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Spermatheca morphology of the social wasp *Polistes erythrocephalus*

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Abstract

The morphology of the *Polistes erythrocephalus* (Latreille) (Hymenoptera Vespidae Polistinae) spermatheca was studied through scanning and transmission electron microscopy. The spermatheca of *P. erythrocephalus* was located closely above the vagina. It consists of a spherical reservoir, a paired elongated gland and a duct connecting the reservoir to the vagina. The duct and reservoir consist of a single epithelial layer. This layer is formed by columnar cells rich in mitochondria. In addition, we observed several basal cell membrane infoldings associated with mitochondria in the reservoir epithelium. These characteristics stressed the possible role of the component cells in exchange processes between hemolymp and spermatheca lumen. The duct and the reservoir epithelia are surrounded by a further epithelial tissue: the spermatheca sheath. This is a layer of spindle-like cells that may contribute to spermatozoa isolation and maintenance. The present work provided the first description of the spermatheca morphology in the reproductive females of *P. erythrocephalus* that can be used as a basis for future specific studies about reproduction, caste or behaviour characteristics of Polistinae.

Key words: insect, reproductive tract, paper wasp.

Introduction

The spermatheca is a complex structure found in the insect female reproductive system, where spermatozoa are stored. In several hymenopterans (e.g. bees, ants and wasps) it consists of four parts: (a) a reservoir, a sac-like structure containing spermatozoa; (b) a duct connecting the reservoir to the vagina; (c) a paired gland involved in the spermatozoa nutrition and (d) a muscle pump involved in the releasing of spermatozoa prior to egg fertilization (Wheeler and Krutzch, 1994; Pabalan *et al*., 1996; Schoeters and Billen, 2000; Martins and Serrão, 2002; Cruz-Landim and Serrão, 2002). In addition to these structures, Martins *et al*. (2005) found a sheath of multiple cells surrounding the spermatheca duct and the reservoir of the paper wasps genera *Polistes*, *Michocyttarus* and *Apoica* (Polistinae). It is supposed to improve the isolation of the spermatozoa from the body cavity, providing a suitable chamber for gamete storage.

The spermatheca reservoir and the spermatheca duct are formed by a single layer of columnar cells that may play a role in the active transport to regulate the spermatheca milieu in the honey bees (Dallai, 1975) and in ants (Wheeler and Krutzsch, 1994; Gobin *et al*., 2006). Since, the current knowledge of spermatheca in Vespidae is scarce the aim of this work was to describe its morphology in the Polistinae specie *Polistes erythrocephalus* (Latreille). Our analyses revealed a new panorama of the morphology of the spermatheca through the use of light, scanning and transmission electron microscopy. We also discussed in detail the implications of the ultrastructural traits of this apparatus in the storage of male gamete in the female of the social wasp *P. erythrocephalus*.

Materials and methods

P. erythrocephalus were collected in the city of Viçosa, state of Minas Gerais, Brazil, and transferred to the laboratory of cell biology of the Federal University of Viçosa.

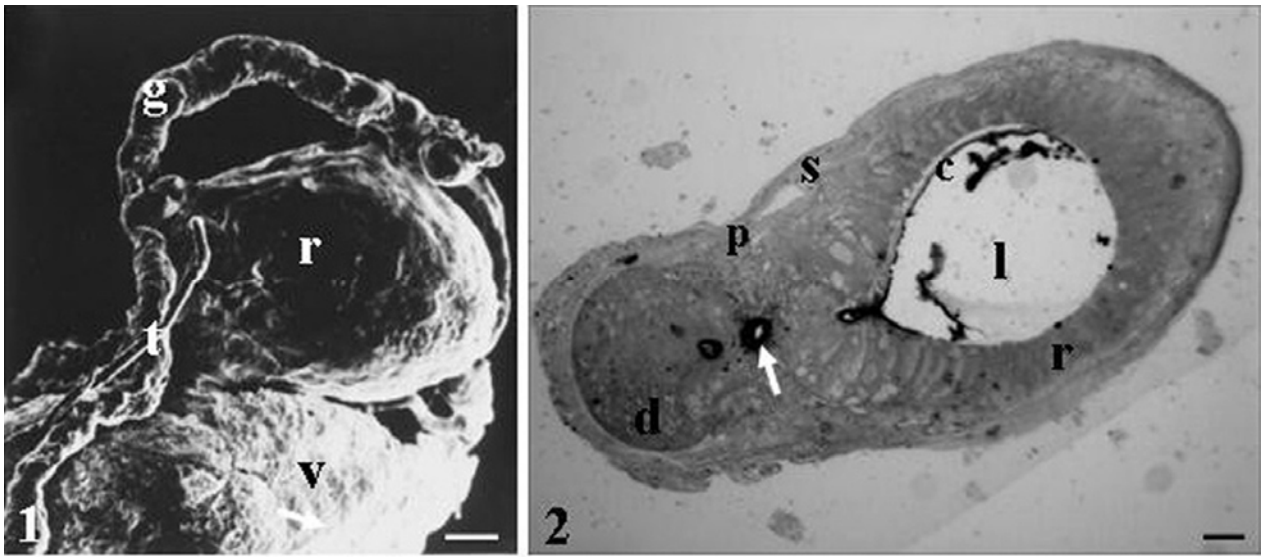
Mated females of *P. erythrocephalus* were dissected in sodium phosphate buffer (PBS) 0.1 M, pH 7.2 and their spermathecae transferred to 4% paraformaldehyde in the same buffer. After fixation, samples were dehydrated in a graded ethanol series, transferred to hexamethyldisilazane, air dried and gold coated in a sputtering device for observation under a scanning electron microscope LEO VP1430. Fertilized females (queens) were separated based on the presence of enlarged ovaries (Martins *et al*., 2005).

Mated females of *P. erythrocephalus* were dissected in PBS and their spermathecea transferred to 2.5% glutaraldehyde in sodium cacodylate buffer 0.1M, pH 7.2. After post-fixation in 2% osmium tetroxide and dehydration in a graded acetone series, tissues were embedded in Epon. Semithin sections were stained with 1% toluidine blue-borax and analyzed with light microscope. Ultra-thin sections were double-stained with 1% uranyl acetate and 1% lead citrate and observed with a transmission electron microscope Zeiss EM 109.

Results

The spermatheca of *P. erythrocephalus* was located closely above the vagina. It consisted of a spherical reservoir, a paired elongated gland and a duct connecting those structures to the vagina (figure 1).

The reservoir and duct of *P. erythrocephalus* spermatheca consisted of a single epithelium with columnar



Figures 1-2. 1) SEM micrograph showing the general view of the *P. erythrocephalus* spermatheca with the paired glands (g) and the oval reservoir (r) above the vagina (v). t- trachea. Bar = 20 µm. **2)** Semi-thin section of *P. erythrocephalus* spermatheca showing the spermatheca reservoir (r) and duct (d), surrounded by the spermatheca sheath (s). Even though we used spermatheca of inseminated females, they had emptied lumen (l) because during microtomy spermatozoa were lost. Arrow- duct lumen; c- cuticle; p- pump. Bar = 20 µm.

cells surrounded by a thick cuticle. However, the duct cuticle was thicker in comparison to the reservoir cuticle. Externally, the reservoir and duct were covered with the spermatheca sheath. Additionally, external muscle fibres were located within the duct and in the reservoir connection, forming the spermatheca pump (figures 2-4).

The spermatheca duct epithelium of *P. erythrocephalus* consisted of columnar cells with nucleus in the median cytoplasm region. Near the reservoir, the duct epithelium cells were closely associated with muscles of the spermatheca pump or with the sheath of cells (figures 3-4). These cells had a round nucleus with cyto-

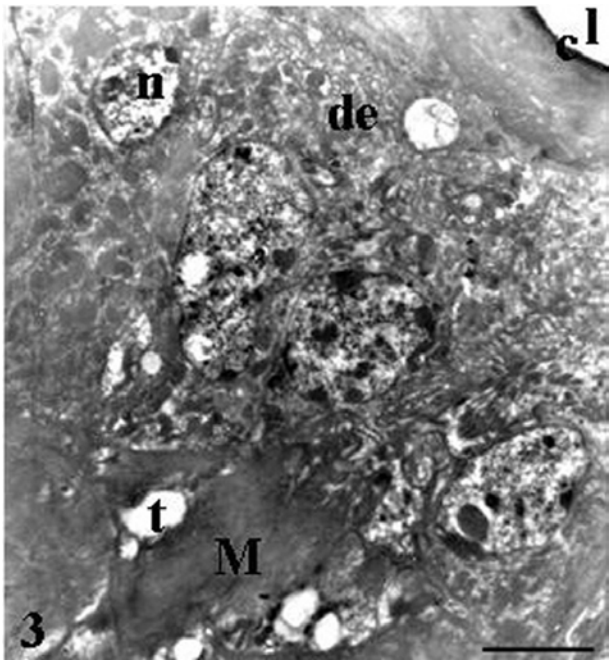


Figure 3. TEM micrograph of the duct of *P. erythrocephalus* spermatheca showing the spermatheca pump muscles (M) below the duct epithelium (de) with basal nucleus (n). c- cuticle. l- duct lumen; t- trachea. Bar = 1 µm.

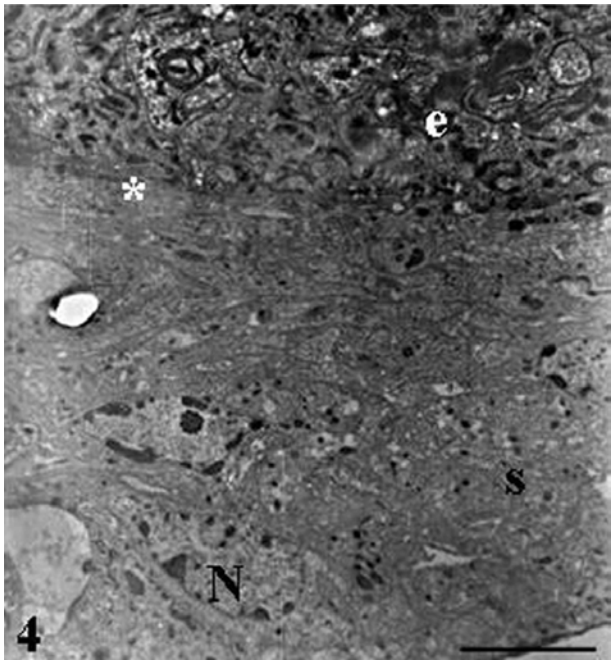


Figure 4. TEM micrograph of the duct of *P. erythrocephalus* spermatheca showing the transition region (*) between the basal region of duct epithelium (e) and the dense and height spermatheca sheath (s) with fusiform cells. N- nucleus. Bar = 5 µm.

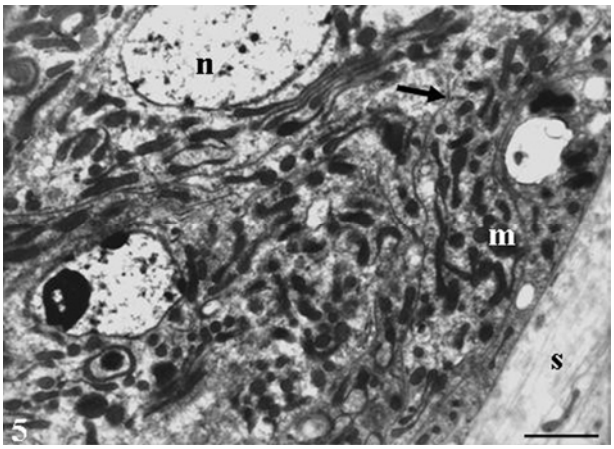


Figure 5. TEM micrograph of the duct epithelium of *P. erythrocephalus* spermatheca showing many mitochondria (m) in the cell cytoplasm. Arrow- cell limit; n- cell nucleus; s- spermatheca sheath. Bar = 1.5 µm.

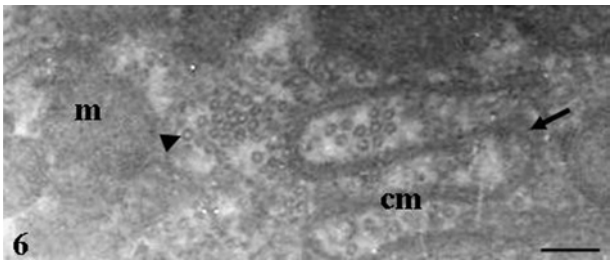


Figure 6. TEM micrograph of *P. erythrocephalus* spermatheca showing the contact region between duct cells with specializations of cell membrane (cm), corresponding to interdigitations (arrow) with microtubules (arrowhead) associated to the cell membrane. mitochondria (m). Bar = 0.1 µm.

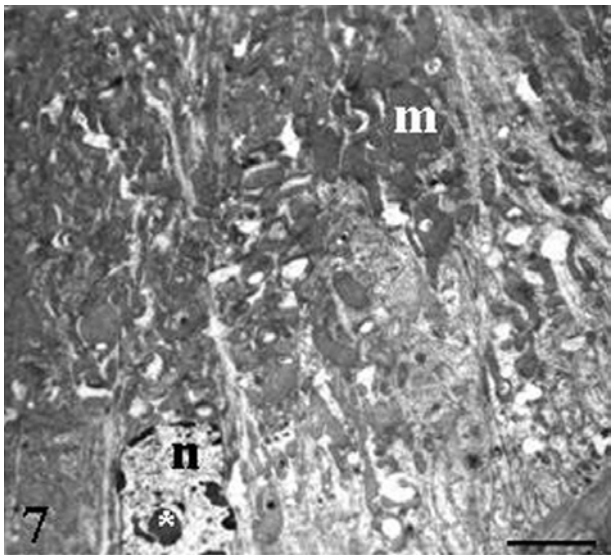


Figure 7. TEM micrograph of the reservoir epithelium of *P. erythrocephalus* spermatheca, showing columnar cells with basal nucleus (n) and cytoplasm rich in mitochondria (m). Note the obvious nucleolus (*). Bar = 1 µm.

plasm rich in mitochondria. Their mitochondria were elongated with enlarged ends and these were clustered and arranged in parallel (figure 5). In addition, the cell to cell contact included interdigitations stiffened by the microtubules (figure 6).

The reservoir epithelium had columnar-tall cells with several mitochondria in their apex (figures 7-8). These cells had a round nucleus located in the middle-basal epithelium cell region in addition to, as well as an obvious nucleolus (figure 7). Moreover, their basal plasma membrane exhibited conspicuous, deep infoldings with associated mitochondria (figure 9).

The spermatheca sheath appeared to be heterogeneous in composition. It consisted of a multilayer of cells, including the muscle fibres and tracheoles attached to duct

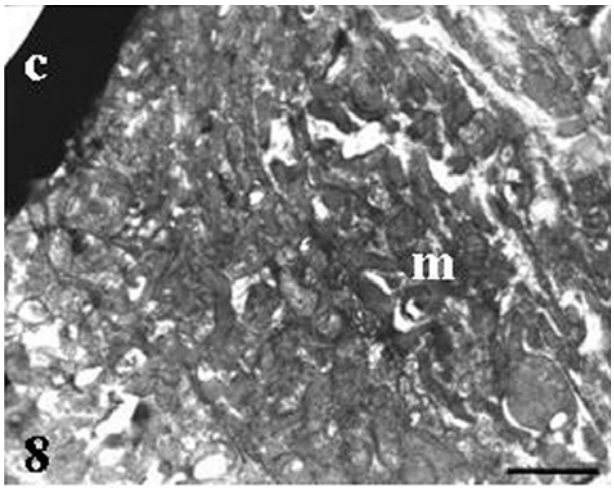


Figure 8. TEM micrograph of the reservoir epithelium of *P. erythrocephalus* spermatheca showing the cell apex filled by mitochondria (m). c- cuticle. Bar = 1.5 µm.

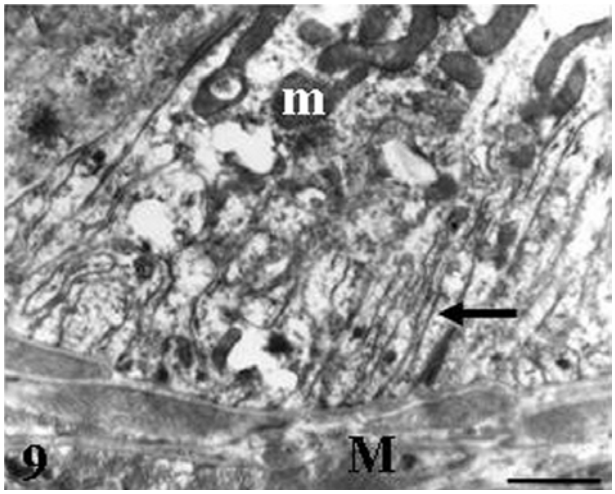


Figure 9. TEM micrograph of the reservoir epithelium of *P. erythrocephalus* spermatheca showing a detailed view of the basal cell region with plasma membrane infoldings (arrow) associated with mitochondria (m). Note that in this region, the cell basal cytoplasm is in close contact with muscle fibres of spermatheca pump (M). Bar = 1 µm.

and reservoir epithelia. These cells were fusiform, forming a dense cellular layer with some enlarged intercellular space and tracheoles among them (figures 10 and 11). These cells had well developed interdigitations and their cytoplasm had few mitochondria (figure 11).

Discussion

The general microanatomy of the *P. erythrocephalus* spermatheca was similar to that described before for other hymenopterans. It consisted of a globular reservoir, a duct, a paired gland, and a muscular pump (Wheeler and Krutzsch, 1994; Pabalan *et al*., 1996; Schoeters and Billen, 2000; Martins and Serrão, 2002; Gobin *et al*., 2006). On the other hand, it had the spermatheca sheath that was found exclusively in the genera *Polistes*, *Michocyttarus* and *Apoica* (Martins *et al*., 2005).

The spermatheca duct and the reservoir were formed by a single epithelium of tightly joined columnar cells, i.e. with little intercellular space between them. These cells had the cytoplasm with several mitochondria. This large quantity of mitochondria was described before in the duct and reservoir epithelia of honeybees and ants. In this case, the large amount of mitochondria has been associated with the active ions transport from the haemoplymph to the spermatheca lumen by means of energy supply. Maybe this role is essential in providing an osmotically suitable environment inside the spermatheca for the spermatozoa storage (Dallai, 1975; Wheeler and Krutzsch, 1994; Schoeters and Billen, 2000).

Besides the large amount of mitochondria, the reservoir epithelium cells had a lot of cell membrane infoldings in their basal region. These membrane infoldings are supposed to increase the cell surface, facilitating the ions uptake from haemoplymph (Gobin *et al*., 2006). The presence of several mitochondria and cell infoldings suggest that the duct and the reservoir epithelium are involved in the transport of substances to the spermathecae lumen. Based on these observations, an osmoregulatory role should be considered for the epithelial cells in *P. erythrocephalus* spermatheca, as described for other social hymenopterans (Tombe and Roppel, 1972; Dallai, 1975; Wheeler and Krutzsch, 1994; Schoeters and Billen, 2000).

It is assumed that the spermatheca sheath contributes to spermatozoa protection, forming a barrier that ensures a suitable environment for spermathozoa storage in Polistinae females (Martins *et al*., 2005). We showed that the spermatheca sheath is formed by juxtaposed cells with interdigitations. These specializations may enhance the cell to cell adhesion.

The ovary mesodermic external sheath protects insects ovaries, but this sheath has many intercellular spaces that allow the transit of substances between ovarioles and haemoplymph (Bünning, 1994). Probably the spermatheca sheath plays a similar role in the ovary external sheath, protecting and allowing the uptake of substances by the spermatheca epithelium from haemoplymph.

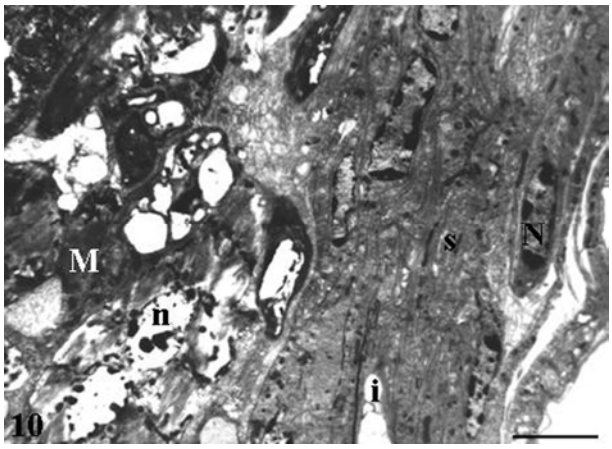


Figure 10. TEM micrograph of the spermatheca sheath (s) of *P. erythrocephalus* spermatheca showing a multilayer of fusiform cells and, beneath, the muscular pump (M) in the region between reservoir and duct. i- intercellular space; n- muscle fibre nucleus; N- fusiform cell nucleus. Bar = 4 μm.

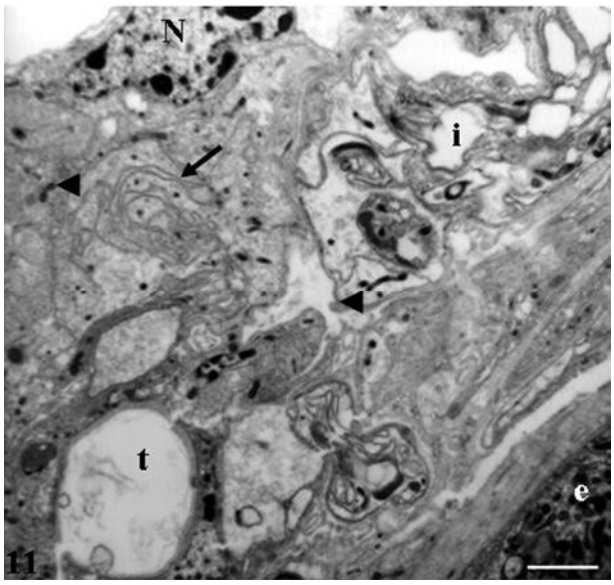


Figure 11. TEM micrograph of the spermatheca sheath of *P. erythrocephalus* spermatheca showing the fusiform cells with plasma membrane infoldings (arrow) and tracheole (t). Arrowhead- mitochondria; e- duct epithelium; i- intercellular space; N- fusiform cell nucleus. Bar = 2 μm.

The present work provided the first detailed description of the spermatheca morphology in the reproductive females of *P. erythrocephalus*. These data can be used as a basis for future studies considering reproduction, caste division or behaviour in Polistinae. It can help understanding how the spermatheca morphology varies between the different levels of the caste hierarchy, and also, if the reproductive differences between queens and workers are associated with their morphology.

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References

BÜNNING J., 1994.- *The insect ovary: Ultrastructure and function*.- Cambridge University Press, Cambridge, UK.

CRUZ-LANDIM C., SERRÃO J. E., 2002.- Ultrastructure of the spermathecal gland of *Melipona bicolor* Lep. (Hymenoptera, Apinae, Meliponini).- *Brazilian Journal of Morphological Science*, 19: 9-16.

DALLAI R., 1975.- Fine structure of the spermatheca of *Apis mellifera*.- *Journal of Insect Physiology*, 21: 89-109.

GOBIN B., ITO F., PEETERS C., BILLEN J., 2006.- Queen-worker differences in spermatheca reservoir of phylogenetically basal ants.- *Cell Tissue Research*, 326: 69-178.

MARTINS G. F., SERRÃO J. E., 2002.- A comparative study of the spermatheca in bees (Hymenoptera; Apoidea).- *Sociobiology*, 40: 711-720.

MARTINS G. F., SERRÃO J. E., FURIERI K. S., 2005.- Notes on the spermatheca of Vespidae and Sphecidae (Hymenoptera).- *Sociobiology*, 45: 119-127.

PABALAN N., DAVEY K. G., PACKER L., 1996.- Comparative morphology of spermathecae in solitary and primitive eusocial bees (Hymenoptera; Apoidea).- *Canadian Journal of Zoology*, 74: 802-808.

SCHOETERS E., BILLEN J., 2000.- The importance of the spermathecal duct in bumble bees.- *Journal of Insect Physiology*, 46: 1303-1312.

TOMBE A. S., ROPPEL R. M., 1972.- Ultrastructure of the spermatheca of the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae).- *International Journal of Insect Morphology and Embryology*, 1: 141-152.

WHEELER D., KRUTZSCH P., 1994.- Ultrastructure of the spermatheca and its associated gland in the ant *Crematogaster opuntiae* (Hymenoptera: Formicidae).- *Zoomorphology*, 114: 203-212.

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