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GLOBODERA ARTEMISIAE (EROSHENKO ET KAZACHENKO, 1972) (*NEMATODA: HETERODERIDAE*) FROM POLAND

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Abstract: *Globodera artemisiae* (Eroshenko et Kazachenko, 1972) was found in Poland in autumn of 2004. The nematodes developed on *Artemisia vulgaris* L. Morphological and morphometric characteristics of the Polish population correspond to earlier known populations from Far East of Russia, Armenia, China, Germany and Sweden. The traditional identification was confirmed by molecular methods. On the basic of rDNA sequences of *G. artemisiae*, deposited in GenBank the product of expected size was obtained. Subsequently, the results were confirmed by sequencing analysis.

Key words: cyst nematodes, *Globodera artemisiae*, identification, distribution

INTRODUCTION

Globodera artemisiae was described from Far East of Russia, as a parasite of *Artemisia rubripes* N. (Eroshenko and Kazachenko 1972, 1983). Later the species was found, as a parasite of *A. vulgaris* L., in Armenia (Pogosjan and Karapatjan 1975), Germany (Sturhan 1968; Sturhan and Krall 1991), China (Cheng et al. 1994) and Sweden (Manduric and Andersson 2004).

In 1998 *G. artemisiae* was recorded from Poland, on basis of rather atypical cysts (Brzeski 1998). Further investigation and examination of slides from Brzeski's collection showed that it was a mistake (Kornobis 2004a, 2004b, unpublished data). Regardless of it, in autumn 2004 more typical population of *G. artemisiae* was found in Poland.

The purpose of this work was to describe morphological and molecular features of nematodes of this population.

MATERIALS AND METHODS

Population of *G. artemisiae* was found in Poznań (XU 20 in UTM grid), in root zone of *A. vulgaris*. Pot experiment confirmed that *A. vulgaris* was a good host for the species.

Morphological and morphometric analysis

Altogether 60 second-stage juveniles and 60 cysts were examined for a number of characters of specific taxonomic value for rapid identification. Juveniles were killed and fixed in 2% formaldehyde and mounted in temporary slides. Vulval cones were prepared as described by Hooper (1985). Observations and measurements were made using Zeiss Axioskop 2+ light microscope, with differential interference contrast equipments.

Molecular identification

DNA isolation and purification**.** The genomic DNA of nematodes was isolated using the Dneasy Tissue Kit (QIAgen) according to the manufacturer instruction.

PCR amplification**.** The total DNA was used for amplification by PCR with specific primers complementary to rDNA sequence (ITS1/2 of rDNA). PCR reaction were performed in total volume of 20 µl. The final mixture contained 1µM primers, 200 µM dNTPs, 1 x PCR buffer (10 mM Tris-HCl, pH 8.8 at 25ºC, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100) and 1 U/25 µl Taq polymerase. The samples were amplified for 35 cycles. Each cycle consisted of the following steps: denaturation at 95°C for 1 min, annealing at temperature shown in Table 1, and extension at 72°C for 1 min. Reaction was performed in a DNA thermal cycler Eppendorf Cycler Personal. Products of amplification were analysed in agarose gel with addition of ethidium bromide and visulalised under UV light.

Primers used for PCR technique for detection of *Globodera artemisieae*

Primer	Primer sequence (5′-3′)	Region of hybridization	Anneaing tempearature [°C]
GloArt1	GCACCGCCAGCGTTTTTCTC	ITS1	–
GloArt2	CAGGCGTGCCAATGGATGTTACTC	5.8S	GloArt 1–2 – 55,7°C
GloArt3	CCTGGCATTGGCGTGTGGTT	ITS1	–
GloArt4	CTGTAAAGCGCGAAAGAAAGCATA	ITS2	GloArt 3–4 – 56.3°C

DNA cloning and sequencing. DNA fragments obtained after PCR were isolated from agarose gel with QiaExII Gel Extraction Kit (QIAgen) and then cloned into pGEM-T-Easy Cloning Vector System (Promega), according to the manufacturer's instructions. *E. coli* strain TG1 competent cells were transformed by electroporation. Clones were verified using X-Gal +IPTG system. Plasmids from positively verified clones were isolated using QIAprep Spin Miniprep Kit (QIAgen) and then automatically sequenced (IBB PAS Sequencing Service).

RESULTS AND DISCUSSION

Morphological and morphometric analysis

Morphometric data of cysts and juveniles are shown in Table 1. The cysts were generally not spherical but tended to be oval, in many cases irregular (Fig. 1). Mature cysts were brightly brown, fresh isolated ones were sometimes white but in distilled water they went to brown during 2–3 days, with similar to *G. rostochiensis* "gold "

phase. Appearance of the fenestral region is shown on Figure 2. The second stage juveniles stylet basal knobs are very different, from slightly forward painting to rounded. The lips had four annules.

Table 1. Morphometric characteristics of the Polish population of *Globodera artemisiae*

Life cycle stage	Character	Mean	Min. – Max.
Cysts	length (μm)	524	384–640
	wide (μm)	425	312–696
	Granek's ratio	1.4	0.6–4.5
Second stage juveniles	length (μm)	461	425–490
	a	25	23–27
	b′	2.6	2.4–2.9
	c	10.5	9.3–11.3
	c′	3.8	2.3–4.6
	hyaline part (μm)	55	46–66

a – ratio of body length to largest body with
b′ – ratio of body length to pharynx length from anterior end to posterior end of glandular lobe
c – ratio of body length to tail length
c′ – ratio of tail length to body width at anus level

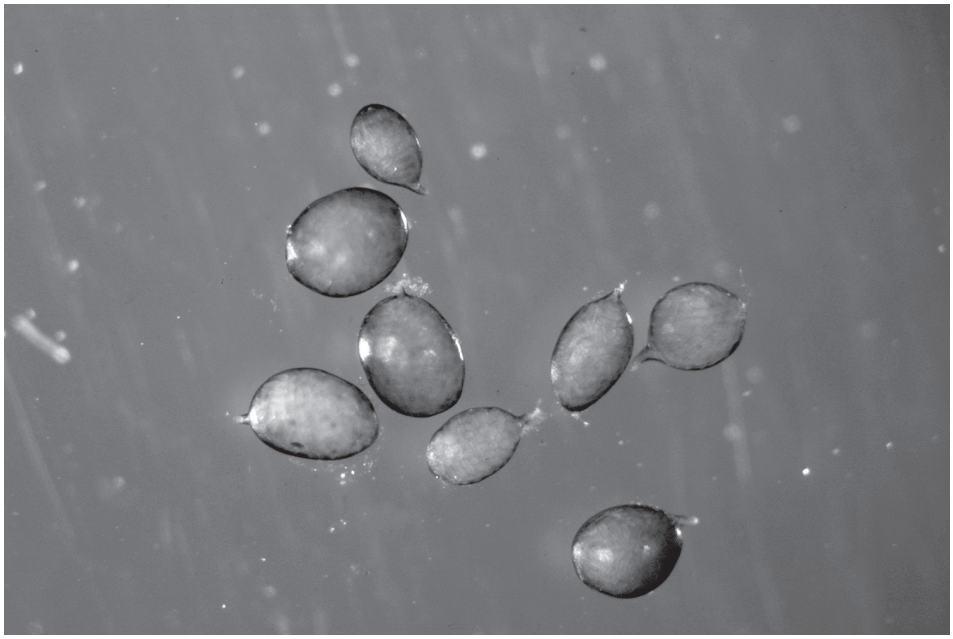

Fig. 1. Cysts of *Globodera artemisiae*

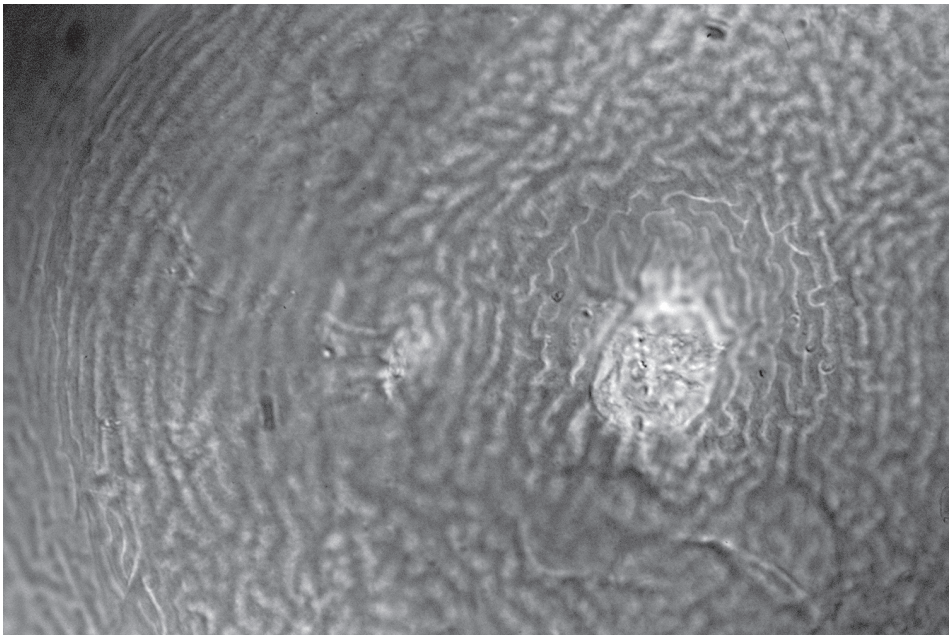

Fig. 2. Fenestral area of cysts of *Globodera artemisiae*

The morphological and morphometric characters of our population were similar to those of the populations from Russia, Sweden, China and Germany, summarized by Manduric and Anderson (2004). Atypical highest value (4.5) of the Granek's ratio was given only by one cyst.

Molecular identification

On the basis of sequences from GenBank of three known *G. artemisiae* populations (from Sweden, Russia and Germany) two primer pairs were designed, that were used for PCR amplification. The applied primers and conditions of reactions (as in methods) gave single and distinct products in PCR, that have been cloned and afterwards sequenced. Results have confirmed that analyzed nematode was indeed *G. artemisiae* (Fig. 3).

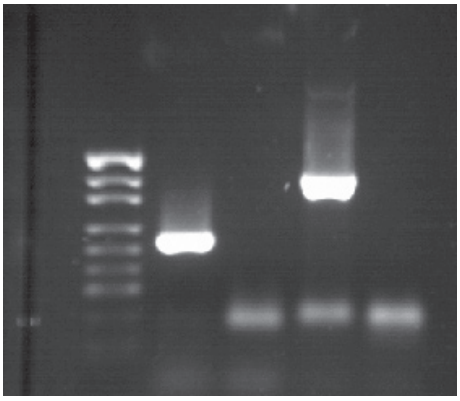

Fig. 3. *Globodera artemisiae* identification by using PCR with primers complementary to ITS1-5.8S rDNA(1) and ITS1-ITS2 (2)

Analysis of ITS1, 5.8S and ITS2 sequences from our speciec, and their comparison with data from GenBank has revealed a high identity level of Polish population of *G. artemisieae* with other populations from Europe which sequences are known.

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POLISH SUMMARY

GLOBODERA ARTEMISIAE (EROSHENKO ET KAZACHENKO, 1972) Z POLSKI

Jesienią 2004 roku znaleziono w Polsce populację *Globodera artemisiae*. Nicienie rozwijały się na *Artemisia vulgaris* L. Morfologiczna i morfometryczna charakterystyka polskiej populacji odpowiada populacjom opisanym wcześniej z Dalekiego Wschodu Rosji, Armenii, Niemiec i Szwecji. Charakterystykę morfologiczną potwierdziła identyfikacja metodami molekularnymi na podstawie rDNA.