

Morphological diagnosis of the eggs of stored-products mites

Zuzana Kucerova · Vaclav Stejskal

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Abstract Eggs of 13 species of common, economically important stored-product pest mites (*Acarus siro*, *A. gracilis*, *Tyrophagus putrescentiae*, *T. brevicrinatus*, *Tyroborus lini*, *Aleroglyphus ovatus*, *Caloglyphus redikorzevi*, *C. oudemansi*, *Lepidoglyphus destructor*, *Glycyphagus domesticus*, *Aeroglyphus robustus*, *Chortoglyphus arcuatus* and *Carpoglyphus lactis*) from four families (Acaridae, Glycyphagidae, Chortoglyphidae, Carpodagliidae) were studied in order to build a diagnostic key. The morphological study dealt with shape, size and surface sculpturing of eggs. Morphological details were visualised using scanning electron microscopy. A key for distinguishing eggs at genus and species level was developed for the major stored-product mites.

Keywords Acari · Egg · Chorion · Scanning electron micrograph · Identification key

Introduction

Some astigmatic mite species are economically important stored-product pests, which frequently occur in stored grain, seed, cereal-based and other food and feed stored commodities mainly in areas with humid climates (Thind and Clarke 2001; Kucerova et al. 2003; Stejskal et al. 2003; Kucerova and Horak 2004; Athanassiou et al. 2005; Sánchez-Ramos and Castañera 2005; Hubert et al. 2006; Palyvos et al. 2008). These pests negatively influence the quality of stored commodities, cause allergenic reactions (Kondreddi et al. 2006) and disseminate toxigenic moulds (Hubert et al. 2004). The serious injuriousness of

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Z. Kucerova (✉) · V. Stejskal
Crop Research Institute, Drnovská 507, Prague 6, 161 06, Czech Republic
e-mail: kucerova@vurv.cz

storage mites is connected with their abundance in stored commodities that is much higher than of insect pests (Stejskal et al. 2003), because of their higher fecundity and speed of development. Fecundity depends on species, diet and environmental conditions. Lifetime egg production per female in, e.g., *Acarus siro* L. (Acaridae) is on average 435 eggs with a maximum of 858 at 15°C and 90% RH (Cunington 1985). *Tyrophagus putrescentiae* (Schrank) (Acaridae) produces about 450–500 eggs/female and completed the generation in 2–3 weeks at 20–25°C and near-saturation humidity (Rivard 1961; Eraky 1995).

In contrast to many storage insects, mite populations are generally difficult to suppress by synthetic pesticides and biocides (Zdarkova and Horak 1974; White and Sinha 1990; Thind and Muggleton 1998; Collins 2006; Nayak 2006; Hubert et al. 2007). Because there are profound differences in susceptibility to acaricides among mite species and developmental stages (Collins 2006; Hubert et al. 2007) the proper mite identification is a serious prerequisite for storage Integrated Pest Management (IPM). The most tolerant developmental stage is the egg, that is also difficult to identify. Both mite eggs and adults are hard to be controlled not only by conventional acaricides, but also with new reduced-risk acaricides such as spinosad or natural monoterpenes (Sanchez-Ramos and Castanera 2001, 2003) and others. This is valid also for modified atmospheres (Conyers and Bell 2003) and fumigants such as methyl bromide, phosphine and sulfuryl fluoride (Jalil et al. 1970; Bowley and Bell 1981; Bell et al. 2004).

Mite eggs are of the centrolecithal type, i.e., the yolk is concentrated in the centre and cytoplasm is peripheral. Cytoplasm is bounded in the viteline envelope which underlies the one- or two-layered shell (endo- and exochorion). There are some differences between species in formation, number and terminology of egg layers according to various authors (Evans 1992; Alberti and Coons 1999). Generally the eggshell is produced by the egg itself and not by the ovarian follicles, as is the case with the chorion of insect eggs (Chapman 1982; Evans 1992). The entire surface of the egg of many mite species is additionally covered by a layer of lipid-rich secretion of accessory glands prior to oviposition (Evans 1992). Although there is some variation in the shape of the eggs of mite species, the majority is ovoid, globular or elliptical. Eggs of storage mites are small (<165 µm long) in comparison with storage insect eggs (>290 µm long).

Morphological studies of various stored-product pests have focused mainly on adult stages (Hughes 1976), whereas developmental stages have received less attention. The identification of stored-product mite eggs is also understudied. Witalinski (1993) described egg shells in several free living and parasitic acaridid mites including some pest mites focusing on their formation and function. Callaini and Mazzini (1984) investigated the egg shell of *T. putrescentiae*.

The aim of this work was to describe the egg morphology of the most economically important storage mite species commonly occurring in Europe and to provide a key for their identification. This paper extends our previous preliminary results (Kucerova and Stejskal 2003), and is part of a broad investigation focused on the diagnosis of stored-product arthropod eggs using scanning electron microscope (SEM)-based techniques (Kucerova 2002; Kucerova and Jokes 2002; Kucerova and Stejskal 2002, 2008).

Materials and methods

Laboratory cultures of the stored-product mite species *A. siro*, *Acarus gracilis* Hughes, *T. putrescentiae*, *Tyrophagus brevicrinatus* Robertson, *Tyroborus lini* Oudemans, *Aleuroglyphus ovatus* (Troupeau), *Caloglyphus redikorzevi* (Zachvatkin), *C. oudemansi*

(Zachvatkin) (Acaridae), *Lepidoglyphus destructor* (Schränk), *Glycyphagus domesticus* (De Geer), *Aeroglyphus robustus* Banks (Glycyphagidae), *Chortoglyphus arcuatus* (Troupeau) (Chortoglyphidae) and *Carpoglyphus lactis* (L.) (Carpoglyphidae) were reared in darkness at 20°C and 85% RH. A mixture of powdered oat flakes, wheat germ and yeast in the ratio 5:5:1 was used as breeding diet. Small breeding cells (1 cm in diameter) were established for egg collection in which five females of each species were allowed to oviposit for 1–3 days and were then removed. The eggs were collected gently with a brush, cleaned in a drop of distilled water, and subjected to examination with a light or electron microscope. The morphological studies included measurements of size (L = length, W = width, and L/W ratio), descriptions of the shape and colour of eggs, and SEM-based documentation of surface structures. Shape and colour was determined observing fresh eggs using a stereomicroscope. Size measurements were taken using a light microscope equipped with an objective micrometer. At least 30 eggs were measured for each species.

Data were analysed by descriptive statistics and differences in egg dimensions among species were displayed by box plots (STATISTICA 8). Another 30 eggs were examined with the SEM. The eggs were placed on special stubs covered with double-sided tape and sputter-coated with platinum in the Sputter Coater SDC 050. The chorion surface was then studied with the JEOL JSM 6400 SEM at magnifications of 500–20,000 $\times$. Surface microsculptures were measured directly from micrographs.

Results

Colour

No significant differences in egg colour were found. All eggs studied were white, partly pellucid and glossy.

Size and shape

The dimensions of the eggs varied slightly within species. The means and 95% confidence interval (CI) for each species are recorded in Table 1. Eggs of *C. lactis* were smallest (<120 μ m long), and *C. redikorzevi* and *G. domesticus* were largest, being significantly larger (>150 μ m long) than those of the other species studied. Egg shape was generally similar for all species: symmetrical and regularly ellipsoid with rounded ends. However, there are differences in the average length/width ratios (Table 1). The L/W ratios of *Acarus* spp., *Caloglyphus* spp. and *Aleuroglyphus* spp. are smaller (mean 1.41–1.55; these eggs are on average shorter: Fig. 1b, l; 2c) than those of other genera of stored-product mites (1.62–1.78; here the shape is more oblong: Figs. 1e, g, i, k, 2a, e, i). Figure 3 illustrates egg size differences among species.

Surface structures

The chorion surface looks generally smooth for all eggs, but SEM magnifications reveal some differences between the species studied. Species of *Acarus* sp., *C. redikorzevi*, *G. domesticus*, *A. robustus* and *C. lactis* have an egg surface devoid of any microsculptures (as in Fig. 1d, m). Egg surfaces in *L. destructor* and *C. oudemansi* are covered with small, sparse irregular tubercles (bumps) (width 0.2–0.5 and 0.5–1.7 μ m, respectively, Fig. 2h, j).

Table 1 Size measurements (μm) and surface sculpturing of stored product mite's eggs (Acari, Astigmata)

Astigmata	<i>L</i>		<i>W</i>		<i>L/W</i> ratio		MS
	mean	95% CI	mean	95% CI	mean	95% CI	
Acaridae							
<i>Acarus siro</i>	125.6	123.0–128.2	83.9	82.9–85.0	1.49	1.47–1.52	a
<i>Acarus gracilis</i>	123.7	121.9–125.5	80.0	79.0–81.0	1.55	1.53–1.57	a
<i>Tyrophagus putrescentiae</i>	122.2	120.1–124.3	69.8	68.6–71.1	1.75	1.71–1.79	p
<i>Tyrophagus brevicrinatus</i>	129.9	127.2–132.6	74.1	72.9–75.3	1.75	1.72–1.79	p
<i>Tyroborus lini</i>	141.5	138.7–144.4	82.6	81.3–83.8	1.72	1.68–1.75	p
<i>Aleuroglyphus ovatus</i>	132.5	130.2–134.8	86.2	84.7–87.7	1.54	1.51–1.56	p
<i>Caloglyphus oudemansi</i>	122.1	117.3–126.9	86.6	83.6–89.7	1.41	1.36–1.46	p
<i>Caloglyphus redikorzevi</i>	155.7	152.1–159.2	109.9	107.4–112.5	1.42	1.39–1.44	a
Glycyphagidae							
<i>Lepidoglyphus destructor</i>	135.0	132.2–137.8	75.9	73.9–77.7	1.78	1.75–1.82	p
<i>Glycyphagus domesticus</i>	154.5	151.7–157.2	88.2	86.9–89.5	1.75	1.72–1.79	a
<i>Aeroglyphus robustus</i>	121.2	118.1–124.2	73.0	71.5–74.5	1.66	1.63–1.70	a
Chortoglyphidae							
<i>Chortoglyphus arcuatus</i>	121.6	119.5–123.6	75.2	73.9–76.4	1.62	1.59–1.65	p
Carpoglyphidae							
<i>Carpoglyphus lactis</i>	115.3	112.7–117.8	67.7	66.4–69.1	1.70	1.68–1.73	a

L, egg length; *W*, egg width; *MS*, microsculpturing on egg surface; a, absent; p, present

Chortoglyphus arcuatus and *A. ovatus* have eggs with a granulated surface composed of spherical tubercles. These tubercles are smaller (0.1–0.25 μm) in *C. arcuatus* (Fig. 2b) and more sparse than in *A. ovatus* (0.1–0.5 μm) (Fig. 2d). The chorion of *T. lini* (Fig. 2f) has a distinct microsculpture formed by two types of granulation. The basal surface of the egg shell has compact dense granulation created with small size granules (0.1–0.3 μm). From this basal surface rise scattered clusters composed of both groups of 2–5 larger spherical granules (tubercles) (0.75–1.35 μm) and separate granules (tubercles) (0.25–1.15 μm). *Tyrophagus* spp. have the most complicated microsculpture. These chorion patterns are formed in *T. putrescentiae* (Fig. 1e, f) with small raised mounds (granules) and larger locular chambers with basal openings. In *T. brevicrinatus* (Fig. 1g, h) there are only locular chambers without the small granules on the chorion surface. The microsculpture differs in size and density depending on the species.

Micropyle and aeropyle

Micropyle and typical aeropyle areas were not found in any eggs studied.

Identification key

The key for identification of storage mite eggs is based on size (see Table 1; Fig. 3) and morphological characters (microsculptures).

Key for the identification of eggs of stored-product pests mites

1	Egg length > 150 μm , without microstructures	2
–	Egg length < 150 μm , with or without microstructures	3
2	Egg width > 100 μm (Fig. 1l, m), L/W ratio < 1.6	<i>Caloglyphus redikorzevi</i>
–	Egg width < 100 μm (Fig. 1k), L/W ratio > 1.6	<i>Glycyphagus domesticus</i>
3	Egg surface without microstructures (as Fig. 1d, m)	4
–	Egg surface with microstructures (as Figs. 1f, h, 2b, d, f, h, j)	5
4	Egg width > 78 μm (Fig. 1a, b)	<i>Acarus siro</i> , <i>Acarus gracilis</i>
–	Egg width < 78 μm (Fig. 1c, d, i, j)	<i>Aeroglyphus robustus</i> , <i>Carpoglyphus lactis</i>
5	L/W ratio > 1.6	6
–	L/W ratio < 1.6	10
6	Egg surface with locular chambers (Fig. 1f)	7
–	Egg surface without locular chambers	8
7	Egg surface with locular chambers and small granules between them (Fig. 1f)	<i>Tyrophagus putrescentiae</i>
–	Egg surface only with locular chambers (Fig. 1h)	<i>Tyrophagus brevicrinatus</i>
8	Egg surface with two distinct types of microstructures (Fig. 2f)	<i>Tyroborus lini</i>
–	Egg surface with only one type of microstructures (Fig. 2b, d, h, j)	9
9	Egg length > 128 μm (Fig. 2i,j), L/W > 1.7	<i>Lepidoglyphus destructor</i>
–	Egg length < 128 μm (Fig. 2a,b), L/W < 1.7	<i>Chortoglyphus arcuatus</i>
10	L/W ratio > 1.47, egg surface with dense rounded granules (Fig. 2c, d)	<i>Aleuroglyphus ovatus</i>
–	L/W ratio < 1.47, egg surface with scarce irregular moulds (Fig. 2g, h)	<i>Caloglyphus oudemansi</i>

Discussion

Differences were found in the egg surfaces of astigmatic species, regardless of their systematic position within families and, in the case of *Caloglyphus*, also within a genus. Six species have no microsculpture at all and seven species have a microsculpture that differed in size, shape and density. According to Witalinski (1993), the egg shell in *Acarus* sp. is a vitelline envelope produced by the oocyte itself and transformed into a one-layer chorion without any microsculpture. Chorions without sculpture were also found in other species studied here (Table 1). On the other hand, in acarid mites of the genera *Tyrophagus* (*T. longior*, *T. perniciosus*) and *Aleuroglyphus*, the egg chorion is additionally coated in the distal portion of the oviduct by an exochorion material that forms the microsculpture (Witalinski 1993). Distinct, regularly-granulated exochorion-type microsculpture was found in *T. lini*, *C. arcuatus* and *A. ovatus*. Differences in the adhesive nature of egg shells were also found, regardless of their type; e.g., the egg surface of *A. robustus* has no microsculpture and is smooth and clean, mostly without particulate impurities. Other eggs without microsculpture, e.g., these of *C. redikorzevi*, *C. oudemansi* and *C. arcuatus*, had surface adhesive and were usually coated with debris and micro-organisms (e.g., Fig. 2g). According to Witalinski (1993) eggs with microsculpture either have an adhesive (*A. ovatus*) or a non-adhesive (*Tyrophagus*) exochorion. Debris stacked on the adhesive surface of eggs could serve as camouflage. The adhesive layer fixes the egg to the

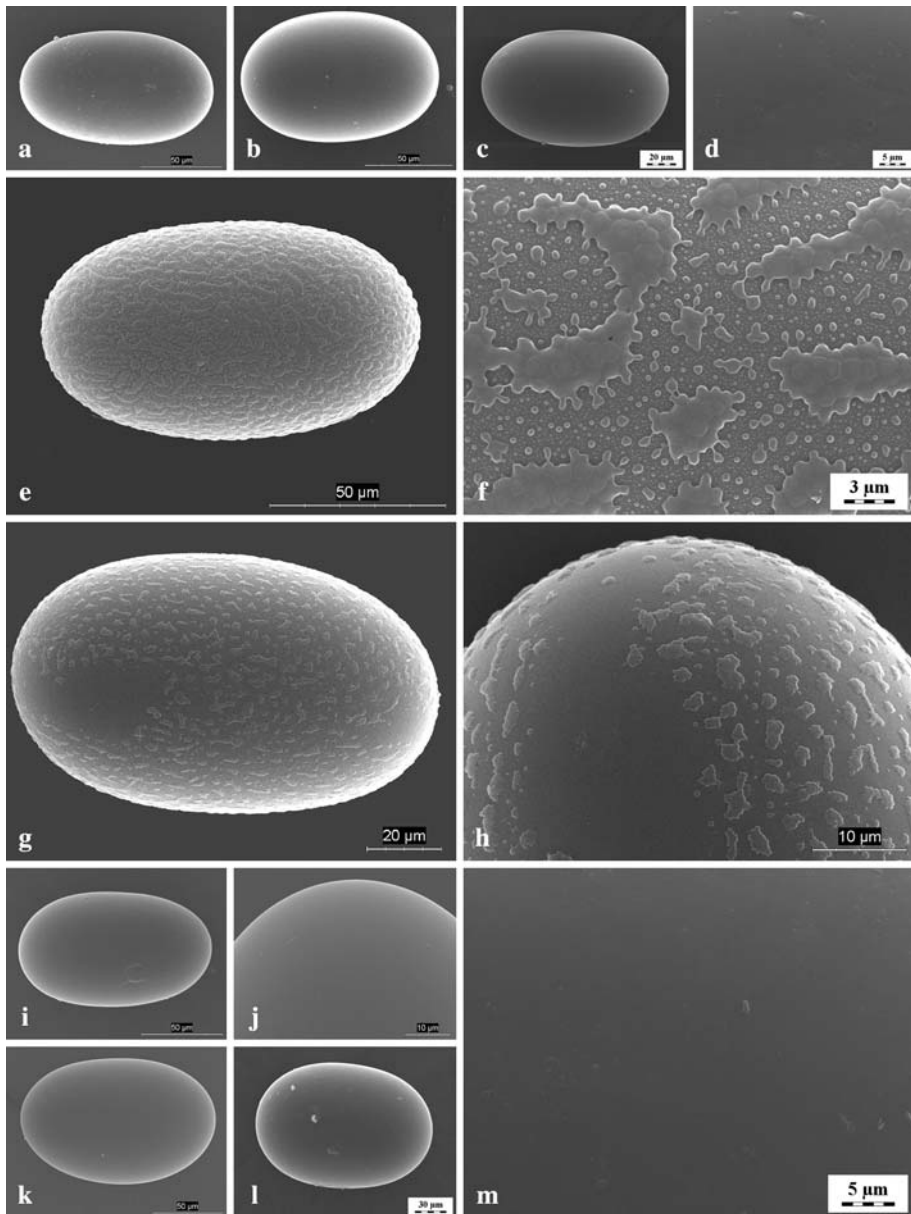


Fig. 1 *Acarus siro* **a** entire egg; *A. gracilis* **b** entire egg; *Aeroglyphus robustus* **c** entire egg, **d** detail of egg surface; *Tyrophagus putrescentiae* **e** entire egg, **f** egg surface sculpturing; *T. brevicrinatus* **g** entire egg, **h** egg surface sculpturing; *Carpoglyphus lactis* **i** entire egg, **j** end of the egg; *Glycyphagus domesticus* **k** entire egg; *Caloglyphus redikorzevi* **l** entire egg, **m** detail of egg surface

substratum (Alberti and Coons 1999). The various adhesive abilities of mite eggs, together with the various surface characters of substrates (Chanbang et al. 2008), can contribute to differences in the diversity and abundance of stored-product mite pests found in several

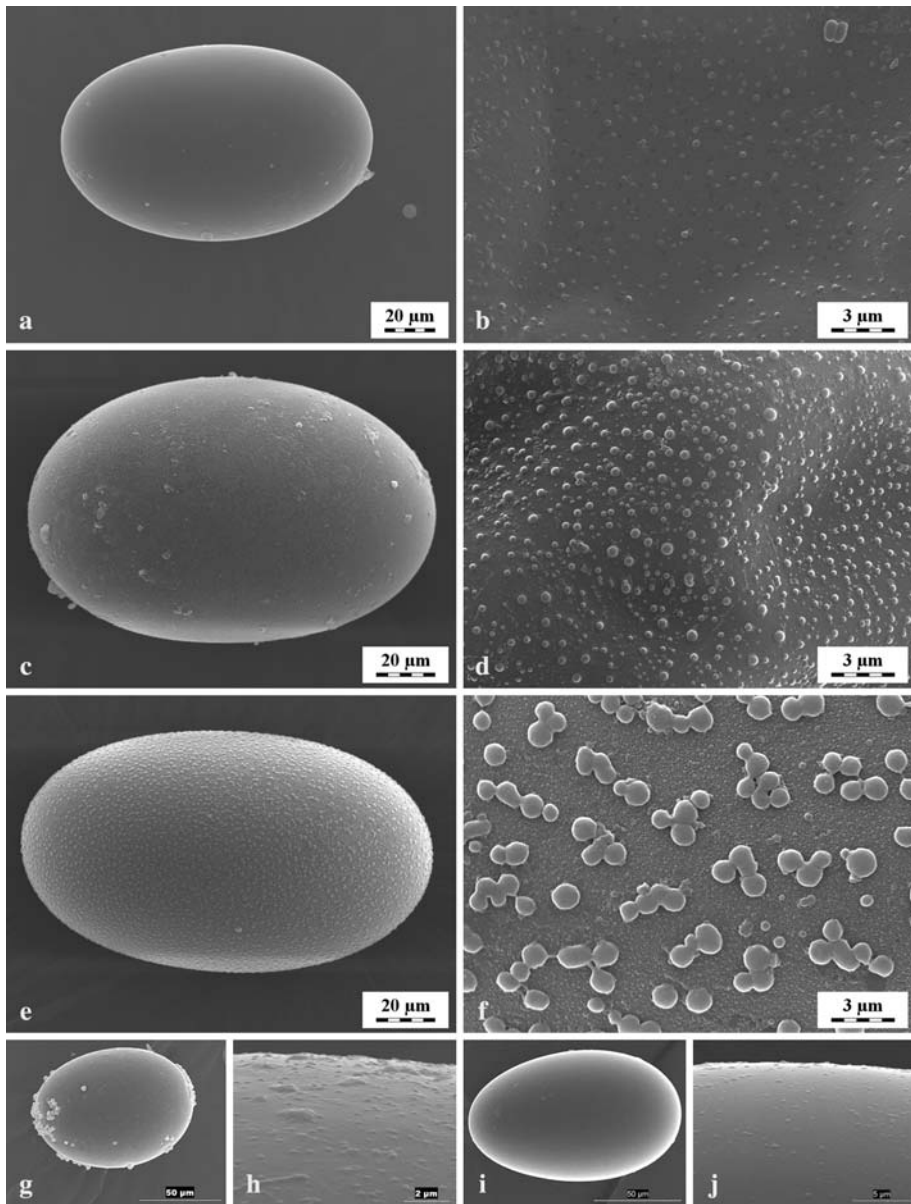
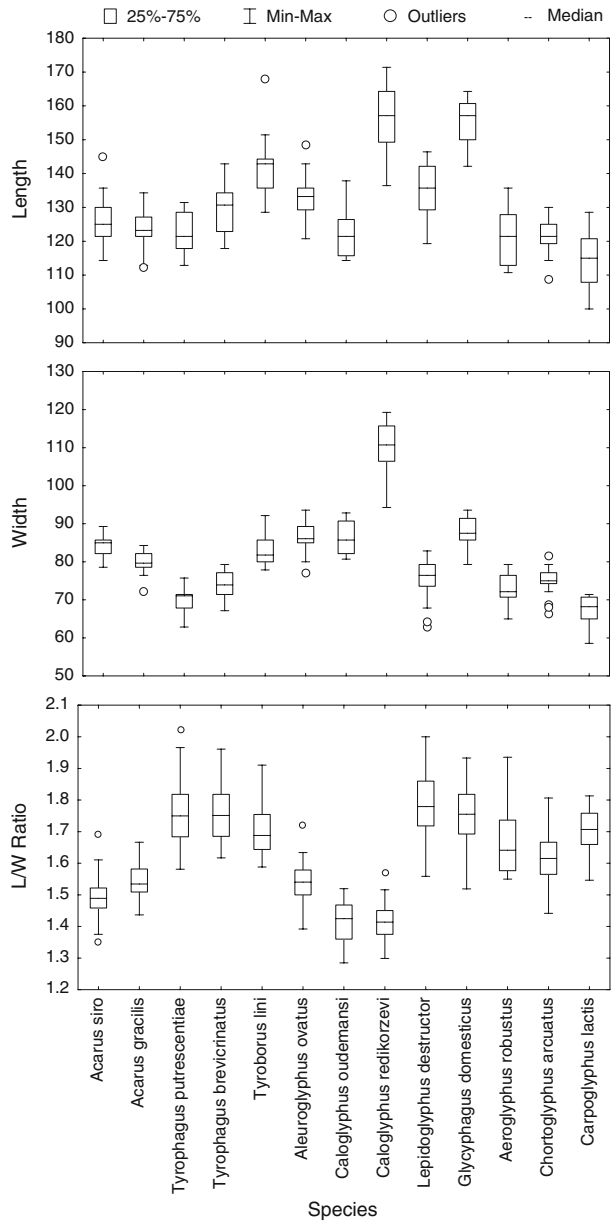


Fig. 2 *Chortoglyphus arcuatus* **a** egg, **b** egg surface sculpturing; *Aleuroglyphus ovatus* **c** entire egg, **d** egg surface sculpturing; *Tiroborus lini* **e** entire egg, **f** egg surface sculpturing; *Caloglyphus oudemansi* **g** entire egg, **h** detail of egg surface; *Lepidoglyphus destructor* **i** entire egg, **j** detail of egg surface

stored grain types (Hubert et al. 2009), depending on storage management protocols. Other interpretations of the function of sculptured egg shells in various mite eggs have been published, but the problem is still not solved completely. For example, *Tyrophagus* spp. egg sculptures may have a respiratory function (Callaini and Mazzini 1984) or serve to reduce water loss (Witalinski 1993). Egg shell structures and their functions should be

Fig 3 Box plots of egg size measurements (length and width [μm] and L/W ratio) of stored-product mite species. Species are arranged according to their systematic position (Hughes 1976). The box plots display the interquartile range, median, minimum and maximum values, and outliers



evaluated in relation to efficacy of various methods of physical and chemical control for major stored-product mite pests.

Significant differences were found also in egg sizes of various species and genera. However, it should be stressed that our measurements are based on laboratory strains. Colloff (1987) reported that eggs from the laboratory populations of the house dust mite *Dermatophagoides pteronyssinus* (Trouessart) (Pyroglyphidae) were larger than the ones

from wild populations because of different diets. Populations of *D. pteronyssinus* taken from natural habitats have been found to show difference also in reproduction and egg development times from those of laboratory populations (Hart et al. 2007). We have not been able to trace any supporting data on egg size variation among various strains of storage mites. Therefore, an effect of diet composition (germ, endosperm, fungi) on egg size of common storage mites under laboratory and storage conditions could be less than the effect of laboratory versus natural diet in the case of dust mites. Nevertheless, this should be tested experimentally by comparing eggs sizes of field and laboratory storage mite populations.

Data for phylogenetic analysis of relationships between mite genera and families (Acaridae) are based on external morphology of adults and nymphs (Klimov 2000; OConnor 2001). It would be valuable to include also eggs morphology to have more comprehensive data for the construction of cladistic trees.

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