

# A computer method for identifying nematode species

## 1. Genus *Longidorus* (Nematoda : Longidoridae)

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### SUMMARY

A computer program to assist in the identification of nematodes is described. The program has been adjusted for identification of *Longidorus* species. It is easy to edit in personal computer, simple to use and can be extended to other genera. The program utilises an index that measures the probability that a character is common to the unidentified specimen and the reference species. The computer output lists the five species most similar to the specimen being investigated. The taxonomist can choose one of these species and if necessary may extend the morphometric range of the chosen species, alternatively, a new species may be created.

### RÉSUMÉ

*Une méthode informatique pour l'identification des espèces de  
nématodes. I. Le genre Longidorus (Nematoda : Longidoridae)*

Les auteurs donnent la description d'un programme d'ordinateur destiné à faciliter l'identification des nématodes. Ce programme a été adapté à l'identification des espèces du genre *Longidorus*. Il est aisé à introduire dans un ordinateur personnel, simple à utiliser et peut être appliqué à d'autres genres. Le programme utilise un index qui mesure la probabilité pour qu'un caractère soit commun au spécimen à identifier et aux espèces de référence. L'ordinateur fournit une liste des cinq espèces les plus semblables au spécimen en étude. Le taxonomiste peut ensuite choisir l'une de ces cinq espèces, en étendant si besoin est, les valeurs des caractères morphométriques de l'espèce choisie; une nouvelle espèce peut également être créée.

Dichotomous keys are not a reliable tool for specific identification in genera with large number of species, high intraspecific variability and interspecific differences relatively small with overlapping occurring between characters. These keys also present other practical flaws : they are rapidly outdated, and are difficult to update; after a number of new species have been described a new key is required; also, as there is need to examine the different characters in a prestablished order, which is often arbitrary, wrong decisions at any level in the key misdirect the user and result in erroneous conclusions (Luc & Dalmasso, 1975; Fortuner & Wong, 1984). However, identifying species by means of polytomous key permits a range of characters to be used simultaneously and the description of a new species can be simply added to the key. Besides a polytomous key seems to be a good basis to prepare a computer program for the identification of species.

Longidorid nematodes include many plant species ectoparasites of arable crops and are widely distributed throughout the world. Their importance has given rise in the last thirty years to a rapid increase in the number of known species (Lamberti, Taylor & Seinhorst, 1975;

Coomans, 1985). *Longidorus* is a well established and defined genus (Hooper, 1980; Coomans, 1985), so very suitable to allow the development of a computer program to help in the identification of nematodes. Therefore, starting, from a polytomous key for identifying *Longidorus* species prepared by Romanenko (1978) and modified by Hooper (1980), a computer program proposed by Fortuner (1983) and a computer technique developed by Boag and Smith (1983, 1984) we elaborated a computer program for the identification of nematodes. The program is described here. At the same time, a revision of the *Longidorus* species described up to date has been carried out in order to apply the program, which is intended to be a tool for the taxonomist.

### Methods

The program was written in Advanced Hewlett-Packard BASIC on a personal computer HP-9020A with a free RAM of 256K and a Winchester rigid disk of 10 Mbytes. The equipment includes an external printer

of 132 columns. The size of the program is 5 828 bytes and that of the data base 12 096 bytes.

#### IDENTIFICATION

The resemblance of species to one another is a property that can be quantified but choice of the method used to measure the resemblance depends upon the information available. Gower (1971) proposed a *General Coefficient of Similarity* that is applicable to a variety of different data types and which can accommodate character weighting. It is defined, for quantitative characters, as :

$$S_B = \frac{\sum W_i (1 - |X_{ij} - X_{ik}| / R_i)}{\sum W_i} \quad (1)$$

where  $W_i$  is the weighting factor,  $|X_{ij} - X_{ik}|$  the absolute value of the difference between species  $j$  and  $k$  for character  $i$ , and  $R_i$  the range. When mixed types of characters are used Gower's coefficient appears to be a satisfactory index for quantifying similarity between two species (Sneath & Sokal, 1973). We adopted the following formula for estimating the similarity :

$$S_B = 1 - \frac{\sum W_i |X_{ij} - X_{ik}| / R_i}{\sum W_i} F_w \quad (2)$$

where  $W_i |X_{ij} - X_{ik}|$  and  $R_i$  are defined as above stated. This coefficient is essentially similar to Gower's but differs by  $F_w$  a correction factor for the missing values (Rey, 1987). It is equal to twice the total number of characters divided by the actual number of characters used plus the total number of characters :

$$F_w = \frac{2 \times (\text{No. of characters})}{(\text{No. of characters} + \text{No. of characters used})}$$

Pinkham and Pearson (1976) suggested an index that may be used with qualitative, semiquantitative or quantitative data and allows weighting. It compares the characters while maintaining their individuality and expressed as a formula gives :

$$S_B = \sum \frac{\min (X_{ij}, X_{ik})}{\max (X_{ij}, X_{ik})} \quad (3)$$

where  $X_{ij}$  and  $X_{ik}$  are the values for species  $j$  and  $k$  for character  $i$ . Although a metric, it is not globally consistent, owing to division by a changing quantity; as a consequence of this, the scale of measurement may change with each pair of species considered (Orloci, 1978).

Any distortion in the measurement scale can be reduced by adding a given quantity to the numerator and denominator which smooths the minimum/maximum ratio for each character. This helps to keep the scale more homogeneous. The quantity to be added

should be the same for each character, not too small in relation with their values as to be meaningless in the similarity ratio but not so big as to outweigh the characters own values. It should take into account all the values for every species and correct the effect of the relative importance of each character due to the internal data structure. A quantity which does this is the standard deviation for a given character therefore we used it with the values from the individual species for each character in the equation :

$$S_{ijk} = \frac{\min (X_{ij}, X_{ik}) + sd_i}{\max (X_{ij}, X_{ik}) + sdi} \quad (4)$$

where  $sd_i$  is the standard deviation for character  $i$  for all the species. The individual similarities for each character of both species are multiplied by the corresponding weight, and added. Subsequently, this sum is divided by the sum of the weights to give the total similarity according to the formula :

$$S = \frac{\sum W_i S_{ijk} F_c}{\sum W_i} \quad (5)$$

$S_{ijk}$  is given by equation (4) and  $W_i$  is the weight for character  $i$ . The correction factor  $F_c$  for the missing values is equal to the total number of characters plus the actual number of characters used divided by twice the total number of characters, and is equal to the inverse of  $F_w$  in formula (2). Double zeroes due either to the absences of the binary characters or to the so coded qualitative characters are considered positive matches.

#### CHARACTERS

Selection of the characters useful for identifying species was based on their relevancy and availability in the published descriptions. They include binary characters (presence or absence), quantitative continuous characters whose real values are used, quantitative discontinuous characters which have been coded and qualitative multistate characters in which, as far as possible, a logical order has been established and then also coded (Abbott, Bisby & Rogers, 1985). Consequently, formulae (2) and (5) are both used for all of the different characters.

As the means give the least stringent and the sample range the most stringent comparisons (Boag & Smith, 1983), the quantitative variables were given maximum and minimum values. A numerical code ranging from 1 to 4 was used for the qualitative characters. For those characters where only one measurement was given, this value was assigned to both the maximum and minimum values of the range. When values were missing, a code of - 1 was given. The maximum and minimum values of the characters of some of the species were extended by using data from other than the original authorities.

Also, where necessary we used measurements obtained from authors drawings and our own observations.

#### WEIGHTING

In the method described here, the non-variable characters with values 0 — 1 acquire a relatively higher importance than the next relatively most important characters, which are the qualitative multistate characters with few states. Similarly the quantitative variables, either discrete or continuous, and within them those which have the highest variability or range in the raw data become less important than the foregoing. Moreover, where data have maximum and minimum values as in the case of the quantitative variables, or they are derived from two characters e.g. ratios  $a$ ,  $b$ ,  $c$ ,  $d$ , and  $V$  they have less importance. With or without weighting each variable individually contributes, with a different potential, to the final result albeit some having more influence than others.

For Moss and Webster (1970), in the absence of logical operational schemes for character weighting, it remains an axiom of numerical taxonomy that characters should contribute equally. Sneath and Sokal (1973) consider equal weighting of all characters is desirable. However, indices like the *General Similarity Coefficient of Gower* (Sneath & Sokal, 1973) and the *Coefficient of Similarity* proposed by Estabrook and Rogers (1966, cf. Legendre & Legendre, 1979) permit arbitrary weighting, i.e. some characters are given more importance than others. Boag and Smith (1984) weight characters useful for identifying species, but do so in ways different to those above; NEMAID program (Fortuner, 1983) permits total freedom of weighting; thus, there is a choice as to the weights to be used or not to use them. But, weighting, like scaling, implies a transformation of the raw data that will affect the final results.

We chose to weight the characters used for the program described here to enhance or decrease their influence on the final result. The weighted values of the variables were estimated by giving them *a priori* weights from 0.1 to 1 according to the importance we considered should be attributed to them in conventional taxonomy. They were then ranked by the *vector projection method* suggested by Orloci (1978) and the arbitrary weights were then adjusted until the characters were in the same order, but not with the same value, as we ranked them initially. These were then checked experimentally by testing them with data from populations of identified species and were corrected when necessary until the results were as expected.

#### THE PROGRAM

The program starts by initialising the necessary matrices. Species names and character weights are included in DATA statements and the raw values of the

characters in the reference file are read from the mass storage unit. The program has five subroutines, four of which are interactively selected by the user via the keyboard : 1) INPUT, entry of the unidentified species data, 2) SIMIL, analysis and display of results, 3) PRINT, printing of the unidentified species values and 4) CORRECT, correction of these data.

The raw data for the unidentified species are entered in a numeric vector in the same order as are the weighting coefficients, using the subroutine *Input* and displayed on the computer screen by the subroutine *Print*. When necessary, the data can be corrected by using the subroutine *Correct*; any incorrectly entered value can be altered. Identification of the species is carried out by the subroutine *Simil* which calculates the similarities between the unidentified species and all the species in the reference file. The similarity indices are calculated according to equation (5), which computes a weighted mean of the similarities which in turn is multiplied by 100 to convert the index to a percentage. If a missing value is found (code = - 1) in the reference file or in the vector for the unidentified species, whilst the program is calculating the sum of the weighted similarities and weights, the program jumps to the next character and consequently neither the weighted similarity nor the character weight are added to their respective variables. Finally, all the indices are stored in a vector and then the five species from the reference file with the highest similarity to the unidentified species are chosen by the subroutine *Find* and displayed on the computer screen : firstly the compendium number in the reference file, secondly the species name and thirdly the similarity index. For comparative purposes a similar output based on formula (2) is presented. The time taken to identify the species is from one to two seconds according to the species estimated.

#### Results and discussion

The program was adjusted for the identification of *Longidorus* species. A reference file of all known species of this genus up to date was prepared (Tab. 1). Characters useful for their identification were selected from various sources (Dalmaso, 1969; Loof & Coomans, 1972; Hooper & Southey, 1973; Romanenko, 1978; Hooper, 1980; Sturhan & Argo, 1983; Coomans, 1985). Therefore, body, odontostyle and odontophore lengths, position of the guide ring and the amphidial pouches, lip region and tail shapes are considered to be the most important characters for species identification. They have been compiled in a table which together with the program listing can be requested from the authors and whose column numbers are explained in detail in Table 2.

Table 2 shows the characters chosen with their codes. The characters are : one non variable (presence or absence of males) three qualitative multistate characters

Table 1

Species list of genus *Longidorus* included in the reference file

Species	Number in the file	Species	Number in the file
<i>L. aetnaeus</i> Roca, Lamberti, Agostinelli & Vinciguerra, 1986	9	<i>L. leptocephalus</i> Hooper, 1961	7
<i>L. africanus</i> Merny, 1966	12	<i>L. longicaudatus</i> Siddiqi, 1962	33
<i>L. apulus</i> Lamberti & Bleve-Zacheo, 1977	32	<i>L. lusitanicus</i> Macara, 1985	62
<i>L. attenuatus</i> Hooper, 1961	14	<i>L. macromucronatus</i> Siddiqi, 1962	49
<i>L. belondiroides</i> Heyns, 1967	27	<i>L. macrosoma</i> Hooper, 1961	52
<i>L. breviannulatus</i> Norton & Hoffman, 1975	20	<i>L. magnus</i> Lamberti, Bleve-Zacheo & Arias, 1982	45
<i>L. caespiticola</i> Hooper, 1961	37	<i>L. martini</i> Merny, 1966	19
<i>L. carpetanensis</i> Arias, Andrés & Navas, 1986	59	<i>L. mirus</i> Khan, Chawla & Seshadri, 1972	17
<i>L. closelongatus</i> Stoyanov, 1964	45	<i>L. moesicus</i> Lamberti, Choleva & Agostinelli, 1983	48
<i>L. cohni</i> Heyns, 1969	42	<i>L. monile</i> Heyns, 1966	3
<i>L. congoensis</i> Aboul-Eid, 1970	11	<i>L. moniloides</i> Heyns, 1966	6
<i>L. crassus</i> Thorne, 1974	43	<i>L. nevesi</i> Macara, 1985	63
<i>L. cylindricaudatus</i> Kozłowska & Seinhorst, 1979	53	<i>L. nirulai</i> Siddiqi, 1965	40
<i>L. diadecturus</i> Eveleigh & Allen, 1982	58	<i>L. olegi</i> Kankina & Metlitskaya, 1983	61
<i>L. distinctus</i> Lamberti, Choleva & Agostinelli, 1983	18	<i>L. orientalis</i> Loof, 1982	31
<i>L. edmundsi</i> Hunt & Siddiqi, 1977	35	<i>L. paraelongatus</i> Altherr, 1974	—
<i>L. elongatus</i> (de Man, 1876) Thorne & Swanger, 1935	22	<i>L. paramirus</i> Darekar & Khan, 1982	16
<i>L. eridanicus</i> Roca, Lamberti & Agostinelli, 1984	56	<i>L. paramonile</i> s'Jacob & Heyns, 1982	4
<i>L. euonymus</i> Mali & Hooper, 1974	21	<i>L. picenus</i> Roca, Lamberti & Agostinelli, 1984	54
<i>L. fasciatus</i> Roca & Lamberti, 1981	39	<i>L. pisi</i> Edward, Misra & Singh, 1964	5
<i>L. fragilis</i> Thorne, 1974	26	<i>L. poessneckensis</i> Altherr, 1974	—
<i>L. globulicauda</i> Dalmaso, 1969	13	<i>L. profundorum</i> Hooper, 1966	28
<i>L. goodeyi</i> Hooper, 1961	34	<i>L. protae</i> Lamberti & Bleve-Zacheo, 1977	15
<i>L. indicus</i> Prabha, 1973	25	<i>L. proximus</i> Sturhan & Argo, 1983	36
<i>L. intermedius</i> Kozłowska & Seinhorst, 1978	44	<i>L. psidii</i> Khan & Khan, 1972	29
<i>L. iranicus</i> Sturhan & Barooti, 1983	50	<i>L. renayii</i> Raina, 1966	2
<i>L. irshati</i> Javed, 1983	24	<i>L. saginus</i> Khan, Seshadri, Weischer & Mathen, 1971	55
<i>L. iuglandis</i> Roca, Lamberti & Agostinelli, 1984	51	<i>L. sylphus</i> Thorne, 1939	23
<i>L. jonesi</i> Siddiqi, 1962	46	<i>L. tanitwha</i> Clark, 1963	47
<i>L. juvenilis</i> Dalmaso, 1969	8	<i>L. tardicauda</i> Merzhevskaya, 1951	38
<i>L. laevicapitatus</i> Williams, 1959	1	<i>L. tarjani</i> Siddiqi, 1962	57
<i>L. latocephalus</i> Lamberti, Choleva & Agostinelli, 1983	10	<i>L. unedoi</i> Arias, Andrés & Navas, 1986	60
		<i>L. vineacola</i> Sturhan & Weischer, 1964	30

(lip and tail region and amphidial pouches shapes), two quantitative coded (body width at guide ring divided by lip region width [ $b/I$ ] and oral aperture to guide ring distance divided by lip region width [ $oa-gr/I$ ] and eleven quantitative continuous characters whose real values are used (de Man indices and total body, odontostyle and odontophore lengths).

The weights assigned to each character are also included in Table 2. We give the maximum weight (2) to the odontostyle length (characters 13 and 14) because of its importance in conventional taxonomy. Also, it has the highest correlation between the maximum and mini-

mum values and being a quantitative variable it presents a very wide range of values which makes it the relatively least important character due to the internal structure of the data. Similarly, we set the weight for lip region shape as (1),  $c'$  index (.9), total body length (.8),  $c$  index (.7), amphidial and tail shape (.6 and .5 respectively) and so on (Tab. 2). The same effect could have been accomplished by using weight values of only half that of the foregoing.

Table 3 shows the printout for results obtained using formula (5) for three populations from our own collection. The expected nematode species was obtained for

Table 2  
Characters, codes and weights used in the program

	Character	Code	Weight	
1- 2	Female body length		0.8	
3- 4	a		0.4	
5- 6	b		0.1	
7- 8	c		0.7	
9-10	c'		0.9	
11-12	V		0.2	
13-14	Odontostyle length		2	
15-16	Odontophore length		0.2	
17	Lip region shape	Distinctly expanded and offset from neck	1	1
		Almost cylindrical, continuous or slightly offset	2	
		Rounded conoid	3	
		Truncate conoid	4	
18	Amphid pouch shape	Elongated, funnel-shaped, not lobed	1	0.6
		Pocket-shaped with two equal lobes	2	
		Pocket-shaped with one lobe longer than the other	3	
19-20	Body width at guide ring level/Lip region width	< 1.5	1	0.3
		1.5 to 2.5	2	
		> 2.5	3	
21-22	Oral aperture to guide ring distance/Lip region width	< 1.5	1	0.4
		1.5 to 2.5	2	
		> 2.5	3	
23	Female tail shape	Conoid	1	0.5
		Rounded	2	
		Digitate	3	
24	Males	Present	1	0.1
		Absent	0	

each population. When using formula 2 similar results were obtained (Tab. 4). The first population was identified as *L. attenuatus*, with a similarity index of 88.11 % (Tab. 3). The relatively low similarity can be explained because the specimens from Spain have longer odontostyle lengths than given in the original description. The five most similar species given in Table 3 are the same as those in Table 4 with population 1.

The largest similarity index was obtained with population 2, *L. intermedius* which gave a value of 94.58 vs 90.04 for *L. indicus*. With this population the similarity index in Table 3 (formula 5) was lower than in Table 4 (formula 2), and the difference between the first and second species was greater with the second than the adopted formula, i. e., it had a higher discriminant capability. Population 3 also was correctly identified by both formula as being *L. caespiticola* with the similarity

index being greatest with formula (5) (Tables 3 and 4).

The proposed program is easy to edit in a personal computer and simple to use, even by non-specialists. However, it has to be regarded as a tool in the identification of species and not as a substitute for a trained, experienced taxonomist. The final decision in the identification of a single specimen or a population is the responsibility of the user. The taxonomist can use this program to help in making a decision as to the identity of the specimen or population being examined. If the similarities to existing species are under a pre-defined limit it is possible to extend the range of an already described species including the new species after checking all the species in the computer output. The final decision to establish a new species should not be taken until type specimens of the most similar species have been examined.

Table 3  
A sample printout from the program when formula (5) has been used

Population 1									
1	7.70	2	8.30	3	177.00	4	197.00	5	17.00
6	19.00	7	94.00	8	108.00	9	2.10	10	2.50
11	52.00	12	53.00	13	95.00	14	99.00	15	50.00
16	54.00	17	1.00	18	2.00	19	1.00	20	1.00
21	2.00	22	2.00	23	1.00	24	1.00		
14	<i>Longidorus attenuatus</i>		88.11						
32	<i>L. apulus</i>		85.22	21	<i>L. euonymus</i>		85.17		
45	<i>L. closelongatus</i>		84.70	30	<i>L. vineacola</i>		84.42		
Population 2									
1	3.90	2	4.30	3	69.00	4	77.00	5	12.50
6	13.70	7	94.00	8	110.00	9	1.10	10	1.30
11	46.60	12	50.00	13	106.00	14	109.00	15	46.60
16	49.50	17	2.00	18	2.00	19	2.00	20	2.00
21	3.00	22	3.00	23	1.00	24	— 1.00		
44	<i>Longidorus intermedius</i>		94.58						
25	<i>L. indicus</i>		90.03	29	<i>L. psidii</i>		89.19		
16	<i>L. paramirus</i>		88.83	53	<i>L. cylindricaudatus</i>		87.24		
Population 3									
1	5.30	2	5.80	3	72.00	4	81.00	5	11.00
6	11.00	7	126.00	8	148.00	9	60	10	86
11	52.00	12	54.00	13	94.00	14	95.00	15	53.00
16	62.00	17	3.00	18	1.00	19	2.00	20	2.00
21	2.00	22	2.00	23	2.00	24	1.00		
37	<i>Longidorus caespiticola</i>		92.15						
34	<i>L. goodeyi</i>		90.97	31	<i>L. orientalis</i>		88.86		
46	<i>L. jonesi</i>		88.66	28	<i>L. profundorum</i>		88.86		

Table 4  
A sample printout from the program when formula (2) has been used

Population 1									
1	7.70	2	8.30	3	177.00	4	197.00	5	17.00
6	19.00	7	94.00	8	108.00	9	2.10	10	2.50
11	52.00	12	53.00	13	95.00	14	99.00	15	50.00
16	54.00	17	1.00	18	2.00	19	1.00	20	1.00
21	2.00	22	2.00	23	1.00	24	1.00		
14	<i>Longidorus attenuatus</i>		86.14						
21	<i>L. euonymus</i>		82.57	32	<i>L. apulus</i>		81.52		
45	<i>L. closelongatus</i>		81.04	30	<i>L. vineacola</i>		80.24		
Population 2									
1	3.90	2	4.30	3	69.00	4	77.00	5	12.50
6	13.70	7	94.00	8	110.00	9	1.10	10	1.30
11	46.60	12	50.00	13	106.00	14	109.00	15	46.60
16	49.50	17	2.00	18	2.00	19	2.00	20	2.00
21	3.00	22	3.00	23	1.00	24	— 1.00		
44	<i>Longidorus intermedius</i>		95.94						
29	<i>L. psidii</i>		91.12	25	<i>L. indicus</i>		91.09		
16	<i>L. paramirus</i>		90.06	53	<i>L. cylindricaudatus</i>		87.25		
Population 3									
1	5.30	2	5.80	3	72.00	4	81.00	5	11.00
6	11.00	7	126.00	8	148.00	9	60	10	86
11	52.00	12	54.00	13	94.00	14	95.00	15	53.00
16	62.00	17	3.00	18	1.00	19	2.00	20	2.00
21	2.00	22	2.00	23	2.00	24	1.00		
37	<i>Longidorus caespiticola</i>		91.14						
43	<i>L. crassus</i>		88.37	34	<i>L. goodeyi</i>		88.29		
27	<i>L. belondiroides</i>		87.65	31	<i>L. orientalis</i>		86.64		

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