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Morphological and molecular characterisation of *Paralongidorus rex* Andrássy, 1986 (Nematoda: Longidoridae) from Poland and Ukraine

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Abstract *Paralongidorus rex* was found for the first time in Poland and Ukraine. This paper describes females and juveniles from four populations of this species on the basis of morphology and morphometrics and provides molecular characterization using 18S, ITS1 and D2-D3 expansion segments of 28S rRNA gene sequences. Morphometrically, females from these populations differed slighty in V ratio (means in four populations: 41.9; 42.7; 46.1; 46.8) and odontostylet length (166.6; 170.6; 191.5; 193.2). Phylogenetic analysis showed that *P. rex* had a sister relationship with *P. iranicus*. PCR-D2-D3 of 28S-RFLP diagnostic profiles with five enzymes are given. Additionally, information on new host plants and map of distribution for *P. rex* are provided. The new record of this nematode species, previously identified as *Paralongidorus* sp. (GenBank: AY601582) from Slovakia, is defined based on comparison of sequences of the D2-D3 expansion segments of 28S rRNA gene. Finally, remarks on the potential importance of this species in grapevine production are given.

Keywords *Paralongidorus rex* · Morphometrics · D2-D3 of 28S rRNA gene · ITS1 rRNA gene · 18S rRNA gene · RFLP

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Nematodes of the family Longidoridae are obligatory plant parasites and are considered as economically important pests. Their importance is further augmented by the fact that 18 or 19 of them (details in Taylor and Brown 1997) are known as vectors of plant viruses. The family consists of seven genera (Decraemer and Robbins 2007), one of them is the genus *Paralongidorus* Siddiqi, Hooper & Khan, 1963. This genus is accepted by nematologists, although its definition and species composition may vary according to the authors (Hunt 1993; Siddiqi et al. 1993; Coomans 1996; Escuer and Arias 1997). Presently, *Paralongidorus* comprises of more than 70 species (Decraemer and Robbins 2007), eight of them, *P. georgiensis* (Tulaganov, 1937) Luc & Doucet, 1984, *P. iberis* Escuer and Arias 1997, *P. litoralis* Palomares-Rius, Subbotin, Landa, Vovlas & Castillo, 2008,

P. maximus (Bütschli, 1874) Siddiqi, 1964, *P. paramaximus* Heyns, 1965, *P. plesioepimikis* Palomares-Rius, Cantalapiedra-Navarrete, Gutiérrez-Gutiérrez, Liébanas and Castillo 2013, *P. remyi* (Altherr, 1963) Siddiqi & Husain, 1965, and *P. rex* Andrássy, 1986 were reported from Europe (Palomares-Rius et al. 2008, 2013). One of these species, *P. maximus,* is known as a virus vector (Jones et al. 1994).

Paralongidorus rex was originally described from Hungary on the basis of two females and one juvenile (Andrássy 1986). Subsequently, one female and twenty-six juveniles of this species were found in other location in Hungary and morphologically characterized by Barsi et al. (2007). Recent report of two females of *P. rex* from India (Bohra 2012) requires confirmation. During nematological surveys, we have found populations of this species with numerous specimens in Poland and Ukraine. In this paper we provide morphological, morphometrical and molecular characterization of several *P. rex* populations.

Materials and methods

Soil sampling and morphological study

Totally 925 soil samples were taken during nematological surveys on occurrence of longidorids in Poland. Three out of seven samples containing *P. rex* were taken for analysis and specimen from these populations were used for detailed morphological and molecular study presented here. From Ukraine, one sample containing this species was under study (Table 1). Longidorids were extracted using the sieving and decanting method (Brown and Boag 1988) but with 100 μm sieves. Nematodes for morphological study were killed by heating, fixed in TAF (Courtney et al. 1955) and then transferred to glycerol as described by Seinhorst (1959). Identification and measurements of specimens from Poland were made by first author (FWK) using Zeiss Axioskop 2 microscope and from Ukraine by the second author (SS) using Olympus BX51 microscope. Specimens for molecular study from Poland were video-captured (De Ley and Bert 2002; De Ley et al. 2005) using a Leica DM5000 microscope equipped with Sony HD camera. Video records of morphological features are available upon a request from the first author. Morphometrical indices d and d' as defined by Brown et al. (1994) were also used: anterior to guide ring length divided by body width at lip region and body width at guide ring divided by body width at lip region, respectively.

DNA extraction, PCR assays and sequencing

Specimens for molecular study were transferred to DESS solution (Yoder et al. 2006). After video-capturing specimens were washed in sterilized, deionized water for one min and used for DNA extraction. DNeasy Blood and Tissue Kit (Qiagen) was used for DNA extraction. DNA was eluted with 100 μl 10 mM Tris–HCl pH 8.0. The following primer sets were used for amplification: D2-D3 expansion fragments of 28S rRNA gene with D2A (5′- ACA AGT ACC GTG AGG GAA AGT TG -3′) and D3B (5′- TCG GAA GGA ACC AGC TAC TA -3′) (Rubtsova et al. 2001), 18S rRNA

Table 1 Populations of *Paralongidorus rex* used in this study

Sample code	Associated plants	Location	Geographical coordinates	GenBank accession numbers of rRNA gene sequences
FK 115	*Acer pseudoplatanus* L.	Poland, Kraków	N50.04760; E19.83630	28S: KJ427790; ITS1 clone 1: KM103254; ITS1 clone 2: KM103255
FK 116	*Populus alba* L.	Poland, Skierniewice	N51.96204; E20.14243	28S: KJ427791
FK 274	*Acer pseudoplatanus* L. with other species in the understory	Poland, Solina	N49.40108; E22.46151	28S: KJ427793; 18S: KJ427794; ITS1 clone 1: KM103256; ITS1 clone 2: KM103257
FK 370	*Acer platanoides* L., *Acer pseudoplatanus,* grasses mainly with *Aegopodium podagraria* L.	Ukraine, Lviv, Vysokyj Zamok park	N49.84887; E24.03966	28S: KJ427792

gene with nSSU_F_07 (5′- AAA GAT TAA GCC ATG CAT G −3′) and nSSU_R_81 (5′-TGA TCC WKC YGC AGG TTC AC-3′) (http://www.nematodes.org/research/barcoding/sourhope/nemoprimers.shtml) and ITS1 with rDNA2 (5′-TTG ATT ACG TCC CTG CCC TTT-3′) (Vrain et al. 1992) and rDNA5.8S (5′-ACG AGC CGA GTG ATC CAC CG-3′) (Cherry et al. 1997). PCR was made in 10 µl final volume reaction containing 5 µl Type-it Microsatellite PCR Master Mix (Qiagen), 0.25 mM of each primer and 4 µl of DNA template. Amplification was performed on Applied Biosystems (Foster City, CA, USA) thermal cycler using the following PCR program: initial denaturation for 5 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 60 s at 50 °C and 60 s at 72 °C and a final extension for 5 min at 72 °C. For 18S PCR conditions as described by Gutiérrez-Gutiérrez et al. (2011). PCR products were directly sequenced using BigDye v3.1 kit according to the manufacturer's instruction (Applied Biosystems, Foster City, CA, USA) and run with DNA ABI PRISM 3130xl genetic analyzer. The additional internal primer nSSU_R_13R (5′- GGG CAT CAC AGA CCT GTT A −3′) (http://www.nematodes.org /research/barcoding/sourhope/nemoprimers.shtml) was also used

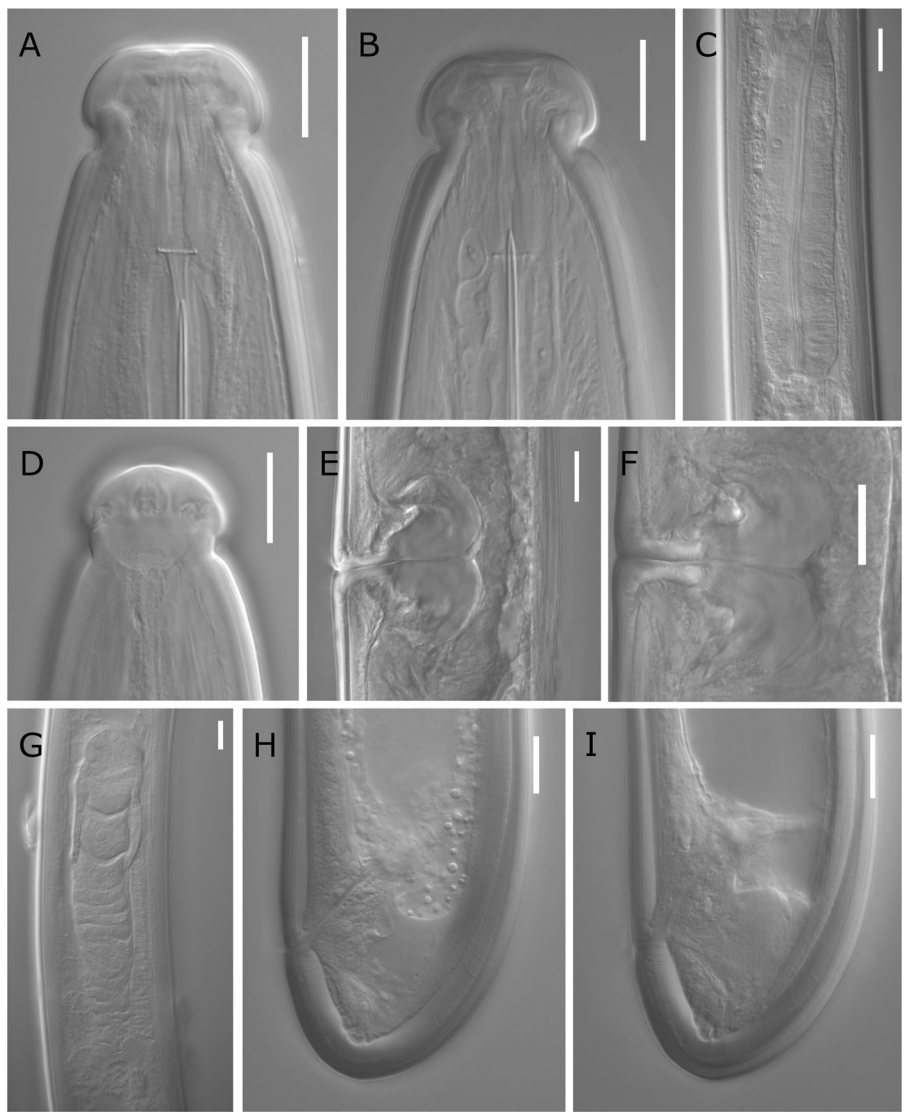

Fig. 1 Microphotographs of *Paralongidorus rex* female. **a**, **b**: Shape of anterior body part; **c**: Pharyngeal bulb; **d**: Shape of amphidial fovea; **e** Vulva region: **f**: *pars distalis* and *proximalis vulvae*; **g**: Reflexed ovary; **h**, **i**,: Tails. Scale bar =20 µm

for sequencing of 18S rDNA PCR product. New sequences were deposited at the GenBank under the following accession numbers: KJ427790- KJ427794 and KM103254- KM103257.

Sequence and phylogenetic analysis

The new sequences of the 18S rRNA, D2-D3 of 28S rRNA and ITS1 rRNA genes were aligned using ClustalX 1.83 with default parameters with their corresponding published gene sequences (He et al. 2005; Palomares-Rius et al. 2008, 2013; Pedram et al. 2012). Outgroup taxa for each data set were chosen according to the results of previously published data. Sequence data sets were analysed with Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) BI analysis for each gene was initiated with a random starting tree under the GTR + I + G model and was run with four chains for 1.0×10^6 generations. The Markov chains were sampled at intervals of 100 generations. Two runs were performed for each analysis. After discarding burn-in samples and evaluating convergence, the remaining samples were retained for further analysis. The topologies were used to generate a 50 % majority rule consensus tree. Posterior probabilities (PP) are given on appropriate clades.

PCR-D2-D3-RFLP

The PCR product of D2–D3 of rDNA was digested by one of the following restriction enzymes: *Alu*I, *Hinf*I, *Bsp*143I (*Mbo*I), *Tru1*I (*Mse*I) or *Rsa*I (Fermentas

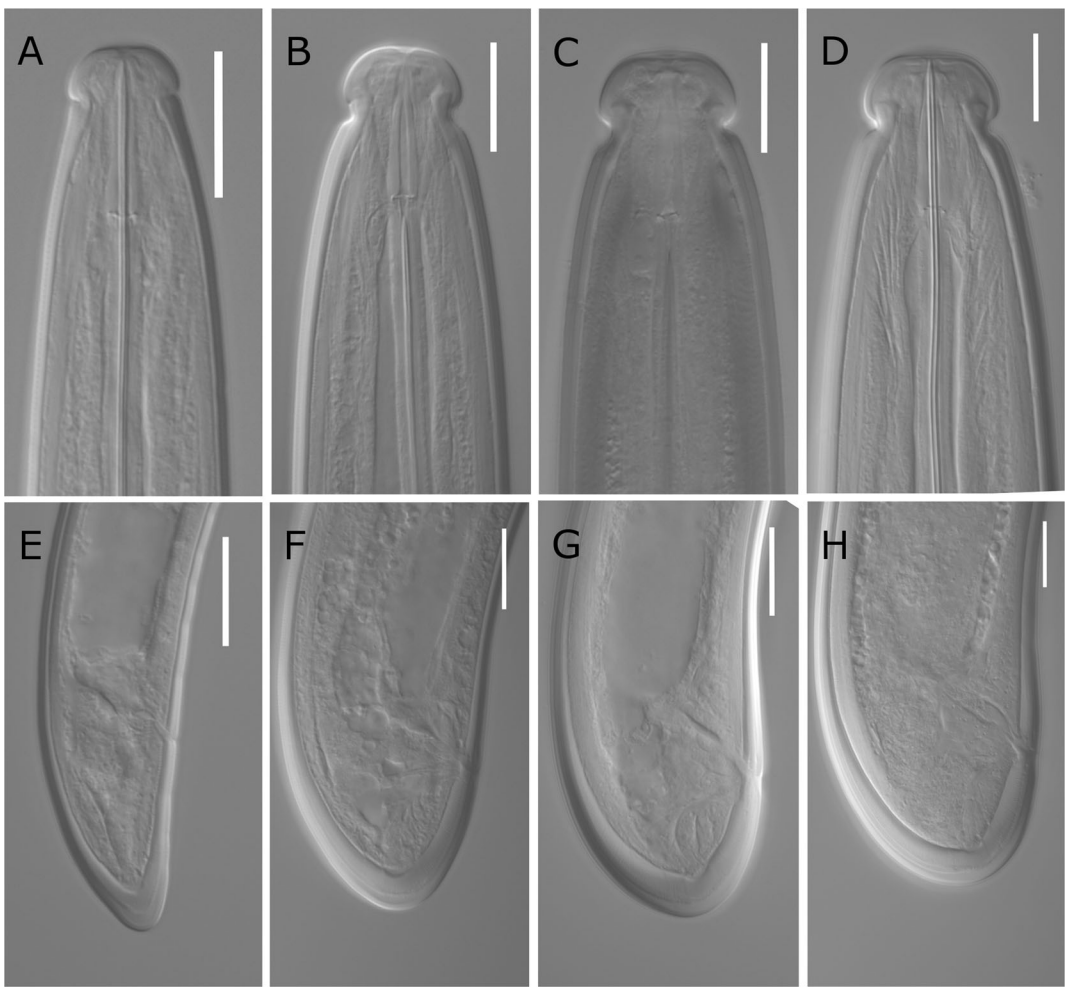

Fig. 2 Microphotographs of *Paralongidorus rex* juveniles. **a-d** and **e-h**: Shape of anterior body part and tail in J1, J2, J3, J4, respectively. Scale bar =20 μm

Table 2 Morphometrics of *Paralongidorus rex* females from Poland and Ukraine. Measurements (μm) and ratios are in the form: mean± standard deviation (range)

Country	Poland			Ukraine
Sample code	FK 116	FK 115	FK 274	FK 370
Location	Skierniewice	Kraków	Solina	Lviv
Character				
n	22	11	8	16
L	9320.7±861.68 (7887–10981)	8461.0±674.8 (7620–9349)	8644.0±676.4 (7608–9790)	9160±909.9 (7446–11263)
a	98.4±7.6 (87.1–112.2)	91.3±4.93 (83.7–98.1)	93.4±4.83 (84.5–99.9)	94.0±4.9 (85.4–107.4)
b	14.4±1.7 (11.2–18.1)	12.7±0.81 (11.8–14.1)	13.7±1.23 (12.4–15.6)	14.3±1.4 (12–17)
c	264.7±31.62 (219.1–337.4)	226.6±25.6 (192.1–274.2)	243.8±24.6 (214.4–284.0)	244.1±26.63 (192–305)
c’	0.53±0.04 (0.44–0.61)	0.58±0.039 (0.5–0.63)	0.55±0.03 (0.5–0.6)	0.53±0.04 (0.45–0.63)
d	1.16±0.054 (1.06–1.25)	1.15±0.035 (1.11–1.21)	1.19±0.06 (1.09–1.25)	1.13±0.052 (1.02–1.22)
d’	1.34±0.027 (1.28–1.4)	1.35±0.032 (1.31–1.4)	1.39±0.034 (1.33–1.43)	1.41±0.06 (1.4–1.5)
V	46.1±1.1 (43.9–48.0)	46.8±1.83 (44.6–51)	42.7±0.82 (41.6–44.0)	41.9±1.28 (38.6–44.3)
Odontostylet length	193.2±6.7 (180–206)	191.5±5.52 (182–198)	166.6±3.42 (163–172)	170.6±4.5 (161–180)
Odontophore length	95.5±8.5 (85–105) n=5	101.7 (99–104) n=3	93.7±6.28 (86–100) n=6	96.9±5.27 (86–103) n=7
Total stylet length	288.5±11.6 (273–303) n=5	290.0 (285–296) n=3	260.7±7.97 (249–269) n=6	265.2±6.87 (256–278) n=7
Anterior end to guide ring	40.5±1.79 (37–43)	39.5±0.9 (38–41)	39.5±2.14 (36–43)	38.8±1.81 (35–42)
Pharyngeal bulb length	177.4±9.8 (165–197)	172.0±13.9 (150–190)	171.4±6.21 (161–178)	185.1±9.1 (166–204)
Pharyngeal bulb width	38.2±1.67 (35–40)	35.0±2.4 (31–37)	36.4±2.56 (32–40)	35.9±1.92 (32–37)
Tail length	35.4±3.26 (28–40)	37.5±2.7 (32–41)	35.6±2.97 (32–40)	37.3±3.89 (31–47)
Hyaline part of tail length	12.0±1.37 (10–15)	12.5±1.18 (11–14)	15.8±1.83 (14–20)	14.6±1.42 (12–18)
Width at level of				
lips	34.8±0.92 (32–36)	34.2±0.75 (33–35)	33.3±0.89 (32–35)	34.3±1.2 (31–36)
guide ring	46.9±1.41 (44–49)	46.3±1.1 (45–49)	46.4±1.77 (44–50)	49.0±1.52 (46–52)
base of pharynx	79.1±3.64 (72–85)	76.3±2.76 (72–80)	76.7±4.57 (70–83)	79.6±3.38 (74–85)
vulva	94.5±2.03 (90–98)	92.6±3.85 (84–98)	92.5±3.93 (86–98)	94.8±5.0 (86–101)
anus	66.6±2.62 (63–72)	65.0±2.6 (59–68)	64.4±3.2 (60–69)	68.5±3.29 (62–74)

Table 3 Morphometrics of *Paralongidorus rex* juveniles from Poland and Ukraine. Measurements (μm) and ratios are in the form: mean±standard deviation (range)

Country	Poland				Ukraine			
Sample code	FK 116				FK370			
Location	Skierniewice				Lviv			
Stage	J1	J2	J3	J4	J1	J2	J3	J4
Character								
n	7	7	3	1	15	16	26	19
L	1717.1±91.29 (1608–1845)	2897.9±437.76 (2345–3559)	3785.2 (3602–4009)	6607	1591±85.6 (1429–1760)	2614±275.1 (2211–3221)	4213±427.2 (3594–5302)	6422±454.4 (5572–7249)
a	51.3±4.0 (47.1–59.5)	59.5±2.48 (57.1–63.9)	71.9 (68.1–75.6)	82.6	46.4±3.84 (37–52)	54.2±3.54 (45–60)	67.5±5.09 (58–81)	79.9±4.7 (69–89)
b	5.4±0.31 (5.1–6.0)	6.9±0.89 (5.8–8.2)	7.6 (6.9–8.7)	11.6	5.1±0.63 (4.3–6.3)	6.8±1.0 (5.3–9.2)	8.2±1.01 (5.1–10.4)	11.1±0.72 (10.0–12.7)
c	48.7±4.88 (42.3–57.7)	89.3±12.22 (75.6–111.2)	117.8 (111.4–128.7)	178.6	48.7±3.73 (44–59)	77.5±8.79 (65–93)	115.5±14.4 (99–161)	171.7±16.7 (144–204)
c’	1.35±0.08 (1.26–1.5)	0.82±0.08 (0.7–0.93)	0.67 (0.62–0.72)	0.6	1.2±0.11 (1.02–1.41)	0.8±0.08 (0.71–1.0)	0.7±0.06 (0.58–0.86)	0.6±0.04 (0.54–0.69)
d	1.42±0.05 (1.33–1.47)	1.36±0.15 (1.27–1.69)	1.20 (1.15–1.25)	1.16	1.5±0.11 (1.29–1.66)	1.2±0.06 (1.12–1.3)	1.1±0.05 (1.01–1.18)	1.1±0.03 (1.03–1.19)
d’	1.36±0.07 (1.27–1.47)	1.43±0.14 (1.35–1.75)	1.37 (1.35–1.42)	1.35	1.46±0.084 (1.36–1.6)	1.39±0.08 (1.26–1.6)	1.42±0.10 (1.2–1.55)	1.37±0.06 (1.33–1.51)
Odontostylet length	93.4±1.99 (90–96)	109.9±2.27 (107–114)	140.0 (140–140)	161	84.1±2.81 (79–88)	104.1±2.5 (98–108)	126.1±5.93 (115–139)	145.8±3.78 (142–155)
Replacement odontostylet length	109.3±1.38 (108–112)	141.4±5.89 (132–148)	167.7 (158–183)	185	100.7±4.0 (92–107)	122.3±4.62 (114–129)	147.2±9.32 (128–176)	168.9±3.62 (162–175)
Anterior end to guide ring	21.3±0.8 (20–22)	26.6±0.79 (26–28)	30.3 (30–31)	36	21.8±1.14 (20–24)	24.0±0.91 (22–26)	28.8±1.14 (27–32)	33.8±1.24 (32–36)
Tail length	35.4±2.7 (32–39)	32.6±3.6 (26–37)	32.3 (28–36)	37	32.5±2.12 (29–36)	36.0±2.8 (29–39)	36.3±2.61 (32–44)	37.2±3.0 (33–43)
Hyaline part of tail length	7.4±0.98 (6–9)	6.7±1.7 (4–9)	8.3 (8–9)	10	6.2±0.91 (5–8)	7.9±0.93 (6–9)	10.1±1.4 (9–15)	12.4±1.37 (10–16)
Width at level of								
lips	14.9±0.38 (14–15)	19.7±1.89 (16–22)	25.3 (24–26)	31	14.6±0.63 (14–16)	20.1±0.81 (19–22)	26.1±1.43 (24–30)	30.9±0.81 (29–32)
guide ring	20.4±0.98 (19–22)	28.0±1.29 (26–30)	34.7 (34–35)	42	22.0±0.91 (20–23)	28.1±1.09 (26–30)	36.2±1.7 (33–39)	43.1±1.76 (40–46)
base of pharynx	33.1±1.95 (31–37)	45.9±5.81 (40–56)	51.3 (50–52)	69	33.2±1.62 (31–36)	45.6±4.0 (37–53)	58.0±3.58 (51–65)	72.3±3.23 (66–78)
mid body	33.6±2.57 (31–39)	48.7±6.86 (41–61)	53.3 (52–55)	80	34.1±2.5 (31–39)	48.1±5.72 (38–60)	62.4±4.29 (54–74)	80.3±5.0 (71–92)
anus	26.3±2.14 (25–31)	39.6±4.89 (33–46)	48.0 (45–50)	62	27.1±1.63 (25–30)	40.1±3.5 (33–48)	51.6±2.72 (47–56)	61.4±3.56 (55–67)

International, Inc.) (Subbotin et al. 2014) in the buffer stipulated by the manufacturer. Digested DNA was run on a 1.4 % TAE buffered agarose gel, stained with ethidium bromide, visualised on UV transilluminator and photographed. The length of each restriction fragment from the PCR products was predicted by a virtual digestion of the sequences using WebCutter 2.0 (www.firstmarket.com/cutter/cut2.html).

Results and discussion

Description of *Paralongidorus rex*

Ilustrations: Figures 1 and 2; measurements: Tables 2 and 3.

Female

Body long and robust, slightly tapering toward anterior end, C-shaped to spiral, always more coiled in the posterior half. Cuticle usually smooth, in some specimens with fine transverse striation, 5–6 μ thick at the level of guiding ring, 4–5 μm along the body, 14–15 and 14–16 μm at half of the tail length at ventral and dorsal side, respectively. Lip region 15–18 μm high, anteriorly usually flat, sometimes slightly convex, laterally widely rounded, separated from the rest of the body by clear constriction followed by 2–4 μm long depression. Cephalic lobes conspicuous. Amphid with slit-like aperture, about two-thirds as wide as lip region, amphidial fovea large, stirrup-shaped, with fine rib-like structure in proximal part. Stylet guiding ring simple, 6.5 (6–7) μm wide. Lateral chord 11.8 (10–15) and 28.0 (25–30) μm wide at the level of base of pharynx and mid-body, respectively. Nerve ring encircling odontophore at its base, 257.6 (240–280) μm from the anterior end. A second nerve ring situated posteriorly, about 1.5 corresponding body width. Pharyngeal bulb occupying 27.3 (24–33)% of total pharynx length, pharyngeal gland nuclei situated as follows: DN 37.8 (35–41), RS_1N

Fig. 3 Distribution map of *Paralongidorus rex* occurrence

57.1 (54–60) and L_1SN 56.6 (52–60)%. Dorsal gland nucleus diameter 3.5–4 μm and latero-ventral nuclei 4.5–5 μm. Vagina occupying 54.6 (49–59)% of corresponding body width, *pars distalis vaginae* and *pars proximalis vaginae* 24.1 (20–28) and 31.1 (27–35) μm long, respectively. Reproductive system with both genital branches equally developed. Anterior and posterior uterus 469.9 (415–580) and 500.9 (380–688) μm long, respectively, with no specific structures or sperm. Strong sphincter between oviduct and uterus. Rectum 46.7 (39–53) μm long. Tail round, with two pores on each side, situated subterminally and slightly shifted to ventral side

Male

Not found

Juvenile

Four juvenile stages were found. Body J-shaped in J1, C-shaped in J2 and J3 and similar to adults in J4. Lips in all stages anteriorly usually flat, sometimes slightly convex, laterally rounded. In J1 lip separated from the rest of body by constriction but without depression. In other stages lips separated by clear constriction followed by depression. Tail conoid in J1, broadly rounded conoid in J2, similar to adults in J3 and J4. Genital primordorium length 33.6 (32–39) μm (n=7) in J1; 48.7 (41–61) μm (n=7) in J2; 53.3 (52–55) μm (n=3) in J3; 102.5 (n=1) in J4.

Distribution and host plants

In Poland, seven (0.76 %) out of 925 soil samples were identified as containing *P. rex.* Distribution map of known population of this species is presented at Fig. 3. Information on plant hosts and geographical coordinates of samples are given in Table 1.

Females from four populations studied here are similar to each other with an exception of some variations in three morphometrical traits: odontostylet length, V ratio value and hyaline part of tail length. In females of populations from Skierniewice (FK116) and Kraków (FK115) odontostylets are longer and V values are higher than in those from Solina (FK 274) and Lviv (FK370) (Table 2). Conversely, hyaline part of tail is clearly longer in populations from Solina and Lviv. Females of populations from Poland and Ukraine are also similar to those from the type population from Hungary (Andrássy 1986), with some differences in V ratio. The value of this ratio (V=47) is similar to

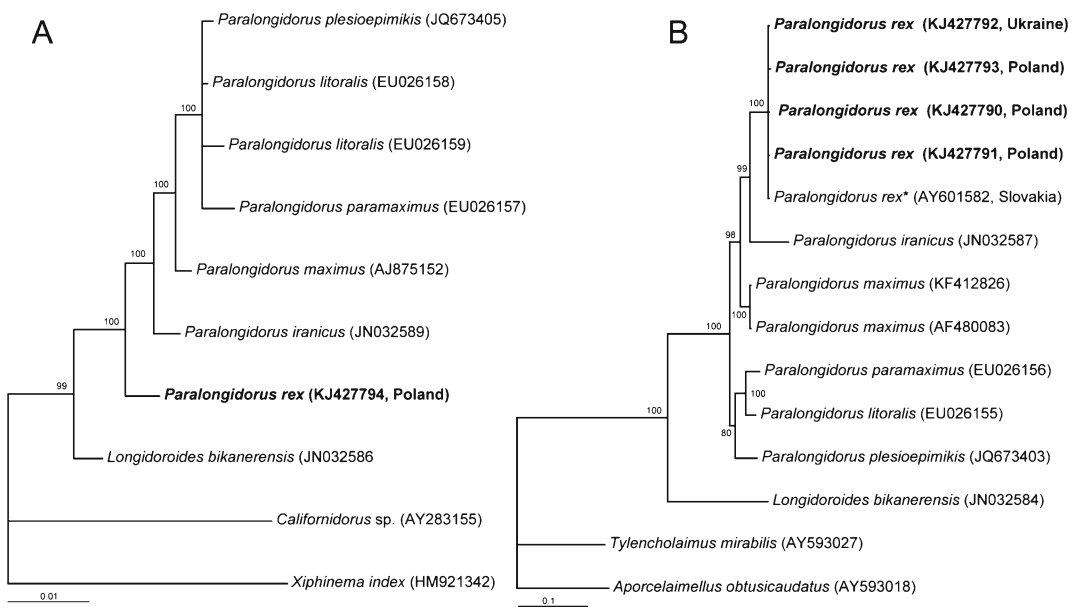

Fig. 4 Phylogenetic relationships within the genus *Paralongidorus.* Bayesian 50 % majority rule consensus trees as inferred from (**a**) the 18S rRNA gene sequence alignment and (**b**) the D2 and D3 expansion segments of 28S rRNA sequences alignment under the GTR+G+I model. Posterior probabilities more than 70 % are given for appropriate clades. Newly obtained sequences in this study are in bold letters, astersik (*) indicates sequence from specimen described as *Paralongidorus* sp. in GenBank (more details in text)

specimens from populations from Skierniewice and Kraków (mean 46.1 and 46.8, respectively) and different from those in Solina and Lviv (42.7 and 41.9, respectively). Odontostylet length in females of the type population (178 and 180 μm in two specimens) is shorter than those for populations from Skierniewice and Kraków (180–206 and 182–198 μm, respectively), slightly longer than the range in population from Solina (163–172 μm) and from Lviv population (161–180 μm).

Juveniles from Poland (Skierniewice) and Ukraine (Lviv) are also morphometrically similar, with an exception of variations in odontostylet and replacement of odontostylet lengths, both of which are longer in population from Skierniewice (Table 3). In the first description given by (Andrássy 1986), single fourth stage juvenile is similar to specimens from our study, with

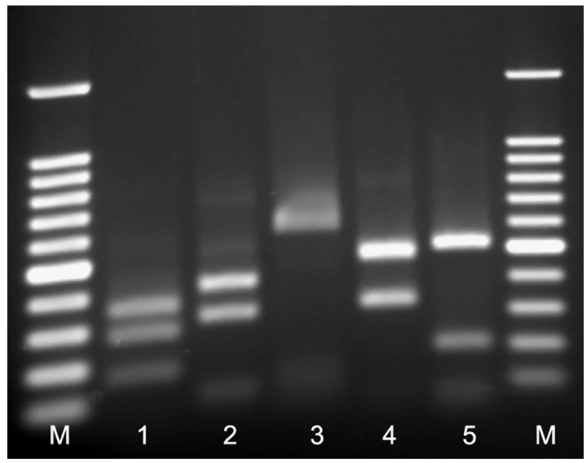

Fig. 6 PCR-D2-D3-28S-RFLP diagnostic profile for *Paralongidorus rex*. M - 100 bp DNA marker (Promega, USA), 1 - *Alu*I, 2 - *Hinf*I, 3 - *Bsp*143I, 4 - *Tru*1I, 5- *Rsa*I

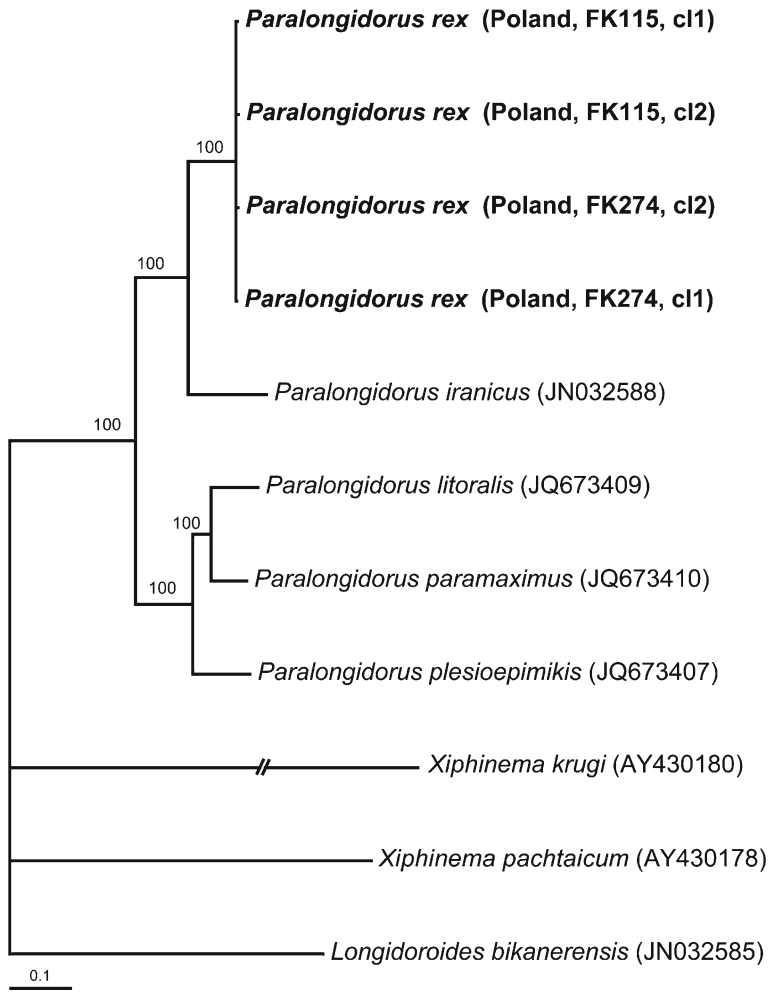

Fig. 5 Phylogenetic relationships within the genus *Paralongidorus*. Bayesian 50 % majority rule consensus trees as inferred from the ITS1 gene sequence alignment under the GTR+G+I model. Posterior probabilities more than 70 % are given for appropriate clades. Newly obtained sequences in this study are in bold letters

an exception of higher "a" index (102 in the type population vs 82.6 and 69–89 in populations from Skierniewice and Lviv, respectively). Descriptions of all juvenile stages were also given by Barsi et al. (2007). In comparison with this Hungarian population, juviniles from Poland and Ukraine are characterised (Table 3) by greater lips width [in J1: 14.9±0.38 (14–15) and 14.6±0.63 (14–16); in J2: 19.7±1.89 (16–22) and 20.1±0.81 (19–22); in J3: 25.3 (24–26) and 26.1±1.43 (24–30); in J4: 31 and 30.9±0.81 (29–32)] vs 12.8 (12.1–13.2) μm in J1; 17.7 (17.2–18.4) μm in J2; 22.5(22.4–23) μm in J3; 26.3 and 28.8 μm in J4. Juveniles from Hungarian population described by Barsi et al. (2007) are similar in odontostylet length [78.4 (76.4–83.3) μm in J1; 94.4 (89.1–98.3) μm in J2; 122.5 (119.0–129.3) μm in J3 and 145.0–145.6 μm in J4] with those from population from Lviv and slightly different with those from Skierniewice population. Identification code of *P. rex* in polytomous key of Escuer and Arias (1997) including data presented here and other available sources (Andrássy 1986; Barsi et al. 2007) is: A1; B1; C4; D3; E1; F5,6; G7; H1,2; I2,3; J1; K6; L1,2,3; M3; N-; O-.

Phylogenetic relationships of *P. rex* with other species

Phylogenetic relationships of *P. rex* with other *Paralongidorus* are presented in Figs. 4 and 5. In the D2-D3 of 28S rRNA and ITS1 rRNA gene trees *P. rex* showed a sister relationship with *P. iranicus.* Sequences of *P. rex* differs from those of *P. iranicus* in 12 bp for 18S rRNA gene (~1650 bp), in 43 pb for D-D3 of 28S rRNA gene (~705 bp) and in 158 bp for the ITS1 rRNA gene (~740 bp). The analysis of the D2-D3 of 28S rRNA gene sequences of three *P. rex* populations from Poland and one Ukraine (FK115 and FK370) revealed that their sequences were identical each other and with that of *Paralongidorus* sp. from the GenBank accession number AY601582 submitted by He et al. (2005). Sequences of the D2-D3 of 28S rRNA gene from FK 116 and FK 274 populations differed in one nucleotide. Therefore, we consider this *Paralongidorus* sp. with AY601582 sequence as a representative of *P. rex* and, thus, constitute a new geographical record. He et al.(2005) reported that that this sample was taken from Czech Republic, however, Dr Shesh Kumari from the Crop Research Institute in Prague informed the first author that location of the sample was in Slovakia, as it has been reported in the GenBank record for the sequence. *Paralongidorus rex* has been previously reported from two localities in Hungary (Andrássy 1986; Barsi et al. 2007), and, thus, in the results of the study, the distribution of this species is extended for Poland, Ukraine and Slovakia.

PCR-RFLP study

PCR-D2-D3-RFLP profiles generated by five enzymes for *P. rex* is given in Fig. 6. Lengths of restriction fragments from RFLP for the D2–D3 of the 28S rRNA gene obtained with using WebCutter 2.0 were: *Alu*I - 347, 284, 180, 14 bp; *Hinf*I - 405, 317, 103 bp; *Bsp*143I - 590, 128, 63, 44 bp; *Tru*1I - 483, 323, 19 bp; *Rsa*I - 512, 204, 70, 33, 6 bp.

Barsi et al.(2007) reported *P. rex* from grapevine and suggested potential importance of this species as a pest of this culture. This importance of this nematode is further augmented by the relative abundance of this species in Poland and probably also in Ukraine. For example, *Paralongidorus maximus* (Bütschli, 1874) Siddiqi, 1964 being closely related species and considering as a potentially important pest for grapevine production was present only in one sample out of about 2700 samples in longidorid survey conducted in Poland (Szczygieł and Brzeski 1985). In our present study *P. maximus* was also rare as it was found only in one sample out of 925 (data not published). Thus, *P. rex* occurs more frequently, at least in Poland, and therefore, further studies are required to elucidate its impact on grapevine production.

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