

## The structure and the chemical composition of the spherites in the cave cricket *Troglophilus neglectus* (Rhaphidophoridae, Saltatoria)

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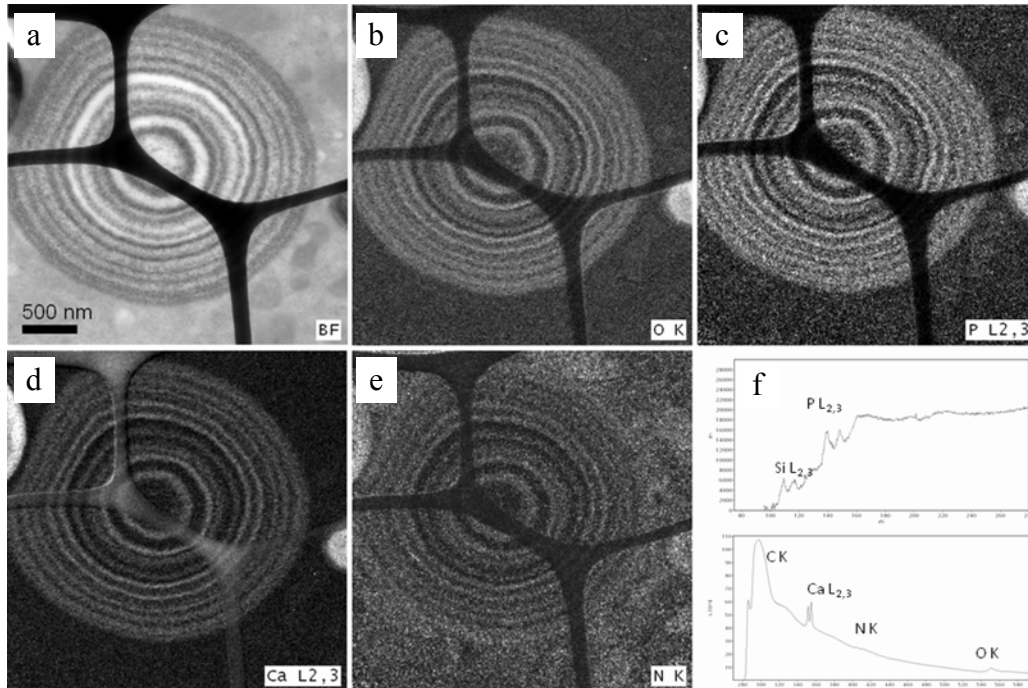
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Cave crickets of the taxon Rhaphidophoridae inhabit mostly tropical and subtropical regions, as well as temperate and boreal ones [1]. More than half of the over 300 described species are cavernicolous, and four dozens of them live in Europe [1]. In *T. neglectus*, different aspects of biology, ecology and physiology, and selected biochemical topics have been studied [2, 3, 4]. The life cycle of *T. neglectus* spans two years. Larvae hatch in May, older larvae spend an inactive 4–6 months overwintering in caves, usually from November until March, and then again an active epigeal ecophase from April till October. They mature in July and die in October. In this contribution, we investigate the cave crickets *T. neglectus* as an appropriate experimental species from which to obtain preliminary information on the structure and chemical composition of spherites in the excretory system of raphidopods. The main goal was to get an insight into possible physiological roles of spherites in the Malpighian cells during the crickets' life cycle through the analytical electron microscopy in natural conditions, irrespective of the crickets' diet. In particular, we focused on their role during the winter quiescence, representing a natural starvation period, which is being studied for the first time in insects.

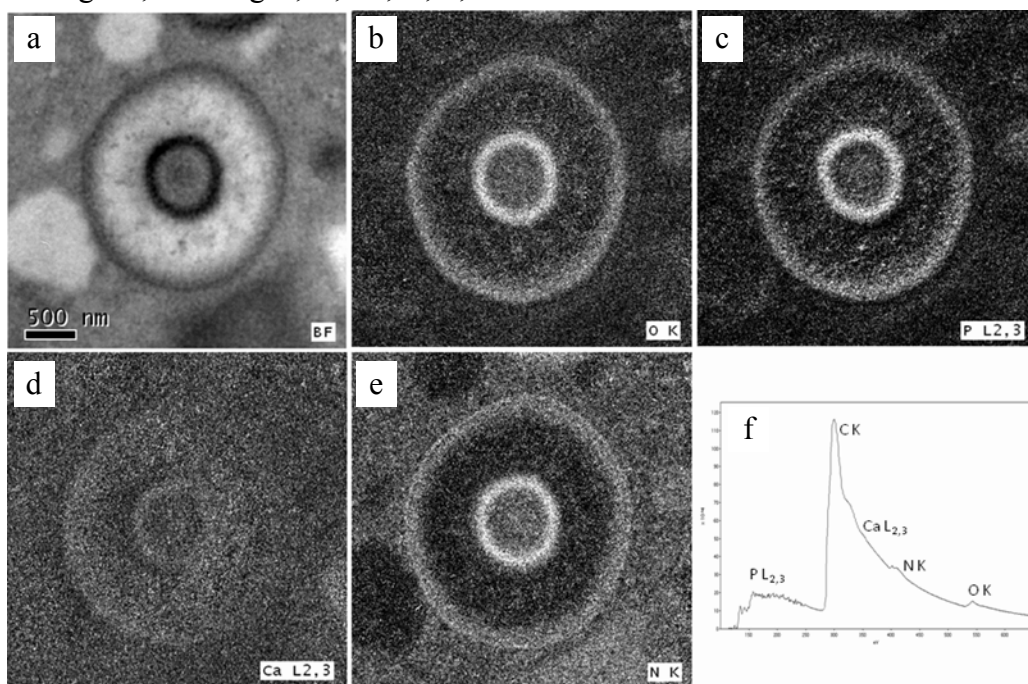
Special interest was directed to the dormant overwintering period when we hypothesized that the primary role of spherites is to supply minerals for basic vital processes. The investigation was carried out by light and transmission electron microscopy (TEM), energy dispersive x-ray spectroscopy (EDXS), electron energy-loss spectroscopy (EELS) and energy filtering TEM.

At the beginning of dormancy in November juveniles, minerals are accumulated in spherites and then decline till March. In one-year-old May larvae, spherites are commonly rich in minerals, and from July on they are progressively exploited in the adults. Spherite destruction starts with apoptosis in senile October individuals. The findings suggest that the mineral supply of spherites is crucial to supporting vital processes throughout the life cycle of *T. neglectus*.

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**Figure 1.** Ultra-thin section of the Malpighian tubule in *Troglophilus neglectus* (November). (a) Bright field TEM image of a spherite, (b) O jump ratio image, (c) P jump ratio image, (d) Ca jump ratio image, (e) N jump ratio image, (f) overview EELS-spectrum of November spherite in Fig. 1a, showing C, O, Ca, N, P, Si.



**Figure 2.** Ultra-thin section of the Malpighian tubule in *Troglophilus neglectus* (October). (a) Bright field TEM image of a spherite, (b) O jump ratio image, (c) P jump ratio image, (d) Ca jump ratio image, (e) N jump ratio image, (f) overview EELS spectrum of October spherite in Fig. 2a showing C, O, N, Ca, P.