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# Phylogenetic Affinities of the *Globodera pallida* Inferred From the mtDNA *cyt-b* Gene Polymorphism

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The mitochondrial cytochrome b gene marker was used to investigate the genetic variability of *G. pallida* populations of different origins and selection on three sources of resistance. **Aim**. To sequence the mitochondrial gene *cyt-b* and to clarify its application as a genetic marker for intraspecific genetic diversity study, phylogenetic analysis and nematode virulence assessment. **Methods.** The cysts of nematodes were used as a source for DNA extraction. Polymerase chain reaction was performed using the specific primers of INRAcytbL and INRAcytbR, followed by the amplified product sequencing. The nucleotide sequences were processed and aligned using software PhredPhrap, CONSED and Clustal W. MEGA-4, DNADIST software package; while PHYLIP and Arlequin were used for statistical analysis. Phylogenetic trees construction and visualization were performed using the software package PHYLIP and TREEVIEW. **Results.** The phylogenetic analysis based on mitochondrial cytochrome b gene sequences has showed that the Ukrainian populations of *G. pallida* were almost identical to other populations from the Europe. Limited genetic variability was observed between *G. pallida* populations distributed in the Europe and Ukraine, accounting for 82.3 per cent ($P < 0.05$) of the genetic variability inferred from the mitochondrial cytochrome b gene polymorphisms within the populations studied. *G. pallida* populations selected on three sources of resistance were similar but not identical indicating that changes in mtDNA haploid type frequency had taken place as a result of the selection regime, but the marker used was not yet applicable for virulence monitoring. **Conclusions.** The obtained data prove the hypothesis that *G. pallida* populations in Ukraine are the result of the continuing spread of the species within the Europe and not the consequence of additional introduction from the South America.

**Key words:** *Globodera pallida*, mitochondrial DNA, cytochrome *b* gene, phylogenetic analysis.

## INTRODUCTION

For a long time the study of evolutionary interrelations within the phylum *Nemathelminthes* was based on the limited quantity of clearly specified morphological features or ecological factors. That sometimes caused various interpretation of similar facts, and also debates, in classification system of the nematodes, in particular [1]. Due to implementation into practice of the new systematic approaches connected to the use of molecular markers, the opportunity to verify existing and to formulate new phylogenetic hypotheses on the new, genetic level appeared [2].

The molecular markers are conditionally divided into three groups: 1) caused by mutations in the anonymous segments of genome, which can be detected by the diverse variants of polymerase chain reaction (PCR), restriction fragments length polymorphism or molecular hybridization; 2) specific and repetitive DNA elements; 3) connected to the nucleotide polymorphisms of the specific genes or non-coding segments of DNA [3].

First steps in the introduction into nematological studies of the third type markers were made by the use of the ribosomal DNA sequence, in particular, the gene, which codes the small sub-unit of ribosomal RNA (SSU rDNA). Revision of 53 species of the nematodes carried out at the end of the 90's of the past century on the basis of this marker allowed the authors to formulate

the phylogenetic hypothesis, which was subsequently widely assigned on resolution of many questions concerning the morphological evolution, systematics and parasitism of nematodes [4, 5].

The contemporary assortment of the third type molecular markers includes the genes not only of nuclear, but also mitochondrial DNA (mtDNA), whose advantages as molecular marker consist of haploidy, maternal inheritance, the absence of recombination and high speed of mutations [6].

The small sizes of mtDNA (in comparison with the nuclear one) make it possible to sequence and analyze complete mtDNA by rapid rates. That is confirmed by the accumulated base of such data in the GenBank: as of the September 2013 it disposed the complete sequences of mtDNA of 45 species of the nematodes [7], while the complete genome is published only for 10 species of nematodes [8].

For the very moment, the opportunity of using mtDNA as molecular marker not only at the level of taxonomic rank, but also populations and individuals has been proven. Thus, some segments of mtDNA are successfully used for distinguishing of the populations (pathotypes) of plant parisitic nematodes [9–11], helminths [12] and free-living nematodes [13].

The compact structure of mtDNA makes it possible to determine its structural organization, together with the information against the nucleotide composition. It can be used in the phylogenetic studies, since it is considered that the rearrangements of 37 typical genes of mtDNA appear to be unique, rare events and easily recognized phenomenon because of the homology of the mitochondrial genes across of multicellular organisms [14]. It is the structural organization of mtDNA that is assumed as the basis of the reproduction of the phylogenetic attitudes of the representatives of such phylum as *Arthropoda, Mollusca, Brachiopoda, Annelida* and others [15].

The similar studies of the plant parasitic nematodes are considerably inferior in their quantity; however, even those infrequent researches, which appeared during two recent decades, showed the special evolution of mtDNA in the nematodes relative to other multicellular organisms. That is confirmed, in particular, due to the structural organization of mtDNA of *Meloidogyne javanica* [10], *Radopholus similis* [16], *Globodera rostochiensis* [17] and *G. pallida* [18–20].

Thus, two latter closely-related species – *G. rostochiensis* and *G. pallida* – have the most complicated among the representatives of the *Metazoa* organization of mtDNA (it has been known now), since it is proven that the mitochondrial genes of these species are located not in one, as usual, but in several separate annular molecules of mtDNA (small circular mitochondrial DNA – scmtDNA) by size of 6.3-9.5 thousand bp each [18–19].

The indicated size of scmtDNA is of insufficient length to encode all mitochondrial genes. It is confirmed with the size of the smallest of mtDNA that is sequenced for the nematode *Onchocerca volvulus*, which is 13.700 bp [21]. As it was confirmed with the investigations conducted by the British scientists, the mitochondrial genes of the potato cyst nematodes were located in scmtDNAs mosaically, being somewhere duplicated in the different molecules. This is correct for the gene of cytochrome b (*cyt-b*) discovered in scmtDNA I and III *G. pallida* [18-20] with the identity of nucleotide sequences up to 99 per cent [20].

*Cyt-b* belongs to the group of the mitochondrial genes, which correspond for the process of the oxidizing phosphorylation, as a result of which adenosine triphosphoric acid (ATF) is accumulated, which is subsequently used by a cell for the energy supply for the various processes of its vital activity. Now the *cyt-b* gene is considered as one among the most studied taking into account its structure and function of the protein, which it codes [22]. In particular, among the mammals, it will soon remain no families, for which it at least the individual section of the *cyt-b* gene would not be sequenced [3].

The first studies on the use of this marker for establishing the degree of the relationship of 32 South American populations of *G. pallida* has been accomplished by Picard *et al.* in 2007 [23]. According to the obtained results five well-suported clades of the nematodes reproducing the distributions of *G. pallida* in the center of its origin – Peru – from the south (around the lake of Titikaka in the central Andes) to the north of the country (which is also confirmed with the aid of other microsatellite markers) are revealed. At that, it has been proven that this nematode expansion was accompanied rather by multiple host-shifts from wild to cultivated potatoes, than by the gradual spread of initial population of *G. pallida* together with the local forms of potatoes.

The last assertion is made by the authors on the basis of the divergence index of the *cyt-b* gene nucleotide sequences estimated in 12 per cent, which is compared to the divergence level of the nematodes *Caenorhabditis briggsae* and *Caenorhabditis elegans*, which, as

assumed, occurred 23-120 million years ago, whereas the propagation of the cultivated potatoes together with the human economic activity is dated not earlier than 5000 [24]. Although the molecular clock can further be clearly calibrated (for example, in comparison with *Heligmosomoides polygyrus* Dujardin, 1845 that has the life cycle similar to *G. pallida*, it is only 3 million years), the difference between these events will nevertheless be too essential so as to reject the proposed hypothesis [23].

In the following research of the same scientists [25] with the application of the same molecular markers the origin of 17 European populations of *G. pallida* is proven from the South American populations of the nematodes distributed around the Lake Titikaka in the central Andes of Peru: between the Arapa, the Canyon of Colca and the Sicuani region. At that, the above discovered *cyt-b* haplotypes of the South American nematode populations in comparison with the European ones and the absence of the geographical clustering for the European populations in the context of their tying into the country of origin of the nematodes sample (the latter is connected by the authors with the existing market for seed potatoes in the countries of the European Union). Thus, the authors confirmed the hypothesis of potato cyst nematodes distribution in the world in the direction "the South America → the Europe → other continents of the world".

During the first stage of our research the *cyt-b* mitochondrial gene fragment was sequenced for the five European (Halton, Luffness, Pa1, Uzhok, Zhornava) and one South American (P5A) *G. pallida* populations [26]. The use of these original given in comparison with the data published by Picard [23] and Plantard [25] allowed to bring identity of *G. pallida* populations of Uzhok and Zhornava from Ukraine and their belonging to the same clade of the European populations of this species.

The purpose of the following stage of the study consisted in the determination of the nucleotide sequence of the *cyt-b* mitochondrial gene fragment for the nematode not only by various geographical origin, but also selection on the potatoes of resistant genotype for explaining the informativeness of this genetic marker in the study of intraspecific genetic variety and phylogenetic similarity of the nematodes from the selected populations and monitoring of changes in the degree of their virulence.

## MATERIALS AND METHODS

The *G. pallida* samples of five European populations (Farcet, Bedale, Newton, Rookmaker, Chavornay), two South American (P4A, P5A), and also three populations of special selection on the potatoes with various sources of resistance to nematode (Farcet_c11305: the selection of the Farcet population on the resistant potato clone of c11305, which contains the nematode resistance geneSv1; Farcet_c8906 (Sv2), Farcet_c12674 (H3)) were chosen as the subject of research.

DNA from the cysts (50 cysts to each population) was extracted applying the improved Pastrik method [27] with additional phenol/chloroform step and ethanol precipitation.

For reaction of amplification the specific primers of INRAcytbL (5′-GGGTGTGGCCTTGTTATTTC-3′) and INRAcytbR (5′-ACCAGCTAAAACCCCATCCT-3′) were used [23]. PCR in total volume of 10 µl was carried out using the commercial reagents by the Promega (UK) on the Perkin-Elmer 9700 Thermal Cycler amplifier (Applied Biosystems, UK) following the procedure: initial denaturing at a temperature of 94 ºC (5 min), 40 cycles of amplification, each included denaturing (95 °C, 19 s), hybridization (50 ºC, 30 s) and elongation (72 ºC, 30 s); final elongation was fulfilled at the temperature of 72 °C during 5 min. The PCR result was interpreted as positive, if the amplicon with length of 872 bp was detected in the analyzed model.

The obtained amplicons were sequenced. For that end, they were preliminarily processed with ExoSap-ITTM according to the producer's recommendation (GE Healthcare, UK). The sequencing was fulfilled using the reagents of DNA Sequencing Kit (Perkin-Elmer ABI PRISM Big Dye, Applied Biosystems) according to the standard procedure of the company-producer on the automatic of sequencer of ABI 377 (Applied Biosystems) with the use of INRAcytbL and INRAcytbR primers [23].

The obtained sequences were processed applying the PhredPhrap [28] and CONSED software [29]. The sequences of the mtDNA *cyt-b* gene of the nematode of five populations from the countries of the Europe and Ukraine (AM409001-AM409005) deposited owing to our previous researches [26], and also published by other scholars for 18 populations of *G. pallida* from the Europe (EF189179, EF189182, EF189187-EF189189, GQ294506-GQ294511, EF189178, EF189180, EF189181, EF189183-EF189186), 32 of the South America (AY851617-AY851648) and 14 of the North America (HQ909338, GQ294493-GQ294505) were used for further analysis. The corresponding sequences of *Globodera rostochiensis* (EF193005) and *Globodera mexicana* (AY851616) were selected as the external group.

The nucleotide sequences were aligned applying the Clustal W software [30]. The data were statisticaly processed applying the MEGA-4 [31], DNADIST from the PHYLIP package [32] and Arlequin software [33]. The phylogenetic trees were constructed, using the PHYLIP software package [32], robustness of which was assessed by bootstrap analysis using 1000 interactions, and visualized with the aid of the TREEVIEW software [34].

## RESULTS AND DISCUSSION

The nucleotide sequence of the mtDNA *cyt-b* gene with length of 872 bp for the nematodes of 10 *G. pallida* populations (Farcet, Bedale, Newton, Rookmaker, Chavornay, P4A, P5A, Farcet_c11305, Farcet_c8906 and Farcet_c12674) have been determined. Additionally the data of the nucleotide sequence of the mtDNA *cyt-b* gene published in the GenBank and totally representing 31 *G. pallida* populations from the Europe, 34 – the South America and 14 – the North America were used for the subsequent analysis.

The study of these nucleotide sequences allowed to reveal 40 haplotypes of the mtDNA *cyt-b* gene of *G. pallida* determined by 220 polymorphic sites, whereas 132 of them proved to be phylogenically informative (Table). The greatest quantity of haplotypes (23 of 40) with the high content of informative polymorphic sites (144 of 200) has been found in *G. pallida* from the region of its origin – the South America.

Among 40 revealed haplotypes of the mtDNA *cyt-b* gene of *G. pallida* only one (Hap_1) was encountered more frequently, namely – in 33 per cent of samples from the nematode populations originated from the Europe and North America. Seven more haplotypes (Hap_12, Hap_13, Hap_21, Hap_25, Hap_28, Hap_30, Hap_34) were typical for 2.5–7.6 per cent of population. However, only one of them (Hap_12) was presented in the nematode populations at least in two regions (the Europe and South America). Other 32 haplotypes are registered in the single cases (1.27 per cent of population).

The haplotypes of the nematodes from all over the world have in common the following: predominance in the nucleotide composition of pyrimidine nucleotide of thymine (T (50.5 per cent) > G> A> C); an increase in the fraction of the nucleotides pair of T + A from the first to the third position of codon from 64.5 per cent to 70.9 per cent and the advantageous use of seven codons, which have 2-3 bases of thymine (48 per cent of the total amount of codons). It is typical for other species of the nematodes. Although, the mechanism of such changes has been not accurately known yet, it is assumed that the corresponding displacement in nucleotide composition can occur as a result of the errors in the replication (hyper-mutation, mutation displacement, mutation pressure) and selection [35, 36].

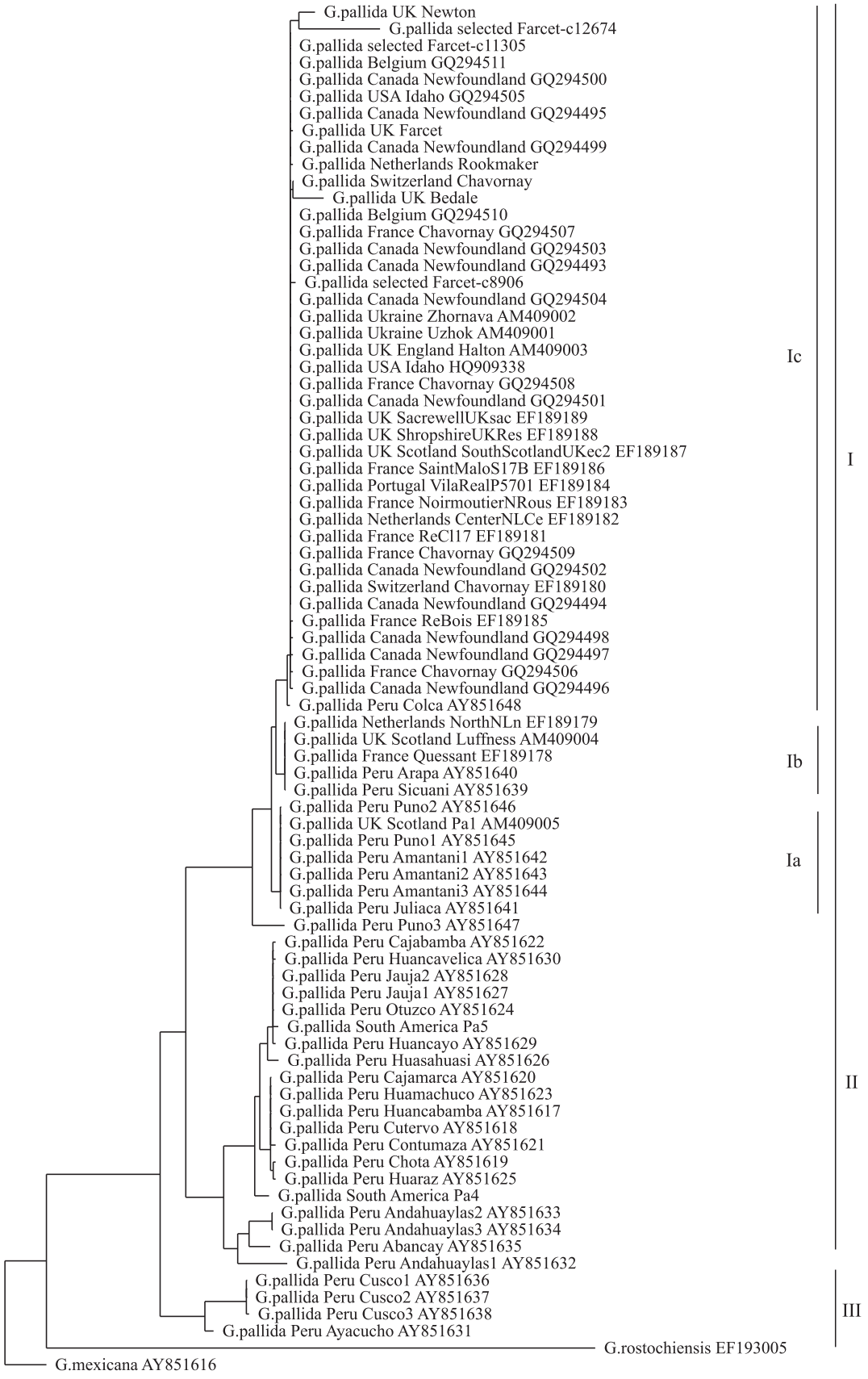
The average haplotype diversity (h) for *cyt-b* gene of *G. pallida* from the investigated populations was at the level of 0.885 ± 0.001, whereas the average nucleotide diversity ($\pi$) proved to be ten-times below and equal to 0.049 ± 0.000. Just as in the first case, it was the largest for the nematodes from the South America ($\pi$ = = 0.067 ± 0.001) and smallest – for the North America ($\pi$ = 0.001 ± 0.0002). This combination of the high haplotype diversity against the background of low nucleotide one gives an indirect evidence on the presence of the closely-related haplotypes in the populations of *G. pallida*, which have been come across expansion recently.

The analysis of the mitochondrial *cyt-b* gene's genetic variety stated the negative value of the Tajima test for the nematode populations from the Europe (D = –2.47) and North America (D = –1.67), although only for the populations of the Europe it was statistically significant (P < 0,05). The similar result is obtained on the Fu test (Fs = –1.07 and –2.29 respectively), which can indicate the various mutation speed for the separate

*G. pallida* mtDNA cyt-b Gene Haplotypes Characteristic and Neutrality Tests' Values

| Region | Populations Quantity | Haplotypes Quantity | Haplotypes Diversity, *h* [41] | Polymorphic Sites Quantity | | Nucleotide Diversity, $\pi$ [41 ] | Tajima Test Value, *D* [38] | Fu Test Value, *Fs* [42] |
|---|---|---|---|---|---|---|---|---|
| | | | | total | informative | | | |
| Europe | 31 | 16 | 0.800 ± 0.006 | 89 | 21 | 0.010 ± 0.000 | −2.47* | −1.88 |
| North America | 14 | 4 | 0.396 ± 0.025 | 3 | 0 | 0.001 ± 0.000 | −1.67 | −2.29 |
| South America | 34 | 23 | 0.961 ± 0.000 | 200 | 144 | 0.067 ± 0.000 | 0.07 | 2.41 |
| Total | 79 | 40 | 0.885 ± 0.001 | 220 | 132 | 0.049 ± 0.000 | −0.95 | 1.07 |

Note. *P < 0.05.




The phylogenetic tree representing the correlations between the *G. pallida* population samples based on the *cyt-b* gene hyplotypes

sites of marker or evidence either selection or species expansion within the region [37, 38].

The greater part of the specified genetic diversity of the mtDNA *cyt-b* gene of *G. pallida* was detected within populations (82.3 per cent, P = 0.040). The variability between the *G. pallida* populations from various regions (the Europe, North America, South America) – 17.9 per cent (P = 0.0001) is considerably less, but proved to be statistically significant, whereas the differences between the populations within these regions were statistically insignificant.

On the basis of the phylogenetic analysis of the obtained data the identity *G. pallida* from the populations of Uzhok and Zhornava disseminated in Ukraine with the representatives from 27 other European populations that together with 14 North American populations form the independent subgroup (Ic) of clade I has been proven (Figure). Two other clades are formed by the populations of *G. pallida* from the South America only. Thus, clade II covers the *G. pallida* populations from the northern part of Peru, whereas clade III contains the *G. pallida* populations from the central and southern part of Peru.

As the result, it affords grounds to assert that *G. pallida* from the populations extended in Ukraine is rather a descendant of the nematode from the European populations, than the separate introduction from the region of its origin – the South America.

The above mentioned topology of the phylogenetic tree remains subject to applying various algorithms of data processing: the method of the nearest neighbor, unweighted pair-grouping method with the arithmetical averaging and the method of maximum parsinomy, that indicates its high authenticity [40]. And although the marker proved to be unfit for the artificially selected nematodes' virulence changes monitoring on various sources of resistance, nevertheless, it makes possible to distinguish populations with the prolonged history of the virulence forming. It is confirmed with the extraction of several highly virulent populations among the European ones, including PA1 and Luffness populations more identical with a number of *G. pallida* populations of the American origin (subgroup Ia and b of clade I, respectively). The differences in the virulence level of the nematodes from these *G. pallida* populations has been also established in our own studies on biological test on the potatoes of five genotypes, which have various sources of partial resistance to the nematodes [26]. Taking into account mentioned above, it allows to assume that several introductions of *G. pallida* occurred to the European part of the continent (in contrast to the opinion of Plantard concerning only one introduction [25]).

The established phylogenetic ties of the studied *G. pallida* populations extended in the countries of the Europe and in Ukraine, and also the probability of several introductions to the region have been subsequently confirmed by the American scientists, who enlarged the diagram of the studies covering the *G. pallida* populations of Great Britain, Romania, the USA, Canada, New Zealand, Chile and Argentina [41].

## CONCLUSIONS

The phylogenetic analysis carried out on the basis of the exploration of the nucleotide sequences of the mitochondrial DNA cytochrome *b* gene of the *G. pallida* showed almost complete identity of the nematodes spread over the territories of Ukraine and countries of the Europe. It confirms the hypothesis that the expansion of *G. pallida* to Ukraine occurred precisely from the territory of the adjacent European countries, whereas the introduction from the South America to the Europe was, probably, repeated. The marker appeared to be unfit for the nematode virulence monitoring.

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**Філогенетичні зв'язки *Globodera pallida*, встановлені за поліморфізмом гена цитохрома *b* мітохондріальної ДНК**

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Ген цитохрома b (*cyt-b*) мітохондріальної ДНК використано для дослідження генетичної мінливості *G. pallida* з популяцій різного географічного походження та селекції на картоплі стійких генотипів. **Мета**. Встановити нуклеотидну послідовність фрагмента мітохондріального гена *cyt-b* для з'ясування інформативності цього генетичного маркера у вивченні внутрішньовидової генетичної різноманітності та філогенетичної подібності нематод з відібраних популяцій та моніторингу змін

ступеня їхньої вірулентності. **Методи.** У молекулярно-генетичних дослідженнях використовували ДНК, екстраговану з цист нематод. Полімеразно-ланцюгову реакцію проводили за використання специфічних праймерів INRAcytbL та INRAcytbR з наступним секвенуванням продукту ампліфікації. Для опрацювання і вирівнювання нуклеотидних послідовностей застосовано програмне забезпечення PhredPhrap, CONSED і Clustal W. Статистичну обробку даних здійснювали за допомогою програм MEGA-4, DNADIST програмного пакету PHYLIP та Arlequin. Філогенетичні дерева будували і візуалізували, використовуючи програмні пакети PHYLIP та TREEVIEW. **Результати.** Філогенетичний аналіз, проведений на основі нуклеотидних послідовностей фрагмента мітохондріального гена *cyt-b* засвідчив майже повну ідентичність нематод *G. pallida*, поширених в Україні та країнах Європи. Встановлено незначну генетичну диференціацію між вибірками досліджуваних популяцій *G. pallida* за фрагментом мітохондріального гена *cyt-b* порівняно до такої на внутрішньопопуляційному рівні (82,3 %; $P < 0,05$). Популяції *G. pallida* після селекції на трьох джерелах стійкості були схожими, але не ідентичними, на що вказують зміни частот гаплотипів мтДНК внаслідок відбору, проте маркер виявився непридатним для повноцінного моніторингу ступеня вірулентності нематод. **Висновки.** Одержані дані підтверджують гіпотезу, за якою експансія *G. pallida* в Україну відбулася саме з території сусідніх європейських країн, а не була окремою інтродукцією виду з Південної Америки.

**Ключові слова:** *Globodera pallida,* мітохондріальна ДНК, ген цитохрому *b*, філогенетичний аналіз.

**Филогенетические связи *Globodera pallida*, определенные по полиморфизму гена цитохрома *b* митохондриальной ДНК**

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Ген цитохрома b (*cyt-b*) митохондриальной ДНК использован для исследования генетической изменчивости *G. pallida* из популяций разного географического происхождения и селекции на картофеле устойчивых генотипов. **Цель.** Установить нуклеотидную последовательность фрагмента митохондриального гена cyt-b для выяснения информативности этого генетического маркера в изучении внутривидового генетического разнообразия и филогенетического сходства нематод из отобранных популяций и мониторинга изменений степени их ви-рулентности. **Методы.** В молекулярно-генетических исследованиях использовали ДНК, экстрагированную из цист нематод. Полимеразно-цепную реакцию проводили с использованием специфических праймеров INRAcytbL и INRAcytbR с последующим секвенированием продукта амплификации. Для обработки и выравнивания нуклеотидных последовательностей применяли программное обеспечение PhredPhrap, CONSED и Clustal W. Статистическую обработку данных осуществляли с использованием программ MEGA-4, DNADIST программного пакета PHYLIP и Arlequin. Построение и визуализацию филогенетических деревьев выполняли с помощью программного пакета PHYLIP и TREEVIEW. **Результаты.** Филогенетический анализ, проведенный на основе нуклеотидных последовательностей фрагмента митохондриального гена *cyt-b*, показал почти полную идентичность нематод *G. pallida*, распространенных в Украине и странах Европы. Установлена незначительная генетическая дифференциация между выборками изучаемых популяций *G. pallida* по фрагменту митохондриального гена *cyt-b* по сравнению с таковой на внутрипопуляционном уровне (82,3 %; $P < 0,05$). Популяции *G. pallida* после селекции на трех источниках устойчивости были подобными, но не идентичными, на что указывают изменения частот гаплотипов мтДНК в результате отбора, однако маркер оказался непригодным для полноценного мониторинга уровня вирулентности нематод. **Выводы.** Полученные данные подтверждают гипотезу, согласно которой экспансия *G. pallida* в Украину состоялась с территории соседних европейских стран, а не была отдельной интродукцией вида из Южной Америки.

**Ключевые слова:** *Globodera pallida,* митохондриальная ДНК, ген цитохром-*b*, филогенетический анализ.

REFERENCES

1. De Ley P., Blaxter M. L. Systematic position and phylogeny // The Biology of Nematodes / Ed. by D. L. Lee.– London: Taylor and Francis, 2002. – P. 1–30.
2. Nadler S. A. Advantages and disadvantages of molecular phylogenetics: A case study of *Ascaridoid* nematodes // J. Nematol. – 1995. – **27**, N 4. – P. 423–432.
3. Bannikova A. A. Molecular markers and modern phylogeny of the mammals // Zh. Obshch. Biol. – 2004. – **65**, N 4. – C. 278–305.
4. Baldwin J. G., Nadler S. A., Adams B. J. Evolution of plant parasitism among nematodes // Annu. Rev. Phytopathol. – 2004. – **42**. – P. 83–105.
5. Dorris M., De Ley P., Blaxter M. L. Molecular analysis of nematode diversity and the evolution of parasitism // Parasitol. Today. – 1999. – **15**, N 5. – P. 188–193.
6. Howe D. K., Baer C. F., Denver D. R. High rate of large deletions in *Caenorhabditis briggsae* mitochondrial genome mutation processes // Genome Biol. Evol. – 2010. – **2**. – P. 29–38.
7. Molnar R. I., Bartelmes G., Dinkelacker I., Witte H.,

Sommer R. J. Mutation rates and intraspecific divergence of the mitochondrial genome of *Pristionchus pacificus* // Mol. Biol. Evol. – 2011. – **28**, N 8. – P. 2317–2326.

8. Kumar S., Koutsovoulos G., Kaur G., Blaxter M. Toward 959 nematode genomes // Worm. – 2012. – **1**, N 1. – P. 42–50.
9. Hyman C., Whipple L. E. Application of mitochondrial DNA polymorphism to *Meloidogyne* molecular population biology // J. Nematol. – 1996. – **28**, N 3. – P. 268–276.
10. Okimoto R., Chamberlin H. M., Macfarlane J. L., Wolstenholme D. R. Repeated sequence sets in mitochondrial DNA molecules of root knot nematodes (*Meloidogyne*): nucleotide sequences, genome location and potential for host-race identification // Nucleic Acids Res. – 1991. – **19**, N 7. – P. 1619 – 1626.
11. Whipple L. E., Lunt D. H., Hyman B. C. Mitochondrial DNA length variation in *Meloidogyne incognita* isolates of established genetic relationships: Utility for nematode population studies // Fundam. Appl. Nematol. – 1998. – **21**, N 3. – P. 265–271.
12. Wu S. G., Wang G. T., Xi B. W., Xiong F., Liu T., Nie P. Population genetic structure of the parasitic nematode *Camallanus cotti* inferred from DNA sequences of ITS1 rDNA and the mitochondrial *COI* gene // Vet. Parasitol. – 2009. – **164**, N 2−4. – P. 248–256.
13. Derycke S., Remerie T., Vierstraete A., Backeljau T., Vanfleteren J., Vincx M., Moens T. Mitochondrial DNA variation and cryptic speciation within the free–living marine nematode *Pellioditis marina* // Mar. Ecol. Prog. Ser. – 2005. –**300**. – P. 91–103. – Access: dx.doi.org/10.3354/meps300091.
14. Boore J. L., Lavrov D. V., Brown W. M. Gene translocation links insects and crustaceans // Nature. – 1998. – **392**, N 6677. – P. 667–668.
15. Boore J. L., Staton J. L. The mitochondrial genome of the sipunculoid *Phascolopsis gouldii* supports its association with *Annelida* rather than *Mollusca* // Mol. Biol. Evol. – 2002. – **19**, N 2. – P. 127–137.
16. Jacob J. E. M., Vanholme B., Van Leeuwen Th., Gheysen G. A unique genetic code change in the mitochondrial genome of the parasitic nematode *Radopholus similis* // BMC Research Notes. − 2009. – 2:192.
17. Gibson T., Blok V. C., Dowton M. Sequence and characterization of six mitochondrial subgenomes from *Globodera rostochiensis*: Multipartite structure is conserved among close nematode relatives // J. Mol. Evol. – 2007. – **65**, N 3. – P. 308–315.
18. Armstrong M. R., Blok V. C., Phillips M. S. A multipartite mitochondrial genome in the potato cyst nematode *G. pallida* // Genetics. – 2000. – **154**, N 1. – P. 181–192.
19. Blok V., Cock P., Hunt M., Hedley P., Morris J., Jones J. Investigations of the expression of the multipartite mtDNA of *G. pallida* // COST872 Meeting (Toledo, Spain, May 2009). – Toledo, 2009. − P. 78.
20. Gibson T., Blok V. C., Phillips M. S., Hong G., Kumarasinghe D., Riley I. T., Dowton M. The mitochondrial subgenomes of the nematode *G. pallida* are mosaics: Evidence of recombination in an animal mitochondrial genome // J. Mol. Evol. – 2007. – **64**, N 4. – P. 463–471.
21. Keddie E. M., Higazi T., Unnasch T. R. The mitochondrial genome of *Onchocerca volvulus*: Sequence, structure and phylogenetic analysis // Mol. Biochem. Parasitol. – 1998. – **95**, N 1. – P. 111–127.
22. Esposti D. M., De Vries S., Crimi M., Ghelli A., Patarnello T., Meyer A. Mitochondrial cytochrome b: Evolution and structure of the protein // Biochim. Biophys. Acta. – 1993. – **1143**, N 3.− P. 243–271.
23. Pimentel D., Lach L., Zuniga R., Morrison D. Environmental and economic costs associated with non-indigenous species in the United States // BioScience. – 2000. – **50**, N 1. – P. 53–65.
24. Thurston H. D. Andean potato culture: 5000 years of experience with sustainable agriculture // Advances in Potato Pest Biology and Management / Eds G. W. Zehnder, M. L. Powelson, R. K. Jansson, K. V. Raman.– St. Paul: Am. Phytopathol. Soc. press, 1994. – P. 6–13.
25. Plantard O., Picard D., Valette S., Scurrah M., Grenier E., Mugniery D. Origin and genetic diversity of Western European populations of the potato cyst nematode (*G. pallida*) inferred from mitochondrial sequences and microsatellite loci // Mol. Ecol. – 2008. – **17**, N 9. – P. 2208–2218.
26. Pylypenko L., Phillips M., Blok V. Characterisation of two Ukrainian populations of *G. pallida* in terms of their virulence and mtDNA, and the biological assessment of a new resistant cultivar Vales Everest // Nematology. – 2008. – **10**, N 4. – P. 585–590.
27. Pastrik K.J., Rumpenhorst H. J., Burgermeister W. Random amplified polymorphic DNA analysis of a *G. pallida* population selected for virulence // Fundam. Appl. Nematol. – 1995. – **18**, N 2. – P. 109–114.
28. Green P. Phrap, version 1.090518. [Electronical resource] // Access: http://phrap.org.
29. Gordon D., Abajian C., Green P. CONSED: A graphical tool for sequence finishing // Genome Research. – 1998. – **8**, N 3. – P. 195–202.
30. Chenna R., Sugawara H., Koike T., Lopez R., Gibson T., Higgins D. G., Thompson J. D. Multiple sequence alignment with the Clustal series of programs // Nucleic Acids Res. – 2003. – **31**, N 13. – P. 3497–3500.
31. Tamura K., Dudley J., Nei M., Kumar S. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0 // Mol. Biol. Evol. – 2007. – **24**, N 8. – P. 1596–1599.

32. Felsenstein J. PHYLIP – Phylogeny Inference Package (Version 3.2) // Cladistics. – 1989. – **5**. – P. 164–166.
33. Excoffier L., Laval G., Schneider S. Arlequin (version 3.0): An integrated software package for population genetics data analysis // Evol. Bioinform. Online. – Publ. online Feb 23, 2007). – **1**. −P. 47–50.
34. Page R. D. M. TreeView: An application to display phylogenetic trees on personal computers // Comput. Appl. Biosci. – 1996. – **12**, N 4. – P. 357–358.
35. Hugall A., Stanton J., Moritz C. Evolution of the AT-rich mitochondrial DNA of the root knot nematode, *Meloidogyne hapla* // Mol. Biol. Evol. – 1997. – **14**, N 1. – P. 40–48.
36. Li M.-W., Lin R.-Q., Song H.-Q., Wu X.-Y., Zhu X.-Q. The complete mitochondrial genomes for three *Toxocara* species of human and animal health significance // BMC Genomics. − 2008. − 9:224. Doi: 10.1186/1471-2164-9-224.
37. Aris-Brosou S., Excoffier L. The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism // Mol. Biol. Evol. – 1996. – **13**, N 3. – P. 494–504.
38. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism // Genetics. – 1989. – Vol. 123. – P. 585–595.
39. Tamura K., Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees // Mol. Biol. Evol. – 1993. – **10**, N 3. – P. 512–526.
40. Nei M. Molecular Evolutionary Genetics.–New York: Columbia Univ. Press, 1987. – 512 p.
41. Madani M., Subbotin S. A., Ward L. J., Li X., De Boer S. H. Molecular characterization of Canadian populations of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida* using ribosomal nuclear RNA and cytochrome *b* genes // Can. J. Plant Pathol. – 2010. – **32**. – P. 252–263.
42. Fu Y. X. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection // Genetics. – 1997. – **147**, N 2. – P. 915–925.