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Protistology

Tuzetia dualis sp. n. (Microsporidia, Tuzetiidae) from the mayfly *Cloeon dipterum* L. (Insecta, Ephemeroptera) in Western Siberia

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Summary

A new species of microsporidia – *Tuzetia dualis* sp. n., from the fat body of *Cloeon dipterum* (Insecta: Ephemeroptera), is described. The species possesses single nuclei throughout the life cycle and develops in direct contact with the host cell cytoplasm. Sporogony is poly- and disporoblastic and only one spore type is formed. Spores are oval and measure 4.2 × 2.8 μm (after fixation and staining). The majority of spores are enclosed within individual sporophorous vesicles, as typical of the family Tuzetiidae. The polar tube is isofilar with 12-13 coils. The following features distinguish Tuzetia dualis n. sp. from other species of the genus *Tuzetia*: a) larger spore size; and b) presence of sporophorous vesicles with coupled spores. *T. dualis* is the third species of this genus found in *C. dipterum*.

Key words: *Cloeon dipterum, Tuzetia dualis* sp. n., Microsporidia, Ephemeroptera, ultrastructure

Introduction

The first species of a microsporidium from Ephemeroptera, *Nosema schneideri* (later assigned to the genus *Tuzetia*), was described nearly one hundred years ago (Leger, Hesse, 1910). This and two additional species of microsporidia from mayflies, also assigned to the genus *Nosema* according to the taxonomic criteria of that time, were found in Central Europe (France), and in North (US) and South (Brazil) America (Kudo, 1924). Later, on the basis of the ultrastructure of the sporogonial stages, these *Nosema* species were transferred to the genus *Tuzetia*. The only taxonomic feature diagnostic for *Tuzetia* was the presence of an individual sporophorous vesicle (SPV) envelope around each spore (Maurand et al., 1971; Maurand, 1973). In a later revision of the genus, Larsson (1983) included 6 species parasitizing Ephemeroptera and amended the diagnosis to include: "Meront, sporont, and spore uninucleate. Merogony results in a production of a small number of uninucleate merozoites, usually 2-8. The sporogonial plasmodium divides into a small number of uninucleate sporoblasts, usually 2-8. Spores are oval or pyriform. Polar filament isofilar. Exospore thin, electron-dense without stratification. The sporophorous vesicle usually traversed by narrow tubules, 20-25 nm in diameter".

Some species of *Tuzetia* exhibit a broad geographic distribution congruent with the distribution of their hosts. For example, a Palearctic ephemeropteran species *Cloeon dipterum* is widely distributed in Europe and Asia and two species of microsporidia, *Tuzetia ecdyonuri* and *T. lipotropha*, have been found parasitizing this insect host, in both Romania and Sweden.

In the present study, we now describe a new species of *Tuzetia* parasitizing fat body cells of nymphs of *C. dipterum* in Western Siberia, Russia.

Material and methods

Infected nymphs of *C. dipterum* were collected in a flood-plain pond within the city of Tomsk in July 1996. This was an occasional finding and no data on the parasite prevalence were available. For the light microscopy (LM) studies, smears of infected insect tissues were fixed with methanol and stained with a Giemsa stain solution. Stained smears were examined with an Axio 10 Imager M1 (Carl Zeiss, Germany) and digital images were acquired with an Variocam camera. Measurements of single and coupled spores (n = 50 each) were performed with Carl Zeiss Axiovision version 4.6.4.

For transmission electron microscopy (TEM), pieces of infected tissue were fixed with a 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer with 4% sucrose for 1-2 h, and postfixed with 1% cacodylate-buffered osmium tetroxide for 1 h. Tissues were dehydrated in an ascending ethanol series and absolute acetone and embedded in epon-araldite. Ultrathin sections were cut using an Ultratome-III (LKB, Poland), stained with 2% uranyl acetate in 50% ethanol and lead citrate for 10-20 min, and examined in a using JEM-100 CX II (JEOL Ltd, Japan) electron microscope at an accelerating voltage of 80 kV.

Repeated surveys for microsporidian infection of mayfly populations around Tomsk in 2003-2004 failed to yield new material, restricting our investigations to light and electron microscope analyses of the material from 1996 (Simakova et al., 2008).

Results

The ultrastructure of a microsporidium *T. dualis* sp.n. (Microsporidia, Tuzetiidae Sprague, Tuzet et Maurand 1977) from fat body of mayfly *Cloeon dipterum* (L.) (Ephemeroptera: Baetidae), collected in Western Siberia, was studied.

DESCRIPTION OF *TUZETIA DUALIS* SP. N.

Host: mayfly *Cloeon dipterum* (L.) (Ephemeroptera), nymphs.

Localization: adipose tissue.

Type locality and collection date: flood plain pond around the city of Tomsk, July 1996.

Morphology of the life cycle stages.

LM. Monomorphic development with formation of spores of one type. All life cycle stages are monokaryotic. Meronts divide by a rosette-like fission with formation of a small number of cells, not exceeding 8. A rosette-like sporogonial plasmodium with 8-12 nuclei forms spores which lie in the SPV envelope individually. A sporogonial plasmodium with 2-4 nuclei produces sporoblasts and spores, coupled within a SPV (Fig. 1, A). Spores are oval. Mean sizes of either single or coupled spores are 4.3 × 2.7 μm (3.7-4.8 × 2.4-3.2) after fixation and staining.

TEM. ***Merogony***. Meronts are round cells about 2 μm in diameter with nuclei up to 1.5 μm in diameter, occupying the major volume of the cell (Fig. 1, B). Nucleoplasm and cytoplasm are of moderate electron density, the nucleoli are not observed. The cell is bounded by a plasma membrane devoid of additional layers. Meronts divide by rosette-like fission producing multiple sporonts (mostly 8).

Sporogony. The of merogony and initiation of sporogony is characterized by the formation of small groups of electron-dense secretory granules on the plasma membrane (Fig. 1, C), later transforming into a thin (up to 50 nm) electron-dense layer, covering the entire surface of the sporont (Fig. 1, D-E). This layer gives rise to a number of narrow tubuli on the exospore's surface which traverse the SPV cavity. The SPV envelope is ornamented with these tubuli (Fig. 2, A, B).

Two types of sporont division are observed. In the first, sporonts divide by rosette-like fission into several single cells with an abundant distribution of electron-dense material encompassing the entire surface (Fig. 1, D-E). In the second, sporont division produces coupled cells, elongated and usually strongly deformed. An amorphous electron-dense layer joins these cells (Fig. 2, D-F) and the mature spores are coupled laterally (Fig. 2, G).

Spore. Sporogony of both types results in formation of identical spores. The spores are monokaryotic, oval in shape, 2.8-3.1 × 1.3 μm in

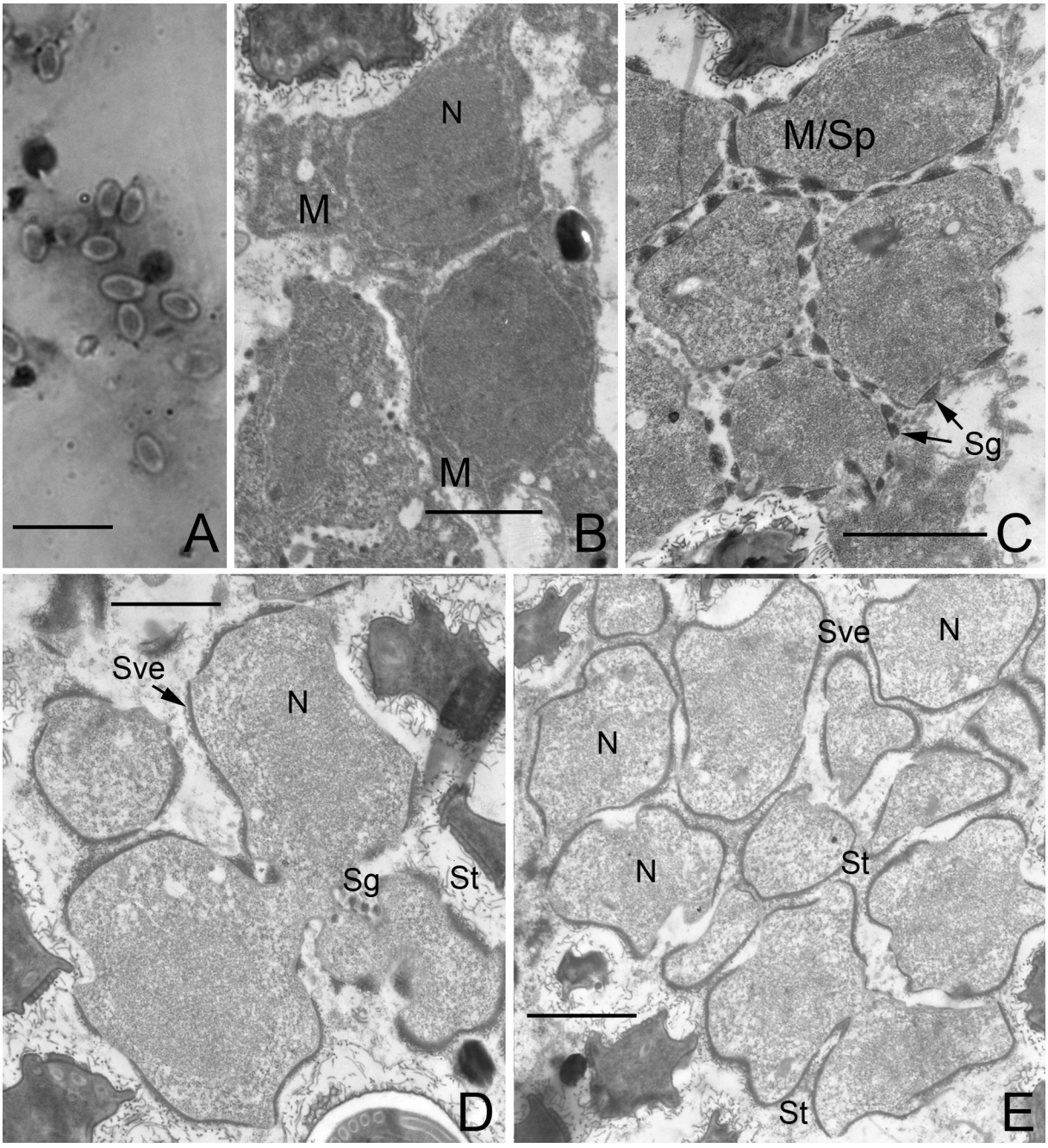

Fig. 1. Light microscopy of stained spores and ultrastructure of prespore stages of *Tuzetia dualis* sp. n. A – single and coupled (arrow) spores; B - meronts with large nuclei; C - sporonts, their surface coat covered by spots of secretion granules; D - tetratomic rosette-like sporogonial plasmodium covered by secretion layer; E - polytomic sporogonial plasmodium covered by secretion layer. *Abbreviations*: M – meront, M-Sp – meront-sporont transitional stage, N – nucleus, Sb – sporoblast, Sg – secretory granules, Sp – sporont, Sve – sporophorous vesicle envelope, St – secretory tubules. Scale bars: A - 10 μm; B-E - 1 μm.

size (Fig. 2, A). The nucleus occupying the spore centre is surrounded by 2-3 rows of polyribosomes. The anchoring disc is fungiform. The polar sac covers the anterior portion of the polaroplast. The lamellar polaroplast is bipartite with loose anterior and dense posterior compartments. The isofilar polar tube usually has 12-13 (rarely 14) coils arranged in one row (Fig. 2, A, G). The polar tube is about 100 nm in diameter (Fig. 2, B). The endospore is up to 100 nm in thickness. The exospore is thin, not stratified; it produces secretory material 10-15 nm in diameter, described by Larsson (1983) as "narrow

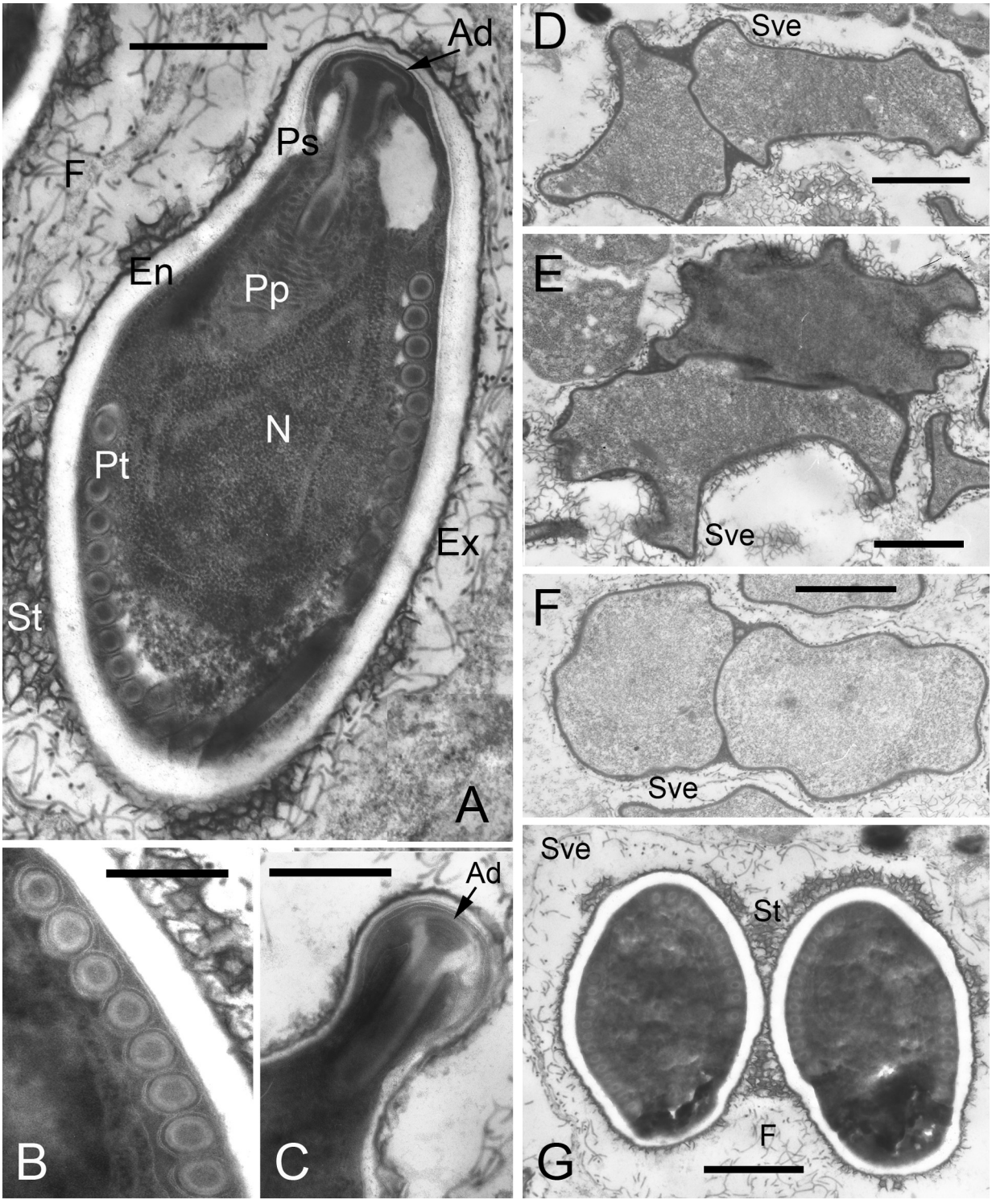

Fig. 2. Ultrastructure of sporogonial stages and SPVs of *Tuzetia dualis* sp. n. A - mature spore with ten coils of polar tube and large nucleus; B - structure of polar tube; C - structure of anterior pole of spore; D, E, F - two sporoblasts of disporoblastic sporogony coupled together by posterior poles or by sides; G - two mature spores in one sporophorous vesicle. *Abbreviations*: Ad - anchoring disc, En – endospore, Ex – exospore, St – secretory material indicated as "narrow tubules" by Larsson (1983) in episporontal space, Pp – polaroplast, Ps – polar sac, Pt – polar tube, Sb – sporoblast, St – secretion tubules. Other abbreviations are as in Fig. 1. Scale bars: A - 0.5 μm. B,C - 0.25 μm. D-G - 1 μm.

Table 1. Characteristics of the *Tuzetia* species from Ephemeroptera.

Species, author, country	Host species, localization	Sporoblast number, sporogony type	Spore size (μm) and shape	Number of PT coils
T. baeticida, Codreanu-Balcescu and Codreanu, 1976, 1982. Romania.	*Baetis vernus,* midgut	2, dumbbell-like	5 (alive), oviform, cylindrical	6
T. ecdyonuri, Codreanu-Balcescu and Codreanu, 1982*; Larsson, 1983**. Romania, Sweden.	*Ecdyonurus venosus**, *Cloeon dipterum* **, adipose tissue, ovaries	4-8, rosette-like	5-5.5 × 2.5-3 (alive.)*. 3.5-4 × 1.5-1.8 (stained)**, pyriform*, ovoid**.	6-7*, 8-9**
T. lipotropha, Codreanu-Balcescu and Codreanu, 1975*. Larsson, 1983**. Romania, Sweden.	*Rhitrogena semicolorata**, *Cloeon dipterum***, adipose tissue	8, rosette-like	6.5 × 3.5 (alive)* 5.9-6.3 × 2.9-3.2 (alive) and 4.0-5.2 × 2.0-2.2 (stained)** pyriform*, ovoid**.	14-15*, 12-14**
T. schneideri, (Leger and Hesse, 1910), Maurand, 1973*, Larsson,1991**. France, Sweden.	*Ephemera vulgata**, *E. danica***, midgut	4 or 8, rosette-like	4 × 2*, 4.9-5.6 × 2.5-2.8 (alive)**	7-9
T. urdae, Larsson, 1983 Sweden.	*Baetis sp.,* adipose tissue	4	3.3-3.6 × 1.3-1.6 (stained)	6
Tuzetia sp., Codreanu-Balcescu and Codreanu, 1982. Romania.	*Baetis vernus,* midgut	2, dumbbell-like	3-3.5	9-11,
T. dualis sp. n. Simakova et al., present paper. Russia (West Siberia).	*Cloeon dipterum,* adipose tissue	2, dumbbell-like and 8-12, rosette-like	4.2 × 2.8 (stained) 2.8-3.1 x 1.3 (EM), oval	13 (12-14)

Notes: Asterisks indicate correspondence between the authors and features given in their works.

tubules", composing a unified tubular net traversing the episporontal space. A typical deformation of the spore during fixation is observed: the spore wall appears concave in regions not supported by the polar tube coils in the anterior part of the spore (Fig. 2, A, C).

Sporophorous vesicle. The SPV envelope, visible as a tubular or filamentous net, gradually detaches from the cell forming a cavity (Fig. 1, E, 2, D-G). The episporontal space is traversed with abundant narrow tubuli connected into reticular structures, accumulating around the spore and/or joining two spores (Fig. 2, A, G). The ratio of single spores to coupled spores was 3:1 (Fig. 2, F, G), and the number of polar tube coils did not vary between single and coupled spores as well.

Discussion

Tuzetia dualis sp. n. represents the second species of microsporidia found in mayflies of the Russian fauna; the other is Pankovaia semitubulata (Simakova et al., 2008).

The morphology of sporogonial developmental stages of *T. dualis* are typical of the genus: single nuclei, division by rosette-like fission, formation of 2-8 spores from one sporont, presence of an individual SPV, secretory material in the form of narrow tubuli, and a reticular SPV envelope. Presently, 6 species of microsporidia, belonging to this genus, have been described from mayflies in Europe (Table 1). Some of the insect hosts from which these microsporidia have been described, including *C. dipterum*, are widely distributed in Eurasia and thus might serve as hosts for one or more species of microsporidia in different geographic regions.

The microsporidium described in this study can be distinguished from other known species by its spore size and unique proclivity to undergo two simultaneous sporogonies. The presence of sporogony with the formation of two spores within a

SPV goes beyond the frames of the genus diagnosis. Nevertheless, the formation of the majority of individual spores within a SPV, the presence of tubular structures and fibrils in the SPV lumen, and a reticular envelope of SPV are diagnostic for *Tuzetia* genus and thus justify its placement of this isolate within the genus.

Diagnosis of *Tuzetia dualis* sp.n.

Monomorphic and monokaryotic throughout life cycle. All stages develop in direct contact with host cell cytoplasm. Stained spores are oval and measure 4.3Ч 2.8 μm. The majority of the spores are enclosed within an individual SPV, but a certain proportion are coupled and enclosed within one SPV.

Type host: mayfly *Cloeon dipterum* (L.) (Ephemeroptera, Baetidae).

Localization: adipose tissue of nymphs.

Type locality: vicinity of Tomsk, Western Siberia.

Type material: Holotype is on a Giemsa-stained slide no. 358/31.07.1996.A, paratype on slide no. 358/31.07.1996.B. Epon-araldite embeddings and negative EM images are stored under respective numbers.

Deposition of types: The holotype is deposited in the Microsporidia Collection, Research Institute of Biology and Biophysics, Tomsk State University, Tomsk, Russia. The paratype is in the Microsporidia Collection, All-Russian Institute of Plant Protection, St. Petersburg-Pushkin, Russia.

Differential diagnosis. Two major distinctions of this species are a) presence of a second type of sporogony and b) comparatively larger spores.

Etymology. The species name reflects dual character of sporogony, being polysporoblastic and disporoblastic with production of single or coupled spores of one type.

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