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MERLINIUS BREVIDENS (SIDDIQI, 1970) AND *MERLINIUS NOTHUS* (SIDDIQI, 1970) NEMATODES DETECTION AND IDENTIFICATION IN FLOWERING PLANTS GROWN IN GREENHOUSE

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Abstract

Merlinius brevidens is detected and identified in the root systems of the Chrysanthemum flowering plants in a greenhouse from Ploieşti, Prahova, Romania; also other species existent in Romania, namely Merlinius nothus was found and identified in Strelitzia plants in a greenhouse from the same locality. The morphobiometrical characteristics mostly used for the separation of the two species involve the body and stylet length, shape of head, the tail shape, the tail and its type, the number of annules found in the head and tail areas, the position of the vulva (V), and the ratio c' in females case. This is the first report for Romania of Merlinius brevidens.

Key words*: Merlinius brevidens and Merlinius nothus, detection and morfobiometrical identification in flowering plants.*

INTRODUCTION

The stunt nematodes are a broadly distributed group of genera and species distributed all over the world in agricultural soils and uncultivated land. Of the 267 now valid species, few have been proven as being pathogenic, but about 8% are known ectoparasites (Anderson and Potter, 1991). Many are part of a complex of plant parasite nematodes in soil around crop plants, they can be thought of a stress factor to their hosts (Anderson and Potter, 1991).

The *Merlinius* genus was introduced by Siddiqi in 1970, these species were previously accommodated in the *Tylenchorhynchus* genus, due to the fact they had six incisures in the lateral fields. Tarjan in 1973 gave a key data and diagnosis of the genus and species of Tylenchorhynchina and discussed with Siddiqi about some characters. În 1973 Tarjan agreed with Siddiqi (1970) that the presence of six incisure in the lateral fields is a consistent and easily recognizable character, so the genus *Tylenchorhynchus* becomes *Merlinius* genus. Handoo et al., (2007) agrees with both Siddiqi (1970) and Tarjan (1973) because their actions led to an easier management of this complex and large group of nematodes. Today the genus *Merlinius* comprises 32 valid species distributed worldwide as parasites of large plants varieties Handoo et al., (2007). The history of the genus *Merlinius* was discussed by Hooper (1978), Fortuner and Luc (1987) including *Merlinius* in the Telotylenchinae subfamily, and subsequently in the Belonolaimidae family. Anderson and Potter (1991) included the genus *Tylenchorhynchus*, *Merlinius* and *Amplimerlinius* in a review of significant species for agriculture, they also presented a good historical past of this nematodes group taxanomy. In 1998, Brzeski included all species of the genus *Merlinius* in *Geocenamus* and gave a key for 19 species and a compendium for 77 species. He concluded that the genus *Geocenamus* may be a collective group, which could be divided into separate genera, but the study of the cephalic structure of several species under electron microscope were necessary before reaching to a conclusion. (Handoo et al., 2007).

As a result of these changes, the development and compilation of a dichotomous key for all species belonging to the *Merlinius* genus has become increasingly difficult.

Many of the species that were included in the *Merlinius* genus were placed in new genera by

different specialists and several species belonging to other related genera were either moved or given other synonyms. The most important character used in distinguishing this genus is the number of lateral lines, comprising species with three or six lateral lines.
The general objective of research is identification of phytoparasitic nematodes fauna in flowering plants grown in Romania. In the present research have been identified two species of *Merlinius* with six lateral lines which are part of the *Merlinius* genus. In 1997, Popovici referred to the existence of the species *Merlinius nothus* in Romania but are not data regarding to description of this species. The description of two species of *Merlinius*, *Merlinius brevidens* and *Merlinius nothus,* based on the morphobiometric characters of these species, is provided.

MATERIALS AND METHODS

The soil samples were taken from the same greenhouse, one from *Chrysanthemum* crop and the other from *Strelitzia* crop. Geographical coordinates (GPS) are: $44^0$56′02.82′′N; $26^0$02′49.18′′ E.
The samples were taken from the root system of the plants and the amount of soil taken was about 500 ml for each cultivated flowering plant variety.
The nematodes density found was approximately 15 specimens for the first species and 20 specimens for the second detected species, for about 500 ml of soil analysed for each flowering plant variety.
The extraction of nematodes from the soil for both species of detected nematodes was done using the COBB method or the buckets method. After the nematode's extraction and recovery from the soil, followed their pre-identification with the Leica DMLB MZ 12.5 stereomicroscope and identification with DIC microscope.
The identification of the species and their measurements were carried out on fresh material in case of *Merlinus brevidens* and permanent slides for *Merlinius nothus*, having a greater number of specimens. In order to make permanent slides the nematodes were stored in a buffer solution of water and formaldehyde and then were placed into glycerine, in order to draw morphometric observations and measurements (Hooper, 1986). The measurements of the analysed samples were done using a Zeiss Axio Imager 2/430004 - 9902 microscope, supplied with Zeiss Axiocam 506 - 426556 digital camera and a Zen 2.6 (Blu edition) incorporated software.

RESULTS AND DISCUSSIONS

Merlinius brevidens (Allen, 1955; Siddiqi, 1970) a new record for Romania

= *Tylenchorhynchus brevidens* (Allen, 1955)
= *Geocenamus brevidens* (Allen, 1955; Brzeski, 1991)

Description
Figure 1 (a-f), Table 1
Female:

The body is straight to strongly arched when relaxed, with the width in the middle between the following values 21.4 (20-23); with fine annules that are about 1µm wide or less.
The lateral lines occupy about a third of the diameter of the body, they present six incisions. The excretory pore (n = 10) has between 105-119 µm, with an average of (110.9±5.0) µm, located at the posterior of the head and at the level of the oesophageal basal bulb. The hemizonide is located in front of the excretory pore at a distance of 1-3 µm from this. The deirids are present, localized in the excretory pore, but most often they are not seen because of the cuticle. The head is not tall, is rounded, continuous or slightly set off against the body, being demarcated by five or six annules. The stylet is 15.7 µm long (15-17), with rounded knobs, 3.5-4.5 µm wide, sloping posteriorly. The oesophagus is 141 (132-147) µm length. The vulva is between 54-59 (56.1±1.3) percent of the body length.
The ovaries extend to the anterior part of the body close to the basis of the oesophagus in pregnant females. The tail is tapered, curved, between 48-65 (53.8 ±5.4) µm, (n = 10) in length. The tail is marked by the presence of 39-62 fine annules, cuticular inclusions being particularly dense at the tip of the tail. Phasmids near or slightly posterior to the middle of the tail.

In case of this species, no males were present in the examined soil.

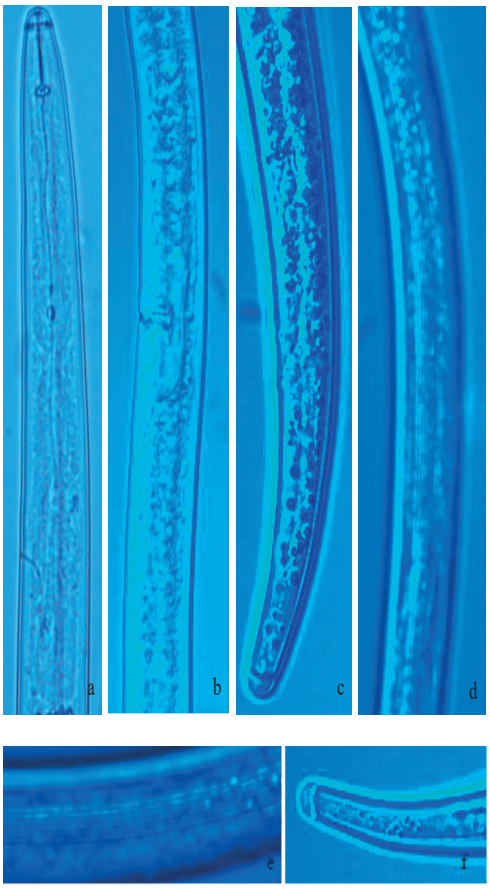

Figure 1 (a-f) *Merlinius brevidens*: (a) Anterior region of female; (b) Vulval region; (c, f) Female tail; (d, e) Lateral field showing six incisures

Merlinius nothus (Allen, 1955; Siddiqi, 1970), existent species in Romania:

= *Tylenchorhynchus nothus* (Allen, 1955)
= *Geocenamus nothus* (Allen, 1955; Brzeski, 1991).

Description
Figure 2 (a-e), Table 1

The female is between the following values n = 11, 747-884 (807.2 ±14.1) µm; the stylet is 15-19 (17.2±1.7) µm is fine to medium, its cone is slightly longer than the shaft, the knobs are elongated to a certain extent having a diameter of 4.5 µm. The cuticle is distinctly annulation, the annules being rounded by 1.1-1.8 µm wide. The lateral lines have six incisions. The head is shorter than the adjacent body, it is rarely continuous, the annules are 6 in number. The deirids are present and located at the level of the excretory pore. The cephalic skeleton is weak. The genital tract can spread to a longer part of the body than the intestine. The vulva has a small cavity between 54-58 (56) percent of the the body length. The sperm tank is off set when it is filled with sperm and can be bilobed. The tail is tapered, having 40 to 46 annules, rounded at the tip, in most cases it is smooth, rarely striated. Tail length for n = 11, (34-55 µm) 47.6 ±7.0. The phasmids are distinct and located in the middle of the tail.
No males of this species were detected in the examined soil samples.

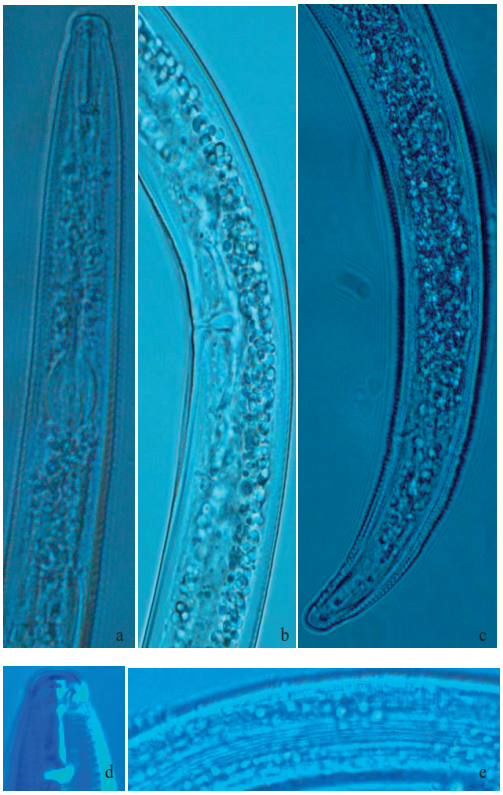

Figure 2 (a-e). *Merlinius nothus:* (a, d) Anterior region of female; (b) Vulva and reproductive structures; (c) Tail region; (e) Lateral field showing six incisures

Merlinius brevidens species detected in *Chrysanthemum* flowering plants is somehow

longer, its length being comprised between (579-778 vs. 540-690 µm).
The stylet length has values comprised (15-17 vs. 14-16 µm), being to a certain extent higher than the original described species.
The vulva is positioned between (54-59 vs. 52-58 %) of the body length. The ratio c' is between 3.6 and 4.3 µm. The annules in the head area are 5 to 6 and the tail ones are 50 to 60. Generally, the morphology of the populations of the two recovered nematode species has similarities that are close to the originally described species.

Table 1. Morphometric characters of the species *Merlinius brevidens* (Allen, 1955; Siddiqi, 1970) and *Merlinius nothus* (Allen, 1955; Siddiqi, 1970) collected from South Romania

Species:	*Merlinius brevidens*		*Merlinius nothus*
Host plants	*Chrysanthemum*		*Strelitzia*
Locality:	Ploieşti		Ploieşti
Characters/ ration[b]	Females	after Allen,1955	Females
n	10	11	11
L (µm)	667.8 ± 61.0 (579 – 778)	540 - 690	807.2 ± 14.1 (747 – 884)
a	31 ± 1.9 (27 - 34)	23 – 27	29.9 ± 2.8 (26 – 32)
b	4.7 ± 0.3 (4.2 – 5.2)	4.2 – 5.2	5.0 ± 0.1 (4.7 – 5.3)
c	12.3 ± 0.6 (11.6 – 13.7)	11 – 13	17.2 ± 1.4 (15 – 24)
c'	4 ± 0.2 (3.6 – 4.3)		2.5 ± 0.07 (1.7 – 3)
V %	56.1 ± 1.3 (54-59)	52 – 58	56 ± 0 (54 – 58)
Stylet length	15.7 ± 0.6 (15 – 17)	14 – 16	17.2 ± 1.7 (15 – 19)
Pharynx length	141 ± 5.8 (132 – 147)		159.5 ± 5.6 (149 – 176)
Excretory por	110.9 ± 5.0 (105 – 119)		133.2 ± 3.5 (127 – 150)
Max. body diam.	21.4 ± 0.9 (20 – 23)		26.9 ± 2.1 (25 – 29)
Anal body diam.	13.9 ± 1.8 (12 – 18.5)		18.4 ± 2.1 (14 – 27)
Tail	53.8 ± 5.4 (48 – 65)		47.6 ± 7.0 (34 – 55)

The abbreviations of the measured characters from table 1 were defined in Siddiqi (2000).

CONCLUSIONS

Merlinius brevidens is reported for first time in Romania in a crop of *Chrysanthemum* flowering plants grown in greenhouse.
The second detected species *Merlinius nothus* was listed for Romania by Moldovan et al, 2007, but in this study, we identified for first time the nematode in association with *Strelitzia* plants.
The species were found in crops of flowering plants grown in the greenhouse from Southern part of Romania, in the Ploiesti region, Prahova county, in autumn of the year 2019.
Both species are plants ectoparasite pests that develop all stages of development in the soil.
Several symptoms were reported in the normal growth and development of plants, which had a smaller growth, showing symptoms of decreased resistance and abnormal development.
The present study was mainly referring to the detection and identification of nematode species harmful to cultivated plants. In order to

provide data on the pathogenicity, biology and dynamics of the populations of these species, more investigation is required.

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