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Morphological characteristics of local entomopathogenic protozoan strains isolated from insect cadavers of certain stored-grain pests in Egypt

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Abstract

The present study has documented, for the first time in Egypt, the natural occurrence of four entomopathogenic protozoans (EPP) among five of the most abundant and damaging insect pests of stored grains or their products. These insect pests (*Laemophloeus* (*Cryptolestes*) *turcicus* (Grouvelle), *Rhyzopertha dominica* (Fabricius), *Sitophilus zeamais* (Motschulsky), *Tribolium castaneum* (Herbst), and *Plodia interpunctella* (Hobner) were infesting lots of crushed-maize grains, wheat grains, and wheat flour, brought, in 2015, from El-Behera Governorate, Egypt. The morphological characteristics, including spore size, of the entomopathogen infective units, spores, of the isolated entomopathogenic protozoans, were closely fit with the description to the following genera: *Mattesia*, *Farinocystis*, *Adelina*, and *Nosema*. The prevalence of these entomopathogens ranged between 9 and 89%. This study seems to be the first report of *Mattesia* sp. on *S. zeamais*; *Adelina* sp. on *L. turcicus* or *R. dominica*, and the second report of *Nosema* sp. on *R. dominica*. The rate of natural infection by the neogregarine, *Mattesia* sp. (tentatively, *M. dispora*), was the highest in *L. turcicus* beetles (89%) followed by that in *P. interpunctella* moths (48%), larvae (40%), and pupae (32%) and then in *S. zeamais* weevils (42%) and *R. dominica* beetles with a low rate of infection (9%). The microsporidian entomopathogen, *Nosema* sp., (tentatively, *N. whitei*) was naturally occurred in 11% of the examined adult cadavers of *R. dominica*. The coccidian entomopathogen, *Adelina* sp., was found, respectively, in 60% and 27% of larval and adult cadavers of *T.castaneum*, while the *Adelina*-natural infection rates in *R. dominica* and *L. turcicus* adult cadavers were 34% and 14%, respectively. A high rate of natural infection with another neogregarine, *Farinocystis* sp. (tentatively, *F. tribolii*), has also been recorded in *T. castaneum* adult (50%) or larval cadavers (36%).

Keywords: Entomopathogenic protozoans, Stored-grain insect pests, *Mattesia*, *Farinocystis*, *Adelina*, *Nosema*, Spore dimensions

Background

Stored products as a unique habitat, present a prime opportunity to use entomopathogenic protozoans (EPP) by distributing infective quantities of the entomopathogen. Hence, controlling stored-product insect pests may be most beneficial in long-term storage since such EPP produce slow-acting chronic infection. Furthermore, they may cause a reduction in host vitality, feeding, fecundity, longevity, and survivorship (McLaughlin 1971; Khan and Selman 1989; Flinn and Schöller 2012; Ramanujam et al. 2014). Weiser et al. (1976) recorded promising examples for certain entomopathogenic protozoans that cause reductions in storage insect pests.

Literaturely, attempts to use entomopathogenic protozoans for microbial control of stored product insect pests are few. Hence, to achieve a desired suppression in populations of such insect pests, actually, more studies are required on protozoan natural infection rates in stored-product insects, and on the host range of candidate EPP. Therefore, such research approaches should be invaded. Although insect pests of stored grains and their products are among

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the most economically important pests in Egypt, there are no records on their naturally occurring EPP. Thus, as a first step in casting the light on this field, the present work was carried out, hopefully, to document the Egyptian strain(s) of the entomopathogenic protozoans, originally isolated from certain stored-grain insect pests, and their natural infection rates, as well as to describe some of the morphological characteristics of their infective units, spores.

Materials and methods

Insect pests

In August and September 2015, lots of 5–10 kg of stored-crushed maize grains, and others of wheat, wheat flour, and rice were brought from a private storehouse of stored-grains in Koom-Hamada, El-Behera, Egypt, to the Insect Pathology Laboratory, Applied Entomology Department, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt. All lots were infested with the most abundant cosmopolitan insect pests of stored grain; namely, *Laemophloeus turcicus* (Grouvelle), *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (Fabricius), *Oryzaephilus surinamensis* (L.), *Sitophilus zeamais* (Motschulsky), and *Plodia interpunctella* (Hübner). Among the crushed-maize grain lots, infested with the flour-mill beetle, *L. turcicus*, two cultures have been observed. *Laemophloeus* beetles of the first culture were less active and completely unable to fly; also, a considerable number of naturally dead or moribund beetles were present. On the contrary, the flour-mill beetles of the second culture were active, good fliers, and a very few number of dead beetles has been observed. The first procedures of Koch's postulates (Kiraly et al. 1970) revealed a severe association of the entomopathogenic neogregarine protozoan, *Mattesia* sp. with *Laemophloeus* adults of only the first culture, whereas those of the second culture showed to be totally free of the protozoan pathogen.

Natural infection rates

Mother cultures infested with *L. turcicus*, *T. castaneum*, *R. dominica*, *S. zeamais*, *O. surinamensis*, and *P. interpunctella*, were maintained in plastic jars (9 × 20 cm) supplied, separately, with crushed-maize grain, wheat, rice, and whole wheat flour. All jars were covered and closed with cotton cloth covers and rubber bands and kept at the laboratory conditions of 25.3 ± 3.8 °C, 84.2 ± 3.7% RH, and photoperiods of ca. 12–14 h. The naturally dead insects of these stock cultures were periodically (weekly) collected and stored in the fridge (4–8 °C) in clean, sterilized vials. These dead insects were individually smeared in Ringer solution and microscopically examined (×100 and ×400 magnification) for *Mattesia* infection or other entomopathogenic protozoans. Before preparing the subject smears, dead insects were thoroughly brushed with 70% ethanol on tissue papers for surface sterilization. *Mattesia*-natural infection rates among the subject insect pests were recorded and compared only to both male and female beetles of *Laemophloeus*. Other entomopathogenic protozoans were recorded too.

Spore measurements

Mattesia, *Farinocystis*, or *Nosema* spore dimensions (length and width, in micrometers, μm) were measured by using the micrometric lens (×400 or ×1000). The spore size was determined in both Lactophenol-mount and Ringer solution-mount preparations of infected cadavers ($n = 50$ or 60; 5 or 6 insects, 10 readings per insect). Infective units of the isolated EPP, in Ringer solution or Lactophenol preparations, were photographed using both light and scanning electron microscope (SEM).

Statistical analysis of data

All means obtained were compared by the adoption of the F test and t test at the 5% level of probability, while all probable comparisons among these means were achieved using Duncan's multiple range test, at the 5% or 1% level by adopting the computer program, SPSS 16.0.

Results and discussion

Natural mortality rates due to certain entomopathogenic protozoans among certain stored-grain insect pests in Egypt

As shown in Table 1, four EPP, *Mattesia* sp., *Nosema* sp., *Adelina* sp., and *Farinocystis* sp., were recorded among five stored-grain insect pests, *L. turcicus*, *P. interpunctella*, *S. zeamais*, *R. dominica*, and *T. castaneum* (Fig. 1).

The rate of natural infection by the neogregarine, *Mattesia* sp. (Fig. 2), was the highest in *L. turcicus* beetles (89%) followed by *P. interpunctella* moths (48%), larvae (40%), and pupae (32%) and then *S. zeamais* weevils (42%) and *R. dominica* beetles with a low rate of infection (9%).

The microsporidian entomopathogen, *Nosema* sp. (Fig. 3), was naturally occurred in 11% of the examined adult cadavers of *R. dominica* (Table 1).

The coccidian entomopathogen, *Adelina* sp. (Fig. 4), was found, respectively, in 60% and 27% of larval and adult cadavers of *T. castaneum*, while the *Adelina*-natural infection rates in *R. dominica* and *L. turcicus* adult cadavers were 34 and 14%, respectively (Table 1).

A high rate of natural infection with another neogregarine, *Farinocystis* sp. (Fig. 5), was also recorded in *T. castaneum* adult (50%) or larval cadavers (36%).

Additionally, on the basis of sex differences, the presence of the entomopathogen, *Mattesia* was also detected and compared only in *L. turcicus* adult males and females (Fig. 1). The findings in Table 2 indicate that there is no remarkable difference between the two sexes

Table 1 Natural mortality rates (%) among certain insect pests of stored grain due to entomopathogenic protozoans

Entomopathogenic protozoan	Insect pest	Natural mortality rate (%)		
Mattesia sp.	*Laemophloeus turcicus* (Grouv.) (Coleoptera: laemophloeidae)	2601/2914	(89)	A
	Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae)	34/71 40/100 17/53	(48) (40) (32)	A L P
	Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidae)	143/339	(42)	A
	Rhyzopertha dominica (F.) (Coleoptera: Bostrichidae)	72/775	(9)	A
Nosema sp.	*R. dominica*	83/775	(11)	A
Adelina sp.	*Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)	73/275 72/120	(27) (60)	A L
	R. dominica	266/775	(34)	A
	L. turcicus	402/2914	(14)	A
Farinocystis sp.	*T. castaneum*	138/275 43/120	(50) (36)	A L

A adult, *L* larva, *P* pupa

toward *Mattesia* disease, where the recorded *Mattesia*-natural infection rates in both of 160 *L. turcicus* females (90.63%) and 144 males (90.97%) were nearly equal (91%). Hence, it seems that the *Mattesia* does not prefer any sex of the flour-mill beetle. A similar observation has been reported by Baki (2016) who found no considerable difference between sexes of *Crioceris asparagi* (Linne) (Coleoptera: Chrysomelidae) toward *Mattesia* infection.

Beetles of *Laemophloeus* spp. (*Cryptolestes*) and *R. dominica*, as well as *P. interpunctella* moth have been

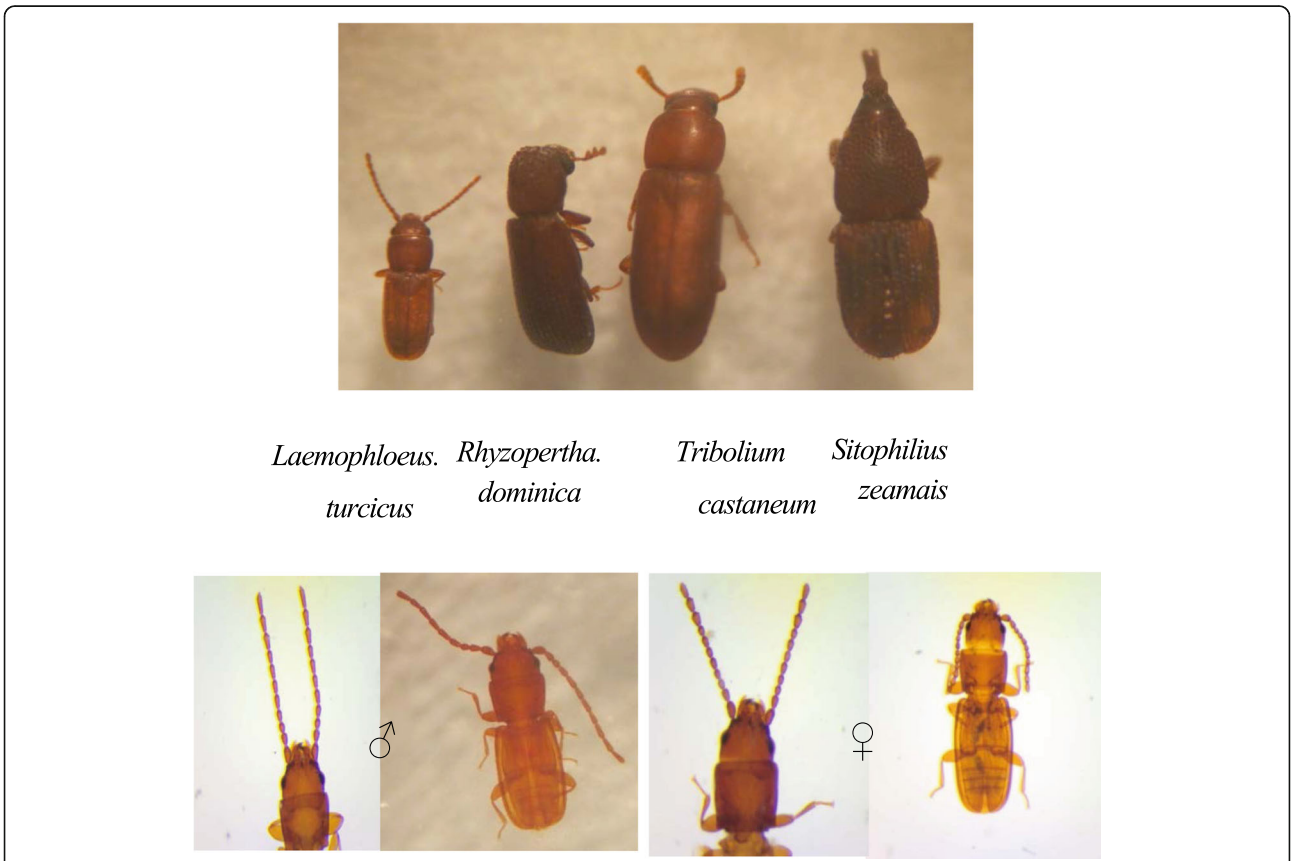

Fig. 1 The coleopterous pests associated with crushed-maize grain-laboratory cultures; (×25 magnification). Below, *Laemophlaeus turcicus* adult male (left, male sign; antennae: filiform) and female (right, female; antennae: moniliform), with anterior end magnified (×100 magnification)

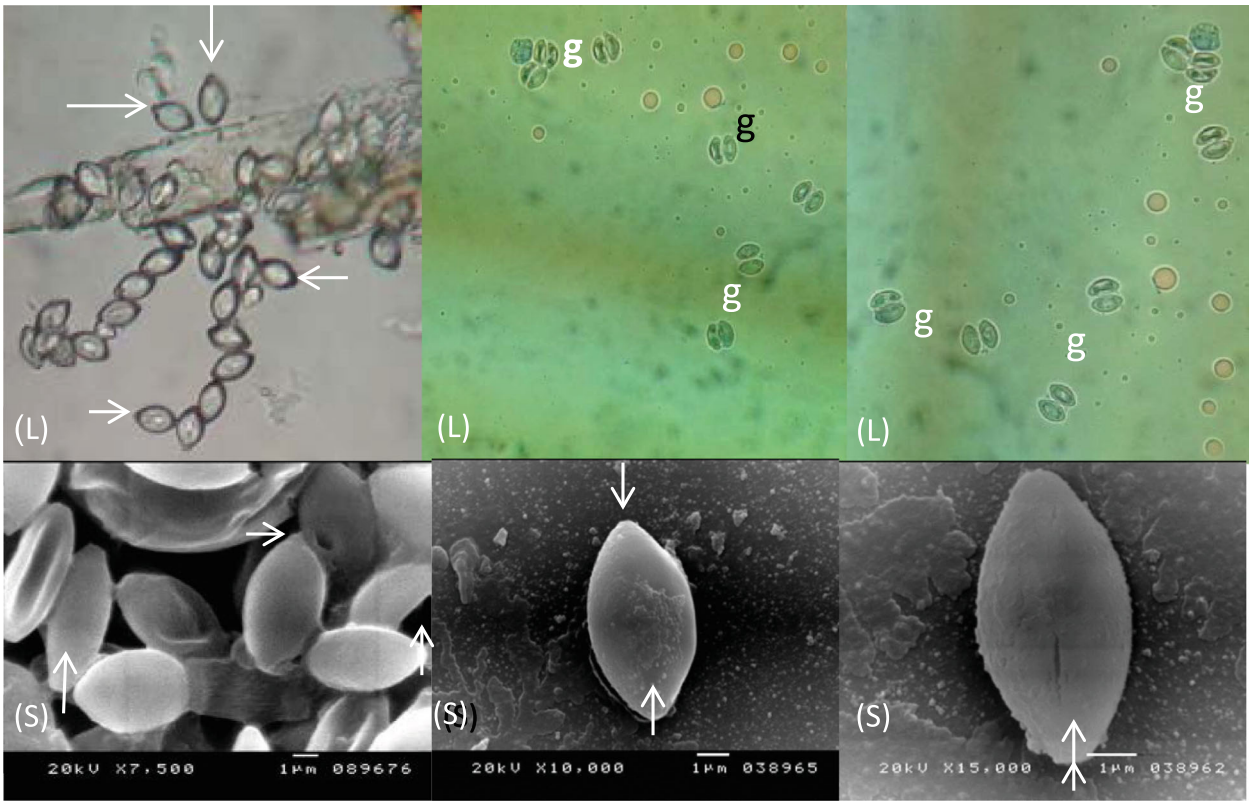

Fig. 2 Light (L) and scanning electron (S) microscope views of *Mattesia*. Note the navicula-shaped spores with polar plugs (arrows) and spore pairs within a gamontocyst (g) (×400 magnification)

reported to be attacked by the neogregarine, *Mattesia* spp. (Finlayson 1950; Lord 2003, 2006). Also, *T. castaneum* is known to be affected by both the neogregarine, *Farinocystis tribolii* (Weiser 1953; Rabindra et al. 1981; Njila and Mwansat 2012) and the coccidian, *Adelina* spp. (Bhatia 1937; Ghosh et al. 2000; Njila and Mwansat 2012).

The literature on host range of the EPP, *Mattesia*, *Nosema*, *Adelina*, or *Farinocystis*, infecting stored-grain insects, presents no records in this concern from Egypt. Therefore, the present study seems to be the first report of *Mattesia* sp. on *S. zeamais*, *Adelina* sp. on *L. turcicus* or *R. dominica*, and *Nosema* sp. on *R. dominica*, in Egypt. Meanwhile, it appears to be the first record for *Mattesia* sp. in *Sitophilus* spp. or *R. dominica*, and *Adelina* sp. in *R. dominica.*

The present naturally occurring *Mattesia*-infection in *L. turcicus* population is congruent with what has been reported by Lefkovitch (1962); on the contrary, Finlayson (1950) mentioned that *L. turcicus* beetles were apparently not susceptible to *Mattesia* infection.

Microscopic examination for *Mattesia*-infected cadavers of the flour-mill beetle, *L. turcicus* reveals that the bodies of these small (1.5 to 2.0 mm) insect pests would act just as reservoirs for the infective units, spores, of such an EPP, *Mattesia* sp. Meanwhile, adopting the procedure of smear or water mount preparations (i.e., microscopic examination of the insect body contents) in order to calculate the natural infection rates or to confirm the presence of the protozoan disease causative agent results, vainly, in a great loss in masses of *Mattesia* and *Farinocystis* spores, or *Adelina* oocysts which could be benefited in their mass production. Therefore, to avoid such a technical loss in the protozoan infective units, another procedure was successfully followed. Herein, the microscopic examination, at a magnification of ×100 or ×400, for *L. turcicus* adults from the ventral side (i.e., via sternites) easily reveals the presence of the characterized lemon-shaped spores of *Mattesia* species. *Mattesia* spores can easily be observed through the sterna of the thorax or the abdomen and sometimes in the head (Fig. 6). Furthermore, the same procedure was followed to confirm the presence of both the *Adelina* oocysts in either *L. turcicus* adult (Fig. 6) or in *T. castaneum* adult cadavers (Fig. 7) and the *Farinocystis* spores of *T. castaneum* adults (Fig. 7). Pereira et al. (2002) recognized the oocysts of *Mattesia* sp. through the cuticle in all body regions, especially the head and the appendages of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae).

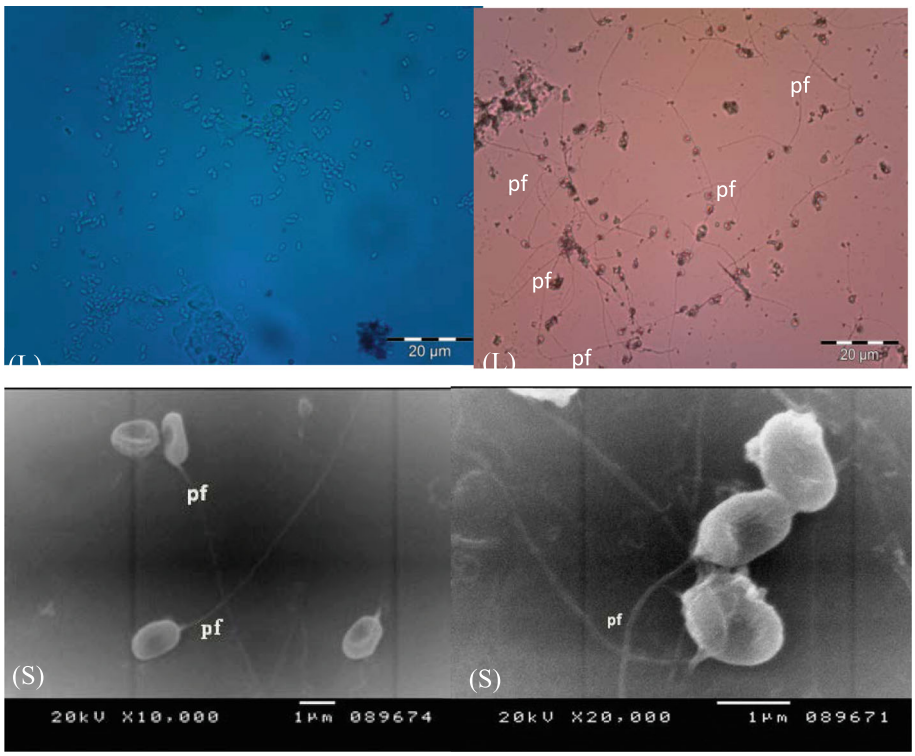

Fig. 3 Light (L) and scanning electron (S) microscope views of *Nosema* spores isolated from *Rhyzopertha dominic*a. Note the extruded polar filament (pf)

This procedure of detecting the causative agent(s) of certain protozoan diseases will certainly save a considerable quantity of insect cadavers that harbor masses of the infective units of certain EPP which would surely be lost during the classical detection procedure by smears or water mounts. Hence, practically, large numbers of protozoan-infected cadavers could be save to act as reservoirs for producing infective units, spores, or oocysts, in large quantities for microbial control of insect pests in storage.

Spore measurements

Mattesia sp.

The *Mattesia* spores, originally isolated from *L. turcicus*, *R. dominica*, *S. zeamais*, and *P. interpunctella* were lemon-shaped (navicular) with obvious polar plugs and also formed in pairs within a gamontocyst or gametocyst (Fig. 2).

The *Mattesia*-spore sizes, as measured from fresh spores, in Ringer solution, or from stained ones, in Lactophenol, are summarized in Table 3. Based on *t* test data (significant at 1% level), the differences between fresh-spore sizes of Ringer solution-mounted spores were not significantly consistent with those of Lactophenol-mounted spores (Table 3).

Mattesia spore dimensions for a sample of 50 spores in Ringer solution mounts were measured from *L. turcicus* adults. Their spore sizes ranged in length from 12.5 to 15 μm and in width from 7.5 to 10 μm; with a mean ± standard deviation (or error) of 14.65 ± 0.88 (or 0.12) × 9.85 ± 0.60 (or 0.08) μm. These spore dimensions, in general, were not significantly different (Duncan's multiple range test, significant at 1% level) from those of *S. zeamais* adults [range, 12.5–15 × 7.5–12.5μm; 14.80 ± 0.70 (or 0.10) × 9.8 ± 0.99 (or 0.14) μm], as well as the corresponding *Mattesia*-spore measurements from *R. dominica* adults [range, 12.5–17.5 × 7.5–10μm; 15.08 ± 0.92 (or 0.13) × 9.25 ± 1.16 (or 0.16) μm] or *P. interpunctella* larvae [range, 12.5–17.5 × 7.5–10μm; 14.45 ± 1.05 (or 0.15) × 9.15 ± 1.20 (or 0.17 μm)]. Exception was recorded for spore widths from *R. dominica* [9.25 ± 1.16 (or 0.16) μm] and *P. interpunctella* [9.15 ± 1.20 (or 0.17) μm] in their fresh, Ringer solution-mounted preparations (Table 3). Their spore widths were significantly not different, but varied significantly, at 1% level, from their corresponding values of *L. turcicus* [9.85 ± 0.60 (or 0.08) μm] or *S. zeamais* [9.8 ± 0.99 (or 0.14) μm] (Table 3).

Meanwhile, the corresponding figures for *Mattesia*-spore dimensions in Lactophenol-mounted preparations for the subject four insect pests were, in general, not significantly different, at 1% level, Duncan's multiple range test (Table 3); except for spore-widths described from *S. zeamais* [8.35 ± 1.20 (or 0.17) μm] which were significantly, at 1% level, wider than those measured from *L. turcicus* [7.9 ± 0.49 (or

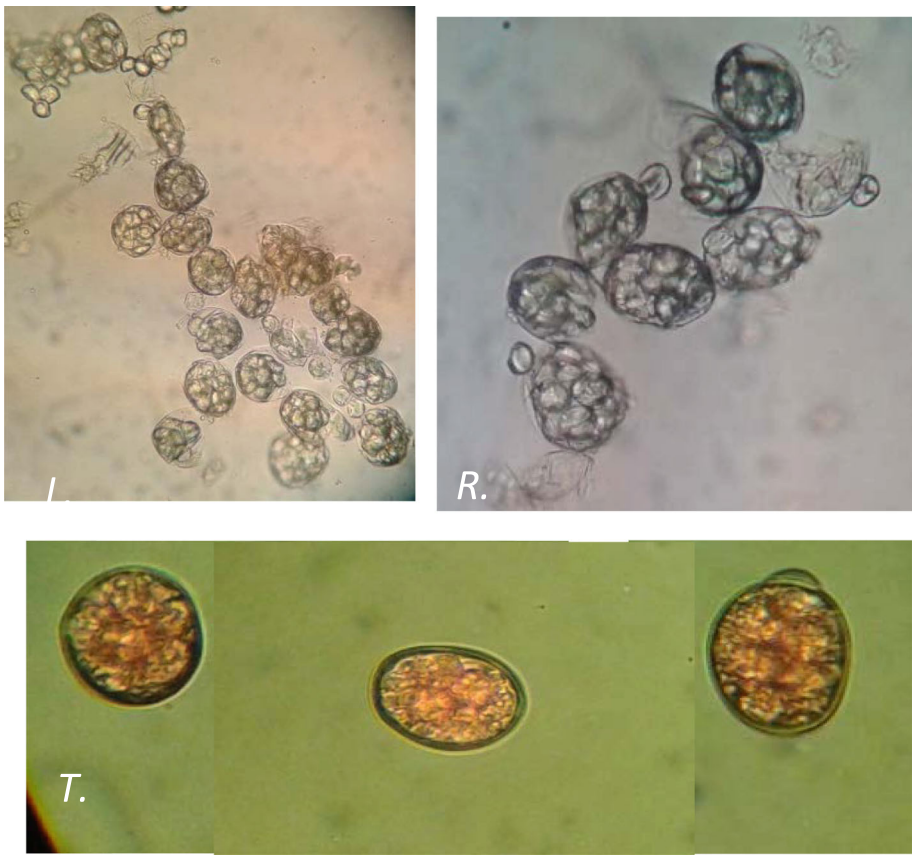

Fig. 4 Light microscope views of *Adelina*-polysporocystic oocysts isolated from *Tribolium castaneum (T.)*, *Rhyzopertha dominica (R.)*, and *aemophloeus turcicus (L.)* (×400 magnification)

0.07) μm], *R. dominica* [7.55 ± 0.35 (or 0.05) μm], or *P. interpunctella* [7.5 ± 0.0 (or 0.0) μm] (Table 3).

Based on the observations by light and scanning electron microscope, the data illustrated in Table 3 and Fig. 2 reveal that the spores (fresh or stained preparations) of the neogregarine entomopathogen which isolated from the subject four insect pests (*L. turcicus*, *S. zeamais*, *R. dominica*, and *P. interpunctella*) have the typical characteristics (i.e., navicular shape, polar plugs, one or two spores in the gamontocyst, and spore dimensions) of the genus *Mattesia* (Order: Neogregarinida; Family: Lipotrophidae).

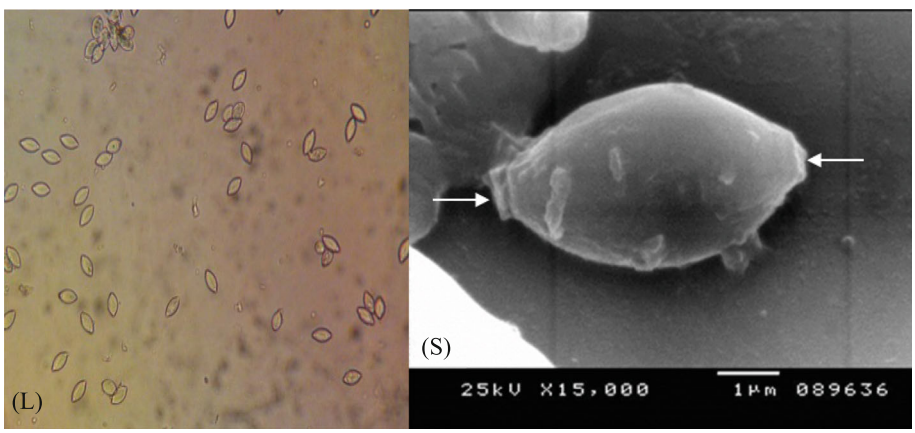

Fig. 5 Light (L) and scanning electron (S) microscope views of *Farinocystis* spores isolated from *Tribolium castaneum* (×400 magnification). Note the polar plugs (arrows)

Table 2 Natural infection rate of *Mattesia* sp. in both sexes of *Laemophlaeus turcicus* adults

Adult beetle sex	No. of examined beetles and *Mattesia*—natural infection rate (%)
Laemophloeus turcicus Female	145/160 (90.63)
Laemophloeus turcicus male	131/144 (90.97)

Levine (1988), Kleespies et al. (1997), and Perkins (2000) noted that the genus *Mattesia* is one of five genera (*Mattesia*, *Farinocystis*, *Lipocystis*, *Lipotropha*, and *Menzbieria*) within the Family Lipotrophidae, in Order Neogregarinida. Members of this Family are characterized by their lemon-shaped (navicular) spores with distinct polar plugs (Fig. 2). On the other hand, Yaman et al. (2012) reported *Mattesia* as the only genus of the Family Lipotrophidae to have one or two spores within a gamontocyst, whereas members of the other four genera have more than two spores in the gamontocyst.

As the lipotrophid entomopathogen is characterized by its lemon-shaped (navicular) spores with polar plugs, and two spores within one gamontocyst, hence, it simply belongs to the genus *Mattesia*. This finding is strongly supported by its corresponding data previously reported by Pereira et al. (2002) from the red imported fire ant, *Solenopsis invicta*; Yaman and Radek (2015) from the great spruce bark beetle, *Dendroctonus micans* (Kugelann) (Coleoptera: Scolytidae); and Baki (2016) from the common asparagus beetle, *Crioceris asparagi* (Linne) (Coleoptera: Chrysomelidae). Also, early, Weiser (1955) emphasized that spores occur in pairs are typical of *Mattesia* species.

On the basis of the size and morphology of the spores, this EPP is identified as belonging to the genus *Mattesia*; meanwhile, in order to know the type species of this genus, *Mattesia*, it was necessary to compare the recorded spore sizes, in Table 3, with those previously described species from the available reports on some coleopterous or lepidopterous stored-product hosts (Table 4).

According to spore dimensions shown in Table 3, *Mattesia*-spore sizes did not, in general, differ significantly among their infected four hosts from stored-grain insect pests. Spore measurements were ranged, in general, between 12.5 and 15.0 μm in length and 7.5–10.0 μm in width, and averagely measured from 14.45 to 15.08 μm by 9.15 to 9.85 μm (fresh-mounts), and from 13.95 to 14.50 μm by 7.50 to 8.35 μm (stained-mounts) (Table 3). These results may indicate that these spores which have been isolated from different stored-product insects, infesting the same arena, are most closely aligned to the same species of the genus *Mattesia*. Comparatively, such spore dimensions are a little bit larger than those previously described (Table 4) for *Mattesia dispora* (12.2 × 6.7 μm) from *Laemophloeus ferrugineus* or *L. minutes* (Finlayson 1950), but to a large extent are matchable with *M. dispora* (15.4 μm in length) from locusts (Žižka 1978). The spores were also, in their fresh or stained mounts, longer (Table 3) than fresh (12.0 × 7.0 μm) or stained (10.0 × 6.0 μm) spores of *M. oryzaephili* from *Oryzaephilus surinamensis* (Ormières et al. 1971), as well as they were longer than those of *M. trogodermae* (11–13 μm) from *Trogoderma granarium* (Hall et al. 1971) (Table 4).

M. dispora, *M. oryzaephili*, and *M. trogodermae* are EPP of the fat tissue of insect pests which inhabit stored products (Liu et al. 1974). Morphologically, the differentiating characteristics, spore size, and shape were helpful in distinguishing, tentatively, the present type species of the genus *Mattesia* from the abovementioned ones. Hence, based on the present results and the published

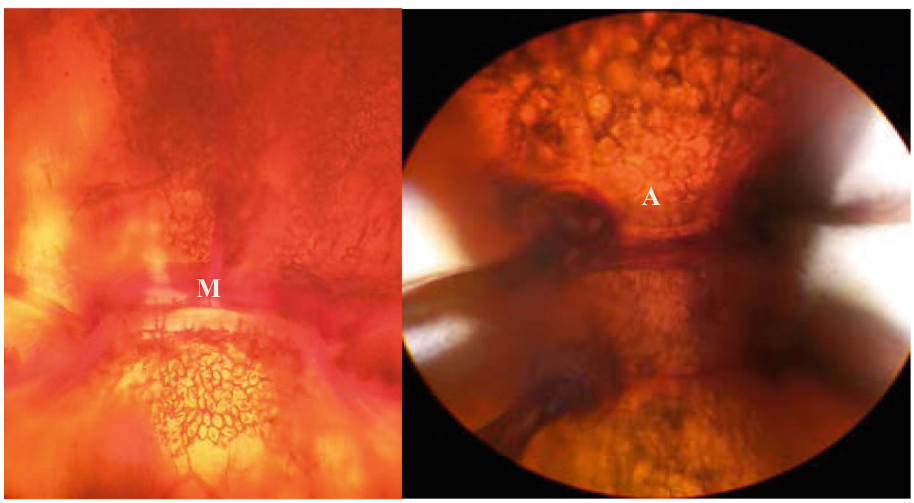

Fig. 6 Light microscope views of *Mattesia* spores (M) and *Adelina* oocysts (A) distinctly seen through the sternites of *Laemophloeus turcicus* beetle (×100 to ×400 magnification)

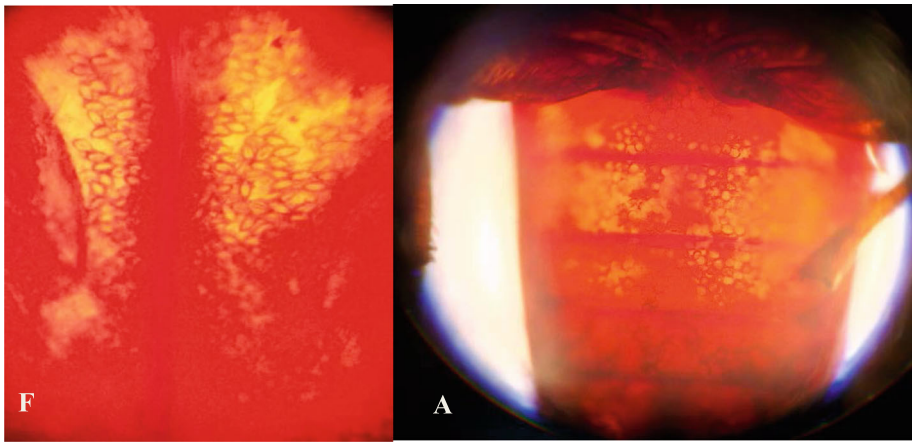

Fig. 7 Light microscope views of *Farinocystis* spores (F) and *Adelina* oocysts (A) distinctly seen via the sternites of *Tribolium castaneum* beetle (×100 to ×400 magnification)

ones, the studied lipotrophid entomopathogen could match *M. dispora*; however, confirming or rejecting this identification certainly needs further ultrastructural study on species classification.

In the available literature, there is no *Mattesia* record, in Egypt, from neither the four insect pests (*Laemophlous*, *Sitophilus*, *Rhyzopertha*, and *Plodia*) nor others which inhabit stored grains or their products. Hence, the present results may provide, for the first time, the Egyptian arsenal of microbial control agents with a promising EPP, *Mattesia*, that may be an important limiting biotic factor for a number of insect hosts in storage. Additionally, the remarkable natural prevalence (9–89%; Table 1) of this entomopathogen, *Mattesia*, along two successive years (2016 and 2017) and to date, among natural populations of the subject four storage insect pests, may reflect an evidence for a

Table 3 Average spore sizes (μm) of *Mattesia* sp., *Farinocystis* sp., and *Nosema* sp. isolated from certain stored-grain insect pests, in fresh and stained smears

Insect pest and its entomopathogenic protozoan	Mean spore size*, length ± SD (SE)** × width ± SD (SE) (range)
Laemophloeus–Mattesia	
Fresh	14.65 ± 0.88 (0.12) *a* × 9.85 ± 0.60 (0.08) *c* (12.5–15) × (7.5–10)
Stained	13.95 ± 1.20 (0.18) *b* × 7.6 ± 0.49 (0.07) *e* (12.5–15) × (7.5–10)
Sitophilus–Mattesia	
Fresh	14.80 ± 0.70 (0.10) *a* × 9.8 ± 0.99 (0.14) *c* (12.5–15) × (7.5–12.5)
Stained	14.40 ± 1.08 (0.15) *b* × 8.35 ± 1.20 (0.17) *f* (12.5–15) × (7.5–10)
Rhyzopertha–Mattesia	
Fresh	15.08 ± 0.92 (0.13) *a* × 9.25 ± 1.016 (0.16) *d* (12.5–17.5) × (7.5–10)
Stained	14.50 ± 1.01 (0.14) *b* × 7.55 ± 0.35 (0.05) *e* (12.5–15) × (7.5–10)
Plodia–Mattesia	
Fresh	14.45 ± 1.05 (0.15) *a* × 9.15 ± 1.20 (0.17) *d* (12.5–15) × (7.5–10)
Stained	14.15 ± 1.20 (0.17) *b* × 7.5 ± 0.0 (0.0) *e* (12.5–15) × (7.5)
Tribolium–Farinocystis	
Fresh	16.95 ± 1.05 (0.15) *g* × 12.0 ± 1.01 (0.14) *s* (15.0–17.5) × (10.0–12.5)
Stained	15.5 ± 1.01 (0.14) *h* × 10.1 ± 1.12 (0.16) *t* (15.0–17.5) × (10.0–12.5)
Rhyzopertha–Nosema	
Fresh	4.32 ± 0.51 (0.07) *m* × 2.04 ± 0.20 (0.03) *k* (4–6) × (2–3)
Stained	3.8 ± 0.40 (0.06) *n* × 2.0 ± 0.0 (0.0) *k* × (3–4) × (2)

*Number of measured spores ranged between 50 and 60 spore. The difference in spore lengths or widths between fresh and stained spores are, in general, significant (*t* test; significant at 5 or 1% level). Data followed by the same letter within column do not differ significantly at 1% level; Duncan's multiple range test
**Standard deviation of the mean (SD) and standard error (SE)

Table 4 Summary of spore sizes of *Mattesia* spp., *Farinocystis* spp., and *Nosema* spp. reported in literature from some coleopterous or lepidopterous stored-product hosts

Entomopathogenic protozoan species	Spore size (μm)	Coleopterous or lepidopterous hosts	References
Mattesia dispora	12.2 × 6.7 μm	*Laemophloeus ferrugineus*	Finlayson (1950)
		L. minutes	
M. oryzaephili	12 × 7 μm (fresh)	*Oryzaephilus surinamensis*	Ormières et al. (1971)
	10 × 6 μm (stained)		
M. trogodermae	11–13 μm (in length)	*Trogoderma granarium*	Hall et al. (1971)
Mattesia sp.	12.5–17.5 × 7.5–12.5 μm (fresh) 12.5–15 × 7.5–10 μm (stained)	*L. turcicus* *S. zeamais* *R. dominica* *P. interpunctella*	The present study (see Table 3)
Farinocystis tribolii	12.00–14.40 × 6.40–8.00 μm	*Tribolium castaneum*	Rabindra and Subramanian (1974)
	13.3–14.3 × 6.7–7.8 μm	*Tribolium garnhami*	Laird (1959)
	15.0–17.5 × 10.0–12.5 μm (fresh) 15.0–17.5 × 10.0–12.5 μm (stained)	*Tribolium castaneum*	The present study (see Table 3)
Nosema whitei	3.8–5.9 × 2.4–3.6 μm (fresh)	*Tribolium castaneum*	Milner (1972)
	2.9–3.8 × 1.7–2.7 μm (stained)		
	4.0–6.0 × 2.3–3.5 μm (fresh)	*T. confusum*	
	3.1–4.1 × 1.6–2.4 μm (stained)		
	3.0–3.9 × 1.8–2.7 μm (stained)	*T. anaphe*	
	4.0–5.2 × 2.5–3.6 μm (fresh)	*Oryzaephilus surinamensis*	
	2.7–3.7 × 1.8–2.7 μm (stained)		
	4.0–6 × 2–3 μm (fresh) 3.0–4 × 2 μm (stained)	*Rhyzopertha dominica*	The present study (see Table 3)

developing epizootic. Therefore, maintaining the protozoan disease in a population is a very requisite demand in this field of control. Fortunately, routes of infection by entomopathogenic protozoans may provide the practical solution of infecting a sufficient proportion of the population, especially through their common method of transmission by scavenger feeding on infected cadavers, where most stored-product insects are cannibalistic (Fig. 8) (Weiser 1963; Kellen and Lindegren 1971; Henry 1981).

On the other hand, this study provides a clear evidence that the isolated *Mattesia* sp. has a relatively broad host range that crosses two orders, Coleoptera and Lepidoptera, which include many damaging stored-grain insects (Lord 2003, 2006). That may reveal the importance of this neogregarine entomopathogen as a promising suppressor for a number of insect hosts in storage.

Farinocystis sp.

During the course of the present study, another naturally occurring lipotrophid entomopathogen, *Farinocystis*, was recorded among cadavers of the red flour beetle, *T. castaneum*. This entomopathogen was found in 50% and 36% of the examined samples of *T. castaneum* adults and larvae, respectively (Table 1). The morphology of the infective units, spores, has been observed with both the light and the scanning electron microscope (Fig. 5). The shape of such lipotrophid spores was navicular, and the surface sculpture was smooth (Fig. 5). The spores were seen as single forms and not formed in pairs within the gamontocyst, as the case of the abovementioned lipotrophid pathogen, *Mattesia* sp. (Fig. 2). Polar plugs were also seen (Fig. 5). By means of microscopic examinations at a magnification of ×100 to ×400, the presence of *Farinocystis* spores in infected cadavers of *T. castaneum* beetles was easily detected through the sternites of these beetles, especially via the abdominal sterna (Fig. 7).

The spores, in their fresh mounts, measure from 15.0 to 17.5 μm in length and from 10.0 to 12.5 μm in width, with an average of 16.95 ± 1.05 (or 0.15) μm by 12.0 ± 1.01 (or 0.14) μm [mean ± SD (or SE); $n = 60$], while in their stained mounts, they measure from 15.0 to 17.5 μm in length and from 10.0 to 12.5 μm in width, with an average of 15.5 ± 1.01 (or 0.14) μm by 10.1 ± 1.12 (or 0.16) μm (Table 3). These spore dimensions (Table 3) were longer and wider than those of *Farinocystis tribolii* (12.00–14.40 μm by 6.40–8.00 μm) from either *T. castaneum* (Rabindra and Subramanian 1974) or *T. garnhami* (13.3–14.3 μm by 6.7–7.8 μm) (Laird 1959) (Table 4). Weiser (1953) was the first to record *Farinocystis tribolii* on *T. castaneum*. The type species *F. tribolii* infects *Tribolium* spp. and *Tenebrio molitor*, while the type species

Fig. 8 Cannibalism (scavenger feeding), among most stored-product insects, as a desirable manner to acquire protozoan infection (×8 to ×40 magnification)

F. tenebrionides infects *Tenebroides muritanicus* (Purrini 1976). According to Sprague et al. (1992) that the host provides a further helpful criterion for differentiating species; therefore, the host affinity is generally recognized as a valid taxonomic character. As the neogregarine parasite, *F. tribolii* has early been described from *T. castaneum* by Weiser (1953); thus, based on the abovementioned criterion, the present *Farinocystis* might be described tentatively as *F. tribolii*; however, further study is needed to confirm or reject this finding. There are no available records on *F. tribolii* from *Tribolium* spp. in Egypt. Therefore, this *Farinocystis* sp. appears to be the first record of such entomopathogen on *T. castaneum* in Egypt. The relatively high rates of the recorded natural infection, 50% or 36%, due to the lipotrophid entomopathogen, *Farinocystis* sp. among *T. castaneum* adults or larvae, respectively (Table 1), could potentially be an encouraging source for the microbial control of this insect pest in storage.

Adelina sp.

The present study has recorded another third EPP, the coccidian, *Adelina* sp., from three storage insect pests: the red flour beetle, *T. castaneum*; the flour-mill beetle, *L. turcicus*; and the lesser grain borer, *R. dominica*. As shown in Table 1, the natural infection rate was low (14%) in the examined samples of *L. turcicus* adults, while those of *T. castaneum* adults and larvae or *R. dominica* adults were higher (27 and 60% or 34%, in respect) than the corresponding rate for *L. turcicus*.

The presence of the characteristic adeleid polysporocystic oocysts in their fresh or stained mounts was the evidence of the coccidian infection (Fig. 4). Meanwhile, by means of the

light microscopy, at a magnification of ×100 to ×400, *Adelina* oocysts were easily seen via the sternal side of infected-adults of both *L. turcicus* and *T. castaneum* (Figs. 6 and 7), but with *R. dominica*-infected adults, this procedure was not helpful as the adult integument is heavily sclerotized. Seemingly, this procedure is useful in mass production program of the entomopathogenic coccidian, *Adelina*, and neogregarines, *Mattesia* or *Farinocystis* from their insect hosts which possess less sclerotized integuments, as in *Laemophloeus* or *Tribolium* adults in the present study.

Three species of the genus *Adelina* have been described from tenebrionid beetles. One is *A. tenebrionis* from *T. molitor* (Sautet 1930); the second one is *A. tribolii* from *Tribolium ferrugineum* (Bhatia 1937); and the third species is *A. castana* from *T. castaneum* (Ghosh et al. 2000). Therefore, to decide whether the present *Adelina* species is belonging to *A. tribolii* or *A. castana* or may be to another different species, future study should be carried out to verify such taxonomal status.

Although *Adelina* spp. have been described from *Tribolium* spp. and other coleopterous pests in storage, as abovementioned, but in the literature, there are no reports on such a coccidian parasite, *Adelina* from *L. turcicus* or *R. dominica*. There is only one report from Kosova, Yugoslavia by Purrini (1977) about *A. tribolii* which was found infecting *L. ferrugineus*. Therefore, this study appears to be the first report of this coccidian entomopathogen on *L. turcicus* and *R. dominica* as well as its first record from *T. castaneum*, *L. turcicus*, and *R. dominica* in Egypt.

Adelina-natural infection rates presented in Table 1 (14–60%) could nominate this entomopathogen as an important third limiting biotic factor for the studied storage insect hosts. Such a relatively broad host range of the coccidian entomopathogen, *Adelina* sp., offers a reasonable possibility for its role to suppress some of the most abundant and damaging insect pests of stored grains or their products. A similar supportive result has been reported by Henry (1981) for the coccidian *A. tribolii* which infects several stored-product pests and causes striking epizootics in *Tribolium* populations. The author recorded infection rates of 68%, indicating that this coccidian pathogen is an important factor in the regulation of laboratory and natural populations of *Tribolium* spp.

Nosema sp.

The microsporidian entomopathogen, *Nosema* sp. (Fig. 3), is the fourth entomopathogenic protozoan recorded in this study. This nosematid entomopathogen has been isolated only from adults of the lesser grain borer, *R. dominica*. As shown in (Table 1), the *Nosema*-infection rate, among the examined naturally dead adults, was low where 83 of 775 adult cadavers were found to be infected by this entomopathogen with a prevalence of about 11%. Based on reviewing the available literature, the microsporidian entomopathogen, *Nosema* sp., observed in this study seemingly is the first record from *R. dominica* in Egypt, but also the second record on the basis of the other countries. Lipa (1968) was the only author that reported *Nosema weiseri* sp. n. from *R. dominica*.

Microscopic examination of *R. dominica* adult cadavers revealed the presence of microsporidian spores. When the infected beetles were squashed in Ringer solution, a huge number of the characteristic *Nosema* spores (Fig. 3) were released on the microscopic slide.

The isolated *Nosema* sp. was characterized for identification according to its spore morphology. When *Nosema* spores were examined by the light or the scanning electron microscope, their shapes appeared to be oval with a smooth surface sculpturing. The polar filament of *Nosema* was also observed (Fig. 3). Fresh spores measured 4.32 ± 0.51 (or 0.07) μm (4–6 μm) in length and 2.04 ± 0.20 (or 0.3) μm (2–3 μm) in width (mean ± SD (or SE) (range), while the corresponding spore dimensions in their Lactophenol preparations were 3.8 ± 0.40 (or 0.06) μm (3–4 μm) in length and 2.0 ± 0.0 (or 0.0) μm (2 μm) in width (Table 3). The differences between spore sizes measured from fresh spores in Ringer solution smears were not consistent with those measured from stained spores in Lactophenol smears (*t* test; significant at 5% level). As seen in Tables 3 and 4, the *Nosema* spore size for *R. dominica*, whether from fresh or stained smears, seemingly not moderately differs from those previously studied by Milner (1972) in other storage insect hosts, *Tribolium* spp. and *O. surinamensis* (Table 4). Yaman and Radek (2003) reported the spore dimension as a good character for comparison *Nosema* species. Unfortunately, the spore size of *N. weiseri* which has been reported by Lipa (1968) from *R. dominica* is not available. Based on the present spore measurements from infected—*R. dominica* adults, in Table 3, as well as those published by Milner (1972) for *Tribolium* spp. and *O. surinamensis* (Table 4); also, taking into consideration the findings of Milner (1972) which reveal that the effect of host species on the *Nosema* morphology, including spore size, is insignificant, therefore, the present study may suggest *Nosema whitei* as a tentative type species of the genus *Nosema*. However, confirming or rejecting this suggestion certainly needs further ultrastructural study on species classification. In this concern, Yaman and Radek (2003) had considered the spore as the most important life cycle stage for the identification of microsporidia by ultrastructural studies or host range studies.

Summarily, the natural occurrence of protozoan diseases, with low or high infection rates (9–89%), among insect pest populations of stored grains is documented in the present study. Four EPP belong to different genera, *Mattesia*, *Farinocystis*, *Adelina*, and *Nosema*, were

recorded, for the first time in Egypt, from economically destructive five stored-product insect pests (*L. turcicus*, *T. castaneum*, *R. dominica*, *S. zeamais*, and *P. interpunctella*). Cadavers of the subject protozoan-infected stored-grain insect pests seemingly act as reservoirs full of huge numbers of protozoan infective units, spores, or oocysts. They could easily be seen through hosts' sterna that possess a less sclerotized integument (e.g., *L. turcicus* and *T. castaneum*). Also, from the microbial control standpoint, a periodic isolation for the local strain(s) of the entomopathogenic protozoans associated with stored-grain insect pests is important for monitoring such promising biological control agents.

Conclusions

Stored insect pests harbor promising varieties of (EPP), including neogregarines (e.g., *Mattesia* spp. and *Farinocystis* spp.), coccidia (e.g., *Adelina* spp.), and microsporidia (e.g., *Nosema* spp.). The protozoan natural infection rates among populations of some coleopterous and lepidopterous stored-grain pests range from relatively low incidences to high or epizootics. From the microbial control standpoint, a periodic isolation for the local strain(s) of the entomopathogenic protozoans associated with stored-grain insect pests is important for monitoring such promising biological control agents. Hence, Egypt could possess its own arsenal of local microbial control agents (i.e., EPP or others) to suppress, through natural or applied measures, the populations of insect pests in storage.

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