

NEMIDSOFT, A Web-based Software as an Aid to the Identification of Nematodes

1. The genus *Merlinius* Siddiqi, 1970 (Nematoda: Tylenchida)

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ABSTRACT

The genus *Merlinius* includes plant-parasitic nematode species and is of great practical importance. This paper describes a web-based identification tool (which has been described by the authors under the name of Nemidsoft) that uses morphological characters and morphometrical data. The characters and data used are the same as the polytomous keys of Brzeski (1998) [1] and Handoo *et al.* (2007) [2]. For each specimen examined, the software compares characters of the unknown specimen with all 32 species of the genus accepted by Handoo *et al.* (2007) [2] that submitted in database. One or more than one species is suggested to the user by the software. Additional data of the suggested species (in some species) such as, images of the species, PDF of full-text or abstract of the reference article is present. A later step of the program has been adopted for analysis of cyst forming genus *Heterodera*.

Keywords

Database, Morphological characters, Morphometrical data, Nemidsoft, Web-based software

1. INTRODUCTION

Biodiversity is the study of the variety of living beings. For the study of biodiversity in the phylum Nematoda, we need to be able to identify nematodes. For biodiversity, identification of nematodes is not limited to the recognition of a few economically important species but it means being able to put a name on any species observed. It also means being able to recognize that a specimen belongs to a new species [3]. The basis of any study of biodiversity is the morphological description of the existing species as they appear to us. Such descriptions must make it possible to identify other specimens of the described species. Once this done, other aspects of the well characterized species can be studied, including its genome, its ecology (relationships with its environment), its biology (feeding reproduction, host-parasitic relationships), its geographical distribution, its interest as a biological marker, and more. But except for some unicellular organisms such as viruses, certain bacteria, etc., the basis of all these studies will always be the morphological description of the species [4]. The large-scale identification must be done by biologists who are not expert taxonomists. It is argued that printed identification aids are too rigid and unreliable, and molecular identification aid is too limited. Only computer identification tools are flexible, reliable and general enough to give a non-taxonomist the possibility to identify any species [3]. Our

knowledge about morphology and anatomy and about the various other scientific fields represent an enormous amount of facts, which must be classified and stored in a way that supports easy retrieval. This is particularly true if the various experts that are interested in biodiversity want to be able to access data in fields with which they are not familiar. Only computer science offers some hope to put this huge mass of knowledge in order and store it in such a way that the data of interest can be retrieved easily [5]. If an universal data format were established for the description and illustrations or photos of new species and used in a database freely accessible through the internet together with properly designed software, such a database could allow any scientists with minimal training in nematode morphology (including molecular biologists) to identify the specimens they are working on. Development of such software would be a difficult task but not an impossible one [3]. For example the Generic Biological Information System Nemys (<http://nemys.ugent.be/index.asp>) or General Identification System Genisys (http://genisys.prd.fr/genisys_home.html) are published in the web. Some of desktop-based programs that developed in plant nematology are: NEMAID [6]; MARINEMA [7]; PARANEMA [7]; DORY [8]; DITYL [9]; USE OF BIKEY 7 [10]. By the way, identification keys for the computer are not always faster than printed ones [11], particularly if non-metrical data are used. A comparison of shapes, the structure of the front region with images in the identification key can only be made by viewing the specimens with a microscope. A printed key can easily be used next the microscope, but the combination of microscope and computer necessary for utilization of identification software has only recently been applied [12]. In this paper, the concepts we will present, have been developed over the last 2 years by a team consisting of one nematologist (ACB) and one computer engineer (AK) from a knowledge-based IT corporation Omni Net of Baran®. The project was called Nemidsoft (Nematode Identification Software). We believe that our software could readily be extended to identification of other biological groups.

The genus *Merlinius* was established by Siddiqi (1970) [13] to accommodate those forms previously in *Tylenchorhynchus* that have six incisures in the lateral field, a small trough-shaped nonprotrusible gubernaculum, and stout spicules with distal ends notched and without large ventral flanges. Tarjan (1973) [14] gave a valuable synopsis key and diagnostic data of the genera and species of Tylenchorhynchinae, and discussed some of Siddiqi's

characters. Tarjan (1973) [14] agreed with Siddiqi (1970) [13] that the six incisure character is consistent and easily recognisable and that establishment of *Merlinius* made the unwieldy genus *Tylenchorhynchus* less cumbersome, and *Merlinius*, therefore, justifiable. The present authors agree with both Siddiqi (1970) [13] and Tarjan (1973) [14] because their action makes it easier to handle this complex and large group of nematodes. At present *Merlinius* comprises 32 valid species of worldwide distribution that parasitize a wide variety of plants. The history of *Merlinius* was discussed by Hooper (1978) [15]. Fortuner and Luc (1987) [16] included *Merlinius* in the subfamily Telotylenchinae in the family Belonolaimidae. In a review of species of agriculturally important *Tylenchorhynchus*, *Merlinius* and *Amplimerlinius*, Anderson and Potter (1991) [17] also presented a good historical background of stunt nematode taxonomy. Brzeski (1998) [1] included all species of *Merlinius* in *Geocenamus* and gave a key to only 19 species and a compendium for 77 species. However, he concluded that the genus *Geocenamus* may be a collective group that could be split into separate genera, but additional investigations, including scanning electron microscope studies of cephalic structure, were needed for more of the species before any action could be taken. A number of taxonomic changes to stunt nematodes were proposed by different workers [18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 16, 30, 31, 32, 33, 34, 35, 36]. In the study of Handoo *et al.* (2007) [2] *Merlinius* is defined as containing only those species with six lines in the lateral field. A desktop computer software based on cluster analysis for determination of *Tylenchorhynchus* and *Merlinius* have been developed [33]. In this study, we design a web-based software not only for *Merlinius* species but for all the genera and their species. Although, the completion of its details will be gradual and time-consuming work. The *Merlinius* section of the software is the same as in the polytomous key of Handoo *et al.* (2007) [2] which have not been replaced, but only supported by the software.

2. METHODS

The software was developed in ASP.NET web language. The database was supported by SQL-Server. Nemidsoft is freely accessible through the web, www.nemidsoft.net. In the software, for every genus, morphological characters (as character and character states) and morphometrical data (as minimum and maximum data states) were defined. For example, in *Merlinius* Siddiqi, 1970 (based on Handoo *et al.* 2007 [2]); diagnostic morphometric data include: L, a, b, c, V, Lip annules, Stylet, Tail annules, c', Spicule and Gubernaculum length; and morphological characters and their states contain Tail shape (conoid, subcylindrical, cylindrical-rounded, sub-cylindrical-rounded); Tail terminus (finely rounded-pointed, broadly rounded, sub-hemispherical, hemispherical, rounded, bluntly pointed, bluntly pointed-indented, truncate, sharply pointed, pointed acute, filiform pointed, pointed); Tail tip (smooth, annulated, smooth-indented, smooth, finely rounded mucro); Lip region (offset, continuous, conoid, conoid-offset, conoid-narrow, narrow). In the program, there is not any limitations in the definition of all species of all genera of nematodes. Nemidsoft is very user-friendly and can be extended to other types of livings such as fungi, insects, etc. More information and more details about the species such as images, movies (about the biology of the nematodes) can be attached to the Wiki page of the species.

3. DESCRIPTION OF THE USER INTERFACE

In Nemidsoft homepage (<http://www.nemidsoft.net>), there are two main icons; **Go to Database** and **Search in Database** (Fig. 1).



Fig. 1. Homepage of the Nemidsoft.

In Go to Database menu the user must fill in the form with his/her username and password to enter and see the database contents. Creation of ID for users is supported by Administrator of the website. Users must send their request to receive the ID through the **Contact** menu. After log in, the user can see the contents of the database such as **Search**, **Scan**, **Genera** and **Users** icons without any possibility to edit or delete them (Fig. 2).



Fig. 2. Database layout of the Nemidsoft.

In **Search** icon, the user can do a general exploring based on the nematode name and find it, to see the morphological properties (Fig. 3).



Fig. 3. The Search section of the program.

In **Scan** menu (the most important menu of the program), the user can choose the genus name via a combo-list. After select the genus and click on **Go**, the user can fill in the scan form based on morphological features. None of the characters were defined as a required matter. By mouse clicking on **Scan**, the software compares the entry with submitted information in the database and finally, one or more than one species are proposed to the user (Fig. 4).

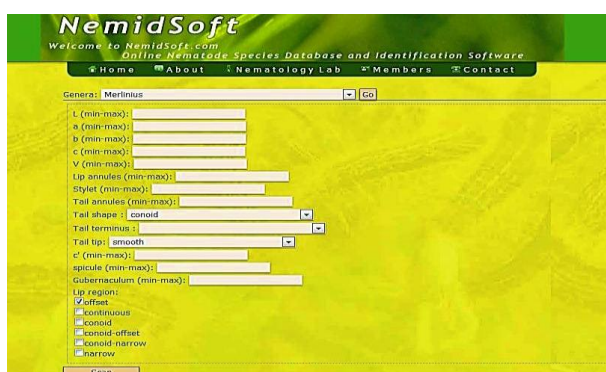


Fig.4. The Scan section with a form that the user must be fill in with morphological features of the unknown species. None of the characters were defined as required icons.

Based on the details of the species, the user can decide to choose the most similar species that it is highly matched to his/her unknown species (Fig. 5).

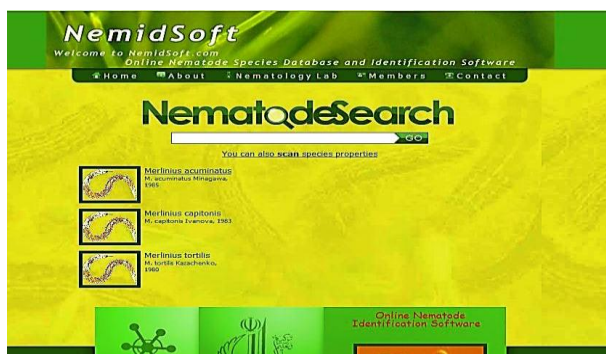


Fig. 5. Search result page of the program.

We try to complete the details of the species via adding the descriptions and photos, but many of the references are unavailable or under copyright rules and we cannot use them in completing the database of the program. So, our trying to complete the program will be a gradual work. In **Genera** icon, user can see the genera that have been defined to the program with their species. Some of Genera labeled with "in progress" that it means the process of completeness of the genera are not finished, and it is under maintenance (Fig. 6).



Fig. 6. The Genera section of the program.

In both genera and species with clicking on the name of the fungus, a page is opened as "wiki page". In this page, properties of the genera and species are present. In **User** icon, every user can see her/his profile and complete and/or edit it and can see the database of the genus. A hypothetical user ID of the site is: email: user@gmail.com; Password: user2012.

4. RESULTS

A serious problem in biodiversity studies is the limited availability and accessibility of data which are widely spread in several books, journals and off-line databases, as well as stored in biological collections, making it very time consuming to compile the necessary information. So, we consider Nemidsoft is useful for the rapid and accurate identification of nematodes. By the way, the program can be extended to some other icons such as **gene sequence search** without substantial costs. In fact, polyphasic taxonomy is better than monophasic only based on morphologic or molecular characters. But, molecular data is not always available. In contrast, morphologic features is easier to obtain than molecular data.

5. DISCUSSION AND CONCLUSIONS

Printed keys are powerful tools that can be used safely as long as they rely on primary identification characters [37]. Limitation of a purely dichotomous key is that each successive dichotomy relies on a single character. If the character used at line L of the key cannot be seen, the identification cannot proceed past line L. Another types of keys is tabular keys. Tabular keys are easy to use with small genera number, but they quickly become too cumbersome when the number of species increases. In the other hand, the paper is a dead medium that cannot be updated, but an electronic medium offers the flexibility needed to give at least some control to the user and to make it easier to update a key [3]. Some of desktop computer identification tools with their approach and scopes are: Nemaaid [6], similarity coefficient

"Gower", *Helicotylenchus*; Marinema [7], multiple entry key, Marine nematodes; Paranema [7], multiple entry key, plant parasitic nematodes; Dory [8], Dorylaimida; Dityl [9], similarity coefficient "Gower", *Ditylenchus*; Use of Bikey 7 [10], Elimination using Excel spreadsheet, Pratylenchidae : Radopholus. The web-based identification programs have many advantages over the desktop-based programs of the late 1980s. First, household computer usage in U.S increased from 22.8% in 1993 to 61.8% in 2003 [38]. In addition, broadband Internet access is becoming more common, and wireless Internet access via cell technology allows access for users in remote areas. Increasingly, common smart phones also provide Internet access without the expense or weight of the laptop; users can either directly access the website or have news emailed to their phone. Another factor favoring web-based systems is the ability to access and integrate or complete data from widely separated locations. For example, definition of many administrators for the website is possible. So, several administrators, probably around the world can enter and edit their data in the Nemidsoft and in the other hand, simultaneous completeness of the program will happen. Perhaps, the greatest advantages of the web-based programs over the desktop-based is that changes in the details of the database at the server instantly propagated to all users at the next log in; so errors can be quickly corrected without having to send out notices the users need to update their systems. One of the web-based nematode database and identification tool is the Generic Biological Information System Nemys, includes keys to genera in some families, but the identification characters used are *ad hoc* characters, each one being defined and valid only for the family considered, which means that the database is far from being generic. In general, the identification process in Nemys seems to be a straightforward elimination of a number of genera every time the user chooses one of the proposed states of one of the characters. Such a system does not degrade gracefully when the user makes a single mistake [4]. Another example of such web-based system is General Identification System Genisys, includes a database schema for morphological characters alone [3]. Genisys must not rely on a single approach but must help the identifier with all possible approaches by providing him/her with a set of tools. The user will then be free to select the tool deemed most appropriate at each step of an identification session [39, 40].

The present software supports the most successful and most general key of the genera, especially the genus *Merlinius* by simplifying the handling of morphological features and morphometrical data. Our aim is to facilitate of the identification of nematode species and to present and further develop software which is easy to handle, useful and easy to obtain (via World Wide Web: www.nemidsoft.net). This simple aim also implies a development for the near future; Nemidsoft will not only support the most important identification key of the genus *Merlinius*, but also of the other genera such as *Heterodera*, *Tylencholaimellus*, *Tylenchorhynchus* and etc. Existing printed references or desktop computerized identification tools cannot be trusted to non-taxonomists for identification of all the specimens collected. A freely accessible identification tool via internet such as the one outlined here, probably can solve this problem. The comparative study of existing programs is presented in Table 1. The biggest technical problem to date have been related to different web browsers. Most of the open source browsers have few problems. Among these, such as Mozilla Firefox, Internet Explorer, Maxthon and Google Chrome, Mozilla Firefox has a high compatibility with

Nemidsoft. Probably, several changes in settings of other browser must be done to ensure compatibility with Nemidsoft.

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Table 1. The comparative study of existing programs.

Advantage or disadvantages	Desktop based or Web-based	Operating System	Approaches of processing and/or language of the program	Graphical Environment	User Management	Being User friendly	Level of nematode identification
***** Program							
NEMAID	Desktop-based	MS-DOS	similarity coefficient "Gower"	weak	Single user only	Moderate	Species level of only the <i>Helicotylenchus</i> genus
Marinema	Desktop-based	MS-DOS	multiple entry key	weak	Single user only	Moderate	Genus level of marine nematodes
Paranema	Desktop-based	MS-DOS	multiple entry key	weak	Single user only	Moderate	Only plant parasitic nematodes
Dory	Desktop-based	MS-DOS	Single query	weak	Single user only	Moderate	Dorylaimida
Dityl	Desktop-based	MS-DOS	similarity coefficient "Gower"	weak	Single user only	Good	Species level of the genus <i>Ditylenchus</i>
Use of Bikey 7	Desktop-based	MS-DOS	Elimination using Excel spreadsheet	moderate	Single user only	Good	Pratylenchidae : Radopholus
Generic Biological Information System Nemys	Web-based	Windows	Straight forward elimination of a number of genera every time the user chooses one of the proposed states of one of the characters	good	Multi-user	Moderate http://genusys.prd.fr/genisys_home.html	keys to genera in some families of nematodes
General Identification System Genisys	Web-based	Windows	Multi-approach (based on user selection)	good	Multi-user	Good http://nemys.ugent.be/index.asp	includes a database schema for morphological characters alone.
NEMIDSOFT	Web-based	Windows	ASP.NET web language (based on two search approach: common and advanced search)	good	Multi-user	Good (easy to handle, useful and easy to obtain (via World Wide Web: www.nemidsoft.net)	Species level of all genera of nematodes (with no limitations)