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MARIONINA SCINTILLANS SP. N., A NEW ENCHYTRAEID SPECIES (ANNELIDA: OLIGOCHAETA) FROM HUNGARIAN GREEN HOUSES

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The article describes *Marionina scintillans* sp. n., a new enchytraeid species found in the green houses of the Botanical Garden of the Eötvös Loránd University Budapest and the Budapest Zoo and Botanical Garden as a result of a comprehensive investigation. This very small new species differs from all the *Marionina* species – which have 1–2 chaetae in all ventral chaetal bundles in the absence of the third pharyngeal glands and in the existence of a pair of small secondary (postseptal) pharyngeal glands in VI close to 5/6 and in a peculiar nephridial degeneration.

Key words: Oligochaeta, Enchytraeidae, *Marionina*, new species, green house, Hungary

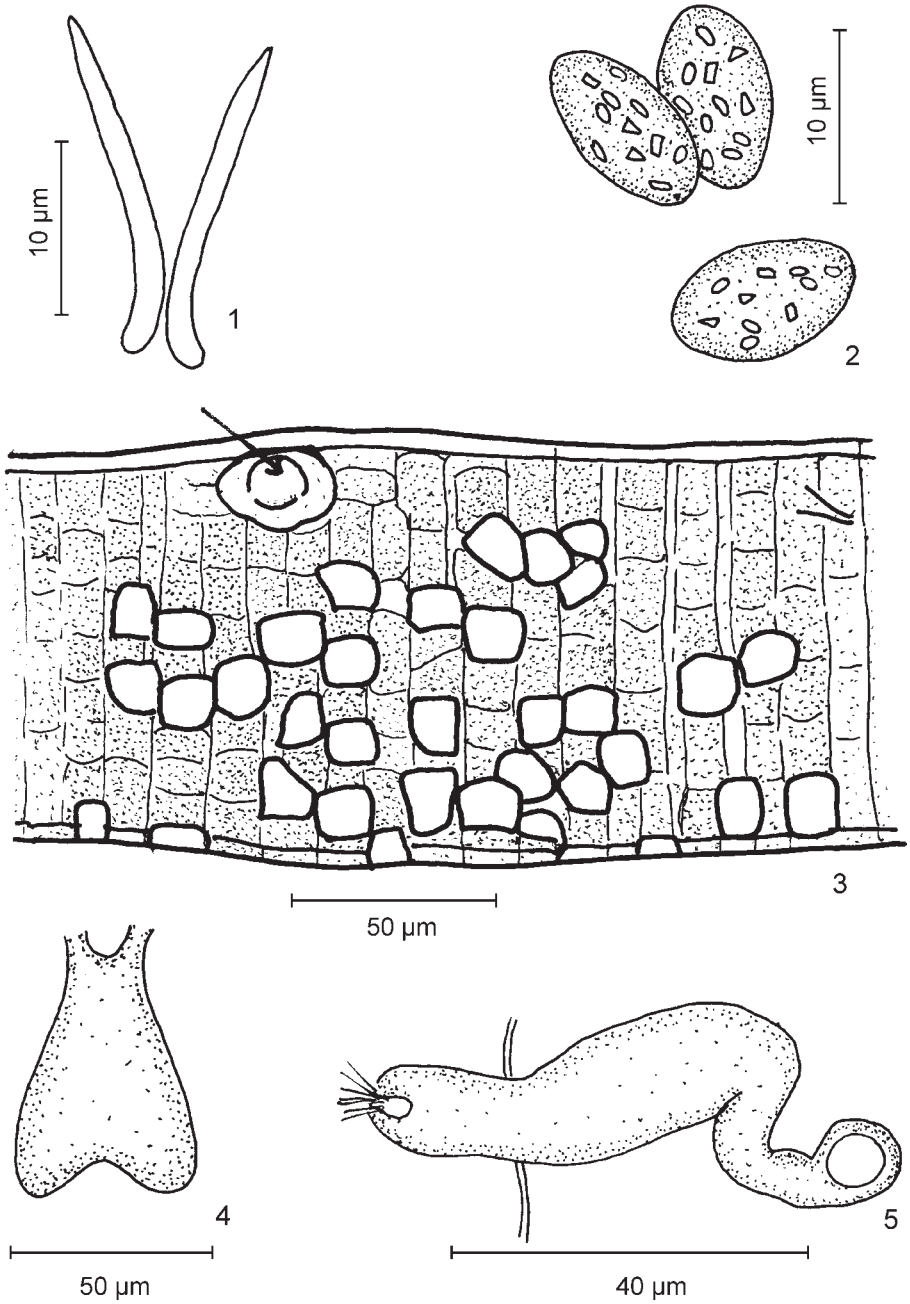
INTRODUCTION

Growing and exhibiting exotic plants in green houses has been very popular in the temperate zone countries for a long period of time. Botanical gardens and green houses import plants frequently from countries situated in tropical, subtropical and Mediterranean climate regions. Due to the accelerated transportation, the survival of soil fauna on the roots of these exotic plants is possible. These newcomer species can settle down in the artificial environment of green houses because of the high and constant temperature and humidity: these factors can ensure optimal life conditions for soil fauna. CSUZDI *et al.* (2008) concerning earthworms and KORSÓS *et al.* (2002) concerning Myriapods gave an account of tropical, subtropical and Mediterranean species, which can spread and survive in Hungary in artificial circumstances like green houses.

Since February 2006 a comprehensive investigation has been in progress with the aim of exploring the enchytraeid fauna of Hungarian green houses. Our new species was found in the course of this investigation.

MATERIAL AND METHODS

Soil samples were collected in the green houses of Eötvös Loránd University and the Budapest Zoo and Botanical Garden. Both were destroyed during the Second World War, so the oldest plants



Figs 1–5. *Marionina scintillans* sp. n.: 1 = chaetae, 2 = coelomocytes, 3 = clitellar region with clitellar glands, lateral view (penial bulb marked with arrow), 4 = brain, 5 = nephridium

Table 1. Two forms of *M. seminuda* and *M. scintillans* sp. n. compared

	M. scintillans sp. n.	*M. seminuda* China	*M. seminuda* New Zeeland
segments	19–23	23–27	22
length (fix.) µm	1.6–2.2	2–3	1.3–1.8
width (fix) anteclit. at clitellum µm	57–100 71–119	75 93	75 90
maximal length of chetae	at the end of body	in segment IV	at the end of body
preclit. nephridia	6/7, 7/8 (8/9 or 9/10) 2–3 mostly unpaired + degenerated sacks	7/8, 8/9 (9/10) 2 (3) pairs	(6/7) 7/8, 9/10 (2) 3 mostly unpaired
pharyngeal glands	4/5 dorsal merging 5/6 dorsal merging 6/7 absent in VI secunder gl.	4/5 dorsal merging 5 /6 dorsal merging 6/7 separate secunder gl. absent	4/5 dorsal merging 5/6 dorsal merging 6/7 separate secunder gl. absent
origin of dorsal vessel	XII	XIII	XIII
coelomocytes	12–18 µm fine and very coarse granules in it	8–10 µm opaque coarse granules in it	not seen

were collected and bought from several foreign countries after the renovations in 1946–1947. Since then the collection has been augmented and revived year by year.

In the majority of cases plants are located in a common container being put into the same soil. For this reason, samples were collected directly from the stem of the plants. Although the air humidity fluctuates between relatively broad values (50–80%), the air temperature as well as soil moisture and temperature are constant. Relative air temperature: 27.5±1.5 °C, soil moisture 63±5%, average soil temperature 22.5±1.2 °C). The values of average soil and air temperature were calculated from data measured every day, from 1st February 2006 until 20th March 2006, except four days (12–14th February and 15th March). The average soil moisture was calculated from 8 samples taken on 8 different occasions.

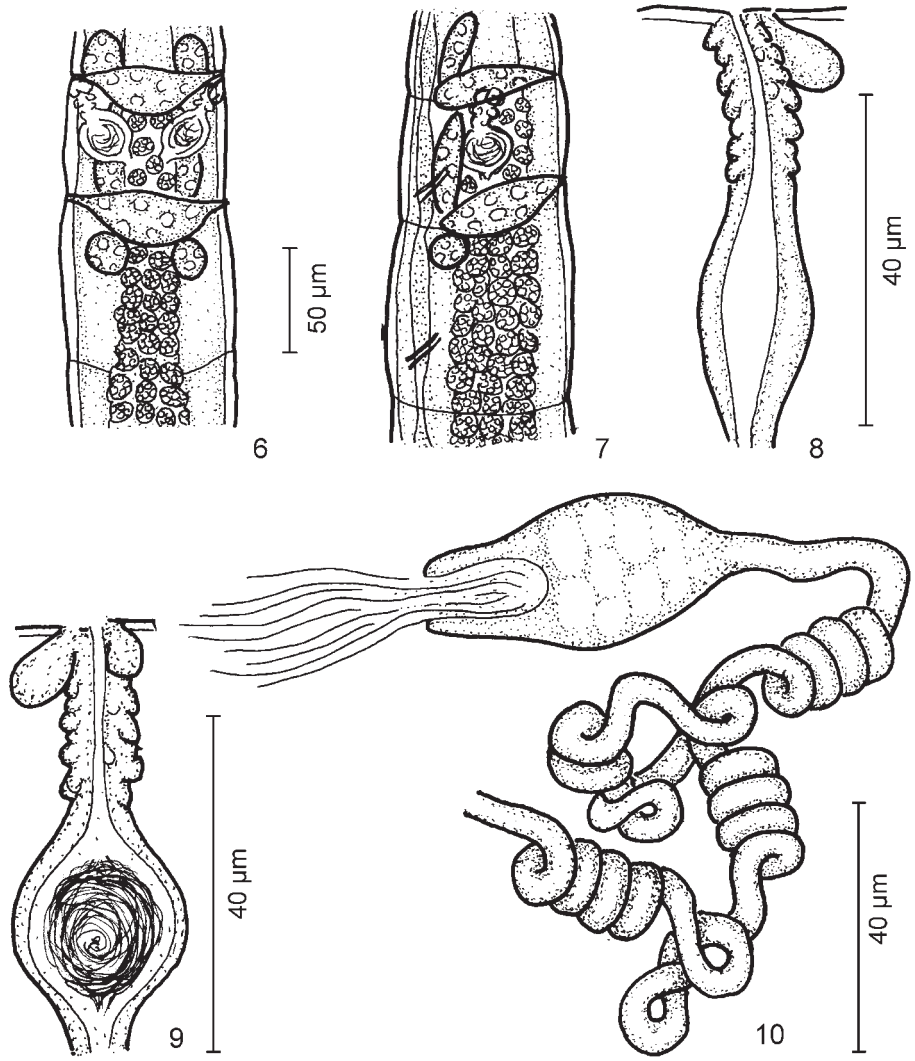
Both qualitative and quantitative samples were collected near *Musa acuminata* (Musaceae), *Roystonea regia* (Arecaceae), and *Paphiopedilum insigne* (Orchideaceae) in the green house of Eötvös Loránd University and near *Phoenix canariensis* (Arecaceae), *Diffenbachia* sp. (Araceae) and *Pandanus* sp. (Pandanaceae) in the Budapest Zoo and Botanical Garden.

Animals were extracted from the soil with the O'Connor's wet funnel method (O'Connor 1962).

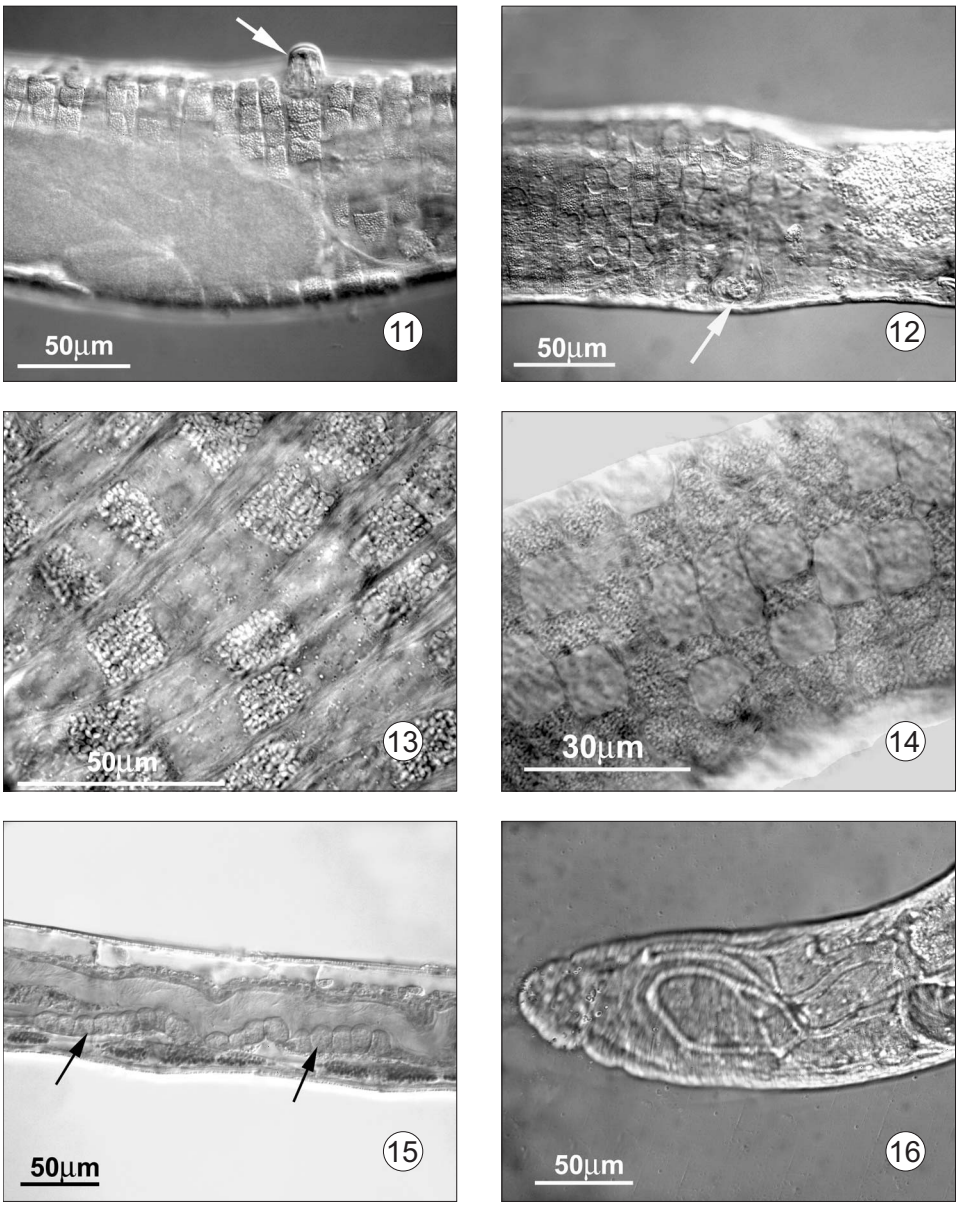
Worms were investigated and measured both alive and preserved since the gradual anaesthesia caused only minor changes in body characteristics of the preserved specimens. The living enchytraeids were put on a slide with a few drops of water and covered with a cover slip because the worms must be immobilized and the body must be flattened in order to make the internal organs visible. Further on the animals were anaesthetized in ascendant concentration of ethanol, and then preserved in 70% ethanol with a 5 percentage of formaldehyde in the solution or in cold and hot Bouin's fluid. A part of the fixed specimens were first stained with a mixed borax-carmine and bromphenolblue (1:1), passed through an ethanol (70% – absolute) dehydratation series and embedded in clove oil, and later mounted in euparal, the others were not stained and preserved in 70% etha-

nol. (From paratypes 37 specimens are stained and whole-mounted in euparal. 38 specimens fixed in 70% ethanol with formaldehyde and 20 fixed in hot Bouin's fluid, than all both preserved in 70% alcohol). Pictures were drawn and photographs were taken of the most important organs by a Zeiss Axioscop 2 microscope, using DIC (Differential Interference Contrast) illumination.

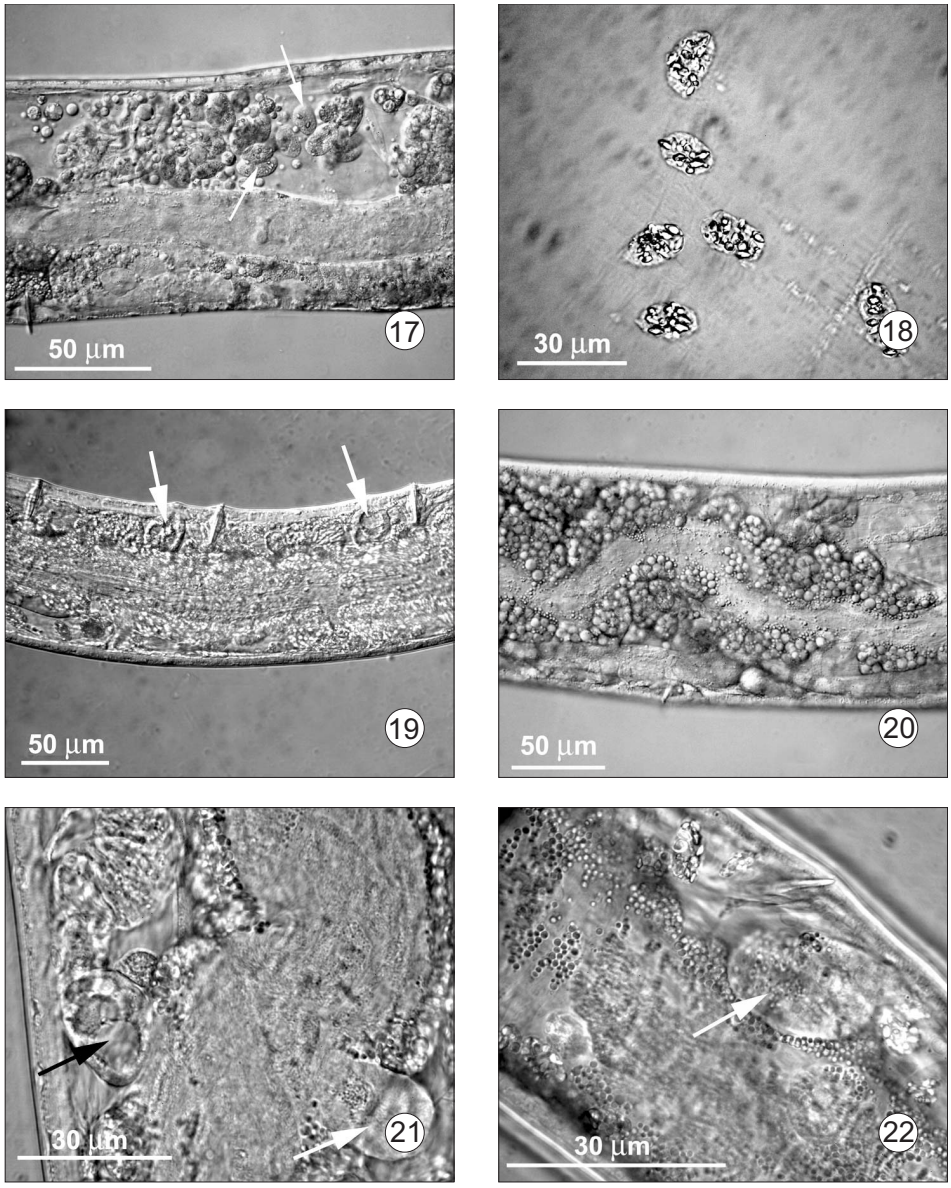
The type material is deposited in the collection of K. DÓZSA-FARKAS at Department of Systematic Zoology and Ecology, Eötvös Loránd University, Budapest. The material was collected by G. BOROS.



Figs 6–10. *Marionina scintillans* sp. n.: 6 = pharyngeal glands, dorsal view, segments III–VI; 7 = pharyngeal glands, lateral view, segments III–VI; 8 = spermatheca (empty); 9 = spermatheca (full with sperm); 10 = sperm funnel and ectal duct



Figs 11–16. *Marionina scintillans* sp. n.: 11–14 = clitellar glands, 11 = granular cells around the penial bulb (bulb marked with white arrow, living worm, lateral view), 12 = photograph of a fixed worm, lateral view (penial bulb marked with arrow), 13 = photograph of a living specimen (dorsal view), 14 = photograph of a fixed and stained specimen (dorsal view); 15 = large epithelial cells (marked with arrow) in the inner ventral wall of the intestine in ½ XVI–XVIII. Photograph of a fixed and stained specimen (lateral view); 16 = photograph of dorsal anterior blood vessel (in living specimen)

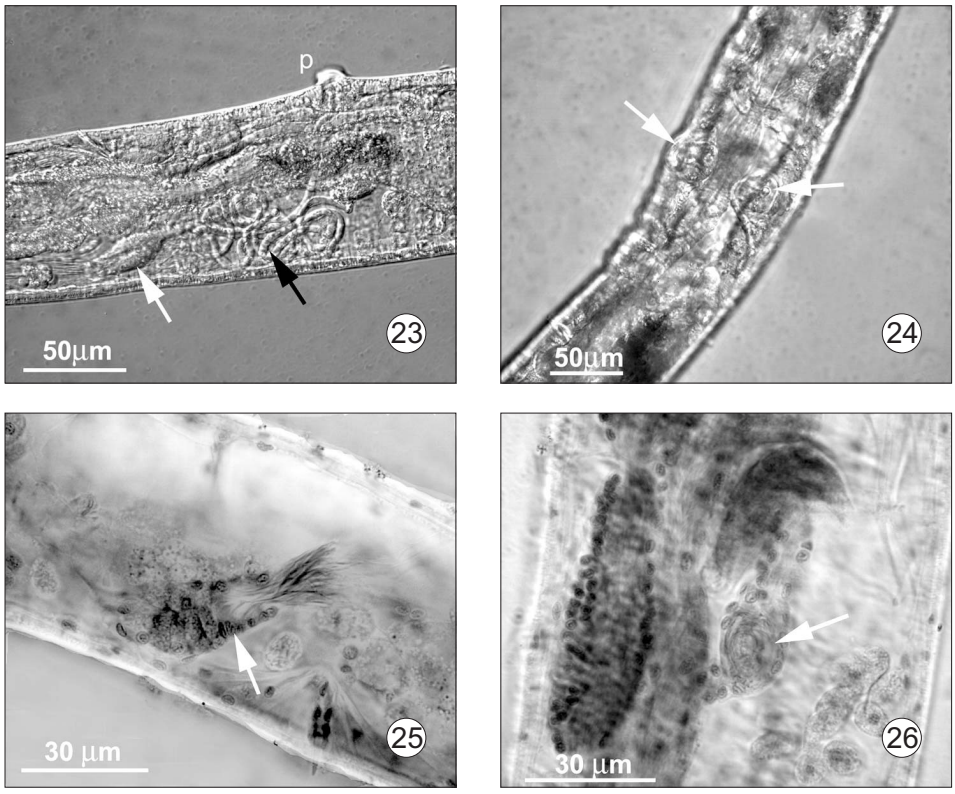


Figs 17–22. *Marionina scintillans* sp. n., photographs: 17 = coelomocytes (marked with arrow) in living enchytraeid worm; 18 = dried coelomocytes on slide glass; 19 = conspicuous vesicles of the nephridial efferent duct at the openings (marked with white arrow) in segments VII and VIII (lateral view, living specimen); 20 = chloragogen cells in VIII (living specimen); 21 = enlargened end of the nephridial efferent duct (the vesicles marked with black arrow) and a degenerated sack of the other nephridia in VIII (marked with white arrow, living specimen); 22 = degenerated nephridial sack in XVI (marked with white arrow, living specimen)

RESULTS

The following species were found in the investigated places: *Enchytraeus dudichi* DÓZSA-FARKAS, 1995, *Fridericia pretoriana* STEPHENSON, 1930, *Henlea nasuta* (EISEN, 1878) and the new species *Marionina scintillans*. Furthermore one species of the genus *Hemienchytraeus* and two species of *Achaeta* turned up; the accurate identification of these worms is in progress.

The new species was found only near *Roystonea regia* in the green house of Eötvös Loránd University and in every sample taken from the Budapest Zoo and Botanical Garden.



Figs 23–26. Photographs of *Marionina scintillans* sp. n.: 23 = sperm funnel (marked with white arrow), sperm duct (marked with black arrow) and penial bulb (p), segments XI–XII (living specimen); 24 = penial bulbs (marked with arrows), ventral view (living specimen); 25 = sperm funnel (marked with arrow) of a fixed and stained specimen; 26 = spermatheca of a fixed and stained specimen. The ampulla full with sperm (marked with arrow)

Marionina scintillans sp. n
(Figs 1–26)

Holotype: Ma 4, fixed cold Bouin's fluid, whole-mounted on slide.

Type locality: green house of Budapest Zoo and Botanical Garden, in the stock of *Phoenix canariensis,* coll. G. BOROS, 08. 03. 2006.

Paratypes: P. 84.1: three specimens, whole mounted together on a slide, previously fixed in cold Bouin's fluid from the green house of Eötvös Loránd University. P.84.2 – P.84.8 twenty-six specimens whole mounted on 7 slides, previously fixed in cold Bouin's fluid, from the Budapest Zoo and Botanical Garden. P.84.9 – P.84.10 eight specimens whole mounted on two slide, previously fixed in ethanol + formaldehyde, from the Budapest Zoo and Botanical Garden. P.84.11 a worm and its flown out coelomocytes dried out and mounted on a slide in euparal from the Budapest Zoo and Botanical Garden. P.84.12 thirty-eight specimens fixed in ethanol + formaldehyde and preserved in 70 % ethanol. P.84.13 twenty specimens fixed in hot Bouin's fluid and preserved in 70 % ethanol all both from the Budapest Zoo and Botanical Garden. All animals were collected on 08.03.2006 and P.84.1 – P.84.6 and P.84.11 was fixed in March 2006, but P.84.7 – P.84.13 (except P.84.11) was fixed in May 2007 from the living worms of the soil samples holding in laboratory.

Etymology: scintillans (lat.) = shiny, bright. Named after the trait of coelomocytes filled with large granules, which are shining in the colours of the rainbow.

Diagnosis. Length 2.0–2.9 mm diameter 81–119 μm (in VIII) (in vivo), segments 19–23. Chetae are straight formula: 0–0 : (0),2–2. Clitellum mostly XII–2/3XIII large hyaline and granular gland cells in about 18–19 rows, ventrally absent. Two pairs of pharyngeal glands merging dorsally at 4/5 and 5/6, with well developed ventral lobes. The third pharyngeal glands absent. One pair of small secondary glands in VI. Preseptal part of nephridia consisting of funnel and nephridial canal, postseptal part elongate, twice as long as preseptal part efferent duct terminal have a conspicuous vesicle at the opening. Pre- and postclitellarly only 2–3 nephridia (paired or single) variable in segments. Most individuals showed unusual nephridial degeneration: the enlargened end of efferent duct constitute a sack which is freely moving in the body cavity. Blood vessel originates in XII, blood colourless. The dorsal anterior blood vessel bifurcation is behind the brain in III. Coelomocytes are elliptical filled with fine and crystal-like larger granules, dark in transmitted light, at the same time shining in the colours of the rainbow. Sperm funnel small, pear–shaped, collar narrower than funnel, sperm duct long and narrow coiled. Penial bulbs small (length 20–25 μm) and compact, seminal vesicle absent. Spermatheca confined to V, connected to oesophagus by short ental ducts, ectal ducts covered by small glands and one or two larger ectal gland at the orifice. Ampulla oval, or spherical when full with sperm (diameter 14–23 μm).

Description. Body colour whitish. Very small species: holotype is 2,16 mm long and 81 μm wide in VIII and 95 μm at clitellum (fixed), segments 21. Body length of paratypes 2.0–2.9 mm,

width 81–119 μm in VIII and 95–130 mm at clitellum (*in vivo*), length of fixed specimens 1.6–2.2 mm and the diameter 57–100 μm in VIII, 71–119 μm at clitellum. Segments 19–23. Two straight chaetae per bundle with distinct ental hook (Fig. 1), equal sized in a bundle, length increasing toward posteriorly body end, 20–22 μm long in preclitellar segments and 28–32 μm at the body end, dorsally all absent, in II and XII (at maturity) missing ventrally too. Chaetal formula: 0–0 : (0),2–2. Epidermal gland cells inconspicuous. Clitellum (Figs 3, 11–14) in XII–2/3 XIII or XII–XIII, gland cells squared, large hyaline and granular cells, arranged in about 18–19 rows, near to penial bulb only granulocytes (Fig. 11) and ventrally absent. In living specimens the granulocytes are conspicuous (Figs 11, 13), in fixed and stained specimens the hyalocytes are better visible (Figs 12, 14). Head pore at 0/I. Brain (Fig. 4) ca 55–80 μm long and 26–32 μm wide (fix.) concave anteriorly and incised posteriorly. Two pairs of pharyngeal glands merging dorsally at 4/5 and 5/6, both with well developed ventral lobes. One pair of small (postseptal) secondary glands occurs in VI behind the septum 5/6 ventro-laterally (Figs 6, 7). The third pharyngeal glands absent. One pair of postpharyngeal bulbs present in III. Transition between oesophagus and intestine gradual, no gut appendages present. Chloragogen cells from V forming a dense layer from VII, their diameter 17–30 μm with large yellow oil body in them (Fig. 20). A longitudinal ridge of tall epithelial hyaline cells noticeable in the inner ventral wall of the intestine in XIV–XVIII, occupying mostly 1,5–2 segments (Fig. 15). Nephridia (Fig. 5) mostly unpaired, preclitellar in 6/7 and 7/8 sometimes in 8/9 or 9/10 too. Preseptal part consisting of funnel and nephridial canal, postseptal part elongate, twice as long as preseptal part. Efferent duct terminal, widen out and have a 10–12 μm wide conspicuous vesicle at the outlet (Figs 19, 21). Postclitellarly only 2–3 nephridia (paired or single) variable in segments (e.g. 15/16 and 19/20 or 14/15 and 16/17). Most individuals showed unusual nephridial degeneration. In some segments one nephridium developed normally while the other is either missing or only the enlargened end of efferent duct is present leading to its pore in front of the chaetal bundles and as a sack it is freely moving in the body cavity (Fig. 21, 22). In other cases both nephridia were reduced, sometimes asymmetrically (e.g. one of them is 20 μm, the other 37 μm). In certain segments no nephridia were present not even in a reduced form. Their distribution greatly varies, however, usually there is one nephridium in 6/7, a complete organ and a reduced "sack" in 7/8 or one sack on each side in VIII, one complete organ in 9/10 and in 15/16, one sack in XI, XIV and XVIII. However, there can be a full organ postclitellary in 13/14, one pair of organs in 16/17 or one organ in 13/14 and 15/16, and two "sacks" in XVIII.

Blood vessel originates in XII, blood colourless. The dorsal anterior blood vessel bifurcation is behind the brain in III (Fig. 16). Coelomocytes (Figs. 2, 17, 18) are elliptical (12–18 μm long in fixed worms), filled with fine and larger (1–3 μm long) granules, dark in transmitted light, at the same time these large crystal-like granules are shining in the colours of the rainbow especially in DIC microscope. In the interesting way these larger granules don't lose their character when the coelomocytes dried out (Fig. 18).

Sperm funnel (Figs 10, 23, 25) small, 30–40 μm long and 18–19 μm wide (fix.), pear–shaped, collar narrower than funnel, spermatozoa ca. 25 μm long. Sperm duct long and narrow (ca. 3 μm wide) coiled, sometimes wound into a spiral in XII. Penial bulbs small (length 20–25 μm) and compact ca. 20 μm distance to each other (Fig. 24). Seminal vesicle absent. Spermatheca (Figs 8, 9, 26) confined to V, connected to oesophagus by short ental ducts. Ectal pores in intersegmental furrow of 4/5, ectal ducts ca. 16–25 μm long and 7–8 μm wide (in fixed specimens) covered by small glands and accompanied by one or two larger ectal gland at the orifice. The ectal glands are almost as wide as the ectal duct diameter. Ampulla oval, or spherical when full with sperm (diameter 14–23 μm). One egg mature at a time.

Remarks. The new species differs from all *Marionina* species by having 2 (rarely one) chaetae in all ventral bundles while they are missing in all dorsal bundles, the absence of the third pharyngeal glands and the possession of a pair of small postseptal (secondary) pharyngeal glands in VI close to 5/6 and the peculiar nephridial degeneration. The closest relative of *Marionina scintillans* appears to be *M. seminuda* XIE et ROTA, 2000 and *M. subterranea* (KNÖLLNER, 1935). *M. subterranea*, however, larger (25 segments, 4 mm long), ventral chaetae are present in II, the first and second pharyngeal glands do not have ventral lobes and the spermatheca bears glands only at the ectal pores. *M. seminuda* was described from China (XIE & ROTA 2000) and a year later from New Zealand (ROTA & MANCONI 2001). It is possible that the latter is a new species because some characters differ from the types (e.g. the size distribution of chaetae, distribution of preclitellar nephridia, absence of seminal vesicle and the coelomocytes were not seen in the New Zealand specimens). The habitats were also considerably different. In China it exists in moist to wet soil along a river while specimens from New Zealand were collected from *Heterorotula* sp. sponge living at 127–145 meter depth in a lake. *Marionina scintillans* sp. n. is similar in many characters, namely the setal formula, the anterior bifurcation of dorsal blood vessel behind the pharynx, the form of the brain (incised posteriorly) and the spermatheca, the well visible postpharyngeal bulbs in the III, and the granular and hyalin clitellar glands arranged in approximately 18 transverse rows, to both variations of *M. seminuda*. In the new species the glandular cells of the clitellum are absent ventrally but this character was not mentioned in the description of *M. seminuda*. *M. scintillans* also differs in some further characters from *M. seminuda* from China and *M. seminuda* from New Zealand as summarized in Table 1.

Further two small *Marionina* species (*M. eleonorae* ROTA, 1995 and *M. praeclitellochaeta* NIELSEN et CHRISTENSEN, 1963) also have two pairs of pharyngeal glands only but neither of them have postseptal glands in VI. Moreover in *M. eleonorae* the pharyngeal glands are free dorsally (ROTA 1995) while those of the *M. scintillans* are fused. Furthermore, *M. eleonorae* has two chaetae in all dorsal bundles and the dorsal blood vessel originates in VIII (ROTA 1995). Similarly, *M. praeclitellochaeta* only bears ventral chaetae but only from II to VI (NIELSEN & CHRISTENSEN 1963).

*

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