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NOTE

Tubular formations extending from parasitophorous vacuoles in gut epithelial cells of cichlid fish infected by *Eimeria (s.l.) vanasi*

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ABSTRACT: An ultrastructural study of the gut epithelium of cultured juveniles of *Oreochromis aurea* × *O. niloticus* hybrids infected with the coccidian *Eimeria (s.l.) vanasi* Landsberg & Paperna in press revealed the presence of numerous tubuli extending from funnels located at the border of the parasitophorous vacuole. These tubuli were most abundant in host cells infected with gamonts, while being fewer, vestigial or absent in cells infected by merogony stages. Such tubular structures have not previously been reported in host cells infected with coccidians.

Cichlid fish in Israel and South Africa were found to be infected with *Eimeria (s.l.) vanasi* Landsberg & Paperna in press. Endogenuous development of this coccidian occurs in both the interior and at the microvillar zone of the mucosal epithelial cells of the intestine (Landsberg & Paperna in press). Stages developing in the microvillar zone closely resemble species of the genus *Epieimeria* (Dyková & Lom 1981, Molnár & Baska 1986). In the present communication we report on the finding of tubular formations in the infected gut epithelial cells not reported so far in any other coccidian infections.

Materials and Methods. Study material from hybrid fry and fingerlings (10 to 25 mm in length) of *Oreochromis aureus* (Steindachner) × *O. niloticus* (L.) was obtained from hatcheries in the Jordan valley, Israel. For transmission electron microscopy (TEM), intestinal samples from the infected fish were fixed in Karnovski for 24 h in 4 °C, rinsed repeatedly in cacodylate buffer (0.1 M, pH 7.4) and postfixed in 1 % osmium tetroxide, in the same buffer, for 1 h. After rinsing in the buffer, the material was dehydrated in ethanol and embedded in Epon. Thin sections cut on a LKB III ultratome with a diamond knife were stained on the grid with uranyl acetate and lead citrate and examined with a Joel 100CX TEM.

Results and Discussion. The cytoplasm of infected cells contained numerous robust straight tubules (60 to 80 nm in diameter) which extended from 160 to 180 nm wide funnels, located at regular intervals along the border of the parasitophorous vacuole (PV) (Fig. 1 to 4). Both the tubule and the wall of the funnel developed from invaginations of the bilaminated wall of the PV. The funnel lumen and the core of the tubule were filled with an electron-dense substance. The core was separated from the tubule and the funnel wall by an electron-lucent interphase. The electron-dense material of the core extended slightly into the lumen of the PV (Fig. 5). This may be suggestive of an excreted liquid substance which coagulated after fixation. In some cells, tubuli were accompanied by numerous mitochondria (Fig. 6).

Tubuli were abundant in epithelial cells infected by early gamonts (Fig. 1), macrogamonts, early stage zygotes (Fig. 2) and microgamonts (Fig. 7). The early stage zygotes were enclosed by an additional pellicular membrane (Fig. 4). Tubuli were sparse or absent from host cells infected by merogony stages (Fig. 8 to 11). In such host cells the PV unit membrane contained vestiges or rudiments of funnels, i.e. a shallow or deep invagination filled by an electron-dense substance (Fig. 8 & 9). Reduction or elimination of the tubular system in some infected cells appeared to coincide with the multiplication of the PV unit membranes (Fig. 9 & 11). Multiplication of membranes was interrupted where a residual funnel occurred along the PV boundary; these funnels remained connected to a single unit membrane (Fig. 9). Funnel vestiges, i.e. invaginations filled with an electron-dense substance, also occurred on the parasitophorous membrane at the host-parasite interphase of meronts established at the microvillar

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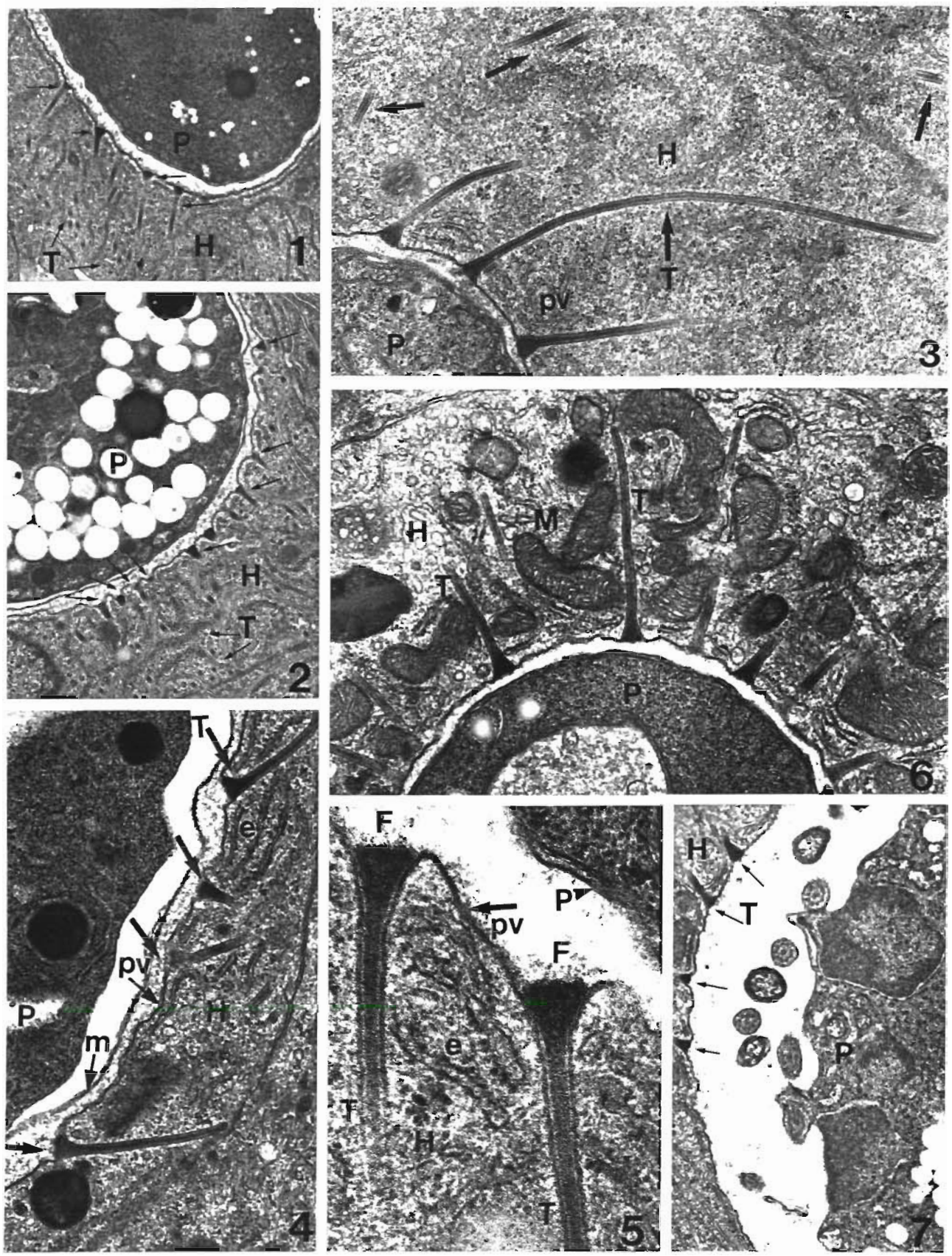
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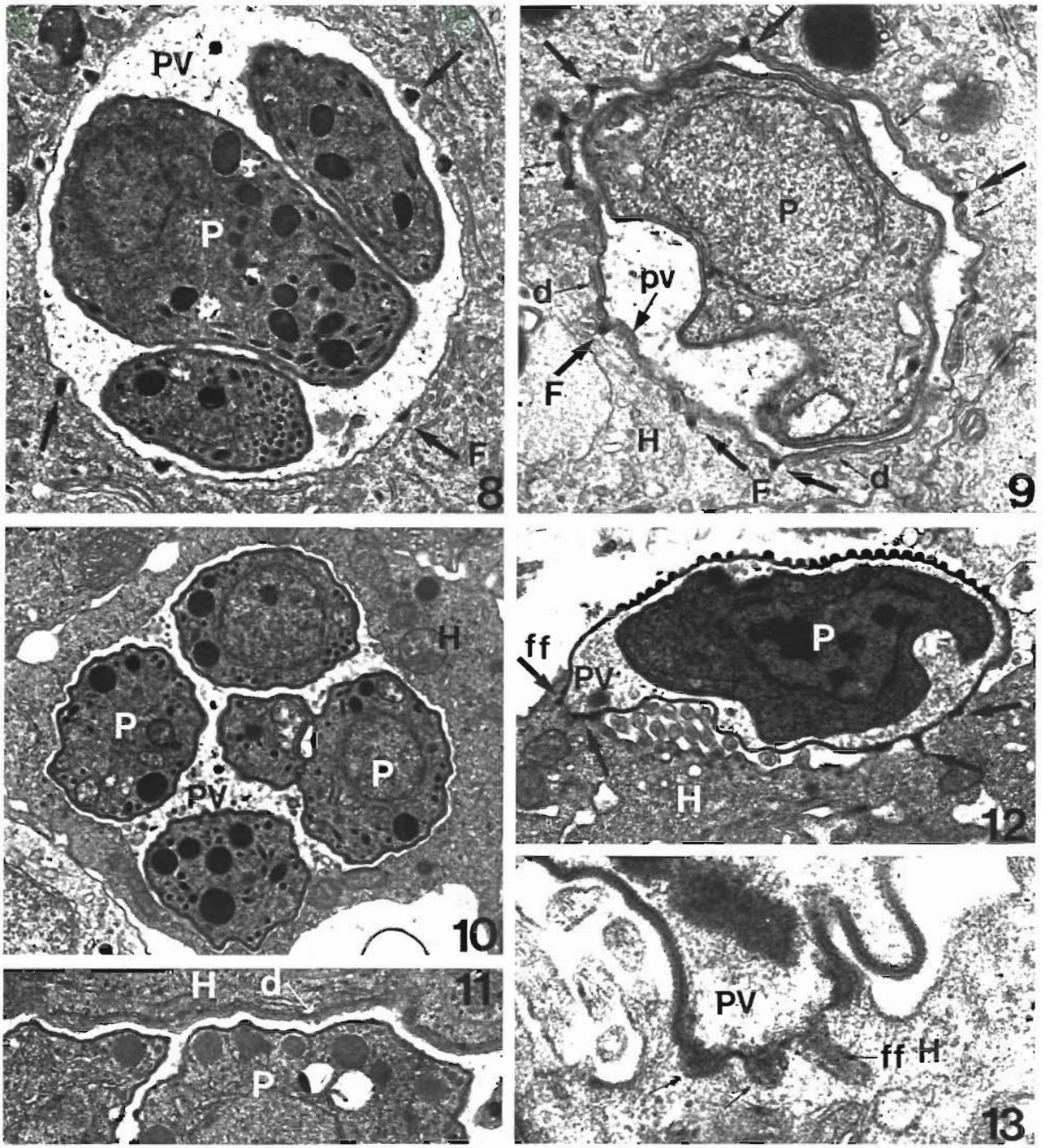

Fig. 1 to 13. *Eimeria (s.l.) vanasi* infecting *Oreochromis aurea* × *O. nilotictus*. Fig 1 Tubular formations in epithelial cell infected by juvenile gamont (× 11 260). Fig. 2. Tubular formations in epithelial cell infected with young zygote (× 10 000) Fig 3 Section showing the length of the tubules (× 22 800) Fig. 4. Higher magnification view of tubuli and funnels draining into PV containing a young zygote (× 25 600). Fig. 5. Highly magnified view of funnels and the proximal part of the tubule (× 80 000). Fig. 6 Tubular formation accompanied by numerous mitochondria in a cell infected by a young gamont (× 26 000). Fig. 7. Tubuli fringing a PV containing a microgamont (× 10 560). Fig 8 Reduced occurrence of funnels on the borders of a PV containing merozoite formation (× 13 700). Fig. 9. Reduction in funnels together with duplication of the PV membrane in cells infected with young meronts (× 20 700). Fig. 10. Absence of funnels and tubuli in cell infected by merozoite formation (× 12 900). Fig. 11. Absence of funnels from PV containing young meronts and bound by multiple membrane wall (× 20 700) Fig 12 Meront located at the microvillar zone of the host cell, vestigial funnels occurring at the interphase between the PV and cytoplasm of the host cell (arrows) (× 16 000). Fig. 13. Highly magnified view of the vestigial funnel associated with PV's of microvillar zone infections (× 55 700)

Abbreviations to figures. d: multimembrane wall of the parasitophorous vacuole, e: endoplasmic reticulum, F. funnel, ff: vestigial funnel; H: host cell; M: mitochondria, m pellicular membrane; p parasite, PV: parasitophorous vacuole, pv: wall of the parasitophorous vacuole; T tubuli

zone of mucosal host cells (Fig. 12 & 13). These invaginations are structurally similar to the parasitophorous protrusions described in *Epieimeria isabellae* (Daoudi et al. 1985) and in *E. anquillae* (Molnár & Baska 1986). This implies the possible existence of a tubular system in *Epieimeria* species. It also suggests a close relationship of this eimerine coccidium to *Epieimeria*.

To the best of our knowledge a tubular system such as that presently described has not been reported in any other intracellular host-parasitic protozoan relationship. The function of this tubular system remains at present unknown. Intravacuolar tubules like those reported in infections by macrogamonts of *Eimeria* and *Isospora* (Michael 1975, Milde 1979) are very distinct from the tubular system reported here. The former tubular system, which was suggested to transfer nutrients from the host cytoplasm into the parasite, appeared to connect the parasite cytoplasm with the host cell.

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