Parasitol Res (1996) 82:279–282

SHORT COMMUNICATION

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Presence of apicomplexan-type micropores in a parasitic dinoflagellate, *Hematodinium* sp.

Received: 20 July 1995 / Accepted: 19 September 1995

Abstract Structures resembling apicomplexan micropores were found by transmission electron microscopy in in vitro-cultured and in in vivo forms of the parasitic dinoflagellate *Hematodinium* sp. from crustacean hosts. Uptake of colloidal gold indicated a cytostomal function for the micropores.

One of the most surprising revelations of molecular systematics is that the apicomplexans (sporozoans), dinoflagellates and ciliates form a single clade (Gajadhar et al. 1991). Although remnants of a plastid in the apicomplexans have been cited as providing a possible connection with the photyosynthetic dinoflagellates (Gardner et al. 1993), not all dinoflagellates possess plastids or plastid remnants. The morphological resemblance between the three groups is slight and their nuclear organisation could not be more different. All that the three groups have in common is the alveolate cortex – hence the adoption of the name Alveolata (Cavalier-Smith 1993) for the clade.

Contemporary classifications recognise the early divergence of the wholly parasitic but poorly known syndinean dinoflagellates (phylum Syndinea, Corliss 1984; also recognised as a subdivision of the division Dinoflagellata by Fensome et al. 1993) from the better-known dinokaryotes (phylum Peridinea, Corliss 1984; subdivision Dinokaryota; Fensome et al. 1993). The latter have chromosomes with characteristic fibrillar banding and no histone protein, whereas the former have more conventional chromosomes with histone proteins. This communication reports the presence of a micropore – a structure characteristic of Apicomplexa – in different stages of the life cycle of a parasitic dinoflagellate belonging to the phylum Syndinea and to the genus *Hematodinium* Chatton & Poisson 1931.

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A species of *Hematodinium* associated with mortality of the Norway lobster, *Nephrops norvegicus*, in the seas around Scotland (Field et al. 1992) has been serially cultivated in vitro at 6–8°C for 3 years using a 10% fetal calf serum in balanced *Nephrops* saline medium (Vickerman et al. 1993). The principal multiplicative stage in culture is the trophont, an aflagellate multinucleate filament, 28–190 μm long (Fig. 1). These filaments show the typical alveolate surface structure and characteristic flexing and longitudinal contraction movements. They multiply by branching and fission, and lack the trichocysts found in the uninucleate flagellate dinospore stage from which they were initially derived.

Transmission electron microscopy of sections of the trophont filaments (processed as described by Field et al. 1992), reveals the presence of micropore-like structures (Fig. 2) scattered among the cortical alveoli. They are seen as caveolae (Fig. 3), ~ 110–200 nm in diameter, with an electron-dense reinforcing sleeve replacing the alveolar sacs in the underlying cortex along the wall of the pit (Fig. 4). Use of colloidal gold and ferritin markers has indicated ingestion of material from the surrounding medium via the micropore (Figs. 5, 6), i.e. that the micropore has a cytostome function in the trophont phase. The role of the micropore in the nutrition of apicomplexans has been reviewed by Senaud et al. (1976) and for *Plasmodium* spp. in particular by Olliaro and Goldberg (1995).

Similar micropores have been demonstrated in stages of development of *Hematodinium* in vivo, notably in circulating stages in generation of the trichocyst-bearing biflagellate dinospore (Fig. 7). Micropores may therefore be a feature of several stages in the life cycle of the syndinean, as they frequently are in coccidians, malaria parasites and other apicomplexans (Scholtyseck and Mehlhorn 1970; Ferguson et al. 1977). To date, micropores have not been observed in mature dinospores, however.

To our knowledge, micropores have not yet been recorded in the alveolate cortex of the dinokaryote dinoflagellates. In ciliates the parasomal sacs, which open close to the ciliary bases, have been shown to be engaged in pinocytosis (Nilsson and Van Deuers 1983). Interesting-

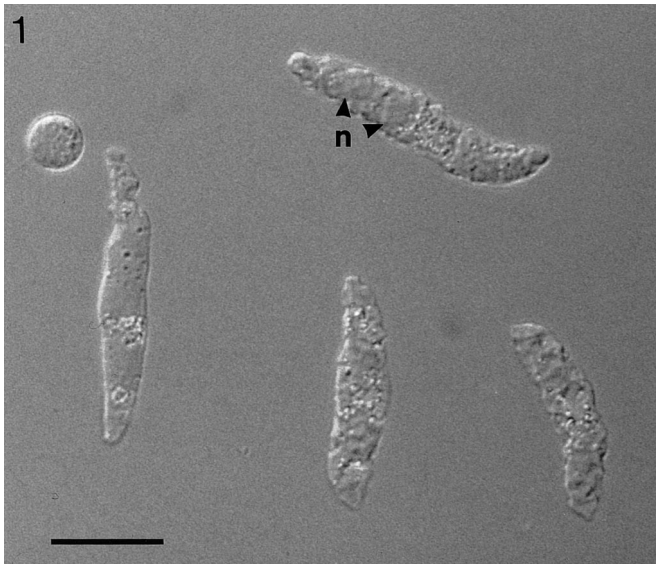

Fig. 1 Filamentous multinucleate trophonts of *Hematodinium* sp. in culture showing a wrinkled surface and nuclei (*n*). Differential interference contrast. Bar = 20 μm

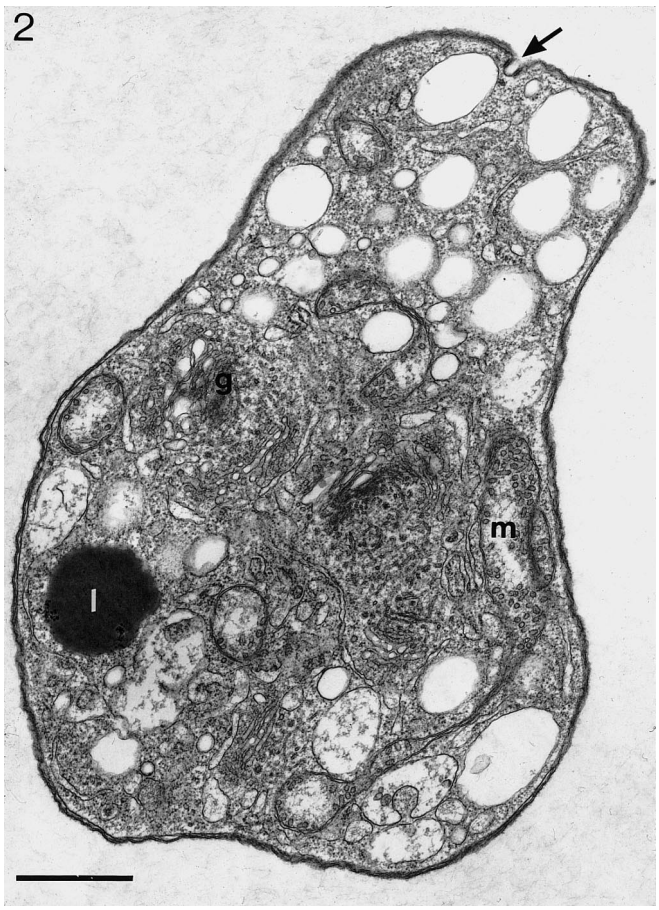

Fig. 2 Culture trophont in transverse section, showing the vacuolated cytoplasm, the absence of trichocysts and a single (*arrow*) micropore. (*g* Golgi apparatus, *l* lipid, *m* mito-chondrion) Bar = 1.5 μm

ly, in the non-ciliated Suctoria and in sedentary ciliates with reduced ciliation (chonotrichs, peritrichs), cortical pores virtually identical in structure to the micropores of apicomplexans and syndinean dinoflagellates will take up ferritin from the surrounding medium (Rudzinska 1977). Micropores therefore appear to be a widespread component of the cortex in the Alveolata. Despite the apicomplexan-like flexing movements, no other apicomplexan feature (e.g. conoid, polar ring, cortical microtubules, rhoptries) has been observed in the trophont of *Hematodinium.*

Acknowledgements This work was supported by the UK Ministry of Agriculture, Fisheries and Food and the Scottish Office Agriculture and Fisheries Department.

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Figs. 2–7 Transmission electron micrographs of thin sections of *Hematodinium* sp.

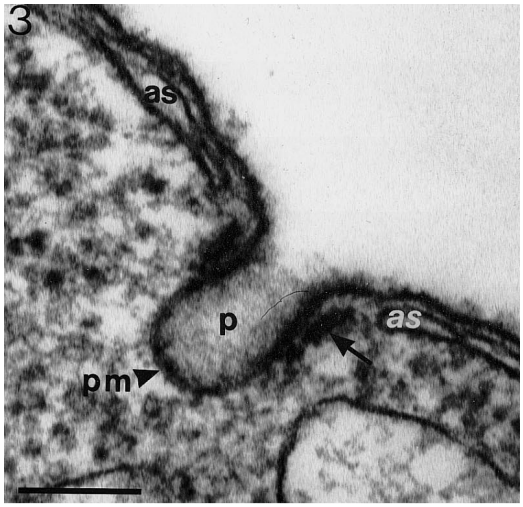
Fig. 3 Vertical section of a micropore. The alveolar sacs (*as*) of the cortex are replaced by an electron-dense sleeve (*arrow*) around the wall of the pit (*p*), which is lined by the plasma membrane (*pm*) only; radial fibrils connect the two structures. Bar = 150 nm

Fig. 4 Horizontal section of a micropore pit, showing an electron-dense sleeve (*arrow*) beneath the plasma membrane (*pm*). (*as* Alveolar sac) Bar = 150 nm

Fig. 5 Unstained section of a culture trophont embedded in LR White resin after incubation for 24 h in a colloidal gold suspension. Three gold particles (*arrows*) are lodged in a micropore pit. (*n* Nucleus) Bar = 1 μm

Fig. 6 Vacuolated cytoplasm of a trophont treated similarly to that shown in Fig. 5. Gold particles (*arrows*) are present in two vacuoles. (*n* Nucleus) Bar = 1 μm

Fig. 7 Section of parasites during microsporogenesis, from the ovary of a naturally infected *Nephrops*. The marked changes in appearance of the chromosomes (*ch*) in nuclei (*n*), characteristic of this stage in the life cycle, and the production of trichocysts (*t*) are evident. A micropore (*arrow*) is also present in a sporogenic cell. Bar = 2.5 μm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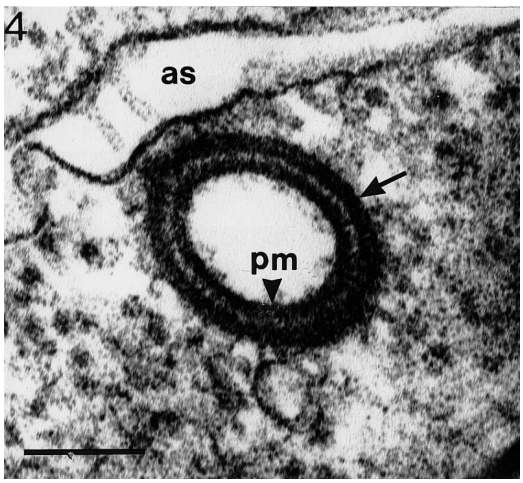
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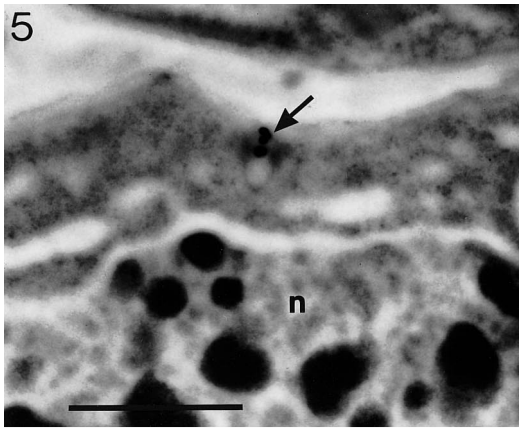
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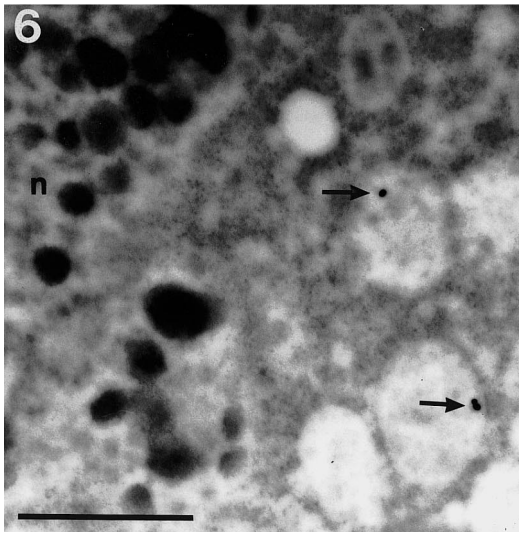
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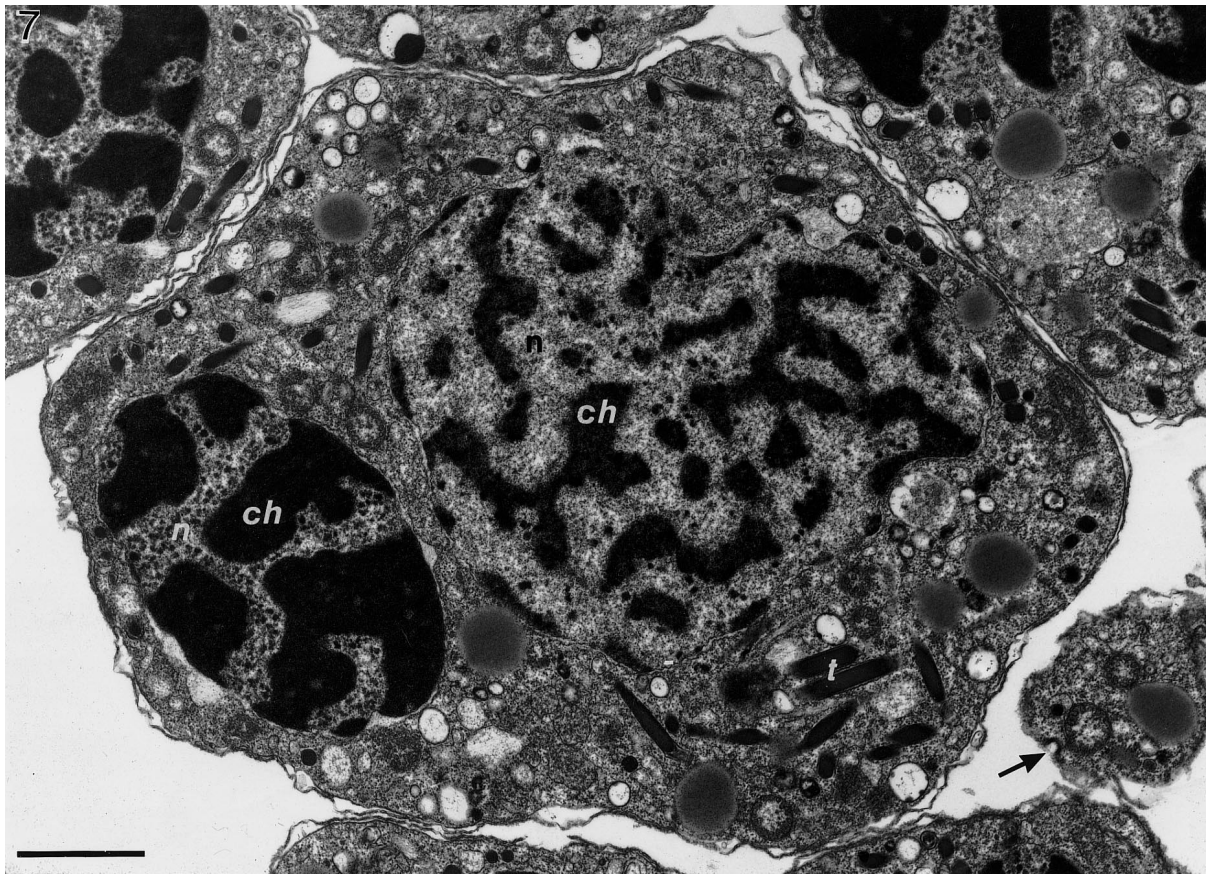
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