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Specified Ultrastructural Data on *Tubulinosema maroccanus* Comb. Nov. (*Nosema maroccanus* Krilova et Nurzhanov, 1987) (Microsporidia) from the Moroccan Locust *Dociostaurus maroccanus* (Orthoptera)

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Summary. *Nosema maroccanus* Krilova et Nurzhanov, 1987 was isolated from the Moroccan locust *Dociostaurus maroccanus* Thunb. in Uzbekistan. Its description, based on one of the first ultrastructural studies of microsporidia from orthopteran hosts, lacked information on the details of cell surface structure, which are considered to be very important in modern taxonomy. We have re-investigated the material upon which the original description of *N. maroccanus* was based and revealed new ultrastructural characters of its cell surface. Late meronts are surrounded with microtubuli, about 20 nm in diameter, attached to the membrane, later replaced with electron dense granules, forming a typical coating of the sporont's surface (the primordium of exospore). Mature spores are also surrounded with a layer of microtubuli. Host cell cytoplasm contains small vesicles with electron-dense cores, up to 50 nm in diameter, surrounding late meronts, sporogonial stages and spores of the parasite. Polar tube is slightly anisofilar. These characters disagree with the diagnosis of *Nosema*, but fit that of the recently established genus *Tubulinosema.* Hence, we transferred the species studied into this genus, establishing *Tubulinosema maroccanus* comb. nov. Analysis of cell surface ultrastructure of two *Tubulinosema* species from Orthoptera showed that they shared two characters not mentioned in the diagnosis of the genus. Therefore, the diagnosis of *Tubulinosema* was modified to include two more characters: presence of vesicles with electron-dense cores surrounding sporogonial stages and presence of microtubuli not only in late meronts, but also, in some species, in spores.

Key words: Microsporidia, *Tubulinosema*, *Nosema*, locust, ultrastructure.

INTRODUCTION

The microsporidium *Nosema maroccanus* Krilova et Nurzhanov, 1987 was isolated from the Moroccan locust *Dociostaurus maroccanus* Thunb. in Uzbekistan. Its description contained fine morphology of the diplokaryon, the bipartite polaroplast and the polar tube (Krilova and Nurzhanov 1987). It was one of the first investigations of *Nosema* species from orthopteran hosts to be conducted at the ultrastructural level, the only other two being that of *N. locustae* from *Locusta migratoria* (Huger 1960), which contained incomplete data, and that of *N. cuneatum* from the grasshopper *Melanoplus sanguinipes*, which was published in the same year as the study of *N. maroccanus* (Streett and Henry 1987). Therefore, comparative analysis of fine

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structure of these microsporidia was not possible at that time. Since then, five other *Nosema* and *Nosema*-like microsporidia from orthopteran hosts have been described at the ultrastructural level: *Nosema pyrgomorphae* (Toguebaye *et al.* 1988, Lange *et al.* 1992), *N. montanae* (Wang *et al.* 1991), *Tubulinosema acridophagu*s (= *N. acridophagu*s) (Streett and Henry 1993), *Paranosema grylli* (= *N. grylli*) (Sokolova *et al.* 1994, 2003), *P. locustae* (= *N. locustae*) (Sokolova and Lange 2002).

The original description of *N. maroccanus* lacks some characteristics that are being used in modern taxonomy of the group. In particular, it contains insufficient information on the cell surface structure. At present the fine structure of the microsporidian cell surface, the interface between the parasite and its host, is generally recognized to be of high taxonomic value (Issi *et al.* 1993, Cali *et al.* 1998, Canning *et al.* 2002, Slothouber Galbreath *et al.* 2004, Franzen *et al.* 2005, 2006a, b).

We have re-examined the material upon which the original description of *N. maroccanus* was based. In this paper we present new ultrastructural data, re-describe this species and transfer it into the recently established genus *Tubulinosema* (Franzen *et al.* 2005). We also propose minor additions to the genus diagnosis.

MATERIALS AND METHODS

The present study is based on re-examination of the material obtained by Krilova and Nurzhanov (1987). Nine specimens (6 males and 3 females) of the Moroccan locust *D. maroccanus* were collected in the desert zone of Uzbekistan in 1985. Two of the females were found to be heavily parasitized by microsporidia, described as *N. maroccanus*. The present study includes re-investigation of the archive Giemsa-stained smears and preparation of new ultra-thin sections and TEM microphotographs.

The protocol for embedding of infected tissues, used by Krilova and Nurzhanov (1987), was as follows: small portions of infected host tissue were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 1–2 h and postfixed in 1.0% buffered osmium tetroxide for 1 h. The tissues were dehydrated in an ethanol series and in absolute acetone and embedded into Epon or Araldite resin. Ultrathin sections were prepared from these embeddings using Reichert Ultracut ultramicrotome (Germany). Sections were stained with 2.0% uranyl acetate in 50% ethanol and with Reynold's lead citrate and examined in Hitachi H-300 electron microscope (Japan).

An attempt to amplify SSU rDNA gene fragment of *T. maroccanus* with *V1f : 530r* primer set using guanidine thiocyanate and phenol/chloroform DNA extracts from archive Giemsa-stained smears, using the modified protocol of Hylis *et al.* (2005), was undertaken.

RESULTS

Light microscopy. In Giemsa-stained smears meronts and sporonts are spherical, with one (rarely two) diplokaryotic nuclei. Chains consisting of three to four cells are sometimes observed. Spores are binucleate, oval or ovoid, 3.8–4.4 × 2.5–3.1 µm in size (the mean value 3.9 × 2.7 µm, n = 50); the spore index (length to width ratio) is 1.4 (Fig. 1).

Transmission electron microscopy. Meronts are oblong or spherical, usually 3.2–3.7 × 2.2–2.5 µm in diameter (mean value 3.5 × 2.4 µm, n = 10), with diplokaryon measuring 2.0 × 1.6 µm (mean value). The electron-transparent cytoplasm contains scattered cisternae of the endoplasmic reticulum. Meronts divide by binary fission; sometimes three or four cells form chains (Fig. 2). In late meronts, an additional external layer is formed, attached to the cell surface and consisting of tubular structures, referred to as small tubuli or microtubuli in Franzen *et al.* (2005), about 20 nm in diameter, parallel to each other, and electron-dense granules, separating these microtubuli (Figs 3, 4).

During transformation from meront to sporont, electron-dense granules become more abundant, forming an interrupted layer on the outer surface of plasma membrane, while microtubuli disappear. Then the electron-dense granules fuse, producing a continuous layer, characteristic of sporonts.

Small vesicles measured up to 50 nm in diameter with electron-dense cores are found in the host cell cytoplasm, surrounding late meronts and subsequent sporogonial stages (Figs 5, 7, 8, 10, 12). In some sections tubular structures of the same diameter as electron-dense cores of vesicles are observed; this suggests that the cores may be, in fact, cross-sections of the tubular structures.

Late sporonts possess a continuous electron-dense external layer on the plasma membrane. Their shape gradually transforms from spherical to ovoid, characteristic of the sporoblast. In the latter the diplokaryotic nuclei are located along the long axis. Late sporoblasts are irregularly shaped and poorly preserved on TEM sections (Fig. 6). Their electron-dense cytoplasm, masking the internal cell structures, obscures further observations.

Young spores are ovoid, 3.0 × 2.1 µm in size (mean value). The endospore is not developed. The primordial extrusion apparatus with the first few coils of the polar

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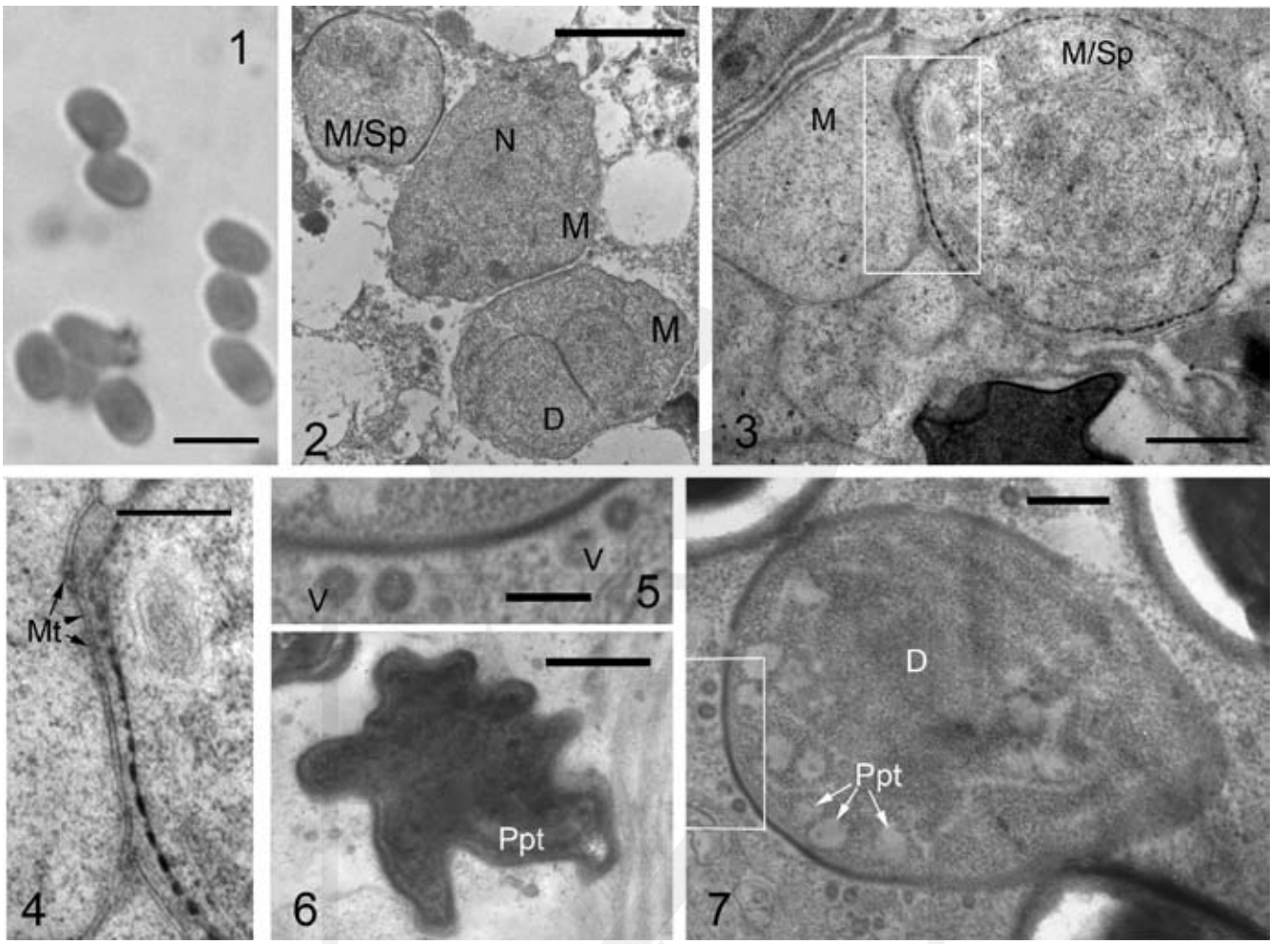

Figs 1–7. Morphology and ultrastructure of *Tubulinosema maroccanus*. Light microscopy. **1** – Giemsa-stained smear with the spores. TEM. Merogonial and sporogonial stages; **2** – chain of meronts; **3** – meront and transitional meront/sporont stage; **4** – an enlarged detail of Fig. 3. Cross-section of microtubuli (arrows) on the surface of meront; **5** – an enlarged detail of Fig. 7. Vesicles with electron-dense cores in the host cell cytoplasm, surrounding the parasite's cell; **6** – sporoblast. Arrows indicate primordium of polar tube; **7** – young spore with primordial polar tube. D – diplokaryon, M – meront, M/Sp – transitional meront/sporont stage, Mt – microtubuli, N – nucleus, V – vesicles with electron-dense cores. Scale bars: 5.0 μm (1), 3.0 μm (2), 1.0 μm (3, 6), 0.5 μm (4, 7), 0.25 μm (5).

tube is visible (Fig. 7). Similarly to the late meronts and subsequent sporogonial stages, the young spores are surrounded by vesicles with electron-dense cores in the host cell cytoplasm (Figs 5, 7).

Mature spores are ovoid and measure 3.2–3.4 × 1.5––1.9 μm (mean value 3.3 × 1.7 μm, n=10) (Figs 8–15). The anchoring disc is 250–300 nm in diameter (Fig. 14). The polar sac covers only the anterior part of the polaroplast, which occupies almost a half of the spore. The polaroplast is bipartite, with the anterior tightly-lamellar part and the posterior tubular part. Diplokaryotic nuclei are located along the long spore axis and are surrounded by 3 rows of polyribosomes. The polar tube is slightly anisofilar; its coils are arranged in one row. Number of coils is from 12 to 15, usually 13. Nine to ten anterior coils are 100–120 nm in diameter, 3–4 posterior coils are 60–80 nm in diameter (Figs 8, 15). The angle between the first coil of the polar tube and the long axis of the spore varies from 70° to 90°. The posterior vacuole is very small. The endospore is relatively thick (up to 150 nm). In the beginning, the exospore of maturing spore is heavily granulated and relatively thick (40––60 nm) (Fig. 7). Then the thick layer of heavily granulated exospore becomes a two-layered structure with the

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Table 1. Microsporidia of the genus *Tubulinosema* and orthopteran microsporidia with *Nosema*-like life cycle.

Microsporidian species	**Type host** and host range	Infected tissue	Spore shape and size (µm)	Polar tube	Polaroplast	Surface ornamentation	References
Tubulinosema ratisbonensis Franzen *et al.* 2005	***Drosophila melanogaster*** (Diptera: Drosophilidae)	FB, Mg, G, Mpt, Mu, N, SG	slightly pyriform 4.2 × 2.5*; 3.9 × 2.4**	9–14, slightly anisofilar	no data	additional layer of membrane-bound small tubular elements (in M)	Franzen *et al.* 2005
T. acridophagus (Henry 1967) Franzen *et al.* 2005	***Schistocerca americana***, *Melanoplus* spp. (Orthoptera: Acrididae), *Helicoverpa zea* (Lepidoptera: Noctuidae)	Mg, G, P, N, FB	ovoid with a narrowed anterior pole 4.1 × 2.6*; 3.9 × 2.5**	10–12, slightly anisofilar	lamellar	plasma membrane covered by a layer of tubular elements, ∅ 35 nm (in M)	Henry 1967, Henry and Oma 1974, Streett and Henry 1993
T. kingi (Kramer 1964) Franzen *et al.* 2005	***Drosophila willistoni*** (Diptera: Drosophilidae)	Mg, G, Mpt, FB	oval 4.3 × 2.6*; 3.7 × 2.1**	10–14, slightly anisofilar	lamellar	surface coat of tubular elements, ∅ 21–37 nm (in M)	Burnett and King 1962, Kramer 1964, Armstrong *et al.* 1986, Franzen *et al.* 2006b
T. maroccanus (Krilova et Nurzhanov 1987)	***Dociostaurus maroccanus*** (Orthoptera: Acrididae), *Calliptamus italicus* (Orthoptera: Catantopidae), Lepidoptera	Generalized infection	ellipsoidal 4.6 × 3.2*; 3.9 × 2.7**	12–15 slightly anisofilar	bipartite: lamellar and tubular parts	additional layer, formed by microtubuli, ∅ ca 20 nm (in M, S); small vesicles, ∅ up to 50 nm with electron-dense cores (in SS, S)	Krilova and Nurzhanov 1987, 1989, present paper
Paranosema grylli Sokolova *et al.* 1994, 2003	***Gryllus bimaculatus*** (Orthoptera: Gryllidae)	FB, H, P, G	ovo-cylindrical 4.5 × 2.2*; 4.3 x 2.0**	15–18, isofilar, arranged in 1–2 rows	bipartite: lamellar and vesicular parts	no specific surface ornamentation	Sokolova *et al.* 1994, 2003
P. locustae (Canning 1953) Sokolova *et al.* 2003	***Locusta migratoria*** (Orthoptera: Acrididae) and over 100 species of orthopteran hosts	FB, may produce generalized infection, never infects MG and G	oval 5.2 × 2.8*	17–18 isofilar	bipartite, two lamellar parts	no specific surface ornamentation	Canning 1953, 1962; Sokolova and Lange 2002
Nosema cuneatum Henry 1971	***Melanoplus*** spp., *Schistocerca americana* (Orthoptera: Acrididae), *Helicoverpa zea* (Lepidoptera: Noctuidae)	P, FB, G, T, Mg, Mpt, N	oval to cuneate 4.8 × 3.4*; 4.4 × 2.9**;	10–12	no data	intermittent layer of vesicles with electron-dense cores (in M, ESt)	Henry 1971, Henry and Oma 1974, Streett and Henry 1987
N. chorthippi[a] Issi et Krylova 1987	***Chorthippus albomarginatus*** (Orthoptera: Acrididae)	FB	3.5 × 1.9*	No data	no data	no data	Issi and Lipa 1968; Issi and Krylova 1987
N. pyrgomorphae Toguebaye, Seck et Marchand 1988	***Pyrgomorpha conica*** (Orthoptera: Pyrgomorphidae)	Mg, FB, Mu	cylindrical 3.9 × 2.0*	7–9	bipartite, two lamellar parts	no specific surface ornamentation	Toguebaye *et al.* 1988
N. montanae Wang, Streett et Henry 1991	***Melanoplus packardii*** (Orthoptera: Acrididae)	FB	ovo-cylindrical 3.1 × 1.5*; 2.8 × 1.4**	5–7	no data	electron-translucent vesicles in close juxtaposition to parasites (in M/St)	Wang *et al.* 1991
N. trilophidiae Wen et Li 1993	***Trilophidia annulata mongolica***, *Oedaleus decorus, Locusta migratoria* (Orthoptera: Acrididae)	SG, Mg, G, H, N, T, FB	3.7 × 1.6*	8	no data	no data	Wen 1996
N. asiaticus Wen 1996	***Oedaleus asiaticus*** (Orthoptera: Acrididae), various orthopteran and lepidopteran hosts	Mg, GC, G, Mpt, FB	ellipsoidal 4.2 × 1.8*	11	no data	no data	Wen 1996

H – haemocytes; FB – fat body; G – gonads; GC – gastric caeca; Mu – muscles; Mg – midgut; Mpt – Malpighian tubules; N – nervous tissue; P – pericardium; SG – salivary glands; T – trachea; M – meronts; LM – late meronts; ESt – early sporonts; SS – sporogonial stages; S – spores; M/St – transitional meronts/sporont stages

* fresh spores, ** fixed and Giemsa-stained spores

[a] *Nosema chorthippi* was not studied at ultrastructural level. TEM characters for this species are given erroneously by Sokolova and Lange (2002, Table 1).

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outer layer of low electron density and the inner one of high electron density (Fig. 11). Later, the electron-dense layer of the exospore of the mature spore is covered by a layer of microtubuli, the structure and diameter of which are the same as in late meronts (Figs 8, 12). Tangential section through the exospore clearly indicates the tubular structure of this layer (Figs 8, 9). The microtubuli sometimes form long bundles, up to 500 nm long, directed into the host cell cytoplasm (Fig. 13). Vesicles with electron-dense cores in the host cell cytoplasm are also found around the mature spores (Figs 8, 10).

DISCUSSION

A concise characteristic of the genus *Nosema* (monomorphic disporoblastic microsporidia, diplokaryotic during the entire life cycle, developing in direct contact with the host cell cytoplasm without a sporophorous vesicle) was for a long time sufficient for taxonomic purposes and no new characters seemed necessary. However, with the emergence of molecular phylogenetic methods and more refined TEM techniques in the last two decades, the genus *Nosema* was split.

The most important morphological characters obtained with the use of improved TEM techniques concern the cell surface ultrastructure. Microsporidia are highly specialized intracellular parasites and all their life processes are intimately intertwined with those of the host cell. The parasite's cell surface is an interface between the microsporidian and its host, and its ultrastructure reflects the specificity of their interactions. The presence of structures incrusting the parasite's cell surface (e.g. microtubuli, electron-dense granules, spiky extensions) served as a basis for establishment of several new genera: *Anncaliia* (Issi *et al.* 1993, Franzen *et al.* 2006a), *Bryonosema*, *Pseudonosema* and *Trichonosema* (Canning *et al.* 2002), *Fibrillanosema* (Slothouber Galbreath *et al.* 2004) and *Tubulinosema* (Franzen *et al.* 2005, 2006b). The rDNA analysis confirmed that these species are distant from the "true" *Nosema* (*N. bombycis* and the closely related species, which occur in *Nosema*/*Vairimorpha* clade in the molecular trees).

Orthopteran hosts are parasitized by representatives of three genera of monomorphic diplokaryotic disporoblastic microsporidia: *Nosema*, *Paranosema* (= *Antonospora*) and *Tubulinosema* (Table 1). *Nosema maroccanus*, re-investigated in this paper, differs from both *Nosema* and *Paranosema* described from orthopteran hosts in the following important characters: the presence of the slightly anisofilar polar tube and the presence of microtubuli on parasite's cell surface and vesicles in host cell cytoplasm surrounding late meronts, sporogonial stages and spores. These characters are typical of *Tubulinosema*. Therefore, we propose to transfer *N. maroccanus* into the genus *Tubulinosema*. Unfortunately, there are no data on its rDNA (our attempts to amplify DNA from the archive samples failed), but, in our opinion, the morphological features presented are sufficient to justify this transfer.

Tubulinosema maroccanus comb. nov. is most similar in its ultrastructure and biological characters to *T. acridophagus*, another former *Nosema* species. Described as *N. acridophagus* Henry 1967 from the grasshoppers *Melanoplus* spp. and *Schistocerca americana*, it was transferred into *Tubulinosema* on the basis of rDNA phylogenetic analysis (Franzen *et al.* 2005). *T. marrocanus* has a slightly anisofilar polar tube. Although the polar tube in *T. acridophagus* was reported to be isofilar, the diameter of the polar tube cross-sections ranged from 75 to 105 nm. Moreover, it can be seen in Fig. 11 (Streett and Henry 1993) that the two posterior polar tube coils in *T. acridophagus* have a smaller diameter than the anterior ones, i.e. it can be regarded as slightly anisofilar as well. The sporogonial stages of these two species are surrounded by small vesicles with electron-dense cores. Both species cause a generalized infection of their hosts and both can invade insects other than orthopterans (e.g. Lepidoptera) and successfully develop in them.

We observed microtubuli in late meronts and spores of *T. maroccanus*, while in the descriptions of *T. acridophagus* (Streett and Henry 1993, as *Nosema*) and *Tubulinosema* spp. (Franzen *et al.* 2005, 2006b) microtubuli were mentioned as structures attached to the surface of meronts only. However, the same characteristic surface structures as in *T. maroccanus* (Figs 8, 11–12) can be also seen in spores of *T. acridophagus* (Streett and Henry 1993, Fig. 11). We consider that the presence of microtubuli around the spores should be added to the diagnosis of the genus.

In our opinion, all *Tubulinosema* species possess one more common character, which is not mentioned in the diagnosis of the genus: the presence of small vesicles with electron-dense cores surrounding the parasite cells. Such vesicles surround late meronts, sporogonial stages and spores of *T. maroccanus* (Figs 5, 7, 8, 10)

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and can be found around the parasite cells in the published images of sporogonial stages of *T. acridophagus* (Streett and Henry 1993, Figs 8–9) *T. ratisbonensis* (Franzen *et al.* 2005, Figs 29, 33), and *T. kingi* (Franzen *et al.* 2006b, Figs 3F, 4, 5).

On the strength of the above, we suggest the following two minor modifications of the diagnosis of the genus *Tubulinosema* (indicated below in bold italic), approved by Dr. Franzen (University of Regensburg, Germany).

DIAGNOSIS

***Tubulinosema* Franzen, Fischer, Schroeder, Schölmerich et Schneuwly, 2005, emend.**

Nuclei are in diplokaryotic arrangement during the life cycle. All stages are in direct contact with the host cell cytoplasm. No sporophorous vesicle present at any point in the developmental cycle. Merogonial and sporogonial division probably by binary fission of cells. No plasmodial stages. Slightly anisofilar polar tube arranged in one or two rows. Small tubuli present on the surface of late meronts ***and in some species on the surface of spores. Sporogonial stages surrounded by small vesicles with electron-dense cores.*** Spores are oval or slightly pyriform. Thick endospore wall, thinner over anchoring disc.

Type species: *Tubulinosema ratisbonensis* Franzen, Fischer, Schroeder, Schölmerich et Schneuwly, 2005

***Tubulinosema maroccanus* (Krilova et Nurzhanov, 1987) comb. nov.**

Monomorphic and diplokaryotic. Merogonial and sporogonial division by binary fission. The cell surface of late meronts and mature spores with an external layer of microtubuli. Late meronts, sporogonial stages and spores surrounded by small vesicles with electron-dense cores in the host cell cytoplasm. Mature spores in fresh preparations ovoid, 4.4–5.0 × 2.5–3.8 µm in size. Polar tube slightly anisofilar, as a rule with 13 (12–15) coils in a single row.

Type host: Moroccan locust *Dociostaurus maroccanus* Thunb. (Orthoptera: Acrididae)

Infection site: generalized infection with spore production in all tissues and organs, including antennae, eyes, ovaries, developing and mature eggs.

Type locality: Uzbekistan, Kashka-Darja region.

Deposition of type specimens: the slides with Giemsa-stained smears are deposited at the State Collection of Entomopathogenic and Phytopathogenic Microorganisms and their Metabolites affiliated to the All-Russian Institute for Plant Protection RAAS (Podbelsky sh. 3, 196608 St. Petersburg, Pushkin, Russian Federation). Deposition number NS-TM-R-1987.

Differential diagnosis (comparison with *T. acridophagus*, another *Tubulinosema* species from orthopteran hosts). The shape and dimensions of spores of these two species differ: fresh spores of *T. maroccanus* (4.6 × 3.2 µm, index 1.4) are larger and broader than those of *T. acridophagus* (4.1 × 2.6 µm, index 1.6). The number of polar tube coils is slightly different: 10–12 in *T. acridophagus* and 12–15 in *T. maroccanus*. Polaroplast of *T. maroccanus* is bipartite, consisting of an anterior lamellar and a posterior tubular part, in contrast to the lamellar polaroplast described in the other species. Propagation of *T. maroccanus* does not induce melanized tumor-like growths at infection loci, characteristic of *T. acridophagus*.

Interactions of *T. marrocanus* with its insect hosts were studied by Krylova and Nurzhanov (1989, as *N. marrocanus*)**.** In its natural host *D. marrocanus a* generalized infection was observed: the spores were found in all the tissues and organs, including eyes and antennae; the eggs were heavily infected, too. Abdomen of the infected individuals was darker than usual and red-brown spots could be seen under the cuticle. There were, however, no signs of tumor growths. Under the laboratory conditions, 2nd instar larvae of the Italian locust *Calliptamus italicus* (Orthoptera: Acrididae) were susceptible to infection with spores isolated from the Moroccan locust. However, *T. maroccanus* spores, either isolated from the original host or passed through the Italian locust, were not infectious to the 2nd instar larvae of the migratory locust *Locusta migratoria* (Orthoptera: Acrididae). On the other hand, *T. maroccanus* spores were found to be infectious to larvae of the cabbage butterfly *Pieris brassicae* (Lepidoptera: Pieridae) and the cabbage armyworm *Barathra brassicae* (Lepidoptera: Noctuidae) (Krylova and Nurzhanov 1989).

Synopsis of the genus *Tubulinosema*

Tubulinosema ratisbonensis Franzen, Fischer, Schroeder, Schölmerich et Schneuwly, 2005
Tubulinosema acridophagus (syn. *Nosema acridophagus,* Henry 1967) Franzen, Fischer, Schroeder, Schölmerich et Schneuwly, 2005
Tubulinosema kingi (syn. *Nosema kingi* Kramer, 1964) Franzen, Fischer, Schroeder, Schölmerich et Schneuwly, 2005
Tubulinosema maroccanus (syn. *Nosema maroccanus,* Krilova et Nurzhanov 1987) comb. nov.

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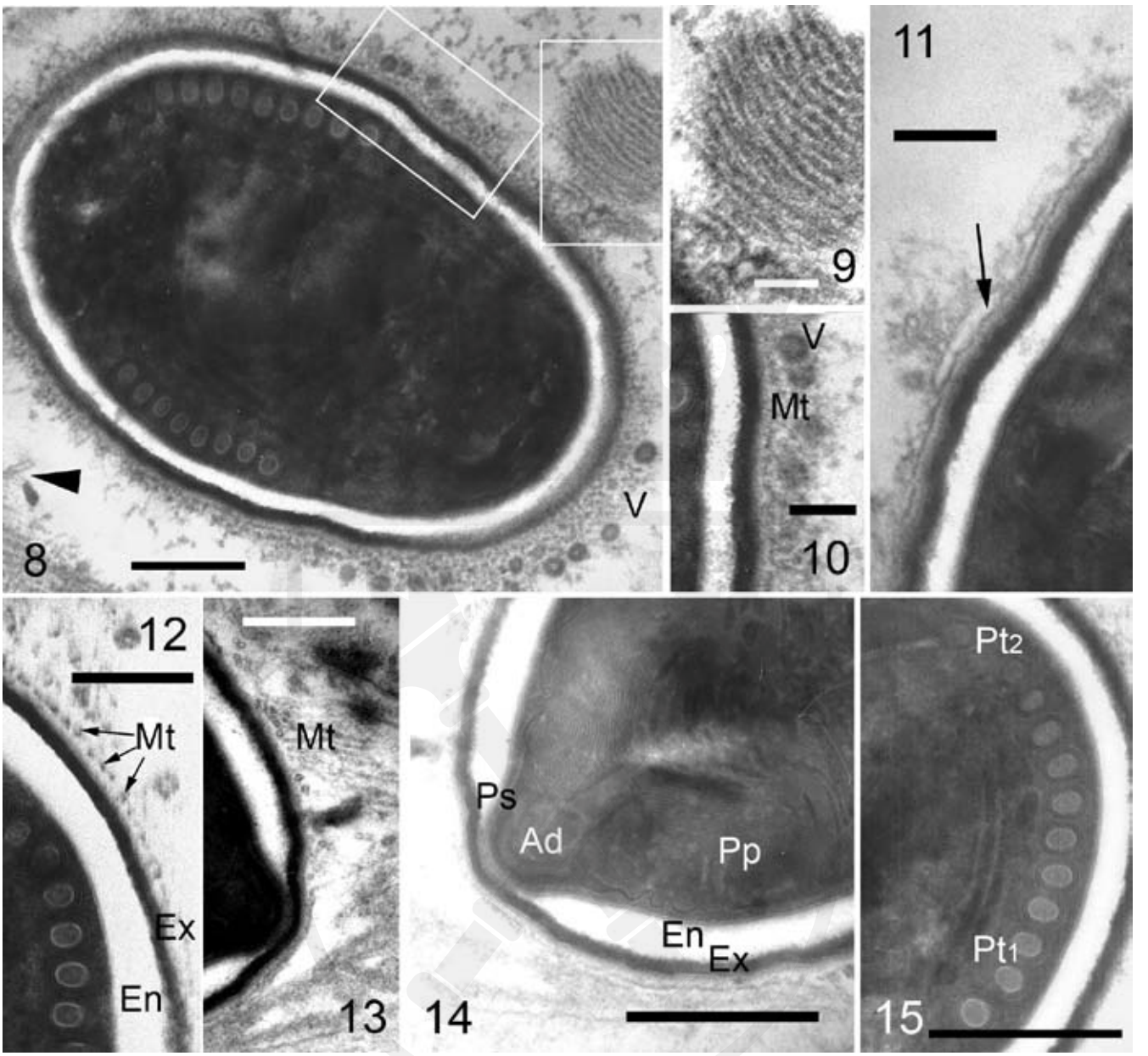

Figs 8–15. Ultrastructure of *Tubulinosema maroccanus*. TEM. Spores. **8** – mature spore with characteristic surface ornamentation. Note external layer composed of microtubuli and vesicles with electron-dense cores surrounding the exospore; **9** – an enlarged detail of Fig. 8. Tangential section through the tubular elements ornamenting the exospore of a neighbour spore; **10** – an enlarged detail of Fig. 8. Small vesicles with electron-dense cores surrounding the spore; **11** – structure of two-layered exospore; **12** – external tubular layer (arrowed) and vesicles adjacent to the exospore; **13** – microtubuli form a bundle, directed into the host cell cytoplasm; **14** – anterior half of the mature spore. Polar sac, anchoring disc and bipartite polaroplast; **15** – slightly anisofilar polar tube with 10 coils of large diameter and 3 coils of smaller diameter in a single row. Ad – anchoring disk, En – endospore, Ex – exospore, Pp – polaroplast, Ps – polar sack, Pt – polar tube, Pt_1 – polar tube coils with greater diameter, Pt_2 – polar tube coils with smaller diameter. Other abbreviations are as in Figs 1–7. Scale bars: 0.5 μm (**8**, **13–15**), 0.25 μm (**9–12**).

Only a few *Nosema* species from orthopteran hosts were studied in detail at the ultrastructural level: *N. cuneatum* (Streett and Henry 1987), *N. pyrgomorphae* (Toguebaye *et al.* 1988, Lange *et al.* 1992) and *N. montanae* (Wang *et al.* 1991). Additional structures ornamenting the cell surface were described in two of them (Table 1): *N. cuneatum* had an intermittent layer around meronts and sporonts, containing small vesicles with electron-dense cores (Streett and Henry 1987, Figs 16–19), and *N. montanae* had vesicular structures in the host cyto-

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plasm in close juxtaposition to the parasite (Wang *et al.* 1991, Fig. 25). In both cases it was suggested that these vesicles were involved in the thickening of the parasite's plasma membrane and the formation of primordial exospore in the course of transition from merogony to sporogony.

On the basis of the material published, we cannot say whether the structures observed in *N. cuneatum* and *N. montanae* are homologous to the microtubuli and the vesicles in *Tubulinosema* species. However, the former species is rather similar in spore morphology (spore shape, size, the number of polar tube coils) to *T. acridophagus* and *T. maroccanus*. This resemblance may suggest that *N. cuneatum* is another candidate for transfer into the genus *Tubulinosema*.

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