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ORIGINAL ARTICLE

A key for the identification of plant-parasitic nematodes of the genera *Longidorus* Micoletzky, 1922 and *Paralongidorus* Siddiqi, Hooper and Khan, 1963 (Nematoda: Longidoridae) occurring in Poland

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Abstract

Plant parasites of the genera *Longidorus* Micoletzky, 1922 and *Paralongidorus* Siddiqi, Hooper and Khan, 1963 comprise a group of plant root ectoparasites, some of which are known as pests of economic importance. Their importance is further augmented by the fact that several species are known to be vectors of nepoviruses. To date 16 species from the genus *Longidorus* and two from *Paralongidorus* have been recorded in Poland. Despite their economic importance in agriculture currently there is no regional key for species identification. This paper presents such a key. The key has many illustrations and is based mainly on traits which are easily observable even by less experienced users. Thus, it should provide a useful tool for both scientists and specialists working in the field of plant protection, soil ecology and zoology as well as for teaching purposes.

Keywords: Nematoda, plant protection, soil ecology, taxonomy, yield losses

Introduction

Plant-parasitic nematodes are known as an important group of pests which threaten global agriculture. However, their impact is probably underestimated as symptoms on plants are frequently non-specific and can be confused with other pathogens or abiotic stresses, for example water or nutrients deficiency (Singh *et al.* 2015). This loss is mainly caused by 250 plant-parasitic species which are considered to be of phytosanitary importance (Singh *et al.* 2013). Two systematic genera in which plant parasitic nematodes occur are *Longidorus* Micoletzky, 1922 and *Paralongidorus* Siddiqi, Hooper and Khan, 1963, both members of the family Longidoridae. To date 18 species from these two genera have been recorded in Poland (Kornobis and Peneva 2011; Kornobis *et al.* 2015; Kornobis *et al.* 2017). Some species recorded from Poland are known as pests of economic importance as well as vectors of plant nepoviruses, e.g., *L. attenuatus* Hooper, 1961 and *L. elongatus* (de Man, 1876) Thorne and Swanger, 1936 (Singh *et al.* 2015). One of the problems with the identification of representatives of both *Longidorus* and *Paralongidorus* is the fact that these genera are rich in species. Presently there are approximately 180 and 90 species, respectively (Gutiérrez-Gutiérrez *et al.* 2018; Liébanas *et al.* 2022). Distinguishing between so many species is not easy, as can be seen in the example of the key to the genus *Longidorus* proposed by Chen *et al.* (1997) and its subsequent supplement (Loof and Chen 1997). In this polytomous key nine morphological and morphometric traits are encoded. The obtained code should subsequently be compared with a list of codes for all known species. In practice this process is surprisingly very time-consuming. Also, since publication of the key, codes for many species have required

changes. This occurs because there is constantly new data on morphometric (and, less often, morphological) traits of many species which broadens known ranges. For example, in the first version of the key the species *Longidorus poessneckensis* Altherr, 1974 (which occurs in Poland) had code A5 B34 C3 D1 E1 F35 G2 H1 I1 (Chen *et al.* 1997). However, after the research of several authors some points had to be changed, for example in A56 B123 and I12 (Kornobis and Peneva 2011). A similar situation has also occurred in some other species. This however leads to an increased level of overlap between codes assigned to two or more species, sometimes to the extent that these species become indistinguishable. Also, it increases the probability of misidentification. A practical solution is the usage of regional keys in which only species occurring in a defined region(s) are taken into account. This approach greatly reduces the number of species which have to be taken into account when constructing the key. This in turn has two main advantages: using the key becomes much faster and the probability of a mistake is considerably smaller. However, a prerequisite for the construction of such a key is sufficient fauna recognition of a given area. Without that the user will frequently face a problem of finding species not included in the key. In this paper a key is proposed for species identification of nematodes of the genera *Longidorus* and *Paralongidorus*. The development of the key was proceeded by over 10 years of intensive faunistic research on the occurrence of both genera in Poland (Kornobis and Peneva 2011; Kornobis 2013; Kornobis *et al.* 2015; Kornobis *et al.* 2017; Kornobis, unpublished) as well as a review of the large body of available literature written by generations of Polish nematologists. Finally, the Introduction would not be complete without mentioning the role of a key based on morphology and morphometry in modern taxonomy where molecular markers are standard. This is especially important if cryptic speciation is taken into account. Such speciation, i.e., speciation without changes in morphology and morphometrics, yet detectable with the use of the molecular markers (or other methods) is taken into account. This phenomenon was reported in Longidoridae (e.g., Palomares-Rius *et al.* 2014; Cai *et al.* 2020). Indeed, a key based solely on morphology is of limited or no use in such cases. However, a reverse situation in which two species are recognizable based on morphology and/or morphometrics and yet exhibit large molecular similarity has also been shown in species *L. intermedius* and *L. piceicola* (Groza *et al.* 2017), both of which occur in Poland. This can cause problems with identification. To illustrate, a simple example can be given: the D2-D3 28S rDNA sequence is commonly used in the taxonomy of many animals including Longidoridae. The *L. intermedius* sequence of that region KT308868.1 (Archidona-Yuste *et al.* 2019) was subjected to BLAST search on the GenBank database on September 8, 2022. The highest match had of course the same sequence, however, the four following places were: *L. piceicola* (LT669801.1), *L. intermedius* (AF480074.1), *L. intermedius* (KF242311.1) and again *L. piceicola* (KY086070.1). It cannot be excluded that future studies will reveal similar situations in other species. These two phenomena, i.e., cryptic speciation versus molecular similarity of species which are separable using morphology and morphometrics illustrate the importance of using the integrative approach in taxonomy, i.e., an approach in which ample data (on morphology, molecular markers, morphometrics and possibly others) are taken into account. The key helps to fulfil that requirement. Furthermore, combined morphological and molecular approaches to identification can prevent misidentification in case of errors in GenBank database, which has been reported in nematodes (e.g., Janssen *et al.* 2017). Another field in which a morphology-based approach to taxonomy is very useful is a quantitative analysis of the number of specimens in soil. Such analyses are of crucial importance for example in phytopathology when assessing if a given species is below or above a damaging threshold or in many other ecological studies. This is particularly important if two or more species from an analyzed group are present in the sample, a situation which is quite common in Longidoridae. To illustrate a simple example can be given: a soil sample which contains two species of the genus *Longidorus*: *L. elongatus* and *L. leptocephalus* in densities of 43 and 19 adults per 100 cm^3 of soil, respectively. Provided adequate training of the person conducting the research, identification and counting of the specimens can be done using the morphological-morphometric approach relatively easily. Using a molecular-based approach the identification can also be done immediately. However, the quantitative part (i.e., assessing the number of each species per 100 cm^3) using solely that approach would be incomparably more technically challenging and expensive. Of course, a mixed approach in which molecular identification precedes counting under a binocular microscope can also be applied. Even here, however, the knowledge of nematode morphology is required simply to distinguish between nematodes, such as *L. elongatus* and *L. leptocephalus* in the given example.

Materials and Methods

The key for species recognition was developed on the basis of data on morphology and morphometrics obtained from two sources. First, data which had been collected by the author (Kornobis and Peneva 2011; Kornobis 2013; Kornobis *et al.* 2015; Kornobis *et al.*

2017; Kornobis, unpublished). Secondly, data which had been obtained from a set of 54 papers written by different authors. These papers included the original description of each species as well as subsequent re-descriptions and/or different records in which data on morphology and morphometrics were provided. While writing the key the use of easy-to-observe traits was emphasized as much as possible. In the first point of the key two traits are used to separate species of the genus *Paralongidorus* from species of the genus *Longidorus*: constriction behind the lips and shape and size of the amphid opening. However, it must be stressed that of these traits only the second one is of systematic importance in differentiating between these genera. Nevertheless, observation of the shape and size of the amphid opening can be difficult in many cases. In contrast, the lack or presence of constriction behind the lips is conspicuous, easily observable and it occurs in both species of *Paralongidorus* occurring in Poland but not in the representatives of the genus *Longidorus*. However, most *Paralongidorus* species (not occurring in Poland) do not have a constriction behind the lips.

Results

The results of this work include a key for species identification of the genera *Longidorus* and *Paralongidorus* occurring in Poland and is presented below. Identification should start from one female, however in some points measurements of additional specimens might be required as well as data on the presence/absence of males.

Key for species identification of the genera *Longidorus* and *Paralongidorus*

1a	Lips separated from the rest of the body by a conspicuous constriction (Fig. 1A, B), amphid opening in the form of a transverse slit*	2
1b	No constriction between the lips and the rest of the body (Fig. 1C–L), amphid opening in the form of a small inconspicuous pore*	3
2a	Ratio V value 36–40**	*Paralongidorus maximus*
2b	Ratio V value 39–51**	*Paralongidorus rex*
3a	Males are either missing or rare in the analyzed population (less than 1 male per 25 females)	4
3b	Males and females occur with a similar frequency in the analyzed population	14
4a	Tail conoid, only on the tip more or less rounded (Fig. 2A–D)	5
4b	Tail in the form of a broadly rounded conoid (Fig. 2E–H)	8
5a	Lips only slightly expanded or not expanded at all*** (Fig. 1C–E), body length 3.4–5.6 mm**	6
5b	Lips more clearly expanded*** (Fig. 1F–G), body length 5–7.4 mm**	*L. attenuatus*
6a	Odontostylet length 59–88 µm**	7
6b	Odontostylet length 78–101**	*L. danuvii*
7a	Ratio c' value 1.0–1.9**	*L. leptocephalus*
7b	Ratio c' value 1.6–2.4**	*L. distinctus*
8a	Ratio a value 138–186	*L. euonymus*
8b	Ratio a value lower	9
9a	Odontostylet length 144–188 µm, anterior body tapering up to about half the distance from the guiding ring to the anterior end (as in Fig. 1H)	*L. piceicola*
9b	Odontostylet length shorter than 144 µm and/or anterior body part different than described in 9a	10
10a	Odontostylet length 128–140 µm, c' > 1	*L. cylindricaudatus*
10b	Odontosylet length and/or c' different	11
11a	Odontosylet length 96–109 µm, lips width 17–20 µm	*L. goodeyi*
11b	Odontosylet length and/or lips width different	12
12a	Odontosylet length 73–103**	*L. elongatus*
12b	Odontosylet length 97–151 µm**	13
13a	Body length ≤ 5 mm (typically 3.5–4.5 mm)	*L. intermedius*
13b	Body length > 5 mm (typically 6–9 mm)	*L. poessneckensis*
14a	Anterior body part tapering evenly, lips not expanded above body contour (close to Fig.1I–J)	15
14b	Anterior body part shaped differently (Fig. 1K–L)	17
15a	Odontostylet length 85–111 µm	*L. caespiticola*
15b	Odontostylet length 119–148 µm	16
16a	Amphidial fovea clearly bilobed	*L. picenus*
16b	Amphidial fovea not bilobed	*L. macrosoma*
17a	Lips width 14–17 µm	*L. artemisiae*
17b	Lips width 19–23 µm	*L. balticus*

*Additional comments on traits used in this point are given in Materials and Methods

**If the value of the feature of the analyzed specimen is in the common interval, the next specimen (specimens) should be measured until the values outside the interval are found

***In practice, observing to what extent lips are expanded can sometimes be difficult. If there are any doubts or problems with decision making at this point, it is strongly recommend to use the second trait, i.e., the body length

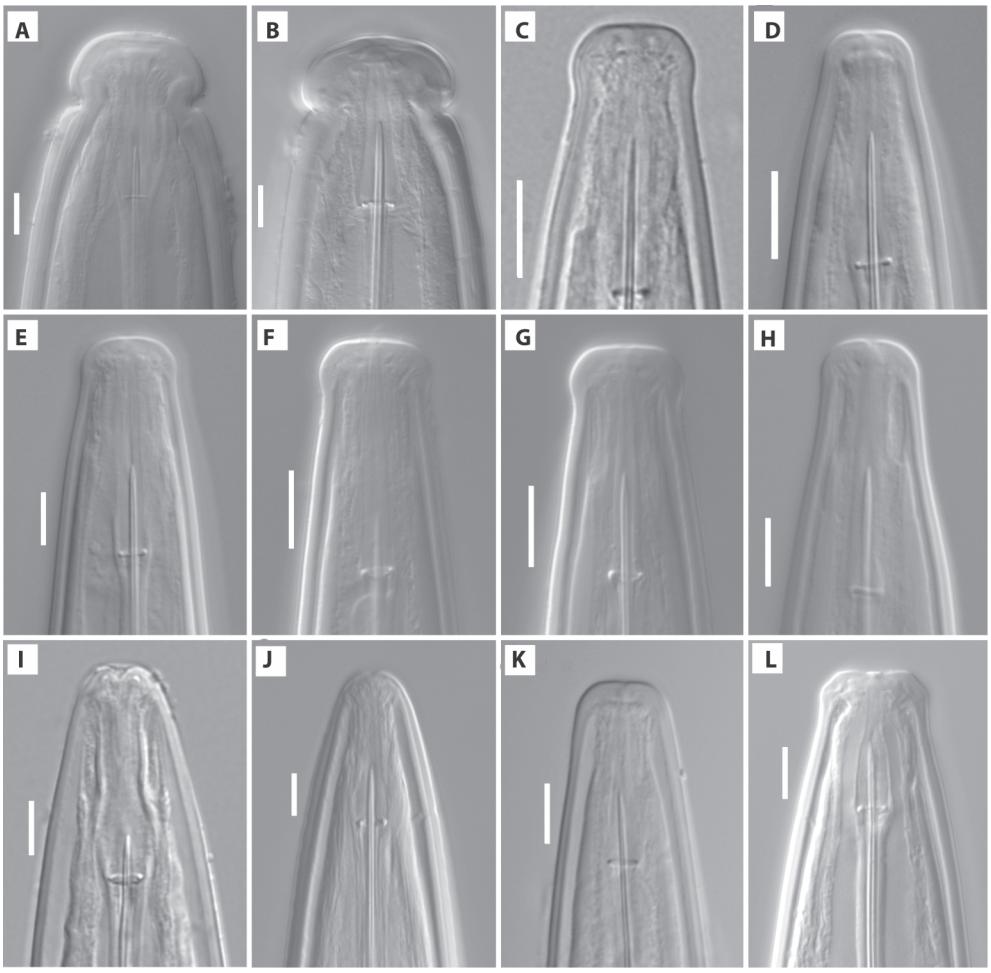

Fig. 1. Anterior body parts of different *Longidorus* and *Paralongidorus* species. Scale bar represents 10 µm

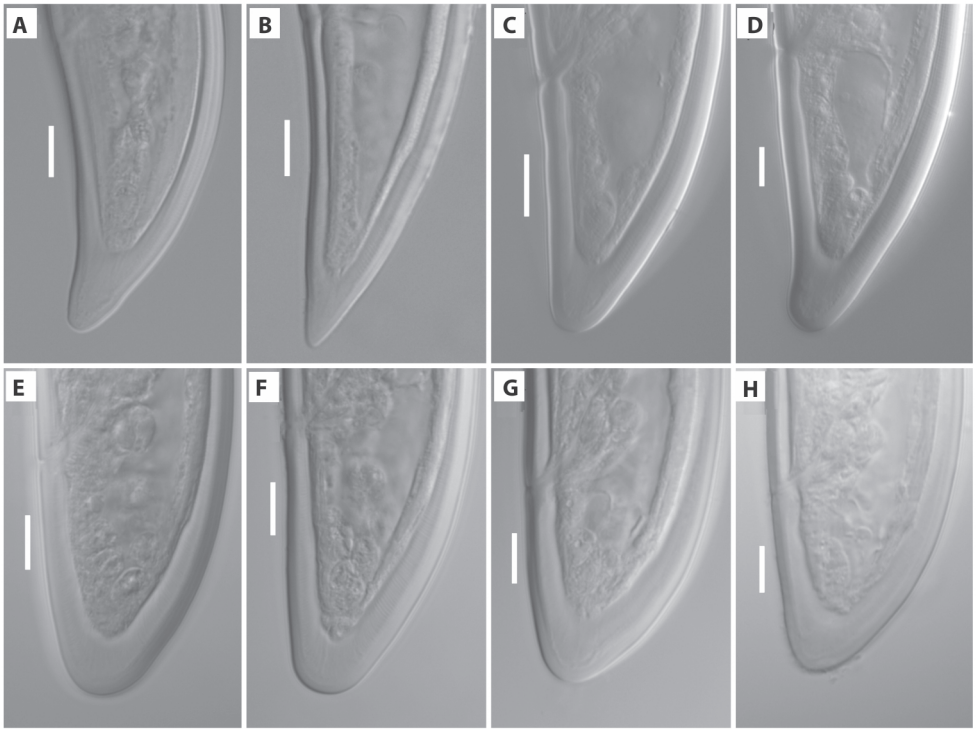

Fig. 2. Tails of different *Longidorus* and *Paralongidorus* species. Scale bar represents 10 µm

Discussion

The presented key enables fast and unambiguous identification of all species of the genera *Longidorus* and *Paralongidorus* known in Poland. The fact that the Polish fauna of both genera is rather well-recognized guarantees that the key will enable identification in the majority of situations. Of course, it does not mean that in Poland there are no unrecorded species belonging to genera *Longidorus* and *Paralongidorus*. Such species could for example be brought with roots of plants imported to Poland or occur infrequently, which makes the detection less probable. If such a situation occurs and specimens from a given population cannot be identified it is recommended to use the key proposed by Chen *et al.* (1997) and Loof and Chen (1997) as well as an overview of the literature published after these papers. However, in practice it is expected that such a situation will happen rarely. Depending on the needs, the results obtained with the key can be further confirmed using molecular methods. With the exceptions of *L. balticus*, *L. cylindricaudatus* and *L. picenus* in GenBank there are comparative sequences for species present in Polish fauna available. Additionally, an effort has been made to make the key presented here as easy to use as possible so that it would be accessible for less experienced users. Therefore, it is believed that it will be a valuable tool for both scientists and specialists working in the field of plant protection, soil ecology, zoology as well as teaching purposes.

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