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Microgemma vivaresi n. sp. (Microsporidia, Tetramicridae), Infecting Liver and Skeletal Muscle of Sea Scorpions, *Taurulus bubalis* (Euphrasen 1786) (Osteichthyes, Cottidae), an Inshore, Littoral Fish

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ABSTRACT. The ultrastructure of a new microsporidian species *Microgemma vivaresi* n. sp. causing liver cell xenoma formation in sea scorpions, *Taurulus bubalis*, is described. Stages of merogony, sporogony, and sporogenesis are mixed in the central cytoplasm of developing xenomas. All stages have unpaired nuclei. Uninucleate and multinucleate meronts lie within vacuoles formed from host endoplasmic reticulum and divide by binary or multiple fission. Sporonts, no longer in vacuoles, deposit plaques of surface coat on the plasma membrane that cause the surface to pucker. Division occurs at the puckered stage into sporoblast mother cells, on which plaques join up to complete the surface coat. A final binary fission gives rise to sporoblasts. A dense globule, thought to be involved in polar tube synthesis, is gradually dispersed during spore maturation. Spores are broadly ovoid, have a large posterior vacuole, and measure 3.6 μm × 2.1 μm (fresh). The polar tube has a short wide anterior section that constricts abruptly, then runs posteriad to coil about eight times around the posterior vacuole with granular contents. The polaroplast has up to 40 membranes arranged in pairs mostly attached to the wide region of the polar tube and directed posteriorly around a cytoplasm of a coarsely granular appearance. The species is placed alongside the type species *Microgemma hepaticus* Ralphs and Matthews 1986 within the family Tetramicridae, which is transferred from the class Dihaplophasea to the class Haplophasea, as there is no evidence for the occurrence of a diplokaryotic phase.

Key Words. Merogony, microsporidia, new species, spores, sporogony, ultrastructure, xenoma.

Bony fish (Osteichthyes) are hosts to several genera of microsporidia (Canning and Lom 1986; Lom and Nilsen 2003). Lom and Nilsen (2003) discussed the ultrastructural diversity of microsporidia infecting fish, including their interactions with the hosts. The genus *Microgemma* was established by Ralphs and Matthews (1986) for *Microgemma hepaticus* Ralphs and Matthews 1986, a parasite in the liver of grey mullet, *Chelon labrosus* Risso. It was characterised by (a) formation of 500-μm diam. xenomas with microvillar surfaces, (b) life cycle stages intermixed in host cell cytoplasm, (c) unpaired nuclei, (d) merogonic plasmodia enclosed within a host membrane and dividing by plasmotomy, (e) multinucleate merogonic plasmodia developing directly into sporogonic plasmodia with loss of the enveloping host membrane, and (f) sporoblasts formed by multiple budding and fragmentation of the sporogonic plasmodia.

During examinations of a collection of marine fish for myxozoan infections, we encountered a microsporidian species causing xenoma formation in the liver and skeletal muscle of sea scorpions, *Taurulus bubalis* (Euphrasen). Some xenomas were actively developing while others, including all of those examined from skeletal muscle, were encapsulated and invaded by phagocytes. The inflammatory reaction has been described (Canning and Curry 2005). According to the above diagnostic characters, we tentatively identified the microsporidia in *T. bubalis* as *Microgemma* sp. and a sequence (AJ252952) of the small subunit (SS) rDNA was included in a phylogenetic analysis of microsporidia from fish, crustacean, insect, and mammalian hosts (Cheney, Lafranchi-Tristem, and Canning 2000). The analysis placed it close to a sequence of *Spraguea lophii* (Doflein 1898). The same SSrDNA sequence was included in maximum-likelihood phylogenies using a wider range of microsporidia (Lom and Nilsen 2003). Their analysis supported the provisional generic assignment to *Microgemma*, because the species clustered with *Microgemma caulleryi* (Van den Berghe, 1940) from sand eels, *Hyperoplus lanceolatus* (Le Sauvage), and *Tetramicra brevifilum* Matthews and Matthews, 1980 from turbot, *Scophthalmus maximus* (L.). This clade was related to a clade containing *Spraguea* spp.

In addition to the type species, *M. hepaticus*, two species have been transferred into the genus, namely *Microgemma ovoideum* (Thélohan, 1895) by Amigó et al. (1996) and *M. caulleryi* (Van den Berghe, 1940) by Leiro et al. (1999). Here, we present data on the development of the microsporidium inducing xenomas in the liver of *T. bubalis*. Similar xenomas in skeletal muscle were all in terminal decline. The morphology has enabled us to confirm the generic assignment to *Microgemma* and name the parasite as a new species, *Microgemma vivaresi* n. sp., in recognition of the outstanding contributions to research on microsporidia of Professor C. P. Vivarès, especially as co-ordinator of the group that elucidated the first complete microsporidian genome sequence, that of *Encephalitozoon cuniculi*.

MATERIALS AND METHODS

Taurulus bubalis collected from Portland Harbour, Weymouth, Dorset, UK, were terminally anaesthetised by immersion in

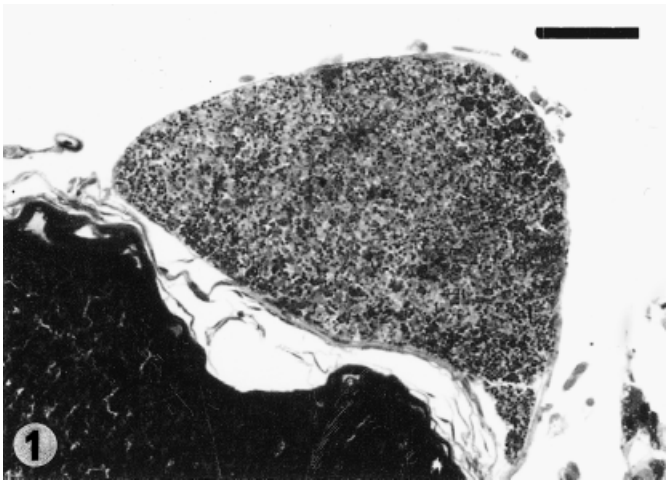

Fig. **1.** Xenoma of *Microgemma vivaresi* n. sp. encapsulated by fibroblasts and separated from normal liver tissue of sea scorpion, *Taurulus bubalis*. Scale bar = 100 μm.

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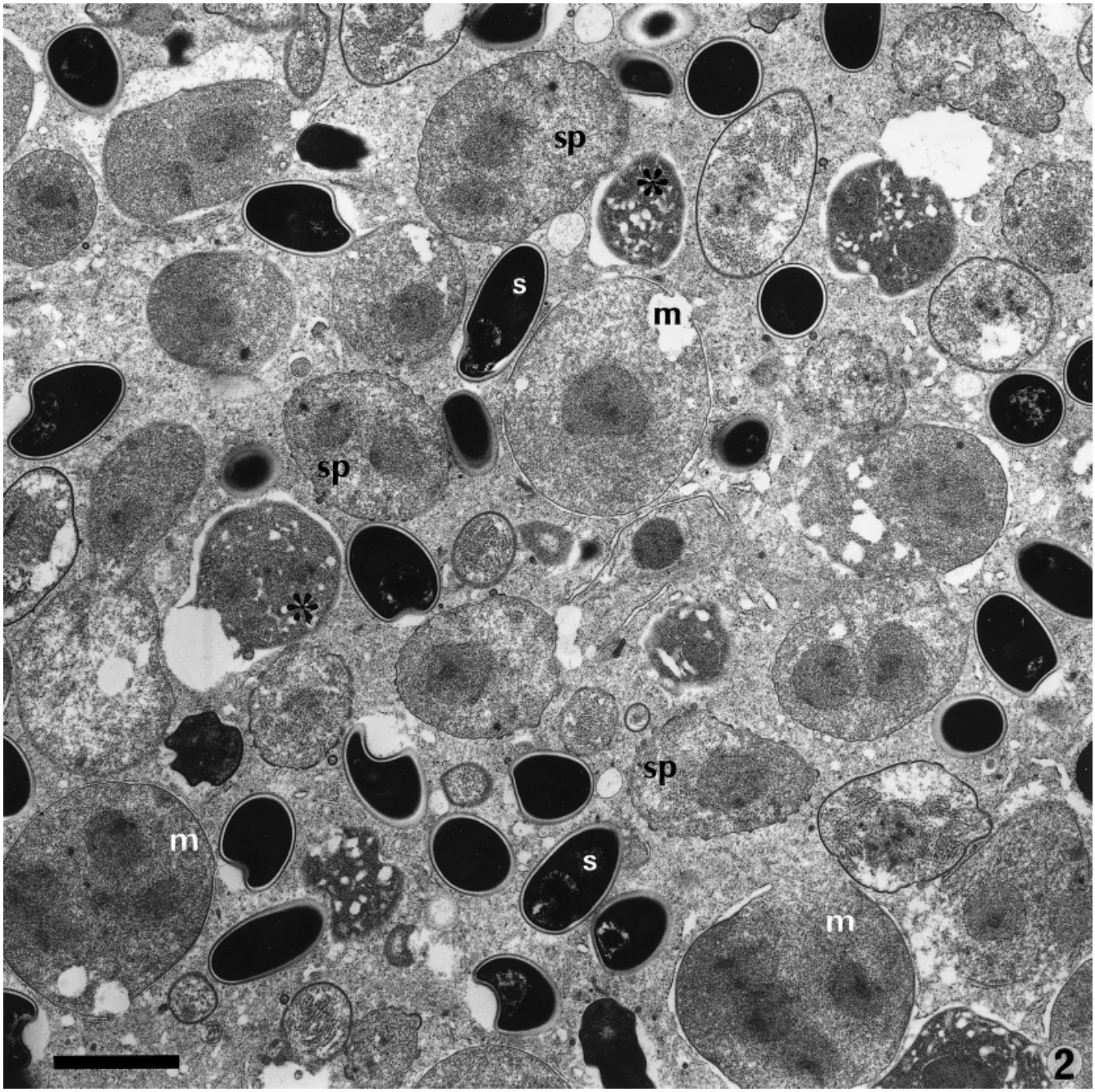
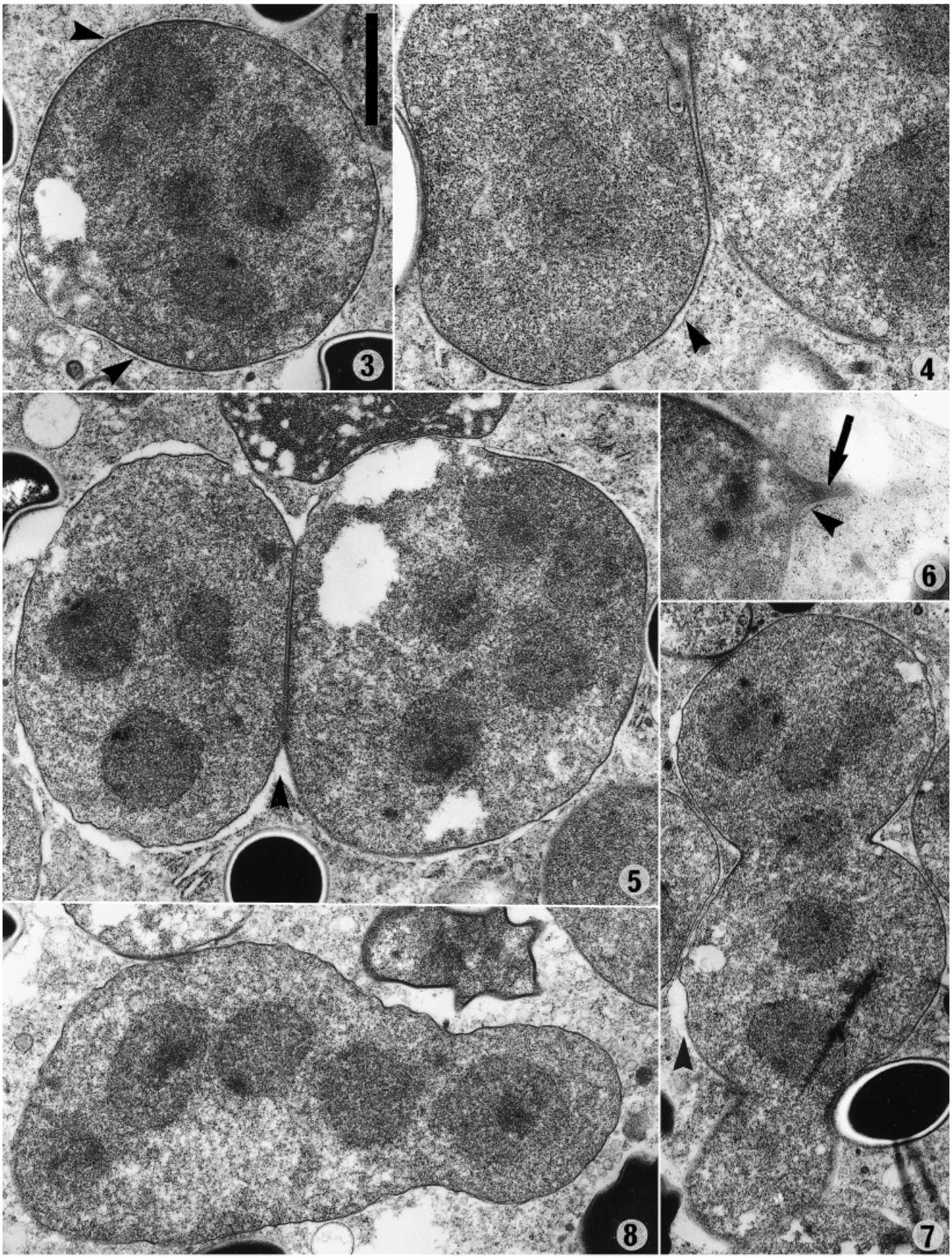


Fig. **2.** Part of the parasitised cytoplasm of a xenoma of *Microgemma vivaresi* n. sp. showing mixed merogonic (m) and sporogonic (sp) stages and spores (s). Unidentified stages, (∗) lie, as do meronts, in a vacuole but have expanded endoplasmic reticulum, more typical of sporonts. In developing xenoma, parasites are restricted to the region of cytoplasm within the hypertrophic reticulate nucleus, which separates non-parasitised peripheral cytoplasm from parasitised central cytoplasm. Scale bar = 3.2 μm.

MS222 (Sandoz) in sea water. Xenomas were excised from liver tissue as cleanly as possible and fixed in 2.5% glutaraldehyde in 0.1 M phosphate or cacodylate buffer (pH 7.2). The cysts were post-fixed in OsO_4 in the same buffers, washed several times in buffer, dehydrated in a graded ethanol series, and cleared in propylene oxide. Some were embedded in epoxy resin, and others in Agar 100 (Agar Scientific). Semi-thin (1.0 μm) sections were stained with Toluidene Blue, and ultrathin sections were stained with uranyl acetate and lead citrate for examination in either a JEOL1210 or AEI EM801 electron microscope at 80 KV. Fresh spores were photographed and measured using Nomarski interference contrast microscopy on a Nikon Eclipse E800 microscope with screen measurement system (Nikon UK, Ltd).

RESULTS

Xenomas still harbouring active merogonic stages could attain at least 200-μm diam. Later stages were encapsulated by fibroblasts and were easily separated from surrounding normal liver cells (Fig. 1). In developing xenomas, all stages of the parasite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were mixed in the centre of the host cell (Fig. 2), separated from a narrow band of parasite-free peripheral cytoplasm by a network formed by the highly branched host cell nucleus (Canning and Curry 2005). Each parasite lay in a rather tightly fitting vacuole. The presence of ribosomes on the vacuolar membrane identified it as a host rough endoplasmic reticulum (Fig. 3). The vacuoles were distended in places around the larger meronts, especially at division planes (Fig. 4, 5). Parasite nuclei were unpaired in all life cycle stages. Meronts were uninucleate or multinucleate, and round or elongate. Their cytoplasm was uniformly granular and poorly endowed with cytoplasmic organelles (Fig. 3). The plasma membrane had a thin surface coat deposit. Division was by binary fission or plasmotomy and was effected by deep invagination into the body (Fig. 4). The division products either remained in close contact within a common vacuole (Fig. 5) before division of the vacuole, or were pulled apart until a narrow isthmus was broken (Fig. 6) and separate vacuoles were formed. Division of elongate forms gave rise to chains of binucleate or uninucleate individuals (Fig. 7).

Transfer to sporogony occurred in uninucleate and multinucleate forms. It was signalled by the disappearance of the vacuole (Fig. 8) and the emergence, at intervals through the plasma membrane, of small secretions of moderate density (Fig. 9). The secretions appear to spread out as plaques on the cell surface (Fig. 10), causing the surface to pucker (Fig. 11). Binary or multiple fission occurred at the puckered stage (Fig. 12–14), giving rise to uninucleate products (i.e. sporoblast mother cells). Deposition of the surface coat was then completed, accompanied by smoothing of the surface contours and expansion of parasite's rough endoplasmic reticulum (Fig. 15). A final division at this stage (Fig. 16, 17) gave rise to uninucleate sporoblasts.

During maturation, sporoblasts became crenated (Fig. 18) and progressively more dense. A dense sphere, up to one-third of the sporoblast width, appeared in the posterior half, and persisted during synthesis of the polar tube and polaroplast (Fig. 19, 20). It became relatively less dense as maturation proceeded, signalled by the interpolation of the endospore between the plasma membrane and the exospore (Fig. 21).

Interspersed between other stages in the host cytoplasm were rare stages, in which the cytoplasm was packed with ribosomes and filled by an extensive system of endoplasmic reticulum (Fig. 2, 22, 23). These had up to three nuclei in one plane of section, a thin surface coat on the plasma membrane, and nearly all lay in a vacuole. The surface characteristics were those of a merogonic sequence but the expanded cisternae of the endoplasmic reticulum are more usually associated with sporogonic stages.

Fresh spores measured $3.57 \pm 0.26\,\mu m \times 2.07 \pm 0.19\,\mu m$ ($n = 30$). They were broadly ovoid with a prominent posterior vacuole occupying up to half of the length of the spore (Fig. 30). Electron microscopy revealed a rather thin exospore measuring 6 nm overlying the endospore, which measured 70 nm, thinning to 30 nm over the sub-apically positioned anchoring disc (Fig. 25, 26). Beneath the spore wall, a narrow band of cytoplasm separated the anchoring disc from the plasma membrane. The anchoring disc, within a narrow polar sac, showed several bands of differing density overlying the expanded base of the polar tube, which measured 140 nm in diam. This part of the polar tube had a central ''plug'' of 90-nm diam. The tube abruptly constricted behind this region, and then it tapered gradually towards the isofilar coiled region where it measured 80-nm diam. The polar tube coiled six to eight and a half times usually in a single row around the posterior vacuole, with eight being the most common number of coils. The posterior vacuole contained flocculent material and sometimes the remnants of the dense sphere (data not shown), which had been universal in the late sporoblasts (see above).

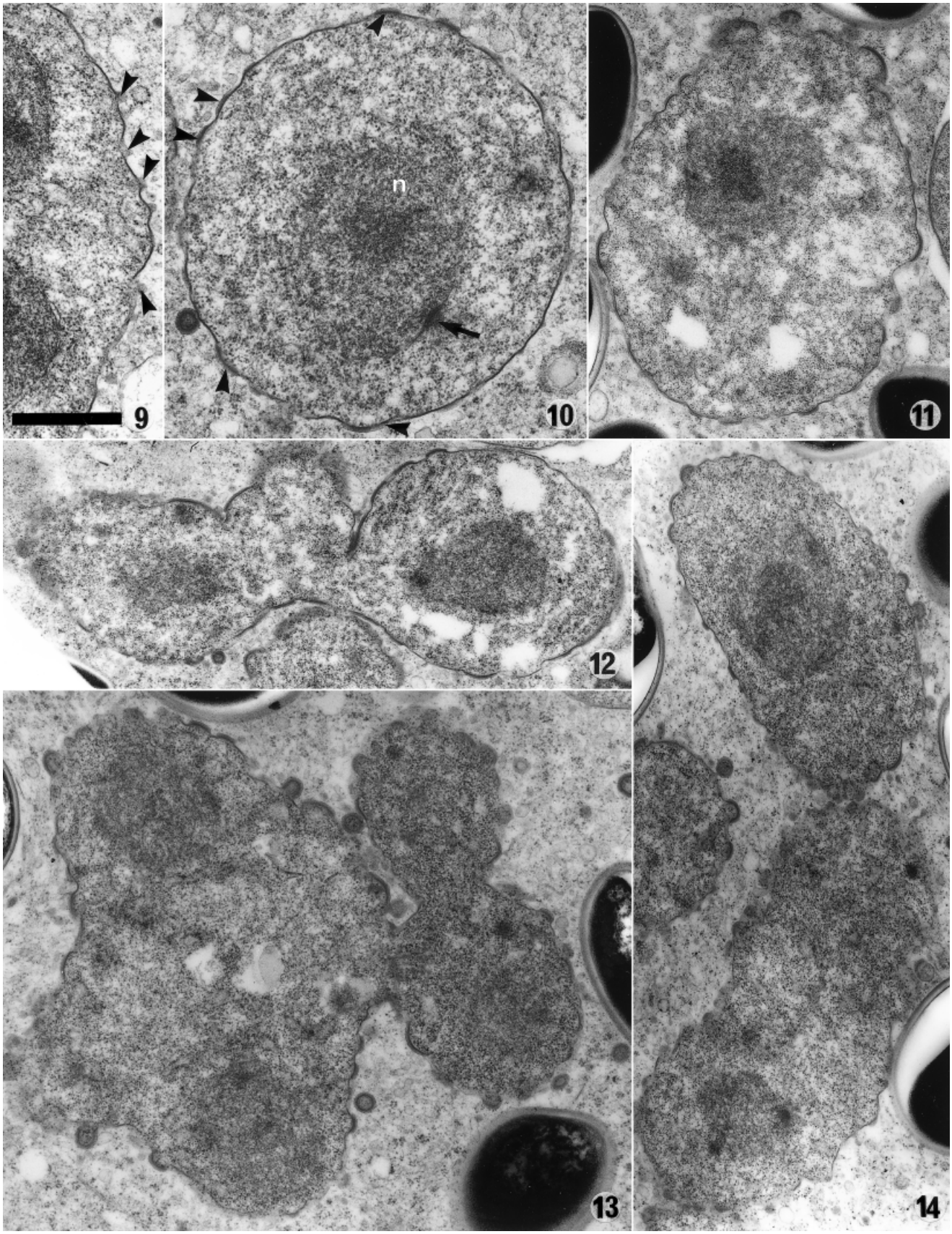
The polaroplast was composed of about 40 tightly packed membranes arranged as doublets, separated by dense material (Fig. 24). The membranes were attached to the anterior end of the polar tube, most of them to the expanded section beneath the anchoring disc, and were directed posteriad-like thick arms. The whole polaroplast structure can be envisaged as a truncated cone with thick laminated walls. In some oblique sections, the ''arms'' appeared connected transversely (Fig. 27). In transverse sections, the circle of lamellae was seen around an area of coarsely granular cytoplasm, through which ran the straight part of the polar tube (Fig. 28). The single nucleus occupied a central position immediately posterior to the polaroplast. Cross-sections of the polar tube revealed a two-membrane sheath around a lucent ring, which in turn enclosed material of moderate density with a denser core. The group of coils was enclosed by abutting ribosomes (Fig. 29).

DISCUSSION

Of the 14 microsporidian genera recorded from fish (Lom and Nilsen 2003), the species herein described from the liver of *T. bubalis* most closely fits the description of *Microgemma*. The type species, *M. hepaticus* Ralphs and Matthews, 1986, infects the liver of grey mullet, *C. labrosus*. *Microgemma hepaticus* induces formation of macroscopic xenomas with a microvillous surface, within which is a parasite-free zone with abundant mitochondria and a reticulate nucleus positioned at the boundary between the parasite-free and infected zones. All stages of merogony and sporogony of *M. hepaticus* are mixed in the central zone and their nuclei are unpaired. Meronts lie within a single cisterna of host rough endoplasmic reticulum, are multinucleate, and divide by binary fission or plasmotomy. Sporonts lose the enveloping rough endoplasmic reticulum, are uninucleate or multinucleate, and are distinguished by the deposition on the plasma membrane of electron-dense plaques, which eventually fuse. Division occurs at the plaque stage by multiple budding, culminating in uninucleate sporoblasts. A dense spherical body in immature spores was illustrated but not mentioned in the text (Ralphs and Matthews 1986). Fresh spores measured $4.2\,\mu m \times 2.4\,\mu m$ and the polar tube had seven to ten coils.

Microgemma vivaresi n. sp. exhibits all of the characters of *Microgemma* except that, in sporogony, the multiple budding does not produce sporoblasts directly but releases sporoblast mother cells, which undergo a final nuclear division and binary fission to produce the sporoblasts. Minor differences exhibited by *M. vivaresi* n. sp. are the smoother xenoma surface lacking microvilli, the more prominent plaques giving a puckered appearance to the sporogonial plasmodia, a slight difference in fresh spore size, and a lower number of polar tube coils. Ultrastructurally, the spores of the two species are indistinguishable except perhaps for the number of polar tube coils. In the absence of transmission experiments, we propose that the minor morphological differences and

Fig. **3–8.** *Microgemma vivaresi* n. sp. in liver of *Taurulus bubalis*. Scale bar in Fig. 3 applies to all figures. **3.** Multinucleate rounded meront showing uniform thickening of the plasma membrane and lying in a close-fitting cisterna of ribosome-studded host endoplasmic reticulum (arrowheads). Scale bar = 1.5 μm. **4.** Merogonic division by deep invagination to separate the products within the vacuole (arrowhead). Scale bar = 3.0 μm. **5.** Close contact between multinucleate division products within a common vacuole (arrowhead). Scale bar = 1.4 μm. **6.** Merogonic division by presumed pulling apart of the division products leaving a temporary isthmus (arrow) between the division products within the host vacuole (arrowhead). Scale bar = 1.0 μm. **7.** Chain formation of binucleate and multinucleate merogonic division products within the host vacuole (arrowhead). Scale bar = 2.0 μm. **8.** Early multinucleate sporont, no longer in a vacuole but lying directly in host cell cytoplasm. Scale bar = 1.4 μm.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the different host warrant the establishment of a new species, which we name *M. vivaresi* n. sp. Unusual stages were found in *M. vivaresi* n. sp. which resembled meronts in having only a slight thickening of the plasma membrane and in lying in a cisterna of host rough endoplasmic reticulum. However, they differed from meronts in showing prolific expansion of their own rough endoplasmic reticulum, a condition more usually associated with the increased synthetic activity of sporonts. Progression to sporogony, signalled by deposition of the future exospore, was not observed in these stages and their role was not determined. It would be useful to re-examine the type species to determine the absence or presence of sporoblast mother cells and the stages with expanded rough endoplasmic reticulum, and to obtain an SSrDNA sequence.

Two other species of *Microgemma* have been described, *M. ovoideum* and *M. caulleryi*. *Microgemma ovoideum* from red band fish, *Cepola macrophthalma*, and shore rockling, *Motella tricirrata*, was first named *Glugea ovoidea* by Thélohan (1895). However, an ultrastructural study of *G. ovoideum* in *C. macrophthalma* by Amigó et al. (1996) revealed the absence of the sporophorous vesicles characteristic of *Glugea* (see Canning et al. 1982) and demonstrated almost all the characters of *Microgemma* as defined by Ralphs and Matthews (1986); Amigó et al. (1996) reported on the formation of sporoblast mother cells but did not observe rough endoplasmic reticulum around meronts. This may have been because of degeneracy of host cell cytoplasm, which might occur when the xenomas are overwhelmed with parasites. Amigó et al. transferred *G. ovoideum* to *Microgemma*. Spores of *M. ovoideum* measured 3.8+0.27 μm × 1.97+0.26 μm and the polar tube had six to eight coils.

Microgemma caulleryi was first described as *Glugea microspora* from the liver of sand eels, *Ammodytes lanceolatus*, now *H. lanceolatus* (see Van den Berghe 1939). As the name *G. microspora* Thélohan, 1891 was a synonym of *Glugea anomala* (Moniez, 1887), Van den Berghe (1940) renamed his species *Glugea caulleryi*. Leiro et al. (1999) transferred *G. caulleryi* to the genus *Microgemma*. They found that xenomas induced by *G. caulleryi* in *H. lanceolatus* lacked the layered capsules and stratification of developmental stages found in *Glugea* xenomas (see Canning et al. 1982) and that, in sporogony of *G. caulleryi*, there were no sporophorous vesicles. Xenomas and development of *G. caulleryi* were in close accord with the features described for other species of *Microgemma* (see Amigó et al. 1996; Ralphs and Matthews 1986). Meronts of *M. caulleryi* were exceptional in being packed with concentric membranes but were in accord with the type species in lying within the host rough endoplasmic reticulum. The rough endoplasmic reticulum was lost around sporonts, which were said to give rise directly to sporoblasts by exogenous budding (Leiro et al. 1999). A dense sphere was not described in sporoblasts. Spores measured 2.6 μm × 1.2 μm (fresh) and the polar tube had seven to nine coils.

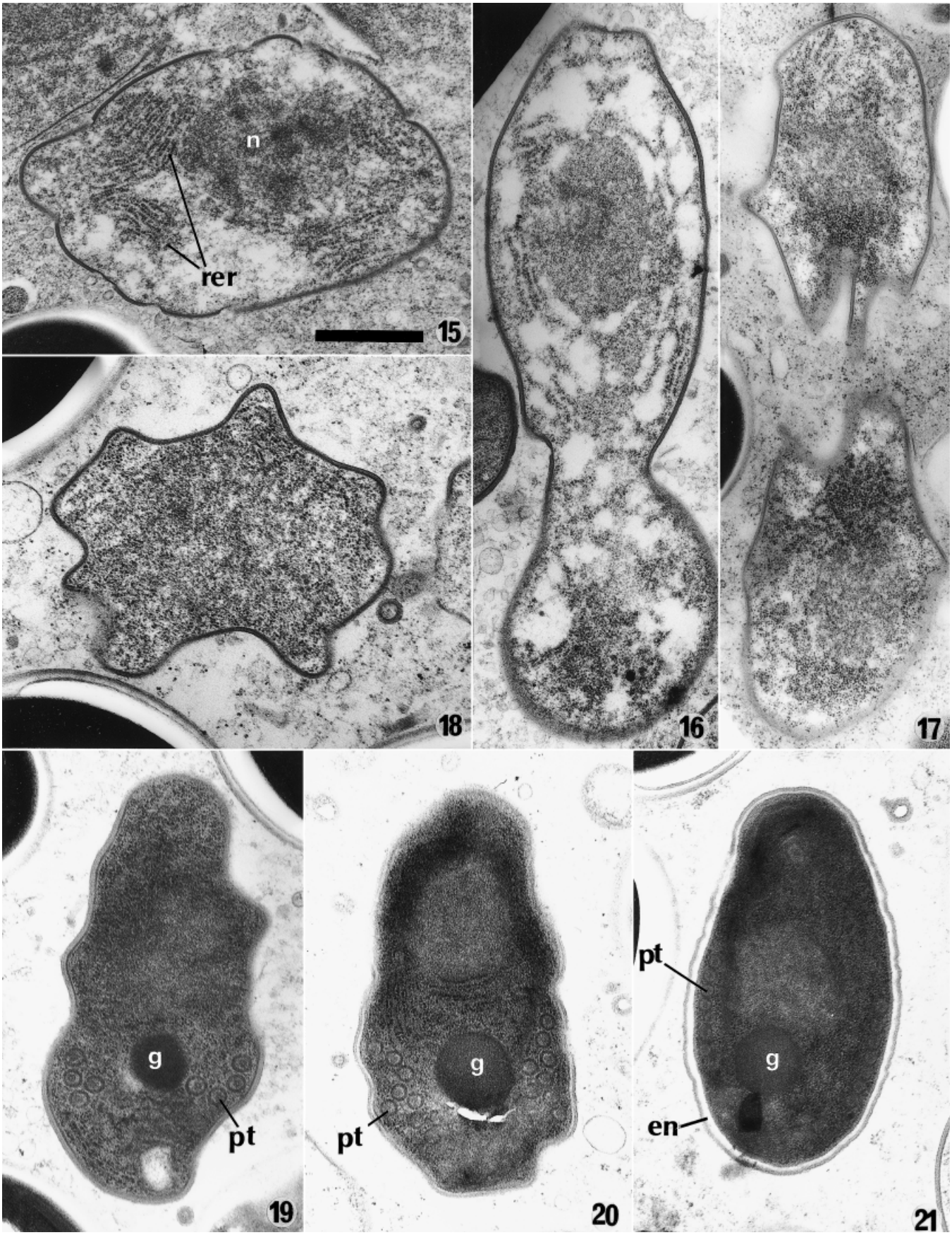
Spores of the four known species of *Microgemma* show virtually no ultrastructural differences and, apart from *M. caulleryi*, which has smaller fresh spores than the others, spore size is too close to be a reliable taxonomic character. The presence of host rough endoplasmic reticulum around meronts, not yet reported in *M. ovoideum*, and the division of sporonts into sporoblasts via sporoblast mother cells, not reported in *M. hepaticus* and *M. caulleryi*, are characters that need re-investigation in the species where they are said to be absent, as these features are of generic importance. *Microgemma vivaresi* n. sp. infecting *T. bubalis* is closest to *M. ovoideum* in spore size, polar tube coil number, and presence of sporoblast mother cells. However, in the absence of cross-transmission experiments, we saw no present alternative to naming it a new species based on its being parasitic in a new host, which is not closely related to the hosts of *M. ovoideum*. It is necessary to point out that *M. ovoideum* has been reported from two unrelated host fishes and, on the basis of host, *M. ovoideum* may represent two species.

These taxonomic difficulties may be resolved by a comparison of rDNA (or other) gene sequences. The difference between *M. caulleryi* and *M. vivaresi* n. sp. (= *Microgemma* sp.) has already been confirmed by their rDNA sequences (Lom and Nilsen 2003). The phylogenetic analyses of Lom and Nilsen (2003) are difficult to interpret because *T. brevifilum* Matthews and Matthews, 1980, a parasite of connective tissue in the musculature of turbot, *S. maximus*, is placed between the two species of *Microgemma*. *Tetramicra brevifilum* has in common with *Microgemma* a similar xenoma structure, unpaired nuclei throughout development, and merogony within a host vacuole, which is lost at the onset of sporogony. Sprague, Becnel, and Hazard (1992) mentioned diplokaryotic meronts and dissociation of the diplokarya in sporogony in their summary of the genus *Tetramicra*, but there is no evidence for this, as diplokarya were neither described nor illustrated by Matthews and Matthews (1980). Diplokarya have not been described in any of the four known species of *Microgemma*.

Characters shown by *T. brevifilum*, which differ from *Microgemma*, are that the sporonts are reported to arise from binucleate (but not diplokaryotic) sporont mother cells, tetrasporoblastic sporogony, the small number of polar tube coils, and the exceptionally large dense inclusion bodies in the spores. Nevertheless, the resemblance between *M. hepaticus* and *T. brevifilum* is striking and the generic separation may have arisen from different interpretations or terminology used to describe the life cycle stages. For example, the binucleate ''sporont mother cells'' of *T. brevifilum* may be equated to merogonic division in chain formation as seen in *M. hepaticus* (see Ralphs and Matthews 1986) and *M. vivaresi* n. sp. (this paper): the host vacuolar membrane appears to be still in place around the ''sporont mother cells'' of *T. brevifilum* and there are no surface coat deposits. Furthermore, the division of *T. brevifilum* sporonts into tetrads may be equivalent to division of the sporont into sporoblast mother cells as described in *M. ovoideum* and *M. vivaresi* n. sp. Sprague, Becnel, and Hazard (1992) have commented that a further division of one of the tetrad products could be interpreted as division of a sporoblast mother

Fig. **9–14.** Early sporogonic development of *Microgemma vivaresi* n. sp. in the liver of *Taurulus bubalis*. Scale bar on Fig. 9 applies to all figures. **9.** Surface of a multinucleate stage with small exudations on the already thickened plasma membrane, signalling the onset of sporogony. Scale bar = 800 nm. **10.** Uninucleate sporont, on which early secretions at the surface have spread out forming plaques (arrowheads). A centriolar plaque (arrow) indicates the onset of division of the nucleus (n). Scale bar = 750 nm. **11.** Early uninucleate sporont showing puckering at the surface in places where surface coat plaques have been deposited. Scale bar = 1.4 μm. **12–14.** Sporogonic division at the puckered stage giving rise to sporoblast mother cells. Scale bar = 1.1 μm (**12**), 1.2 μm (**13**), 1.0 μm (**14**).

Fig. **15–21.** Late sporogonic development of *Microgemma vivaresi* n. sp. in the liver of *Taurulus bubalis*. Scale bar in Fig. 15 applies to all figures. **15.** Sporoblast mother cell showing smoothing of the surface contours as the surface coat is completed, increased rough endoplasmic reticulum (rer), and signs of chromosome condensation in the nucleus (n). Scale bar = 0.7 μm. **16, 17.** Division of binucleate sporoblast mother cells into sporoblasts. Scale bar = 0.9 μm (**16**), 1.0 μm (**17**). **18.** Early sporoblast: shortly after presumed separation from sporoblast mother cells, the sporoblasts are crenated. Scale bar = 8.1 μm. **19–21.** Sporoblast development involving formation of a dense globule (g) of gradually decreasing density, possibly providing material for the synthesis of the polar tube (pt). In Fig. 21 the endospore (en) is clear. Scale bar = 8.1 μm.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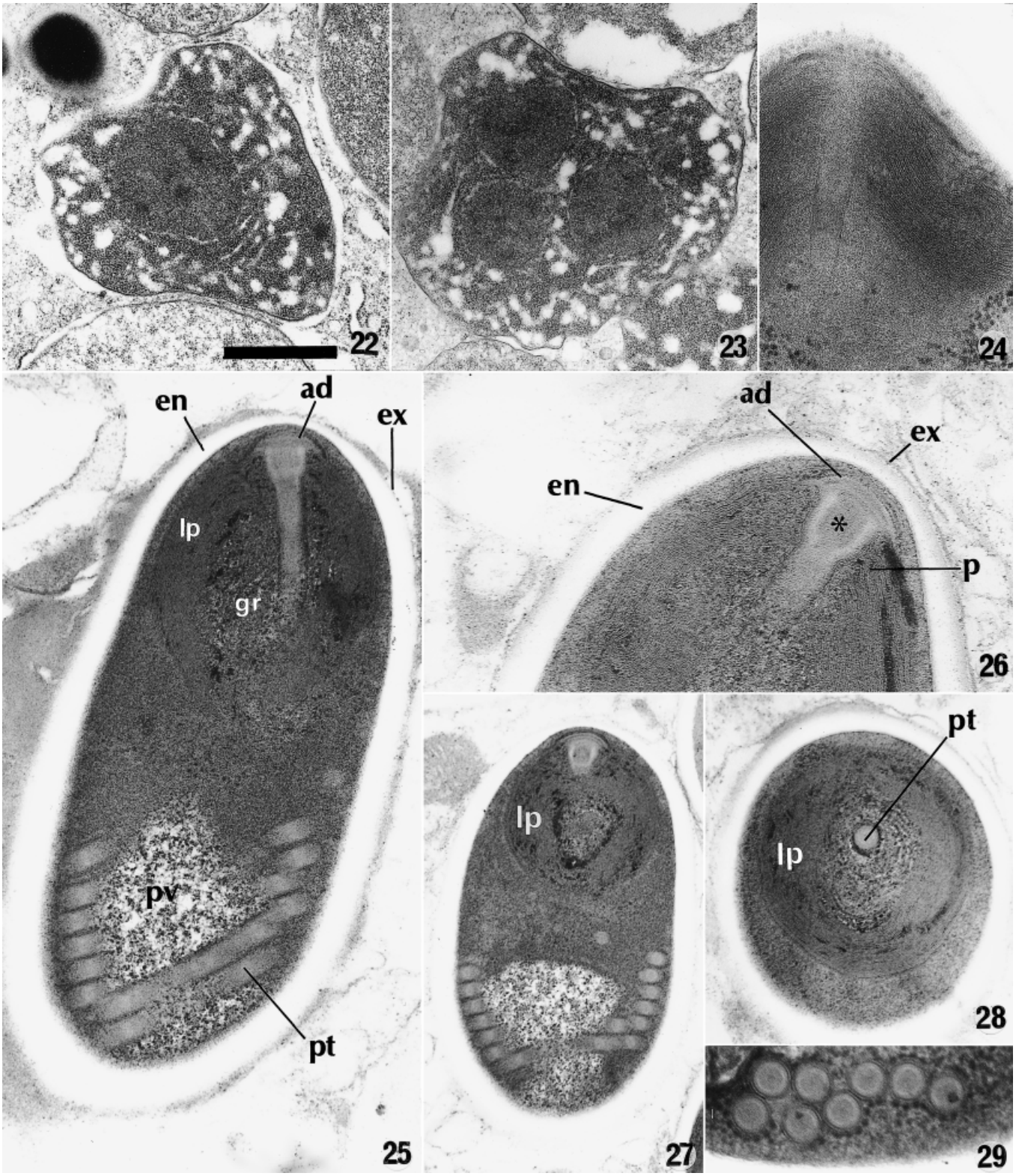
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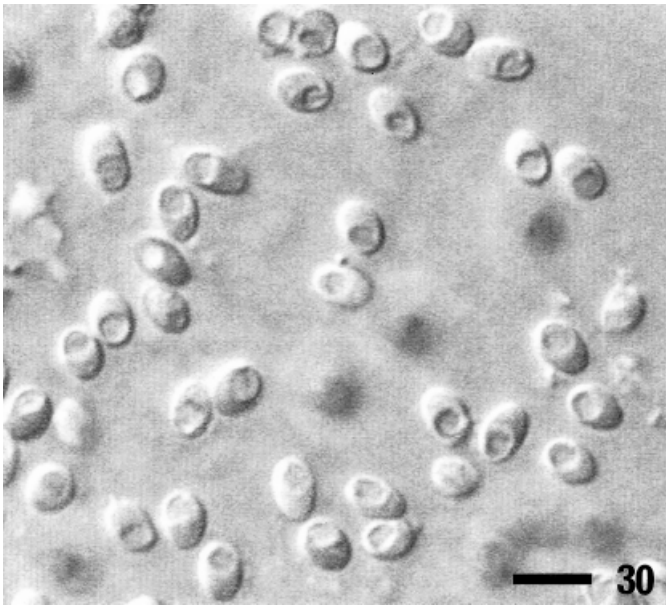

Fig. **30.** Fresh spores of *Microgemma vivaresi* n. sp. dispersed from xenoma in the liver of *Taurulus bubalis*. Scale bar = 5.0 μm.

cell into two sporoblasts. An SSrDNA sequence has not been obtained for *M. hepaticus* (the type species) and this would be enlightening in resolving the taxonomic relationships. Whether or not there are two genera, the species clearly belong to the same Family *Tetramicridae* Matthews and Matthews 1980.

TAXONOMIC SUMMARY

Phylum Microsporidia Balbiani 1882
Class Haplophasea Sprague, Becnel and Hazard 1992
Order Glugeida Issi, 1986
Family Tetramicridae Matthews and Matthews 1980
Microgemma vivaresi n. sp.

Generic diagnosis. With the characters of the genus as defined by Ralphs and Matthews (1986), with the addition of the division of sporonts into sporoblasts via sporoblast mother cells.

Specific diagnosis. Spores broadly ovoid, measuring 3.57+0.26 μm × 2.07+0.19 μm when fresh. Polar tube with expanded base attached to the anchoring disc, constricting abruptly, then tapering to form six to eight and a half coils in a single row. Dense globule in the posterior region of sporoblasts disappears in mature spores.

Type host. *Taurulus bubalis* (Euphrasen, 1786).

Type locality. Portland Harbour, Weymouth, Dorset, UK. Bearing: 50°35′2″N, 2°26′47″W. British Ordnance Survey Map Reference SY68701 07642.

Site of infection. Liver and skeletal muscle.

Ribosomal sequences. GenBank Accession Number AJ252952.

Etymology. In recognition of the outstanding contributions to the study of microsporidia of Professor C. P. Vivarès.

Type material. Hapanotypes deposited in the Natural History Museum, London. Semi-thin Toluidene Blue-stained sections cut from a developing xenoma of *M. vivaresi* n. sp. (block H98–70): Registration Number 2004 : 10 : 1 : 1. Semi-thin Toluidene-Blue sections cut from an encapsulated xenoma of *M. vivarasi* n. sp. (Block H98–216): registration Number 2004 : 10 : 1 : 2. Liver tissue of *T. bubalis* infected with xenomas of *M. vivaresi* n. sp. embedded in Agar 100 resin (block H98–70): Registration Number 2004 : 10 : 1 : 3.

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Fig. **22–29.** *Microgemma vivaresi* n. sp. in the liver of *Taurulus bubalis*. Scale bar in Fig. 22 applies to all figures. **22, 23.** Unidentified stages with greatly expanded endoplasmic reticulum and surface characteristics of meronts. These intermingle with typical meronts and sporonts (cf. Fig. 2). Scale bar = 1.2 μm (**22**), 1.4 μm (**23**). **24.** Part of the lamellar polaroplast of a spore showing the arrangement of membranes in pairs with dense material in between. Scale bar = 300 nm. **25.** Slightly oblique section of a mature spore showing overall structure: ad, anchoring disc; en, endospore; ex, exospore; gr, granular region within lamellar polaroplast; lp, lamellar polaroplast; pt, polar tube; and pv, posterior vacuole. Scale bar = 600 nm. **26.** Anterior end of mature spore showing exospore (ex), endospore (en), subapical anchoring disc (ad), with bands of different density and polaroplast membranes (p) attached to the anterior enlarged part of the polar tube with dense core (*). Scale bar = 360 nm. **27.** Oblique section of spore showing the lamellar polaroplast membranes (lp) appearing to unite posteriorly around the granular region. Scale bar = 920 nm. **28.** Transverse section of a spore showing the lamellar polaroplast (lp) enclosing the granular region, through which runs the straight section of the polar tube (pt). Scale bar = 525 nm. **29.** Transverse sections of the polar tube showing their associated ribosomes. Scale bar = 250 nm.