorous vesicle. Often, 4–6 developing spores per sporont could be observed in a single ultrathin section; the maximum was 10 (Figs 4, 7, 10). Based on this, we estimate the number of spores per sporont at 10–20. The nuclei of early stage sporoblasts were relatively large and rounded (ca. 2 µm) and fairly electron-dense (Fig. 7). Often, a plaque of electron-dense nucleoplasm supported the nuclear envelope at one side.

The process of endogenous spore formation is proposed as follows (summarized as a scheme in figure 12): several nuclei are formed and accumulate in the center of the sporont (Figs 7, 12a, b). Membrane cisterns – probably endoplasmic reticulum – appear in the cytoplasm (Figs 7, 12b). They encircle the nuclei at some distance and thus separate the nuclei plus their surrounding cytoplasm from each other. As a result of the membranous demarcations, a layer of loose cytoplasm remains in the periphery of the sporont, while the central portion becomes compartmentalized (Figs 7, 12b). The central, ball-like aggregate is delimited by two membranes. These outer membrane cisterns are continuous with the cisterns between the evolving sporoblasts. In their interior, the cisterns are filled with a moderately electron dense material. The process of compartmentalization is not absolutely synchronized. Some compartments

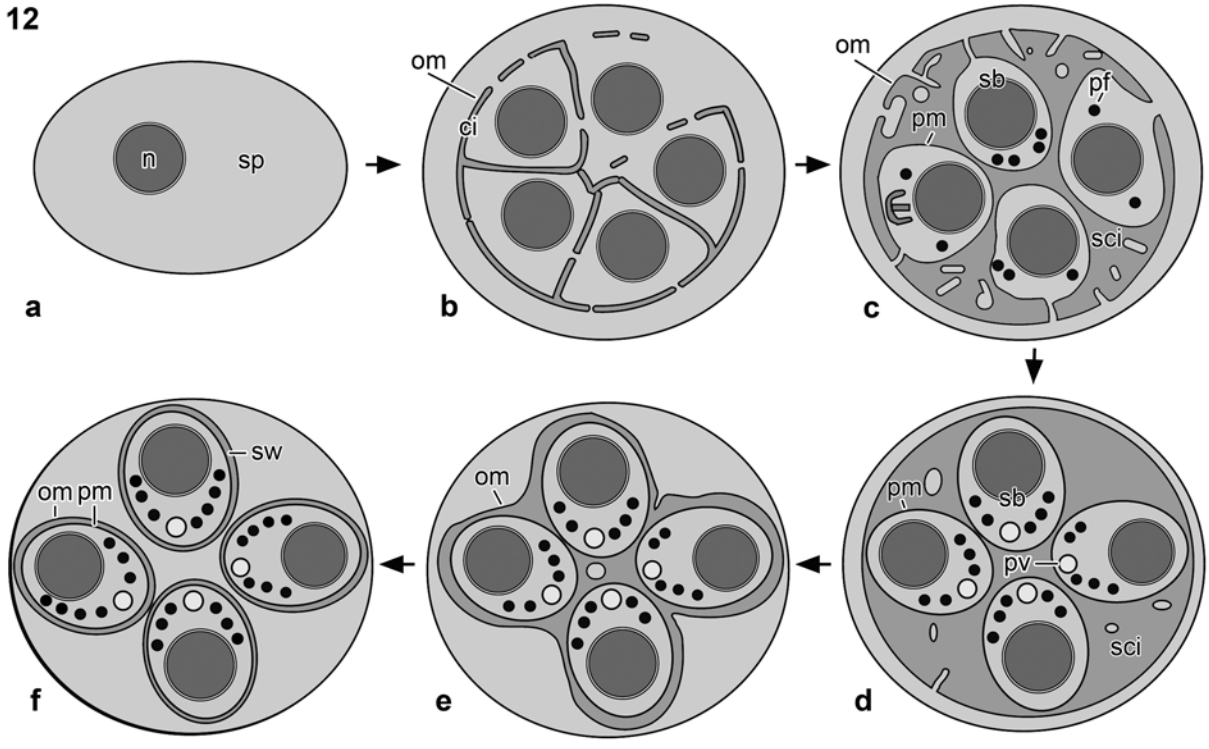


Fig. 12. Scheme summarizing the processes during sporogony (see text). ci – cisterns, n – nucleus, om – outer membrane of (former) cistern, pf – polar filament, pm – plasma membrane of sporoblast/spore, pv – posterior vacuole, sb – sporoblast, sci – swollen cistern, sp – sporont, sw – spore wall.

are nearly closed, but at the same time, other nuclei are still free in the sporont cytoplasm (Figs 7, 12b). Each compartment grows to a monokaryotic sporoblast.

As development proceeds, the two membranes originally bordering the evolving sporoblasts separate from each other, i.e. the flat cisterns grow to more voluminous structures (Figs 8, 9, 12c). The outer peripheral membrane of the ball-like sporoblast aggregate thereby encircles the complete aggregate and each sporoblast has only one membrane left as a border (= inner membrane of cistern = future plasma membrane of sporoblast; Figs 8–10, 12c). Material is produced, filling the space between the sporoblasts; this space is identical to the lumen of the swollen cisterns. The material is a bit denser than the peripheral sporont cytoplasm and finely granulated. The peripheral cytoplasm of the sporont remains in contact with the sporoblast aggregate by cytoplasmic bridges (Figs 8, 9, 12c). It is not clear from the sections whether all bridges are continuous or whether vesicles with peripheral cytoplasm are also pinched off and transported through the lumen of the swollen cisterns. The status of the endogenous spore formation is now as follows: the developing sporoblasts are bordered by one membrane and are nested in a central aggregate which itself is bordered by one membrane and which is filled with granulated material. This complete aggregate lies within the loose cytoplasm of the mother sporont cell that is bordered by its original plasma membrane. The sporont is still found intracellularly in the gut epithelium cell.

During further maturation, the sporoblasts become ovoid. The membrane of the aggregate approaches the surfaces of the sporoblasts, meaning that the lumen of the swollen cisterns begins to shrink again (Figs 10, 12e). The slightly granular material of the aggregate is thereby transformed to a condensed layer surrounding the sporoblasts as a young spore wall. This condensation leads to a disintegration of the central aggregate. The sporoblasts are now separated from each other and are found free in the sporont cytoplasm (Figs 11, 12f). Only a few spores are produced inside the sporont; no further division takes place.

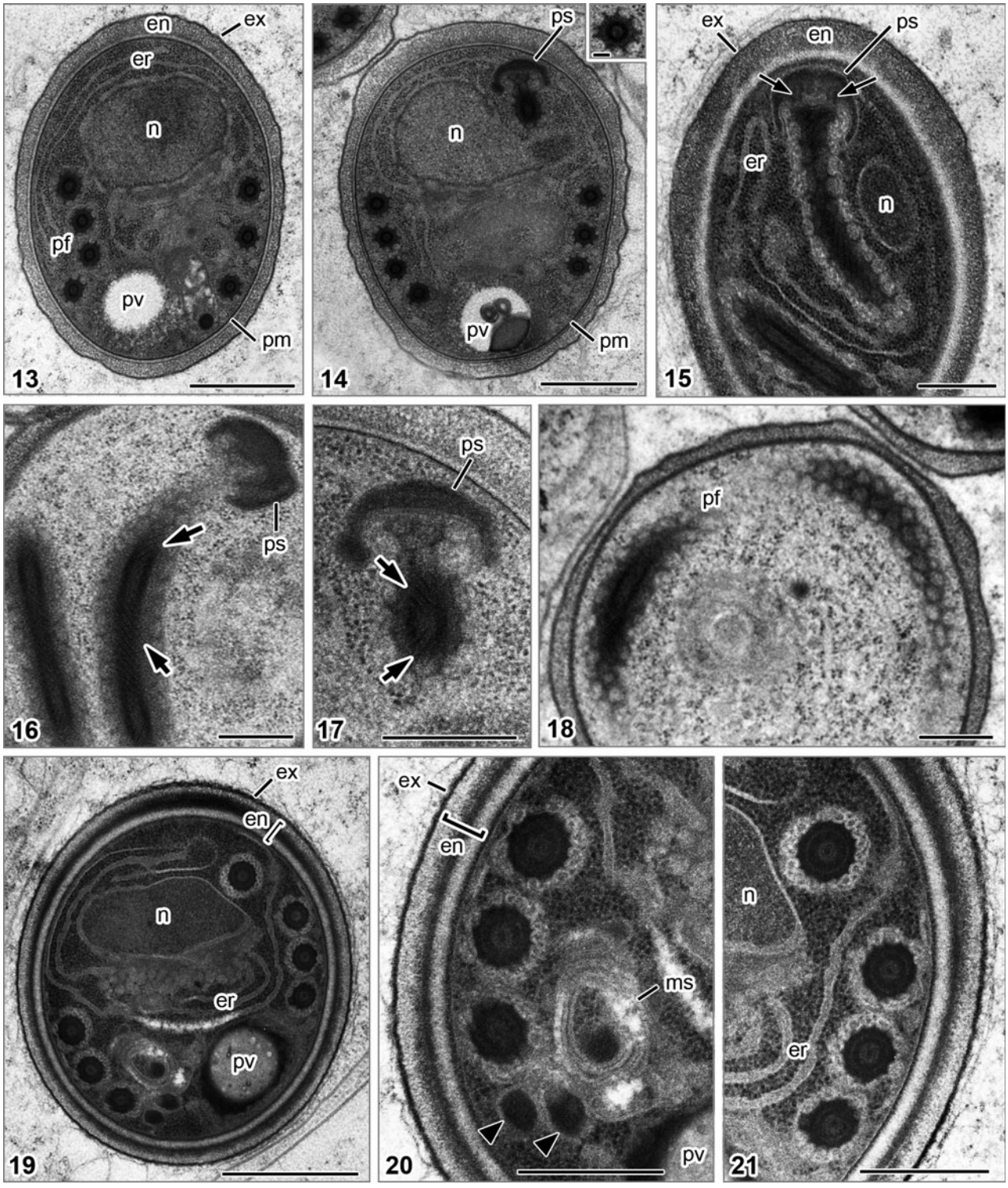
First signs of the extrusion apparatus appeared relatively early during sporulation. Even before all nuclei of the sporont were properly distributed to sporoblasts, early stages of polar filaments and polar sacs appeared in the cytoplasm in the already demarcated sporoblasts (Figs 7, 12c). In cross-sections, the newly arising filament had a small diameter (ca. 0.15 µm) and was enveloped by a membrane (Fig. 7 inset). Later, up to four windings of the filament were formed (Figs 9–11). Anteriorly they were connected to a polar sac (Fig. 9). At the other cell pole, a posterior vacuole arose (Figs 11–13, 18).

Nearly mature and mature spores

The developing spores were of minute size, measuring about 3.6 (3.2–4) µm × 2.6 (2.3–2.8) µm (n = 9) and had a broad ovoid form. We observed only one size class of spores. During the development of the spore wall, an outer, dark, slightly wavy exospore was formed (Figs 13–21). The endospore was firstly homogenous granular (Figs 13–18) but during maturation, a central electron-dense layer arose (Figs 19–21). In mature spores, the exospore had a thickness of about 20 nm, and the three-layered endospore measured about 240 nm. A plasma membrane supported the endospore from inside (Figs 11, 13, 14). The nucleus was situated anteriorly, more or less centrally alongside the polar filament. Its form appeared to be irregular and more elongated than rounded (ca. 1.4 × 0.7 µm), with the longer axis lying perpendicular to the longitudinal axis of the spore (Figs 13, 14, 19). The karyoplasm was mostly homogenously granulated. Sheaths of endoplasmic reticulum sometimes surrounded the nucleus and may be connected to the nuclear envelope (Figs 13, 14, 19, 21). The cytoplasm of the maturing spores became dense, packed with free ribosomes.

The most prominent structure of the spore was the polar filament, which differed in arrangement and structure from typical polar filaments. It arose subapically at a polar sac and was completely coiled in three to four turns; an anterior straight part was missing (Figs 13–15, 19). A membrane was not seen bordering the mature filament (Figs 16, 20, 21). Our measurements of the diameter of the polar filament include only the dark core and not the surrounding 50–80 nm thick (mean 70 nm) honeycomb-like structure. The diameter of the mature filament had a greater diameter (230 nm) in the first turn than in the following turns (190 nm) (n = 20). Thus, the polar filament is of the anisofilar type. In cross-sections, the construction of the polar filament can be seen: an electron-dense core with several sub-layers was surrounded by a lucent construction. The substructure of the core showed some variation with 5–7 more or less fine sublayers of dense or medium dense material (Figs 20, 21). The innermost material and one prominent middle layer were always dense. The outermost layer was also dense and formed little rounded compartments of lucent material at its surface. Thus, in cross-sections, peripheral spikes – the lateral borders of the compartments – gave the filament a star-like appearance (Figs 13, 14, 19–21). The number of spikes increased from nine in young spores (Figs 13, 14) to 16 in mature spores (Figs 19–21) and seemed to be dependent on the diameter of the central filament core. Grazing sections of the lucent surface structures revealed a honeycomb-like pattern: bright rounded compartments enclosed by dark walls (Figs 16–18). The honey-comb layer is missing at the very last part of the filament (Fig. 20). A further detail was visible in grazing sections. The most prominent electron dense layer of cross-sectioned polar filaments showed two levels of striations in grazing sections (Figs 16, 17). The striations within one level were arranged in parallel and run at an angle of about 90 degree to the striations of the second level and 45 degree to the core of the polar filament.

Membranous structures resembling a traditional polaroplast were not formed at any stage. The only clear membranous structure at the anterior cell pole was the polar sac, to which the polar filament was attached. Its form resembled a strongly arched hat of a mushroom with a thickened apical



Figs 13–21. Cellular structures of nearly mature (**Figs 13–18**) and mature spores (**Figs 19–21**). en – endospore, er – endoplasmic reticulum, ex – exospore, ms – membranous structure, n – nucleus, ps – polar sac, pf – polar filament, pm – plasma membrane, pv – posterior vacuole. **Figs 13–15 –** Longitudinal sections of nearly mature spores with anterior nucleus, subapical polar sac and 3–4 coils of the polar filament. Electron-dense material bridges connect the polar sac with the anterior part of the polar filament (arrows). **Figs 16, 17 –** Grazing sections of polar filament depicting two levels in the dark core layer with fine striations running at a 90˚ angle (arrows). **Fig. 18 –** More superficial grazing section of the polar filament shows honeycomb-like structure. **Figs 19–21 –** The spore wall consists of a thin, electron-dense exospore and a thick endospore composed of three layers (bright, dark, bright). The anisofilar polar filament has a wider diameter anteriorly and misses the honeycomb-like structure at the very end (Fig. 19). An unknown membranous structure is present. Scale bars Figs 13, 14, 19 – 1 µm, Fig. 14 inset – 0.1 µm, Figs 15–18, 20, 21 – 0.5 µm

part (ca. 160 nm) and a thinner peripheral margin (ca. 30 nm; Figs 14 – 17). The widest diameter of the polar sac was 0.6 µm. The polar sac was filled with electron dense material that was most dense in the thin margin and the anterior lining of the thickened apical part (Figs 14–17). Between the upper and lower margin of the apical hat part, the material was a bit brighter. A bright structure filled the gap between the end of the dark core of the polar filament and the center of the polar cap. At its border, darker material bridges the polar cap with the polar filament (Fig. 15). A typical anchoring disc was not present inside the polar sac.

Discussion

Life cycle and cytology

The atypical microsporidium herein described is clearly a new species belonging to the Chytridiopsida (syn. Minisporida). For example, the polar filament is short, a polaroplast and merogony are generally missing, and sporogony is the only means of replication. Chytridiopsids are generally parasites of the gut epithelium of insects, myriapods, mites and earthworms (Larsson 2014). Beard *et al.* (1990) suggested that the absence of merogony (in *Nolleria pulicis*) may be explained with a high efficiency of transmission so that there is no need for complicated life cycles. The authors called the type of sporogony in *Nolleria* a "multiple division by vacuolation" and the result a "polysporophorous vesicle". A similar mode of sporogony by vacuolation also applies to our feather mite microsporidium and several other species, such as *Chytridiopsis typographi* (Purrini and Weiser 1985), *Buxtehudea scaniae* (Larsson 1980), *Enterozytozoon bieneusi* (Desportes *et al.* 1985), and *Pseudopleistophora szollosi* (Sprague 1977b; Szollosi 1971). Beard *et al.* (1990) suggested that the vacuoles may function in the release of metabolic products and in forming the inner membrane of the polysporophorous vesicle (the outer one is the plasma membrane of the sporont). The inner membrane of the vesicle is suggested to degenerate during completion of sporogenesis. In contrast, in the new microsporidium, the fused membrane cisterns and their contents seem to be finally transformed into the spore wall.

The polar filament of most members of the Chytridiopsida is characteristically covered by a honeycomb-like surface layer of little chambers (Canning and Vavra 2000). Additionally, in some species, e.g. *Chytridiopsis trichopterae* (Larsson 1993), *Intexta acarivora* (Larsson *et al.* 1997), and *Nolleria pulicis* (Beard *et al.* 1990) an outer membrane was described to enclose the polar filament and its surface layer. Vavra (1976) suggested that this alveolar layer may represent a "special kind of polaroplast". A lamellar/vesicular polaroplast is generally missing in atypical microsporidia. We did not observe a membrane around the surface compartments of the mature polar filament in our sections. Rather, the compartments are bordered by material from the outer layer of the electron-dense core of the polar filament. It is possible that the material of the honeycomb-like layer does play a role in the discharge of the polar filament, but we are skeptical as to whether it is a homologue to the polaroplast. Purrini and Weiser (1985) were able to show a small lamellar polaroplast close to the polar cap of the filament in *Chytridiopsis typographi*. Thus, the polaroplast may be strongly or completely reduced in chytridiopsids, and the honeycomb-structure could fulfill a similar function (e.g. with swellable substances). Since the polar filaments are quite short in this protist group, a lower pressure than usual may be enough to extrude the filament.

The nuclei of sporoblasts contained electron-dense plaques at the inner side of the nuclear envelope. Similar structures were also observed in several other Chytridiopsida and interpreted as nucleoli (Tonka *et al.* 2010).

Systematic affiliation

The described microsporidium from the feather mite *F. rostratus* is an atypical microsporidium. However, all arachnid microsporidia described so far – except one – express the normal spore morphology, i.e., have a long coiled polar filament and a voluminous polaroplast. These typical microsporidia were identified as members of the genera *Cryptosporina*, *Gurleya*, *Napamichum*, *Nosema*, *Oligosporidium*, *Pleistophora*, *Thelohania* and the unclassified genus *Microsporidium* (Becnel *et al.* 2002; Larsson *et al.* 1997). The *Pleistophora* species should almost certainly be reclassified, since this genus typically infects vertebrates such as fish, amphibians, or reptiles. To date, only one atypical microsporidium has been described from mites, *Intexta acarivora* (Larsson *et al.* 1997). Larsson *et al*. (1997) found it in the gut epithelium of the mould mite *Tyrophagus putrescentiae* (Astigmata: Acaroidea: Acaridiidae).

Atypical microsporidia have been classified differently in the past (detailed compilation in Larsson 2014). In 1977, Weiser united the atypical microsporidia (Metchnikovellidae, Chytridiopsidae, Hesseidae) in the class Metchnikovellidea ("normal" microsporidia = class Microsporididea). Sprague (1977a), Issi (1986) and Voronin (2001) divided the atypical microsporidia into metchnikovellideans and taxa belonging to the more advanced microsporidia. For example, Sprague (1977a) put the Metchnikovellidae in an own class Rudimicrosporea, and the other atypical microsporidia in the order Chytridiopsida within the class Microsporea (together with "normal" species of the order Microsporida). Later, Sprague changed the name Chytridiopsida into Minisporida (Sprague 1982). In the present paper, we keep the original name Chytridiopsida since a "minimal development of the accessory spore organelles" – to which the name Minisporida refers – is also true for the Metchnikovellidea. The metchnikovellids are not included in the further considerations since they are exclusively hyperparasites of gregarines in annelids and e.g. possess only a rudimentary polar filament in the form of a short, thick, manubrium shaped tube (Sprague 1977a).

Table I: Characterization of members of the family Chytridiopsidae (no merogony, no polaroplast, monokaryotic, in gut epithelium)

(Species) Genus	Host	Spore	Polar filament			Exospore (ex)/ Endospore (en)	Intracellular location of sporogony stages	References
			Honey-comb	Number of coils	Iso-/ Anisofilar			
Sheriffia brachynema	*Biomphalaria glabrata* (Mollusca, Gastropoda)	small spherical, monomorphic	yes, tuberculate layer, no outer membrane	1–2	isofilar	en and ex	multinucleate plasmodia in thin-walled cysts	Larsson 1014; Richards and Sheffield 1970; Sprague *et al.* 1972
Chytridiopsis spp.	Insecta, Myriapoda	spherical (1.5 µm), monomorphic (dimorphic in *C. typographi* and *C. trichopterae*)	yes, enclosed by outer membrane	2–3	isofilar	thin ex and en	free sporogony and multinucleate plasmodium in thin-walled or thick-walled cysts (sacs); close association with host cell nucleus	Larsson 1993, 2014; Manier and Ormières 1968; Purrini and Weiser 1985; Sprague 1977; Sprague *et al.* 1972
Nolleria pulicis	*Ctenocephalides felis* (Insecta, Siphonaptera)	spherical (1.9–2.5 µm), monomorphic	yes, 9 chambers per ring enclosed by outer membrane	2	isofilar (Ø ca. 300 nm)	no en, thin ex	multinucleate plasmodial aggregate within sporont; polysporophorous vesicle; 100–150 spores; max. 50 per ultrathin section	Beard *et al*. 1990
Intexta acarivora	*Tyrophagus putrescentiae* ("Acari", Astigmata)	spherical to slightly ovoid, microspores Ø 1.3–1.7 µm, macrospores Ø 1.5–2.3 µm	yes, 12 tubular chambers per ring enclosed by outer membrane	2–3 in microspores, up to 9 in macrospores	anisofilar	well-developed en (85–130 nm), thin ex (16 nm)	multinucleate plasmodial aggregate within sporont (polysporophorous vesicle); max. 6 spores per ultrathin section	Larsson *et al.* 1997
Acarispora falculifera nov. gen., nov. spec.	*Falculifer rostratus* ("Acari", Astigmata)	broad ovoid, monomorphic, 3.6 (3.2–4) µm × 2.6 (2.3–2.8) µm	yes, 9–16 roundish chambers per ring, no outer membrane	3–4	anisofilar (Ø 230/190 nm)	thick en (240 nm), thin ex (20 nm)	multinucleate plasmodial aggregate within sporont; max. 10 per ultrathin section, estimated 10–20 spores per sporont	Present study

Thus, the new, atypical microsporidium can be classified within the order Chytridiopsida, which is characterized by spherical or broad oval spores, a short polar filament, a thin or absent endospore and a missing polaroplast and Golgi (Sprague 1982; Weiser 1983). The compilation of families and genera within the Chytridiopsida diverges to some extent for different authors, partly depending on the known genera at that time. Sprague (1977a) describes the three families Chytridiopsidae (*Chytridiopsis*, *Steinhausia*), Hesseidae (*Hessea*), and Burkeidae (*Burkea*). According to Weiser (1983) the three families Chytridiopsidae (*Chytridiopsis*, *Steinhausia*), Hesseidae (*Hessea*, *Burkea*), and Buxtehudeidae (*Buxtehudea*) are included. In 1992, Sprague *et al.* contained four families in the Chytridiopsida: Chytridiopsidae (*Chytridioides*, *Chytridiopsis*, *Nolleria*), Buxtehudiidae (*Buxtehudea*, *Jiroveciana*), Enterocytozoonidae (*Enterozytozoon*; without chytridiopsid characters) and Burkeidae (*Burkea*).

This microsporidium of the feather mite most probably belongs to the Chytridiopsidae because it clearly differs from the genera of the other families. The genus *Hessea* possesses diplokarya, merogony, and sporulates in thick-walled spore sacs (Ormières and Sprague 1973). *Burkea* has a long polar filament, merogony, and sporulates in large spore sacs in the musculature and epidermis of oligochaetes (de Puytorac and Tourret 1963; Burke 1970). *Buxtehudea* also has a long polar filament (8–11 coils), a weekly developed polaroplast, and enveloped plus free sporogony (Larsson 1980, 2014). *Jiroveciana* and *Chytridioides* are insufficiently known by light microscopy only and have therefore been omitted (Jírovec 1940; Larsson 1980; Trégouboff 1913).

The family Chytridiopsidae was created by Sprague, Ormières and Manier in 1972. Table I summarizes the characteristics of (the sufficiently known) genera currently included in the Chytridiopsidae. Sprague *et al.* (1972) defined the features of the family as follows: Sporogonial plasmodium develops in intimate contact with the host cell nucleus, often causing a deep cup-shaped depression in it; produces either 2 kinds of cysts (thick walled durable cyst and fragile membrane) in the same host or only a membranous cyst of varying degrees of toughness. Spores spherical to elliptical; endospore rudimentary or absent; polaroplast rudimentary or absent; polar filament short to medium length. At that time, the authors included only *Chytridiopsis* and the newly created genus *Steinhausia* [n. comb. of *Chytridiopsis ovicola*, *Haplosporidium* (*Chytridiopsis*) *mytilovum*, and *Coccospora brachynema*] in the family Chytridiopsidae. However, the taxonomic position of *Steinhausia* within the Chytridiopsida and its general characteristics were unclear. While *S. brachynema* shows typical features of chytridiopsid microsporidia (Richards and Sheffield 1971), the type species *S. mytilovum* has e.g. a polaroplast, a long polar filament coiled in 2–3 rows, diplokarya and a merogony phase (Sprague *et al.* 1972). In 2014, Larsson gives a detailed reevaluation of the genus *Steinhausia*. He comes to the conclusion that the genus *Steinhausia* with the species *S. mytilovum* (Field 1924), *S. ovicola* (Léger and Hollande 1917) and *S. spraguei* (Kalavati and Narasimhamurti 1977) has to be removed from the chytridiopsid microsporidia. He further decides to introduce a new genus name, *Sheriffia*, for the true chytridiopsid species *S. brachynema*. Since this parasite only infects molluscs, a close relation to the mite microsporidium is unlikely. In addition, there are morphological differences such as the isofilar polar filament and the tuberculate structure of its honey-comb layer in *Sheriffia brachynema*.

Meanwhile two further genera, *Nolleria* (1 species; Beard *et al.* 1990) and *Intexta* (1 species; Larsson *et al.* 1997) were newly described and affiliated with this family. Beard *et al.* (1990) extended the definition of the family Chytridiopsidae by stating that merogony is lacking and that the polar filament is isofilar. However, *Intexta* and the new microsporidium have anisofilar filaments but there are several examples of families including members of both filament types (e.g. Thelohaniidae, Pleistophoridae, Tuzetiidae; Sprague *et al*. 1992). Thus, the criterion "isofilar filament" is questionable in the diagnosis of a family but generally useful for a genus (Larsson *et al.* 1997). However, in polymorphic microsporidia even the same species may have one spore type with an isofilar filament and another spore type with an anisofilar filament (e.g. *Amblyospora dyxenoides*; Canning and Vavra 2000). Therefore, the diagnostic value of the filament type should not be overestimated. Recently, Larsson (2014) emended the diagnosis of the family Chytridiopsidae. He removed the characters, "close association with the host cell nucleus" and "production of thick-walled spore sacs" from the family diagnosis since they are restricted to the genus *Chytridiopsis* and he also withdrew the character "isofilar filament". Larsson (2014) added that there are two sequences of sporogony (free and enveloped) and coined the term "polysporophorous spore sac" for the enveloped sporogony stages which include the plasma membrane of the sporont. However, to our knowledge, the presence of free sporogony is restricted to the genus *Chytridiopsis* and thus is no general feature of the family Chytridiopsidae. There are some more features differentiating the genus *Chytridiopsis* from the feather mite microsporidium: spores are smaller (1.5 µm) and spherical, isofilar filament with only 2–3 coils, thin or missing endospore (Larsson 2014; Manier and Ormières 1968; Purrini and Weiser 1985; Sprague 1977a, b; Sprague *et al.* 1972). Also *Nolleria pulicis* has spherical spores with an isofilar polar filament with only 2 turns and misses an endospore. Further, *Nolleria* produces more (100–150) spores per sporont, which are separated by fusion of numerous large vacuoles instead of flat cisterns (Beard *et al.* 1990).

Our microsporidium most closely resembles *Intexta acarivora* (Larsson *et al.* 1997). Both species infect gut cells of mites. Their life cycle is similar: merogony is missing, and sporogony takes place in a central aggregate within the sporont. The polar filament is completely coiled and of the anisofilar type. There are, however, also a number of differing features. Gut cells infected by *Intexta* are not hypertrophic. *Intexta* has two types of spores (micro- and macrospores), not one, and

both have a smaller diameter than our microsporidium. In *Intexta* spores, the flat nucleus is localized to one side of the spore with its longitudinal axis parallel to the longitudinal axis of the spore (≠ perpendicular position), and its nucleoplasm is denser in the periphery (≠ homogenous nucleoplasm). The lucent construction surrounding the dark core of the polar filament consists of "tubules" in *Intexta* but of rounded compartments in our species conferring a honeycomb-like pattern in grazing section. The endospore of mature spores is lucent in *Intexta* but has an electron-dense layer within a moderate dense endospore in the new species. Taking all these features together, it seems reasonable to create a new genus, i.e. *Acarispora*, for the microsporidium of the feather mite.

Diagnosis

***Acarispora* gen. nov. Radek and Alberti 2014**

Only sporogony in life cycle; sporont directly in host cell cytoplasm; no thick-walled cyst formed; endogenous spore formation (polysporophorous vesicle) by disintegration of a multinuclear plasmodial aggregate within sporont – membrane cisterns delineate cytoplasm plus a nucleus; monomorphic and monokaryotic spores; honeycomb-like compartments around core of polar filament; no polaroplast.

***Acarispora falculifera* sp. nov. Radek and Alberti 2014**

Sporogony: About 10–20 spores endogenously produced within a sporont.

Spores: Broad ovoid, 3.2–4 × 2.3–2.8 µm, thick endospore (240 nm) with median electron-dense layer, thin exospore (20 nm), anisofilar polar filament (Ø 230/190 nm), completely coiled in four turns, honeycomb-like structure around electron-dense core of polar filament showing 9–16 chambers in cross-sections, nucleus centrally located.

Host tissue: Gut epithelium of colon, hypertrophy of infected cells.

Transmission: No information, spores in gut lumen suggests a possible horizontal route of transmission.

Type host: The pigeon feather mite *Falculifer rostratus* Buchholz 1869 (Astigmata, Falculiferidae) collected from the domestic pigeon *Columba livia* f. *domestica.*

Type host locality: Near Greifswald, Germany.

Type species: Ultrathin section of a female *Falculifer rostratus* deposited at the Biology Center of the Upper Austrian Museum, J.-W.-Klein-Strasse 73, 4040 Linz, Austria, under the type number 2014/24.

Etymology: Genus name *Acarispora* refers to mites ("Acari") as the host group and to the formation of spores ("spora"). Species name based on the host genus *Falculifer*.

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