



**10-ый МЕЖДУНАРОДНЫЙ
НЕМАТОЛОГИЧЕСКИЙ СИМПОЗИУМ
И НАУЧНАЯ ШКОЛА
ПО ТЕОРЕТИЧЕСКОЙ
И ПРИКЛАДНОЙ НЕМАТОЛОГИИ
ДЛЯ МОЛОДЫХ УЧЕНЫХ**

01–05 июля 2013
Голицыно – Большие Вязёмы

**МАТЕРИАЛЫ
СИМПОЗИУМА**

**10th INTERNATIONAL
NEMATOLOGICAL SYMPOSIUM
AND SCHOOL ON NEMATOLOGY
FOR YOUNG SCIENTISTS**

July 01–05, 2013
Golitsyno – Bolshie Vyazemy

PROCEEDINGS

**MOLECULAR-TAXONOMIC STUDY OF TWO PINWORM SPECIES OF THE GENUS *PSEUDONYMUS*
(OXYURIDOMORPHA: THELASTOMATOIDEA: PSEUDONYMIDAE) FROM WATER BEETLES
(COLEOPTERA: HYDROPHILIDAE) OF THE ASTRAKHAN STATE RESERVE**

Guzeeva E.A.¹, Kosevich I.A.¹, Spiridonov S.E.¹

¹ Centre of Parasitology, A.N. Severtsov Institute Ecology and Evolution RAS, Moscow, 119071,
Russia; E-mail: guzeyeva@mail.ru, s_e_spiridonov@rambler.ru

Parasitism of thelastomatid nematodes in the hindgut of hydrophilid beetles was reported from different regions of the world. Currently, thelastomatids of water beetles constitute the family Pseudonymidae. Taxonomic relationships of separate genera and species within this family are still not completely resolved, and additional characters for species identification are urgently needed. Two species of the genus *Pseudonymus* Diesing, 1857 were collected from the hindgut of a hydrophilid beetle (*Hydrophilus piceus* L.) sampled in the River Volga delta. Two species of thelastomatids were recovered, identified according to morphological features as *Pseudonymus islamabadi* (Basir, 1941) and *P. hydrophili* (Galeb, 1878). Pronounced sexual dimorphism prevents secure identification of con-specific males and females, and previously such judgments were based solely on comparative occurrence of the specimens of both sexes in dissected hosts. Sequences of the D2-D3 segment of the LSU rDNA for two morphological types of males and females were obtained and compared (alignment length 636-637 bp – nucleotide difference between two species – 44 bp). Complete identity of LSU sequences confirmed that females with strongly swollen the first, fifth and seventh cuticular annuli of the anterior end and 85×45 µm eggshells and males with narrowed tail with short spine appendage and 25 µm long spicule belong to *P. islamabadi*, whereas females with the only one widened annulus and 70×40 µm eggshells and males with rounded posterior end and spicule length of 27 µm belong to *P. hydrophili*. Morphological features of two *Pseudonymus* species from lower Volga are illustrated with SEM. Supported by RFBR 12-04-10100-k.

**THE HISTORY OF THE USDA NEMATODE COLLECTION AND ITS DATABASE: VALUABLE RESOURCES
FOR TAXONOMIC RESEARCH AND IDENTIFICATION**

Handoo Z.A.¹, Mowery J.D.¹, Chitwood D.J.¹

¹ USDA ARS Nematology Laboratory, 10300 Baltimore Ave., BARC-West, Bldg 10A, Beltsville, MD,
20705, USA; E-mail: zafar.handoo@ars.usda.gov;

Mention of trade names or commercial products in this publication is solely for the purposes of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture.

Nematodes constitute an important group in the animal kingdom, and are highly adaptable and capable of surviving in any environment. So far, only a small percentage of the total species are known, and as new species are discovered, they reveal previously unknown agricultural problems (Golden, 1988). Systematics collections are a fundamental research resource, as they contain the specimens upon which all biological research is validated. These resources serve as a major asset for taxonomic research and are used for a wide variety of scientific and regulatory purposes including the facilitation of agricultural trade, the continued protection of agriculture against economically dangerous invasive species, and the advancement of nematode taxonomy and scientific research, including nematode identifications.

The U.S. Department of Agriculture Nematode Collection (USDANC), in Beltsville, Maryland, USA, is one of the largest and most comprehensive nematode repositories in the world. The Collection provides an enduring and stable foundation for the field of nematology and is essential for research on taxonomy, systematics, morphology and identifications. Nematology research in the United States originally began in the late 1800's, and involved many USDA nematologists later recognized as pioneers in the field of nematology. N. A. Cobb worked at USDA for 25 years, during which time he made significant contributions to the taxonomy of nematodes, and is recognized as the "Father of Nematology" in the United States. Other pioneering USDA nematologists include G. Steiner, G Thorne, B. G. Chitwood, J. R. Christie and A. L. Taylor, among others (Golden, 1977). For many years the collections of the USDA nematologists remained as individual personal collections that were not well organized or maintained; unfortunately, many valuable specimens deteriorated or were misplaced and collection records lost during these years. To remedy this problem, soon after the initiation of the current nematode taxonomy program, A. Morgan Golden established the USDA Nematode Collection in 1960 to serve as an official permanent repository that is well organized and contains detailed records of all specimens (Golden, 1962). A preventative maintenance program was also established so that aging slides can be periodically remounted as needed, and the process of salvaging material from the early USDA nematologists remains an ongoing effort. Over time the Collection has steadily expanded, and now includes over 45,000 slides and vials. The growth of the Collection has been facilitated by the deposition of new type specimens from scientists throughout the world, the continuous deposition of specimens received from worldwide sources for identification purposes, as well as the incorporation of personal collections received from individual scientists (Handoo *et al.*, 1998a). The nematode taxonomy program, which provides nematode identification services, has been a large factor in contributing to the success of the Collection. Every year more than 500 samples of nematodes are processed and identified, most of which are from regulatory agencies throughout the United States, and the nematodes from the majority of these samples are deposited into the USDA Nematode Collection.

The Collection consists of 7 constituent divisions of slides and vials, most of which are stored in fire-proof safes (Fig. 1 & 2). While the majority of specimens are plant-parasitic, the Collection also includes many free-living, insect-parasitic, marine and freshwater nematodes. The constituent divisions of the Collection are the Type Collection with 6,748 slides and 613 vials, the General Collection with 18,707 slides and 7,243 vials, the Thorne Collection with 6,600 slides, the Steiner Mermithid Collection with 2,303 slides, the Mass Collection with 1,035 slides and 1,102 vials, the Gates Collection with 356 slides, and a Demonstration Collection of 87 museum jars.

In the early days of the Collection, essential details of each specimen were recorded and filed in a card catalog system, and duplicate records were filed according to species, host, and origin (Handoo *et al.*, 1998b). However, all records are now digitized and are stored and maintained in a computer database. Currently the database is configured so that any new records are automatically uploaded to a USDA server and are then instantly searchable and available to the public at <http://nt.ars-grin.gov/nematodes/search.cfm>. This instant upload configuration represents a great improvement over previous upload methods and greatly improves the timeliness that information can be made available to the public. The database currently contains over 39,000 entries with essential data on nematode host and distribution recorded for each species.

The unique specimens and records in the Collection are extremely valuable and have been used to resolve billion-dollar issues involving agricultural trade. One of the oldest slides in the Collection is of *Mononchus longicaudatus*, from Australia, that was prepared by N.A. Cobb in 1890 (Golden & Huettel, 1990). Identification of new or potentially harmful species of nematodes is important to the success to modern agriculture and aids in the development of quarantine or regulatory procedures to prevent their spread. In 2006, for example, when the pale cyst nematode

was discovered in Idaho, the immediate implementation of a federal quarantine on this serious pest helped prevent the spread of this species in the United States; thereby saving millions of dollars in annual crop losses. The study of original type material of *Globodera pallida* on deposit in the Collection was invaluable in confirming the final identification of the Idaho material (Skantar *et al.*, 2007). Furthermore, systematists often must study type collections thoroughly, and future generations performing comprehensive revisionary work will also need to re-examine type specimens. The Collection serves as a valuable asset especially in regards to revision of earlier described material. For example, during an examination of archival material, several nematode genera from the Collection were found attacked by the bacterial parasite, *Pasteuria*. This revealed substantial information on this important pathogen and helped in the discovery of a new species of bacteria (Sayre *et al.*, 1988). The Collection contains unique and interesting specimens and invaluable information about nematodes, which scientists throughout the world have regularly incorporated into their research and subsequent publications.

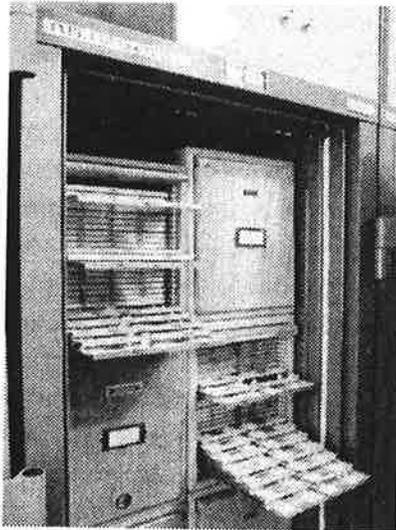


Fig. 1. Slides of type specimens organized in wooden slide boxes inside a fire-proof safe.

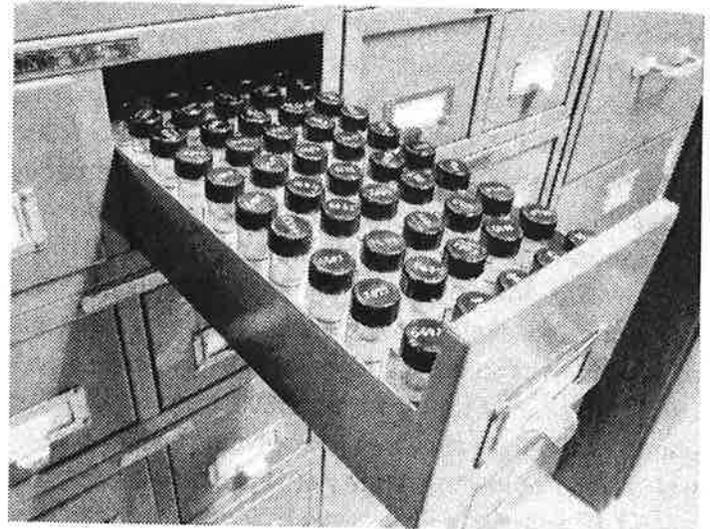


Fig. 2. Vials of nematodes on deposit in the USDA Nematode Collection

Since it is not always possible for scientists to personally visit the Collection, specimens are available for loan for limited periods at the responsibility of a recognized scientist or institution. The utmost care should be taken when handling these valuable specimens and all material should be promptly returned after examination. Scientists are encouraged to visit the Collection in order to study material in person. New type specimens and other material are always welcomed and appreciated when deposited into the Collection.

REFERENCES

- GOLDEN, A. M. 1962. Transfer of type specimens of certain plant nematodes to a new collection. *Nematologica* 8: 84-85
- GOLDEN, A. M. 1977. USDA Nematode Collection. In: *Systematic Collections of the Agricultural Research Service*. pp. 61-64. USDA ARS, Miscellaneous Publication No. 1343, 84 pp.
- GOLDEN, A. M. 1988. Perspectives in nematology. In: *Advances in plant nematology* (M. A. Maqbool, A. M. Golden, A. Ghaffar, and L. R. Krusberg. Eds.). pp. 15-22. M/S Shamim Printing Press, Karachi, Pakistan.
- GOLDEN, A. M. & HUETTEL, R. N. 1990. The USDA Nematode Collection and its oldest slide. *Nematology Newsletter* 36: 20-21.
- HANDOO, Z. A., GOLDEN, A. M. & ELLINGTON, D. M. S. 1998a. Nematodes - USDA Nematode Collection. In: *Systematic Collection of the Agricultural Research Service, revised* (Lichtenfels, J.

- R., J. H. Kirkbride, Jr., and D. J. Chitwood. Eds.). pp. 48-52. USDA ARS, Miscellaneous Publication No. 1343, 77 pp.
- HANDOO, Z. A., GOLDEN, A. M. & ELLINGTON, D. M. S. 1998b. Type specimens on deposit in the United States Department of Agriculture Nematode Collection. *Journal of Nematology* 30: 108-158.
- SAYRE, R. M., STARR, M. P., GOLDEN, A. M., WERGIN, W. P., & ENDO, B. Y. 1988. Comparison of *Pasteuria penetrans* from *Meloidogyne incognita* with a related mycelial and endospore-forming bacterial parasite from *Pratylenchus brachyurus*. *Proceedings of the Helminthological Society of Washington* 55: 28-49.
- SKANTAR, A. M., HANDOO, Z. A., CARTA, L. K., & CHITWOOD, D. J. 2007. Morphological and molecular identification of *Globodera pallida* associated with potato in Idaho. *Journal of Nematology* 39: 133-144.

PHYLOGENY, TAXONOMY AND EVOLUTION OF REPRODUCTION WITHIN THE NEMATODE GENUS PRATYLENCHUS: A MULTI-GENE APPROACH COMBINED WITH KARYOTYPING

Janssen T.¹, Karszen G.², Couvreur M.¹, Bert W.¹

¹ *Nematology Unit, Ghent University, Department of Biology, Ledeganckstraat 35, 9000 Ghent, Belgium; E-mail: Toon.Janssen@UGent.be*

² *Plant Protection Service, Nematology section, P.O Box 9102, 6700 HC Wageningen, The Netherlands*

Root-lesion nematodes of the genus *Pratylenchus* (Filipjev 1936) are an important pest in economic crops (Sasser and Freckman 1987). They have a worldwide distribution (except for polar regions) and can parasitize on a very wide range of plants. At the moment the genus counts about 70 described species, but identification of the different species based on morphology remains problematic. Within the genus reproduction strategies vary from amphimixis over meiotic to mitotic parthenogenesis. Also karyotypes are highly variable and polyploidy is certainly present and probably associated with asexual reproduction (Roman and Triantaphyllou 1969). Current study is based on as much as species as possible, sampling will be done at type locations if possible, and all populations are studied from multiple and complementary perspectives. Morphology is documented using light and electron microscopy and mode of reproduction and karyotypes are elucidated by in vitro staining of reproductive systems. This information will be analyzed in a multi-gene comparative evolutionary perspective to obtain a better insight in evolution of reproductive strategies, genome evolution and phylogenetic relationships. The obtained data and outcomes will be highly valuable towards a complete integrative taxonomy of the genus *Pratylenchus*, the first results will be presented here.

VALIDATION OF THE FLASH-PCR METHOD FOR IDENTIFICATION *GLOBODERA ROSTOCHIENSIS* (WOLL.) BEHRENS

Khudyakova E.A.¹, Sudarikova S.V.¹

¹ *All-Russian Plant Quarantine Centre, 140150, Moscow region, village Byikovo; E-mail: vniikr@mail.ru*

A research on recognition of reliability (validation) of cyst nematode diagnostics employing a commercial diagnostic set produced by the company «Agrodiagnostika» (Moscow) was conducted. The following indicators of validation were defined according to the EPPO PM Standards 7/84, RM 7/98:1. The analytical sensitivity. In a series of experiments it was shown that the detection limit of viable cysts was at least five larvae or eggs per sample, and not less than 50 larvae or eggs per sample for non-viable cysts. 2. Analytical specificity. As a result of the