Journal of Nematology 49(2):207–222. 2017.
© The Society of Nematologists 2017.

Numerical Taxonomy Helps Identification of Merliniidae and Telotylenchidae (Nematoda: Tylenchoidea) from Iran

Reza Ghaderi, Habiballah Hamzehzarghani, and Akbar Karegar

Abstract: Numerical taxonomy was used for identification and grouping of the genera, species, and populations in the families Merliniidae and Telotylenchidae. The variability of each of 44 morphometric characters was evaluated by calculation of the coefficient of variability (CV) and the ratio of extremes (max/min) in the range of 1,020 measured females. Also correlation and regression analyses were made between characters to find potential collinearities. Hierarchical cluster analysis (HCA) was used for (i) grouping 21 genera in the superfamily Dolichodoroidea based on literature data coded for states of 18 diagnostic characters, and (ii) for grouping Iranian populations belonging to selected genera. Furthermore, STEPDISC analysis was used for (i) grouping 11 genera of Merliniidae and Telotylenchidae based on the measurements of 35 characters from 1,007 Iranian female specimens, and (ii) grouping measured females of eight species of *Amplimerlinius* and *Pratylenchoides*. The multivariate data analysis approach showed robust enough to summarize relationship between morphometric characters and group genera, species, and populations of the nematodes and in particular help to identify the genera and species of *Amplimerlinius* and *Pratylenchoides*.

Key words: Amplimerlinius, correlation, hierarchical cluster analysis, *Pratylenchoides*, principal component analysis.

The families Merliniidae Siddiqi, 1971 and Telotylenchidae Siddiqi, 1960 are among the most problematical taxa in Tylenchida Thorne, 1949\Tylenchomorpha De Ley and Blaxter, 2002. There is no overall agreement on the taxonomic positions of the included taxa at the species and\or genus levels or even higher ranks among nematologists (Fortuner and Luc, 1987; Maggenti, 1991; Brzeski, 1998; Siddiqi, 2000; Decraemer and Hunt, 2006, 2013; Andrássy, 2007; Geraert, 2011; Hunt et al., 2013). Some attempts have been made to clarify taxonomical problems within these families using morphological (Sturhan, 2011, 2012; Ghaderi and Karegar, 2014a, 2014b) or molecular (Ghaderi et al., 2014) approaches, but taxonomic identification of the several included members has remained difficult and uncertain. Erection of the superfamily Dolichodoroidea sensu Siddiqi (2000) or the family Dolichodoridae sensu Decraemer and Hunt (2006) for covering all "awl" (dolichodorids), "sting" (belonolaimids), and "stunt" (tylenchorhynchids) nematodes appears no longer justified according to the recent studies (Subbotin et al., 2006; Sturhan, 2012; Ghaderi et al., 2014). The concept of the "large genus" for *Tylenchorhynchus* Cobb, 1913 is one of the most controversial problems in Telotylenchidae. Fortuner and Luc (1987) considered *Bitylenchus* Filipjev, 1934, *Neodolichorhynchus* Jairajpuri and Hunt, 1984, *Telotylenchus* Siddiqi, 1960, and several other genera as synonyms of *Tylenchorhynchus*; although this idea was followed by Brzeski (1998), others (Siddiqi, 2000; Decraemer and Hunt, 2006, 2013; Andrássy, 2007; Geraert, 2011; Hunt et al., 2013) rejected that, partially or completely. For many years, the genus *Pratylenchoides* Winslow, 1958 was thought to be closely related to pratylenchids, but recent morphological (Sturhan, 2011, 2012) and molecular (Bert et al., 2008; Holterman et al., 2009; van Megen et al., 2009; Panahandeh et al., 2014; Ghaderi et al., 2014) information strongly support placement of *Pratylenchoides* within Merliniidae. Many other taxonomic problems of Merliniidae and Telotylenchidae have been discussed in Furtuner and Luc (1987), Sturhan (2012), Ghaderi et al. (2014), and Azizi et al. (2016). More studies on morphological characters and the range of intraspecific variation (accompanied by molecular analyses) are required for achieving a better and more comprehensive view on the taxonomy of these nematodes.

Numerical taxonomy (or phenetics) was largely developed and popularized by Sneath and Sokal (1973), as a response to the call for a more objective taxonomy. This approach consists of applying various mathematical procedures to numerically encoded character state data for the organisms under study. The products of these operations were often taken to be "unbiased" indicators of the similarity or difference between the taxa, which were in turn used to arrange taxa in a hierarchy (Quicke, 1993). The word "character" has many different meanings in taxonomy, but the general idea requires a character to be characteristic sufficiently to be used to differentiate, classify, or identify taxa. The domain of possible qualitative states or the range of possible quantitative values is called "character states" or "character values," respectively (Diederich et al., 1997).

Multivariate data analysis techniques are classified into two main supervised and unsupervised groups and may at the same time be predictive and/or descriptive. Unsupervised methods perform the job of clustering, whereas supervised ones classify the data sets. The basic difference between clustering and classification is that in the clustering the data, points are unlabeled, assuming no prior knowledge of the previous grouping of samples. In classification, the data points have "labels" i.e., there are predefined groups. For instance, hierarchical clustering separates the more similar unlabeled

Received for publication March 28, 2017.
Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz, 71441-65186, Iran.
E-mail: rghaderi@shirazu.ac.ir.
This paper was edited by Eyualem Abebe.

data points (samples) from an experiment and group them tightly close together. In contrast, in a classification method, a training set (a portion of data or a different dataset) is used to discover the unknown grouping pattern. Methods such as HCA, principal component analysis (PCA), factor analysis (FA), and canonical discriminant analysis (CDA) comprise unsupervised methods that are far more useful than supervised ones (Goodacre et al., 2004).

Different methods of numerical taxonomy including HCA, FA, PCA, and multiple regression analysis have been used for identification of plant-parasitic nematodes at different taxonomic ranks, e.g., in *Tylenchus* (Blackith and Blackith, 1976), *Helicotylenchus* (Fortuner et al., 1984), *Rotylenchus* (Zancada and Lima, 1985; Cantalapiedra-Navarrete et al., 2013), *Caloosia* (Fortuner, 1993), *Xiphinema* (Lamberti and Ciancio, 1993; Lamberti et al., 2002; Gozel et al., 2006), *Longidorus* (Ye and Robbins, 2004, 2005), Criconematina (Subbotin et al., 2005), *Heterodera* (Abdollahi, 2009), *Meloidogyne* (Mokaram Hesar et al., 2011), Criconemoides (Chenari Bouket, 2013), and *Paratylenchus* (Akyazi et al., 2015). The present study aims to provide a concise description of patterns of the morphological and morphometric similarities and differences in data obtained from the Iranian populations of Merliniidae and Telotylenchidae. Finally, considering current morphological and molecular information as a basis, our study attempts to address some taxonomic complications in these families.

Materials and Methods

Nematode samples: Nematode isolates were collected from the rhizosphere of different plants in fields, orchards, plantations, and meadows from different localities in Iran (Fig. 1) during 2010 to 2013. Nematodes were extracted from soil samples using the tray method (Whitehead and Hemming, 1965). Specimens were killed and fixed by hot FPG (4:1:1, formaldehyde: propionic acid: glycerol), processed to anhydrous glycerol (De Grisse, 1969), and mounted in glycerol on permanent slides. All measurements were taken by a light microscope Zeiss III, equipped with Dino-eye microscope eyepiece camera and its software Dino Capture version 2.0. Nematode species were identified based on the morphological and morphometric characters, using identification keys (Geraert, 2011, 2013).

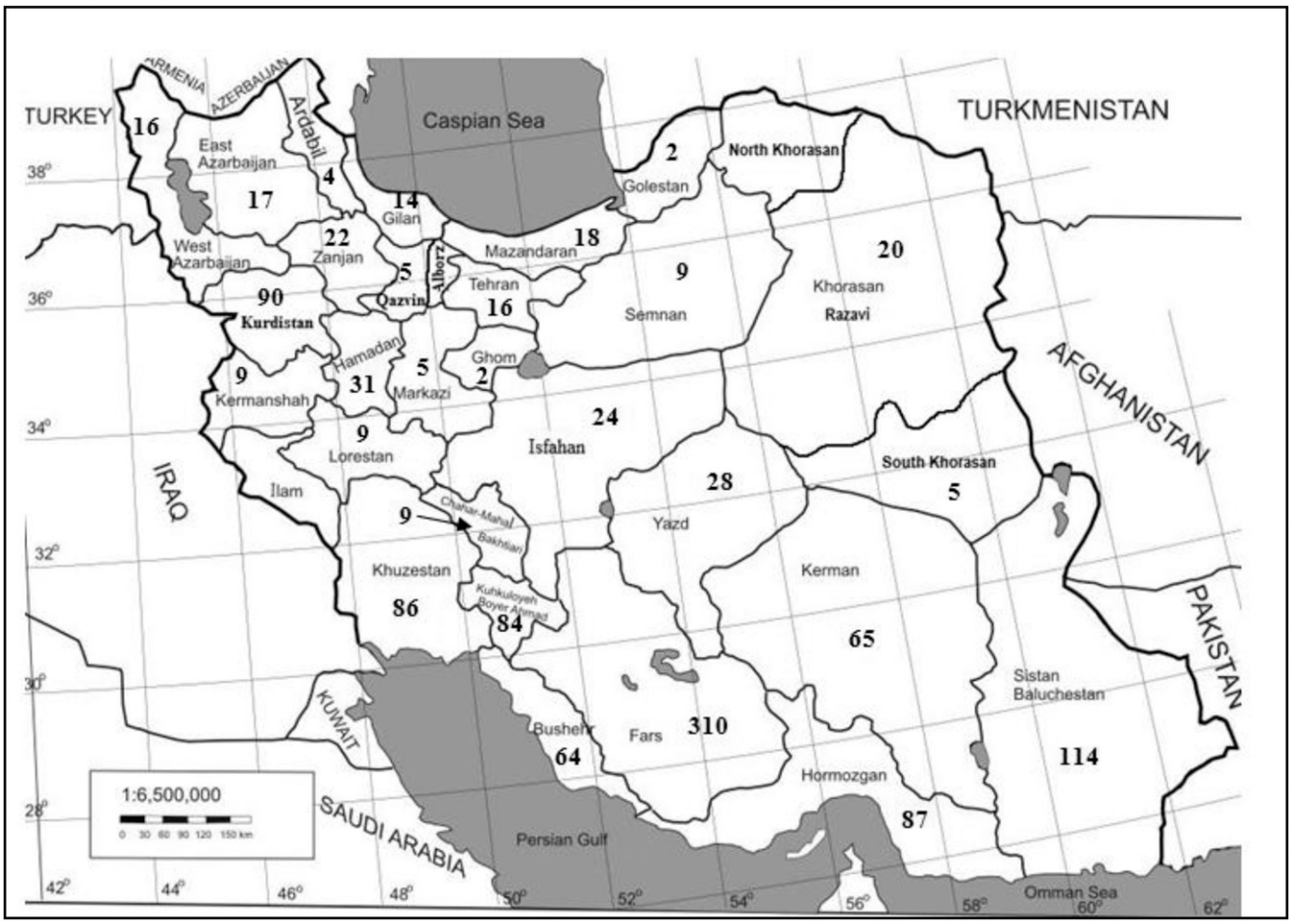

Fig. 1. The number of collected samples (from the rhizosphere of different plants) from Iranian provinces during 2010 to 2013.

Evaluation of morphometric characters: In total, 1,020 females belonging to 197 populations of 31 species from 11 genera of the families Merliniidae and Telotylenchidae, collected from Iran, were used for the present study. For each female, 44 morphometric indices and ratios (Tables 1,2) were used in the calculations. The variability of each morphometric character was estimated by calculation of the CV and the ratio of extremes in the range of measured females (Max/Min). Furthermore, the Max/Min ratios of eight characters (Table 3) were estimated for reported populations in the literature plus those recovered in the present study (more than 6,600 female specimens belonging to 641 populations of 227 species and 25 genera). The used indices and abbreviations are in accordance with those which explained in Siddiqi (2000).

Correlation and regression analysis of characters: Correlation of the 44 morphometric characters was determined with SPSS software (SPSS for windows, version 16.0; SPSS Inc., Chicago, IL) using two-tailed Pearson correlation coefficient. The correlation between eight pairs of the measured characters (which considered to be more informative in taxonomic studies of the families Merliniidae and Telotylenchidae) was evaluated graphically. Scatter plots were made and the regression line was fitted to the data of each pair of the characters.

Multivariate data analysis: HCA is a completely unsupervised method that measures similarity between two observations and then assigns the observation to the cluster of observations to which it has more similarity (Johnson, 1998). HCA was performed using between-group linkage method with the SPSS 16 software for grouping the two sets of data: (i) 21 genera included in the families Merliniidae and Telotylenchidae, and (ii) the studied populations in the genera *Amplimerlinius* Siddiqi, 1976, *Geocenamus* Thorne and Malek, 1968, *Merlinius* Siddiqi, 1979, and *Pratylenchoides*. For grouping genera, the most important diagnostic characters were selected from the literature (Fortuner and Luc, 1987; Siddiqi, 2000; Geraert, 2011; Sturhan, 2012) and their states were defined for each character (Tables 4,5). For grouping studied populations in the present research, common morphometric indices and ratios in nematology (Tables 1,2) were measured and used for cluster analysis.

PCA reduces a set of colinear variables into a small number of hidden orthogonal (uncorrelated) principal components (PC). The basic assumption of PCA is that there exist a number of latent variables or PC accounting for the correlations among observed quantitative variables. Variation in all correlated measured characteristics, accounted for by the same latent PC, is summarized in the PC in as much as the initial data can be represented using a few new uncorrelated PC (Johnson, 1998; Johnson and Wichern, 2002). PCA was performed (i) on the sampled populations (1,007 female individuals belonging to 190 populations of 31 species from 11 genera) in order to determine the morphometric discrimination among genera, and (ii) on the sampled populations of *Amplimerlinius*, *Geocenamus*, *Merlinius*, and *Pratylenchoides* in order to delimit included species in each genus. The analyses were based on the 35 characters (Table 6). PCA was performed with the PRIN-COMP procedure of SAS (Statistical Analysis System, version 9.2) to produce a set of variables (PC) that were linear combinations of the original variables. The new variables were ranked according to the amount of variation accounted for.

As grouping in the 3 dimensional (3D) PC space was inadequate, CDA, another dimension-reduction technique which develops linear combinations of the measured variables or so called CAN (canonical) vectors summarizing between-class variation, was used to increase the resolution of the clustering pattern by minimizing the within cluster variance and maximizing the between cluster variance (Johnson, 1998). The STEPDISC procedure was used to select a subset of the PC outputted from PCA to perform a stepwise discriminant analysis for use in discriminating among the classes. The new subset of PC is selected in a way to maximize the resolution of clustering pattern. Subsequently, CANDISC procedure of SAS was used to compute squared Mahalanobis distances (distances in variance scale) among sample (species for example) means. The values of canonical variables were used to plot all samples in a canonical 3D space of three CAN vectors, to aid in the visual interpretation of group differences or similarities. The loading of a measured variable (metabolite) to each CAN vector is used to explain its influence on grouping criteria.

Results

Variability of morphometric characters: The results of the present study indicated that the CV has a comparable range for the majority of the measured indices and ratios in the subfamilies Merliniinae Siddiqi, 1971, Pratylenchoidinae Sturhan, 2012, and Telotylenchinae Siddiqi, 1960, but showed a different tendency in certain characters among the subfamilies. For example, anus–phasmid distance and phasmid from anus/tail length % showed a higher CV in Merliniinae and Pratylenchoidinae than that in Telotylenchinae (the CV is 17.1, 18.4, and 7.3 for anus–phasmid distance and is 12.5, 11.9, and 7.6 for phasmid from anus/tail length % in Merliniinae, Pratylenchoidinae, and Telotylenchinae, respectively). The data steadily showed that phasmid from anus/tail length % has a higher stability than anus–phasmid distance; in other words, the position of phasmids can be attributed to the tail length. Therefore, these differences may be due to lower variation in the tail length of Telotylenchinae compared to the two other subfamilies (10.7, 13.3, and 7.9 in Merliniinae, Pratylenchoidinae, and Telotylenchinae, respectively).

TABLE 1. Coefficient of variability (CV) of the morphometric characters of 1,020 measured females of the genera and subfamilies in Merliniidae and Telotylenchidae from Iran.

Character\genus or subfamily	*Amplimerlinius*	*Geocenamus*	*Merlinius*	*Nagelus*	*Paramerlinius*	*Bitylenchus*	*Neodolichorhynchus*	*Paratrophurus*	*Trophurus*	*Tylenchorhynchus*	*Pratylenchoides*	Merliniinae	Telotylenchinae
Number of specimens (*n*)	125	259	203	10	23	64	27	11	39	160	99	620	301
Body length (*L*)	9.6	7.9	9.8	7.6	5.4	8.0	6.6	3.4	7.1	8.0	10.0	8.7	7.3
Body length/width (*a*)	6.2	7.5	7.4	5.6	6.8	7.8	5.9	13.0	6.8	7.5	9.0	6.9	7.6
Body length/pharynx length (*b*)	7.7	7.0	7.9	7.5	5.5	7.4	7.0	4.1	5.7	7.6	8.9	7.4	6.9
Body length/tail length (*c*)	9.7	7.8	7.5	10.7	7.7	8.8	6.7	5.4	7.1	7.6	9.6	8.4	7.5
Tail length/anal body length (*c*')	9.9	9.5	10.7	14.4	9.6	11.2	6.4	9.1	6.9	10.3	11.3	10.3	9.3
Vulva position/body length % (*V*)	2.4	2.7	3.0	1.6	2.9	2.3	1.2	3.9	2.8	1.8	3.3	2.6	2.1
Stylet length	3.7	4.1	5.8	2.9	3.5	4.8	1.9	3.4	4.6	4.8	3.8	4.4	4.2
Conus length	5.4	5.6	9.8	3.1	5.3	6.2	5.7	5.9	8.1	6.9	5.3	6.5	6.6
Conus length/stylet length % (*m*)	3.1	3.6	6.3	3.6	3.0	4.3	5.1	3.4	4.8	3.9	3.6	4.2	4.3
Dorsal gland orifice (DGO)	13.7	15.1	20.1	16.0	15.6	17.2	16.4	20.7	16.3	15.5	13.6	16.2	16.6
Pharynx length	5.2	7.0	7.7	2.6	4.4	4.8	2.6	4.7	5.0	8.0	7.9	6.2	5.7
Median bulb from anterior end	4.8	6.3	7.2	3.7	4.2	5.1	5.6	5.4	4.5	6.2	6.2	5.8	5.5
Median bulb from anterior end/pharynx length (MB)	2.8	3.5	3.8	2.5	4.7	2.8	5.3	3.4	2.7	4.3	5.5	3.4	3.8
Pharyngeal median bulb width	6.2	7.1	8.0	6.2	4.9	7.3	6.9	3.3	5.2	6.4	8.1	6.9	6.3
Pharyngeal basal bulb length	6.8	10.7	-	4.2	8.2	5.8	7.7	4.8	5.7	8.9	-	8.4	7.2
Pharyngeal basal bulb width	6.6	7.7	-	7.8	8.2	5.8	-	-	4.1	-	-	7.4	-
Secretory–excretory pore from anterior end	5.0	6.4	8.0	4.5	4.9	5.5	6.4	4.4	5.1	5.5	7.4	6.2	5.5
Hemizonid from anterior end	4.9	6.0	7.5	4.8	4.8	7.7	6.9	6.3	5.9	8.1	7.3	5.9	7.3
Nerve ring from anterior end	4.6	6.5	7.7	3.6	3.8	8.2	6.7	3.3	7.2	7.6	6.0	5.9	7.2
Anterior end-vulva distance	9.1	7.4	9.6	7.6	5.2	9.7	7.1	4.9	10.7	8.7	9.9	8.4	8.7
Anterior end-anus distance	9.7	8.1	9.9	7.9	5.5	10.2	9.8	7.2	7.8	9.5	10.1	8.9	9.3
Vulva–anus distance	11.6	10.2	11.8	9.0	7.6	10.2	7.9	8.4	9.3	7.7	12.2	10.8	8.6
Tail length	10.9	9.5	12.0	11.4	8.6	7.8	4.4	15.3	8.9	7.5	13.3	10.7	7.9
Tail/vulva–anus distance	10.1	9.4	9.5	13.6	7.3	7.5	4.2	18.6	10.4	7.5	10.5	9.8	8.3
Body width (BW)	8.5	8.1	9.0	9.0	6.4	8.2	6.3	12.6	6.5	9.0	10.9	8.4	8.3
Vulval body width (VBW)	10.0	8.1	9.3	8.6	7.2	9.3	7.0	15.2	8.5	8.9	11.7	9.0	9.1
Anal body width (ABW)	10.9	7.6	11.8	9.4	5.8	10.0	11.1	15.9	11.3	11.8	12.1	9.7	11.5
Phasmid body width (PhBW)	11.8	7.6	13.5	12.0	6.8	8.7	10.3	15.4	8.3	11.2	12.0	10.7	10.4
Vagina length	13.9	8.8	11.4	13.1	9.8	10.0	-	12.0	10.6	-	9.1	11.4	-
Vagina/VBW	13.1	10.7	9.2	7.6	10.3	7.6	-	8.7	9.6	-	56.9	10.7	-
Lateral field width	9.9	11.1	12.2	13.5	12.6	4.9	4.1	3.1	4.7	5.1	14.3	11.3	4.7
Lateral field/BW %	7.6	8.0	9.7	9.4	9.5	8.1	6.1	9.2	9.7	7.2	9.5	8.6	7.8
Lip region width	5.4	3.8	5.2	3.9	3.9	16.5	9.2	8.5	9.5	9.1	5.5	4.6	10.9
Lip region height	9.2	6.5	7.8	6.7	4.6	11.0	13.3	25.3	10.6	13.5	8.0	7.5	13.4
Annuli width	6.9	9.9	10.4	10.5	8.3	17.2	17.3	23.4	20.4	19.8	10.1	9.1	19.2
Tail annuli	10.4	12.0	13.5	12.3	12.5	12.6	14.3	19.0	16.2	16.2	14.4	12.0	15.3
Phasmid from anus	17.5	19.9	14.8	13.0	17.7	8.0	6.6	3.4	7.1	8.0	18.4	17.1	7.3
Phasmid from anus/tail length %	10.4	16.8	9.6	11.1	16.9	7.8	5.9	13.0	6.8	7.5	11.9	12.5	7.6
Hyaline length	9.1	-	-	-	-	-	-	10.5	11.7	12.1	15.3	9.1	11.5
Hyaline length/tail length%	11.2	-	-	-	-	-	-	-	12.8	-	-	11.2	12.8
Stylet length/lip region width	5.2	5.0	6.8	5.5	4.7	6.7	4.8	3.8	5.9	5.2	5.0	5.6	5.5
Pharyngeal basal bulb length/width	8.0	11.8	-	9.1	9.4	-	-	-	-	-	-	10.0	-
Post uterine sac (PUS)	-	-	-	-	-	-	-	-	19.2	-	-	-	19.2
PUS/VBW	-	-	-	-	-	-	-	-	17.4	-	-	-	17.4

TABLE 2. Ranges (Maximum/Minimum) of the morphometric characters of 1,020 measured females of the genera and subfamilies in Merliniidae and Telotylenchidae from Iran.

Character\genus or subfamily	*Amplimerlinius*	*Geocenamus*	*Merlinius*	*Nagelus*	*Paramerlinius*	*Bitylenchus*	*Neodolichorhynchus*	*Paratrophurus*	*Trophurus*	*Tylenchorhynchus*	*Pratylenchoides*	Merliniinae	Telotylenchinae
Number of specimens (*n*)	125	259	203	10	23	64	27	11	39	160	99	620	301
Body length (*L*)	1.39	1.45	1.50	1.29	1.26	1.37	1.23	1.12	1.31	1.36	1.51	1.42	1.32
Body length/width (*a*)	1.31	1.44	1.35	1.18	1.28	1.31	1.21	1.53	1.32	1.33	1.39	1.35	1.32
Body length/pharynx length (*b*)	1.31	1.35	1.35	1.31	1.24	1.33	1.22	1.15	1.28	1.27	1.38	1.33	1.27
Body length/tail length (*c*)	1.40	1.41	1.37	1.42	1.34	1.40	1.26	1.23	1.42	1.38	1.46	1.39	1.36
Tail length/anal body length (*c'*)	1.46	1.59	1.58	1.46	1.48	1.53	1.24	1.36	1.39	1.50	1.55	1.53	1.44
Vulva position/body length % (*V*)	1.09	1.13	1.15	1.06	1.10	1.10	1.06	1.14	1.10	1.08	1.14	1.12	1.09
Stylet length	1.14	1.23	1.27	1.10	1.16	1.19	1.07	1.13	1.18	1.19	1.16	1.20	1.16
Conus length	1.25	1.31	1.55	1.09	1.28	1.26	1.19	1.23	1.34	1.28	1.24	1.34	1.27
Conus length/stylet length % (*m*)	1.14	1.17	1.38	1.12	1.13	1.18	1.16	1.11	1.21	1.14	1.16	1.22	1.16
Dorsal gland orifice (DGO)	1.70	1.90	2.22	1.67	1.83	1.94	1.63	1.80	1.96	1.94	1.70	1.91	1.89
Pharynx length	1.30	1.37	1.39	1.09	1.21	1.20	1.09	1.20	1.23	1.36	1.37	1.32	1.25
Median bulb from anterior end	1.28	1.33	1.33	1.13	1.19	1.24	1.17	1.17	1.18	1.28	1.30	1.29	1.23
Median bulb from anterior end/pharynx length (MB)	1.12	1.21	1.23	1.07	1.23	1.12	1.18	1.13	1.10	1.17	1.24	1.18	1.15
Pharyngeal median bulb width	1.26	1.33	1.44	1.26	1.23	1.29	1.27	1.08	1.26	1.30	1.35	1.33	1.27
Pharyngeal basal bulb length	-	1.58	-	1.11	1.40	1.27	1.23	1.16	1.20	1.39	-	-	1.29
Pharyngeal basal bulb width	-	1.42	-	1.32	1.35	1.20	-	1.00	1.08	-	-	-	-
Secretory–excretory pore from anterior end	1.28	1.35	1.40	1.18	1.23	1.23	1.21	1.13	1.18	1.27	1.31	1.32	1.23
Hemizonid from anterior end	1.23	1.25	1.34	1.19	1.22	1.36	1.23	1.27	1.27	1.35	1.27	1.27	1.32
Nerve ring from anterior end	1.24	1.35	1.37	1.12	1.16	1.37	1.23	1.11	1.32	1.34	1.27	1.29	1.31
Anterior end-vulva distance	1.41	1.42	1.49	1.29	1.24	1.43	1.25	1.18	1.53	1.41	1.50	1.41	1.39
Anterior end-anus distance	1.39	1.45	1.51	1.28	1.26	1.53	1.37	1.30	1.34	1.44	1.52	1.43	1.42
Vulva–anus distance	1.48	1.59	1.63	1.27	1.39	1.43	1.32	1.35	1.51	1.39	1.68	1.53	1.40
Tail length	1.42	1.55	1.69	1.52	1.36	1.36	1.16	1.59	1.48	1.39	1.70	1.54	1.38
Tail/vulva–anus distance	1.48	1.49	1.53	1.50	1.39	1.32	1.21	1.72	1.62	1.36	1.49	1.49	1.40
Body width (BW)	1.48	1.45	1.39	1.31	1.35	1.33	1.22	1.50	1.27	1.42	1.54	1.42	1.35
Vulval body width (VBW)	1.55	1.46	1.45	1.33	1.36	1.38	1.22	1.52	1.37	1.39	1.63	1.47	1.37
Anal body width (ABW)	1.61	1.44	1.63	1.36	1.30	1.45	1.36	1.69	1.56	1.55	1.58	1.53	1.51
Phasmid body width (PhBW)	1.63	1.47	1.75	1.49	1.27	1.43	1.34	1.63	1.31	1.49	1.58	1.59	1.44
Vagina length	1.67	1.46	1.66	1.50	1.45	1.51	-	1.31	1.45	1.45	1.38	1.58	-
Vagina/VBW	1.71	1.67	1.49	1.29	1.50	1.38	-	1.24	1.37	1.34	1.48	1.59	-
Lateral field width	1.40	1.54	1.66	1.53	1.59	1.22	1.13	1.09	1.21	1.21	1.60	1.54	1.19
Lateral field/BW %	1.40	1.42	1.51	1.34	1.43	1.36	1.21	1.27	1.46	1.30	1.50	1.44	1.32
Lip region width	1.20	1.17	1.24	1.15	1.15	1.77	1.33	1.24	1.37	1.39	1.23	1.20	1.45
Lip region height	1.35	1.34	1.36	1.18	1.18	1.57	1.38	2.00	1.47	1.66	1.35	1.33	1.59
Annuli width	1.28	1.58	1.57	1.50	1.35	2.01	1.74	2.32	2.25	2.51	1.38	1.47	2.22
Tail annuli	1.41	1.68	1.68	1.38	1.81	1.58	1.58	1.93	1.93	1.96	1.69	1.59	1.81
Phasmid from anus	1.81	2.34	1.78	1.57	1.93	1.37	1.23	1.12	1.31	1.36	2.04	1.94	1.32
Phasmid from anus/tail length %	1.45	2.06	1.51	1.35	2.03	1.31	1.21	1.53	1.32	1.33	1.67	1.68	1.32
Hyaline length	1.40	-	-	-	-	-	-	1.49	1.56	-	1.87	-	1.53
Hyaline length/tail length %	1.44	-	-	-	-	-	-	-	1.60	-	-	-	1.60
Stylet length/lip region width	1.21	1.25	1.33	1.20	1.17	1.28	1.14	1.10	1.24	1.23	1.22	1.25	1.22
Pharyngeal basal bulb length/width	-	1.61	-	1.38	1.37	-	-	-	-	-	-	-	-
Post uterine sac (PUS)	-	-	-	-	-	-	-	-	1.88	-	-	-	1.88
PUS/VBW	-	-	-	-	-	-	-	-	1.75	-	-	-	1.75

TABLE 3. Ranges (maximum/minimum) of the eight important morphometric characters for females of the genera in Dolichodoridae sensu Geraert, 2011, reported worldwide (literature) or collected during the present study.

Genus\character	S	P	I	Stylet length	Body length (*L*)	Body length/ width (*a*)	Body length/ pharynx length (*b*)	Body length/ tail length (*c*)	Tail length/ anal body length (*c'*)	Vulva position/ body length % (*V*)	Tail annuli
Amplimerlinius	18	47	>420	1.16 (1.04–1.48)	1.32 (1.03–1.70)	1.34 (1.01–1.84)	1.27 (1.07–1.60)	1.35 (1.05–1.84)	1.43 (1.14–1.88)	1.10 (1.02–1.22)	1.39 (1.05–1.95)
Belonolaimus	6	25	1,143	1.41 (1.21–1.77)	1.53 (1.29–1.89)	1.55 (1.25–2.04)	1.54 (1.17–1.94)	1.59 (1.20–1.94)	1.76 (1.38–2.38)	1.17 (1.08–1.34)	-
Brachydorus	3	3	39	1.13 (1.11–1.15)	1.23 (1.17–1.28)	1.22 (1.19–1.26)	1.24 (1.17–1.33)	1.52 (1.24–2.00)	1.17	1.10 (1.04–1.15)	-
Carphodorus	1	1	10	1.07	1.15	1.11	1.10	1.10	1.11	1.03	-
Dolichodorus	16	18	271	1.17 (1.03–1.37)	1.32 (1.08–1.56)	1.32 (1.09–1.53)	1.34 (1.06–1.81)	1.54 (1.09–2.39)	1.41 (1.24–1.70)	1.11 (1.04–1.22)	1.51 (1.28–1.74)
Geocenamus	4	11	110	1.24 (1.05–1.48)	1.40 (1.10–1.83)	1.35 (1.26–1.56)	1.46 (1.31–1.57)	1.41 (1.10–1.62)	1.40 (1.15–1.50)	1.12 (1.02–1.21)	1.36 (1.12–1.60)
Histotylenchus	3	4	67	1.15 (1.09–1.26)	1.21 (1.09–1.30)	1.30 (1.11–1.50)	1.34 (1.12–1.50)	1.43 (1.24–1.65)	1.06	1.18 (1.12–1.26)	1.21
Ibipora	1	1	17	1.20	1.30	1.58	1.45	1.29	1.63	1.15	-
Macrotrophurus	1	1	17	1.29	1.79	1.27	1.62	1.51	-	1.12	-
Meiodorus	1	1	15	1.13	1.22	1.25	1.17	1.27	1.18	1.08	1.22
Merlinius	17	124	696	1.20 (1.07–1.47)	1.37 (1.01–2.01)	1.32 (1.06–1.70)	1.30 (1.04–1.63)	1.33 (1.07–1.77)	1.49 (1.10–2.30)	1.11 (1.04–1.24)	1.58 (1.19–2.31)
Morulaimus	8	8	>150	1.22 (1.17–1.29)	1.31 (1.20–1.40)	1.38 (1.29–1.54)	1.42 (1.19–1.69)	1.46 (1.30–1.84)	1.61 (1.30–1.90)	1.09 (1.00–1.16)	1.58 (1.29–1.75)
Nagelus	6	31	246	1.23 (1.09–1.40)	1.48 (1.23–1.87)	1.44 (1.25–1.76)	1.44 (1.18–2.12)	1.45 (1.27–1.80)	1.68 (1.20–2.36)	1.15 (1.08–1.22)	1.90 (1.25–2.55)
Neodolichodorus	7	7	84	1.08 (1.00–1.14)	1.27 (1.06–1.42)	1.25 (1.08–1.36)	1.29 (1.00–1.70)	1.34 (1.11–1.68)	1.80 (1.60–2.10)	1.11 (1.04–1.19)	1.67
Neodolichorhynchus	6	12	141	1.24 (1.10–1.44)	1.30 (1.18–1.49)	1.37 (1.26–1.61)	1.37 (1.17–1.74)	1.44 (1.21–1.85)	1.46 (1.29–1.75)	1.13 (1.06–1.24)	1.56 (1.08–2.27)
Paramerlinius	9	16	181	1.17 (1.04–1.39)	1.39 (1.13–2.02)	1.30 (1.10–1.42)	1.34 (1.14–1.64)	1.40 (1.07–1.88)	1.42 (1.13–1.61)	1.11 (1.01–1.17)	1.59 (1.18–1.96)
Paratrophurus	12	16	>220	1.16 (1.00–1.44)	1.32 (1.04–2.31)	1.34 (1.03–2.00)	1.28 (1.04–1.72)	1.38 (1.06–1.84)	1.41 (1.04–1.76)	1.10 (1.02–1.26)	1.62 (1.10–2.38)
Pratylenchoides	29	65	>850	1.17 (1.00–1.45)	1.43 (1.05–2.27)	1.41 (1.08–1.91)	1.43 (1.11–1.94)	1.38 (1.06–1.83)	1.54 (1.13–2.10)	1.12 (1.02–1.38)	1.73 (1.04–2.77)
Quinisulcius	4	13	79	1.25 (1.13–1.38)	1.33 (1.17–1.63)	1.40 (1.22–1.65)	1.40 (1.10–2.06)	1.41 (1.30–1.52)	1.59 (1.20–2.13)	1.13 (1.06–1.18)	1.66 (1.13–3.00)
Sauertylenchus	1	1	15	1.14	1.41	1.26	1.59	1.30	1.33	1.12	-
Scutylenchus	14	94	650	1.24 (1.12–1.43)	1.38 (1.11–1.64)	1.36 (1.19–1.80)	1.43 (1.14–1.77)	1.38 (1.12–1.57)	1.58 (1.15–1.88)	1.15 (1.04–1.25)	1.73 (1.27–2.37)
Telotylenchus	3	3	29	1.11 (1.04–1.19)	1.17 (1.04–1.41)	1.14 (1.06–1.31)	1.14 (1.03–1.33)	1.19 (1.07–1.31)	1.13	1.06 (1.02–1.10)	1.14
Trichotylenchus	12	13	118	1.12 (1.00–1.25)	1.21 (1.09–1.32)	1.21 (1.09–1.42)	1.19 (1.00–1.42)	1.22 (1.06–1.42)	1.35 (1.16–1.56)	1.08 (1.02–1.18)	1.90 (1.15–3.77)
Trophurus	6	14	145	1.18 (1.07–1.31)	1.26 (1.06–1.41)	1.33 (1.06–1.68)	1.34 (1.12–1.81)	1.39 (1.13–1.72)	1.50 (1.22–1.71)	1.10 (1.06–1.13)	1.47 (1.32–1.63)
Tylenchorhynchus	39	112	>900	1.16 (1.05–1.42)	1.31 (1.01–1.82)	1.34 (1.03–1.83)	1.31 (1.00–1.68)	1.41 (1.01–2.33)	1.45 (1.04–2.25)	1.10 (1.00–1.31)	1.64 (1.10–3.33)
Mean of averages	227	641	>6,600	1.19	1.34	1.33	1.35	1.38	1.44	1.11	1.55
Range of averages				1.07–1.41	1.15–1.79	1.11–1.58	1.10–1.62	1.10–1.59	1.06–1.76	1.03–1.18	1.14–1.90
Range of extremes				1.00–1.77	1.01–2.31	1.01–2.04	1.00–2.12	1.01–2.39	1.04–2.38	1.00–1.38	1.04–3.33

S = number of the species; P = number of the populations; and I = number of the individuals included in calculations.

TABLE 4. Morphological character states for the Merliniidae and Telotylenchidae genera.

Diagnostic characters	Character states and their code numbers
1) The number of longitudinal incisures in the lateral field at mid-body of females	0: three, 1: four, 2: five, 3: six
2) The number of longitudinal incisures in the lateral field at mid-body of fourth-stage juveniles	0: three, 1: four, 2: five, 3: six
3) The number of longitudinal incisures in the lateral field at deirid level of females	0: without deirid, 1: four, 2: six
4) Longitudinal incisures or striae apart from the lateral field	0: absent, 1: only at pharyngeal region, 2: at whole-body length
5) The areolation of the lateral field	0: indistinct, 1: distinct
6) Cephalic region structure	0: simple, without radial grooves or indentation, 1: divided by radial grooves, 2: with dorsoventral indentation
7) Perioral disc	0: indistinct, 1: distinct
8) Amphidial aperture	0: on labial region, 1: after labial region
9) The conical part of the stylet	0: symmetrical, 1: asymmetrical
10) Stylet type (according to Fortuner and Luc, 1987)	0: normal (20–40 μm), 1: very long (more than 80 μm), 2: attenuated (20 μm or less)
11) Pharyngeal glands position to the intestine	0: offset, 1: slight overlapping, 2: distinct and longer overlapping
12) Female reproductive system	0: didelphic, 1: monodelphic
13) Post-rectal sac	0: absent, 1: present
14) Refractive inner cuticle layer at tail end	0: indistinct, 1: distinct
15) Hyaline layer at tail end	0: normal, 1: thick and distinct
16) Female tail shape	0: pointed, 1: conical, 2: subcylindrical, 3: cylindrical
17) Bursa	0: normal, 1: notched or tri-lobed
18) Sexual dimorphism at anterior end (reduced stylet and pharynx in males)	0: absent, 1: present

The lowest CV was observed for *V*, MB, *m*, stylet length, and stylet length to lip region width. On the other hand, dorsal gland orifice (DGO) and post-uterine sac (PUS) showed the highest CVs. However, this high variation may be partially related to difficulties in their microscopic observability. Some characters including *a*, *b*, *c*, and body length showed a reliable variation and can be used for species discrimination in the studied subfamilies, but the two characters that have been extensively used in the previous taxonomic works, namely *c'* and the number of tail annuli, have lower reliability and thus their use should be limited for taxonomic purposes.

Among the measured characters of the present specimens, vulva position, stylet length, and conus/stylet length (*m*) showed the lowest values of Max/Min ratios. The analysis of the literature data also indicated that *V* and stylet length have the lowest intraspecific variability (mean of the Max/Min ratios are 1.11 and 1.19, respectively), followed by body length, *a*, *b*, and *c*

TABLE 5. Matrix of morphological character states for the Merliniidae and Telotylenchidae genera.

Genus\character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Amplimerlinius	3	3	2	0	0\1	0	0	0	0	0	0	0	0	0	1	3	0	0
Geocenamus	3	1	0	0	0\1	1	1\0	0	0	0\2	0	0	0	1	0	1\2	0	0
Macrotylenchus	3	1	1	0	1	0\1	0\1	0	0	1	0	0	0	0	1	3	0	0
Merlinius	3	1	1\2	0	0\1	1	0\1	0	0	2	0	0	0	1	0	0\1\2	0	0
Nagelus	3	3	2	0	0	0	0	0	0	2	0	0	0	0	1	0\1	0	0
Paramerlinius	3	3	1	0	0\1	0\1	0	0	0	0	0	0	0	1	0	1\2	0	0
Scutylenchus	3	1	0	2	0\1	1	1\0	0	0	0	0	0	0\1	1	0	0\1\2	0	0
Pratylenchoides	1\3	1	1	0	0\1	0	0	0	0	0	0\1\2	0	0	0	1	1\2	0	1
Bitylenchus	1	1	0	0	1	2	1	0	0	2	0\1	0	1	0	1	2\3	0	0
Histotylenchus	1	1	0	0	1	0	0	0	1	0	2	0	1	0	1	2\3	0	0
Neodolichorhynchus	0\1	0\1	0	2	1\0	2	0	0	0	2	0	0	0\1	0	0	1\2	0\1	0
Paratrophurus	1	1	0	0	0	0	0	0	0	2	0\1	0	0	0	1	3	0	0
Quinisulcius	2	2	0	0	0\1	0	0	0	0	2	0	0	0	0	0	1	0	0
Sauertylenchus	1	1	0	0	1	1	1	0	0	0	0	0	1	0	0	2	0	0
Telotylenchoides	1	1	0	0	0	0	0	0	0	0	2	0	0	0	1	3	0	0
Telotylenchus	1	1	0	0	0\1	2	0	0	0	2	1\2	0	0	0	0	1\2	0	0
Trichotylenchus	0	0	0	0	1	0	0	0	0	2	2	0	0	0	0	3	0	0
Trophurus	1	1	0	0	0	0	0	0	0	2	0\1	1	1\0	0	1	2\3	0	0
Tylenchorhynchus	0	1	0	0\1	1\0	0	0	0	0	2	0\1	0	0\1	0	0\1	0\1\2\3	0	0
Uliginotylenchus	1	1	0	0	1	0	0	0	0	2	1	0	0	0	0	2\3	0	0
Macrotrophurus	1	1	0	0	0	0	0	1	0	1	0	0	0	0	1	3	0	0

TABLE 6. CAN scores of the diagnostic characters used for identification of species in the genera *Amplimerlinius* and *Pratylenchoides*, as well as for identification of genera in Merliniidae and Telotylenchidae (those with higher loads than 0.4 highlighted in bold).

	Amplimerlinius			*Pratylenchoides*			Genera		
Character\genus	CAN1	CAN2	CAN3	CAN1	CAN2	CAN3	CAN1	CAN2	CAN3
Body length (*L*)	0.068	**−0.461**	−0.233	**−0.485**	−0.044	**0.612**	**0.493**	−0.082	0.036
Body length/width (*a*)	−0.304	**0.466**	**0.553**	−0.073	−0.123	**0.427**	−0.045	−0.041	−0.034
Body length/pharynx length (*b*)	−0.002	0.002	−0.027	−0.114	**−0.754**	−0.065	−0.021	0.107	0.009
Body length/tail length (*c*)	−0.099	−0.228	0.025	0.039	−0.389	−0.310	−0.027	**−0.456**	**−1.199**
Tail length/anal body length (*c'*)	−0.066	−0.062	−0.001	0.008	−0.223	**−0.640**	−0.052	−0.002	0.032
Vulva position/body length % (*V*)	0.036	−0.115	0.083	0.011	**0.454**	**−0.597**	−0.063	0.110	−0.145
Stylet length	**0.916**	0.139	0.063	**0.405**	**0.558**	**0.629**	0.246	−0.025	0.334
Conus length	0.201	−0.062	0.155	0.132	0.189	**0.534**	0.294	−0.240	−0.004
Conus length/stylet length % (*m*)	**−0.952**	**−0.572**	**0.475**	**−0.544**	−0.210	0.093	**−0.428**	0.277	**0.473**
Dorsal gland orifice (DGO)	**−0.408**	**0.662**	**0.589**	−0.311	**0.743**	**−0.409**	0.195	−0.219	−0.198
Pharynx length	0.140	0.082	−0.024	0.031	**−0.437**	0.268	0.350	0.023	−0.015
Median bulb from anterior end	0.352	**0.489**	**0.498**	**0.435**	**−0.504**	**0.769**	0.313	**0.563**	−0.395
Median bulb from anterior end/pharynx length (MB)	0.092	0.320	0.300	0.377	−0.104	**0.663**	0.386	−0.155	**−0.409**
Median bulb width	**0.765**	**−0.575**	−0.188	**−0.431**	0.051	0.323	0.247	−0.392	**0.504**
Secretory–excretory pore from anterior end	0.347	**−0.426**	0.109	**0.467**	**0.631**	**0.503**	0.171	−0.059	0.377
Nerve ring from anterior end	**0.916**	−0.157	**0.583**	0.249	0.027	**0.750**	−0.030	−0.118	−0.094
Anterior end-vulva	0.249	−0.177	0.262	−0.263	−0.057	0.097	**0.477**	0.372	−0.147
Anterior end-anus	**−0.457**	**−0.696**	−0.135	**−0.493**	−0.017	0.157	**0.582**	0.240	0.046
Vulva–anus distance	**−0.707**	**−0.519**	−0.397	−0.230	0.040	0.060	0.105	−0.132	0.192
Tail length	**0.525**	0.235	−0.098	0.009	−0.027	**0.455**	−0.048	−0.119	**0.464**
Body width	0.189	0.128	**−0.714**	0.009	**−0.475**	−0.256	0.086	−0.214	−0.145
Vulval body width	**0.433**	**−0.555**	**0.545**	**0.538**	0.035	−0.201	−0.211	0.161	0.080
Anal body width	**0.583**	**1.155**	−0.122	−0.072	0.126	−0.206	0.042	**0.445**	0.310
Phasmid body width	**−0.503**	**0.404**	−0.239	0.236	0.018	−0.060	0.284	**0.447**	**−0.643**
Lateral field width	-[a]	-	-	0.266	**1.064**	**−0.495**	-	-	-
Lateral field/BW %	-	-	-	0.099	−0.277	−0.316	-	-	-
lip region Width	**0.447**	**−0.423**	**−0.507**	**0.818**	**−0.565**	0.130	0.238	−0.010	**0.747**
lip region height	**0.419**	−0.331	**−0.506**	0.001	0.242	0.165	−0.078	0.210	−0.121
Annulus width	-	-	-	−0.083	**−0.473**	−0.279	-	-	-
Tail annuli	-	-	-	**1.227**	**0.995**	−0.144	-	-	-
Phasmid from anus	0.208	**−0.419**	−0.183	0.093	**−0.459**	0.186	−0.112	0.263	0.384
Phasmid from anus/tail length %	−0.357	**0.640**	**−0.604**	0.394	**−0.979**	**0.499**	−0.074	**0.718**	0.132
Stylet length/tail length (%)	**1.353**	**−0.641**	0.321	**−1.607**	0.315	−0.260	**0.532**	**−0.616**	**0.451**
Stylet length/lip region width	−0.032	0.132	0.154	**−0.485**	−0.044	**0.612**	0.001	−0.294	−0.149
Tail annuli/tail length	0.208	**−0.419**	−0.183	−0.073	−0.123	**0.427**	−0.184	**1.180**	0.202

[a] Not measured.

ratios (mean of the ratios 1.34, 1.33, 1.35, 1.38, respectively), but *c'* ratio and tail annuli showed higher levels of intraspecific variability (mean of the Max/Min ratios are 1.44 and 1.55, respectively).

Correlation and regression analysis among characters: The details of correlation among diagnostic characters were not shown here, but some remarkable notes are discussed. The data would seem to suggest that body length (and other relative characters such as anterior end-vulva distance, anterior end-anus distance, and vulva–anus distance) has a close relationship with stylet length, pharynx length, the position of secretory–excretory pore, and with body diameter at different parts of the nematode. Also, strong positive correlations were found between stylet length with the distance of median bulb from the anterior end (r = 0.823), stylet length with median bulb width (r = 0.868), as well as MB with median bulb width (r = 0.885). Also as can be seen from the regression lines (Fig. 2), it may be concluded that there is a typical close relationship between almost all studied characters, except for body length with tail length (R^2 = 0.506), and tail length with the number of tail annuli (R^2 = 0.129), which showed poor or very poor correlation, respectively.

Clustering of the taxa: The used characters and matrix of the morphological characters are shown in Tables 4 and 5, respectively. HCA of the selected diagnostic characters of the genera in Merliniidae and Telotylenchidae by using average linkage method yielded a dendrogram (Fig. 3) that shows several significant agreements with morphological and molecular findings.

The results of our HCA well supported the separation of the genera included in Telotylenchidae from the genera in Merliniidae. The genus *Bitylenchus* is supported as a sister taxon to the genus *Sauertylenchus* Sher, 1974, and separate from *Tylenchorhynchus*. The genus *Neodolichorhynchus* was located at the base of the Telotylenchidae cluster. The genera *Trophurus* Loof, 1956, *Paratrophurus* Arias, 1970, and *Macrotrophurus* Loof, 1958 (with thick cuticle at tail end) showed close

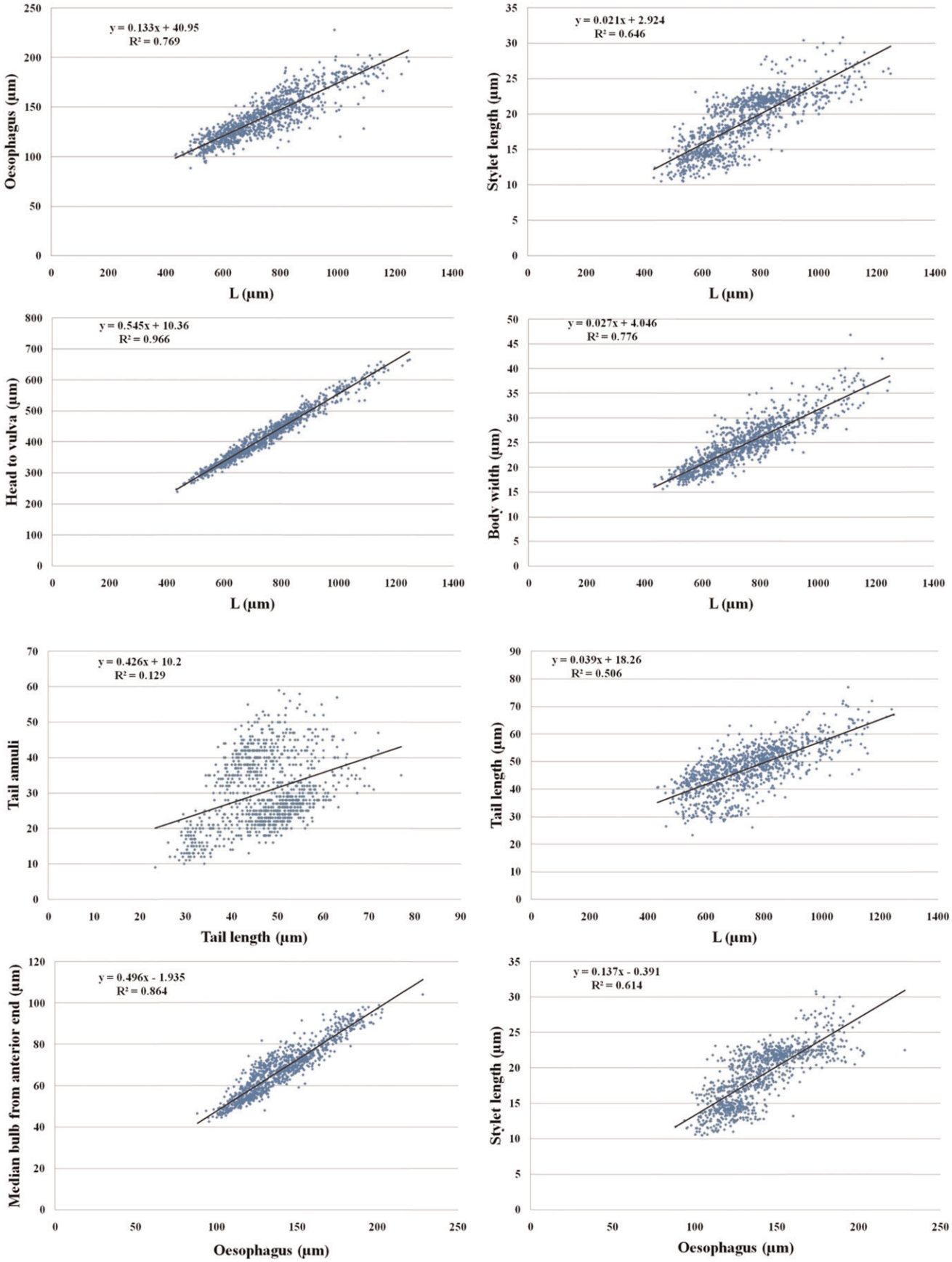

FIG. 2. Relationship between several important characters in the families Merliniidae and Telotylenchidae. Each of the dots scattered around the correlation line is representative of one of the 1,020 measured females in the present study.

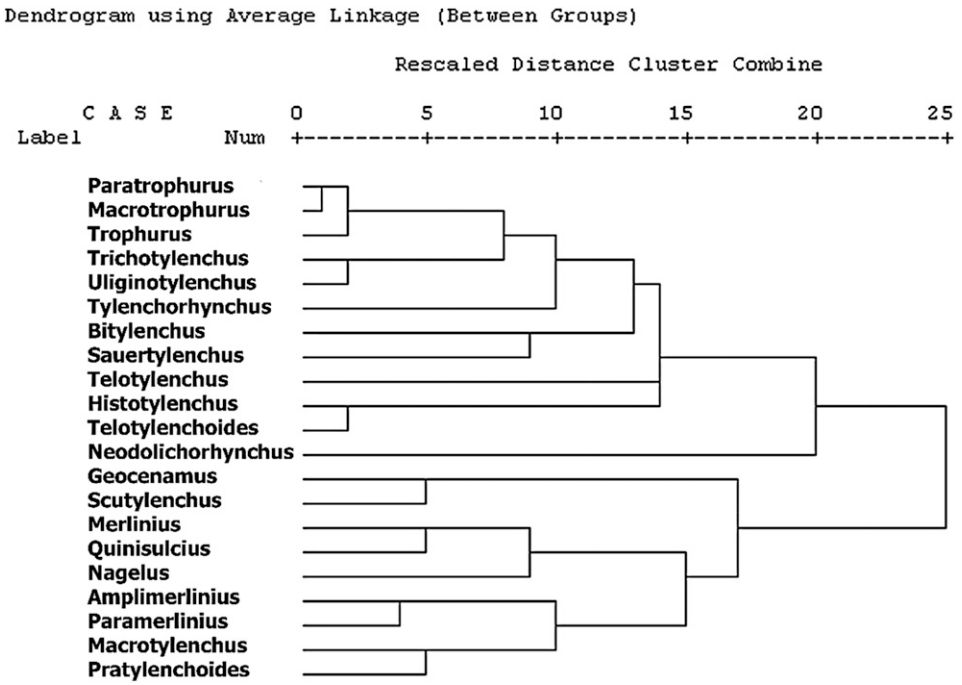

FIG. 3. Grouping of the genera in Merliniidae and Telotylenchidae based on the 18 coded character states, extracted from the literature. Dendrogram was generated based on hierarchical cluster analysis of characters. The scale represents the Euclidean distance in canonical space.

relationships with each other. In the subfamily Merliniinae from Merliniidae, *Scutylenchus* Jairajpuri, 1971, and *Geocenamus* (the only genera without deirids) formed a separate and distinct cluster from other genera including *Merlinius*. The genera *Nagelus* Thorne and Malek, 1968 and *Merlinius* were clustered together and separated from another cluster including *Paramerlinius* Sturhan, 2012, and *Amplimerlinius*. The genera *Macrotylenchus* Sturhan, 2012 and *Pratylenchoides* formed a cluster at the base of the dendrogram.

Furthermore, HCA was used for clustering the populations collected during the present study and was successful in separating species of *Amplimerlinius* (Fig. 4) and *Pratylenchoides* (Fig. 5), but not for those of *Geocenamus* and *Merlinius* (data are not shown). In the genus *Amplimerlinius*, species with different ranges of the stylet length were separated from each other, so *A. macrurus* (Goodey, 1932) Siddiqi, 1976, and *A. uramanatiensis* Ghaderi and Karegar, 2014 (species with 25–31 µm stylet length) comprised the basal cluster and *A. globigerus* Siddiqi, 1979, and *A. paraglobigerus* Castillo, Siddiqi, and Gómez-Barcina, 1990 (species with 20–25 µm stylet length) formed the other cluster. In the genus *Pratylenchoides* (Fig. 5), *P. crenicauda* Winslow, 1958, and *P. erzurumensis* Yüksel, 1977 (species with shorter overlapping of the pharyngeal glands) formed a cluster distinct from *P. ritteri* Sher, 1970 and *P. utahensis* Baldwin, Luc, and Bell, 1983 (species with longer overlapping of the pharyngeal glands).

STEPDISC analysis of the taxa: To sort morphometric characters relating to genera (in Merliniidae and Telotylenchidae) and species (in *Amplimerlinius*, *Geocenamus*, *Merlinius* and *Pratylenchoides*), and to identify their possible natural grouping based on new reduced dimension orthogonal space, the 35 morphometric characters common to all populations (see Table 6) were subjected to PCA. The female individuals of the included species (and/or genera) were plotted in a reduced space consisting of the first three PC accounted for virtually all (PC1 = 39%; PC2 = 36%; PC3 = 25%) the variance in the original morphometric characters with significant PC loadings (data not shown). PC scores

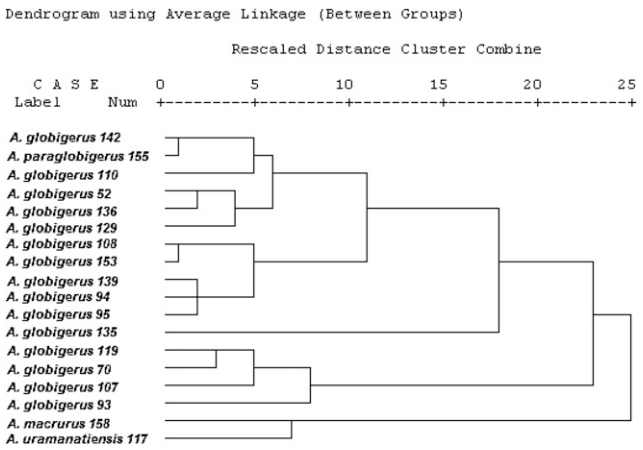

FIG. 4. Grouping of the populations of four species of *Amplimerlinius* based on morphometric characters. Dendrogram was generated based on hierarchical cluster analysis of characters. The scale represents the Euclidean distance in canonical space. Numbers after the species names are sample codes.

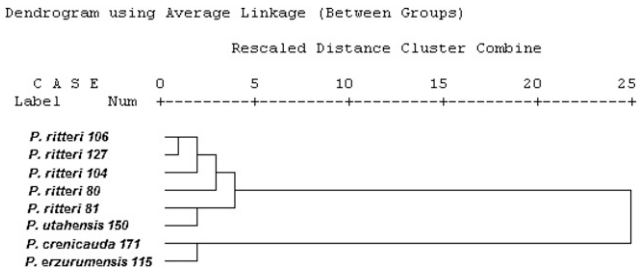

FIG. 5. Grouping of the populations of four species of *Pratylenchoides* based on morphometric characters. Dendrogram was generated based on hierarchical cluster analysis of characters. The scale represents the Euclidean distance in canonical space. Numbers after the species names are sample codes.

measure similarities among species (and/or genera), whereas the PC loadings represent a measure of the contribution of morphometric characters in PC scores and may be used to identify key characters. A 3D scatter plot (PC1 × PC2 × PC3) of taxon PC scores indicated a clustering of the taxons. CDA was applied to a significant subset of 35 PC selected by running STEPDISC procedure of SAS. The first two CAN vectors accounted for ≥98% of the variance in the 35 morphometric characters (CAN1 = 85% and CAN2 = 13%). Each CAN vector is an indirect linear combination of 35 morphometric characters and summarizes part of the variance observed in the taxa (species/genera).

The CAN1 vector grouped the four species of *Amplimerlinius* into two separate clusters: group1 (=*A. macrurus* and *A. uramanatiensis*) with high CAN1 scores and group2 (=*A. globigerus* and *A. paraglobigerus*) with low CAN1 scores. Therefore, the CAN1 vector was considered to explain the differences between group1 and group2 (Fig. 6). The CAN2 and CAN3 vectors, on the other hand, further classified group1 and group2 (separating subgroups *A. macrurus* from *A. uramanatiensis*, and *A. globigerus* from *A. paraglobigerus*) by assigning higher CAN2 (and CAN3) scores to *A. macrurus* and *A. globigerus*. As the 2D CAN space provided sufficient separation of the four species of *Amplimerlinius*, the morphometric characters with high loadings to the CAN1 and CAN2 vectors that identify different taxa were ranked in a descending order of loadings. Of 31 characters, 14 and 17 loaded significantly ($L > 0.4$) to the CAN1 and CAN2 vectors, respectively, with 10 characters (*m*, DGO, median bulb width, anterior end-anus, vulva–anus distance, vulval body width, anal body width, phasmid body width, lip region width, and stylet length/tail length %) being common to both (Table 6).

Four species of *Pratylenchoides* can also be well separated in 2D canonical space of CAN1 × CAN2. CAN1 vector identified two clusters labeled as group1 and group2; group1 (=*P. crenicauda* and *P. erzurumensis*) with the highest CAN1 scores and group 2 (*P. ritteri* and *P. utahensis*) with moderate (*P. ritteri*) to low (*P. utahensis*) CAN1 scores. CAN2 vector further classified group1 into *P. crenicauda* with low CAN2 scores and

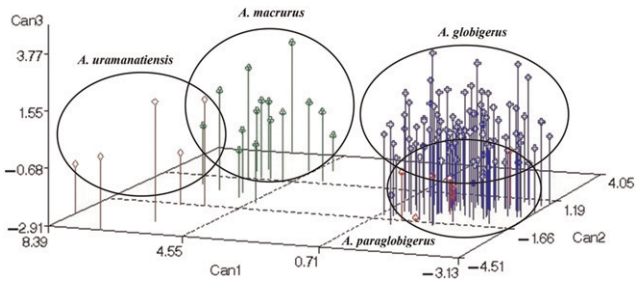

FIG. 6. Scatter plot based on projections of the first three significant CAN vectors of canonical discriminant analysis (CDA) of morphometric characters of 125 female individuals of four species in *Amplimerlinius*. The 3D canonical space was derived from CANDISC analysis of PC outputted from principal component analysis of 31 morphometric characters. The CDA identified the species as three groups (*A. uramanatiensis*, *A. macrurus*, and *A. globigerus* + *A. paraglobigerus*) across CAN1 and CAN2 vectors in the CAN1-CAN2 plane and CAN3 partially separated *A. globigerus* and *A. paraglobigerus* from each other.

P. erzurumensis with high CAN2 scores (Fig. 7). Morphometric characters with high loadings to the CAN1 and CAN2 vectors that identified different species of *Pratylenchoides* were ranked in a descending order of loadings. Out of 35 characters, 12 and 14 loaded significantly ($L > 0.4$) to the CAN1 and CAN2 vectors, respectively, with 5 characters (stylet length, median bulb from anterior end, secretory–excretory pore from anterior end, tail annuli, and phasmid from anus/tail length %) being common to both (Table 6).

Morphological relationships among 1,007 female individuals (belonging to 190 populations of 31 species and 11 genera) in the Merliniidae and Telotylenchidae were investigated in the 2D canonical space. Eleven genera of the two families were well separated in 2D canonical space of CAN1 × CAN2 and formed six genera groups as follows: G1 (*Merlinius*), G2 (*Bitylenchus*), G3 (*Tylenchorhynchus* and *Paratrophurus*), G4 (a disperse pattern of *Trophurus*, *Neodolichorhynchus*, *Geocenamus* and *Nagelus*), G5 (*Pratylenchoides*), and G6

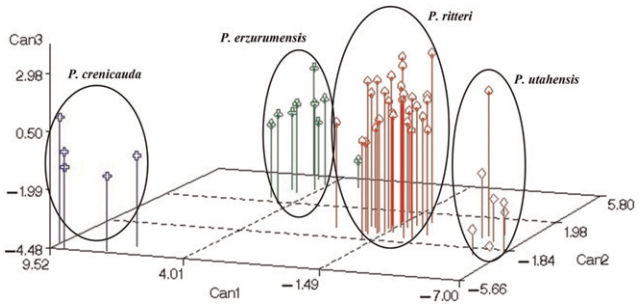

FIG. 7. Scatter plot based on projections of the first three significant CAN vectors of canonical discriminant analysis (CDA) of morphometric characters of 125 female individuals of four species in *Pratylenchoides*. The 3D canonical space was derived from CANDISC analysis of PC outputted from principal component analysis of 35 morphometric characters. The CDA identified the species as four distinct groups, especially across CAN1 and CAN2 vectors in the CAN1-CAN2 plane.

(*Paramerlinius* and *Amplimerlinius*). CAN1 vector identified three clusters labeled as G1, G2+G3, and G4+G5+G6 with high, moderate, and low CAN1 scores, respectively. CAN2 vector further classified G2 from G3 and also G6 from G4 and G5, with high CAN2 scores for the formers (Fig. 8). Further separation of these six groups was not possible, which indicates intermediate measures of genera separators due to possible close evolutionary relationship. The morphometric characters with high loadings to the CAN1 and CAN2 vectors that identify different groups of genera in the Merliniidae and Telotylenchidae were ranked in a descending order of loadings. Out of 31 characters, 5 and 7 loaded significantly ($L > 0.4$) to the CAN1 and CAN2 vectors, respectively, with only one character (stylet length/tail length %) being common to both (Table 6).

Discussion

Variability of morphometric characters: Considering CV and Max/Min ratio as viability criteria, the results of the present study revealed that certain morphometric characters such as vulva position, stylet length, *m*, *a*, *b*, and *c* ratios have acceptable reliability for taxonomic works, but some others including *c'* and the number of tail annuli have lower reliability and thus their using for taxonomic purposes should be limited. The ranges of variation can sometimes proceed to more than three in the number of tail annuli in the same species or even same population. However, in these cases, cuticular annuli on the ventral side of tail are usually compounded with each other to produce larger and wider annuli. This typically occurs in *Tylenchorhynchus clarus* Allen, 1955, which has 12 to 20 annuli on the ventral surface of tail, but their numbers reduce to 6 to 10 annuli after combination. For this reason, *T. variannus* Mavlyanov, 1978 was synonymized with *T. clarus* by Ghaderi and Karegar (2016). Fortuner (1984) noted that in the genus *Helicotylenchus* Siddiqi, 1971, certain characters such as vulva position, *a*, *c*, and *c'* ratios have reliable intraspecific variation, but *b* and *b'* ratios show higher levels of variation. He also discussed that the variability of *c* ratio is generally higher than the variability of tail length and thus, it is best not to use *c* ratio, but to use the actual length of the tail. The results of the present study indicated that tail length may be considered as a more stable character than *c* and *c'* ratios in the most genera of Telotylenchidae, but it has higher variability than *c* and *c'* ratios in *Trophurus*, *Paratrophurus*, and the genera of Merliniidae (Tables 1–3).

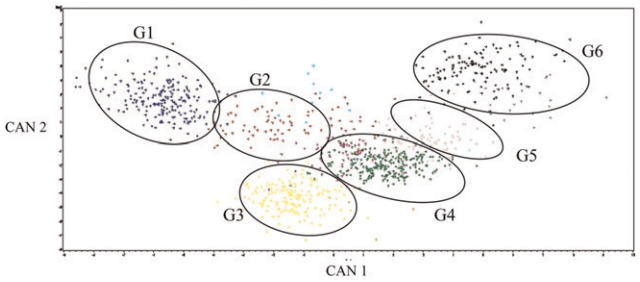

Fig. 8. Scatter plot based on projections of the first two significant CAN vectors of canonical discriminant analysis (CDA) of morphometric characters of 1,007 female individuals belonging to 190 populations of 31 species in 11 genera. The 3D canonical space was derived from CANDISC analysis of PC outputted from principal component analysis of 31 morphometric characters. The CDA identified the genera as six groups: *Merlinius* (G1), *Bitylenchus* (G2), *Tylenchorhynchus* and *Paratrophurus* (G3), *Trophurus*, *Neodolichorhynchus*, *Geocenamus* and *Nagelus* (G4), *Pratylenchoides* (G5), and *Paramerlinius* and *Amplimerlinius* (G6).

Relationships between characters: Analysis of the correlation among diagnostic characters revealed that although there was a close relationship between the studied characters, body length with tail length and tail length with the number of tail annuli showed very poor correlation. In these two sets of characters, the regression line did not pass through the origin, and therefore they can be used in taxonomic works by precaution. Fortuner (1982), with studying populations of *Ditylenchus myceliophagus* Goodey, 1958, discussed that the regression line almost passes through the origin in the regression lines of the two constituents of *a*, *c*, and *c'* ratios, but that is not true for *b* ratio. Fortuner (1984) found that in the genus *Helicotylenchus* Steiner, 1945, the pairs of characters for *a*, *c*, *c'*, and *V* ratios present a high correlation with each other, but other ratios (e.g., *b*, *b'*, and *o*) are based on unrelated pair of characters. Fortuner and Maggenti (1991) found that most characters describing the size of the nematodes in *Hirschmanniella oryzae* (Soltwedel, 1889) Luc and Goodey, 1963 and *H. belli* Sher, 1968 (including *a*, *b*, *c*, and *c'* ratios, and also lengths of body, tail, stylet, and pharynx), as well as number of tail annuli, are highly correlated to each other. Geraert (2006) discussed on the correlation between some selected characters in the representatives of Tylenchida, including Merliniidae and Telotylenchidae. He noted that there is a good relationship between body length and body diameter, body length and pharynx length, and body length and stylet length, with their related regression lines passing through the origin in the genera of Merliniidae and Telotylenchidae. He further noted that regarding correlation between body length and pharynx length, Telotylenchidae and Tylenchidae Örley, 1880 have different regression lines, although certain genera in Telotylenchidae (e.g., *Macrotrophurus* and *Sauertylenchus*) fall within the extension of the Tylenchidae regression line. However, all studied genera had a Telotylenchidae–stylet relationship (Geraert, 2006), suggesting that a constant ratio can be found between body length and stylet length.

Validity of taxa grouping based on multivariate clustering: The results of our HCA were comparable to recent phylogenetic analyses (Ghaderi et al., 2014) and well supported the separation of the genera included in

Telotylenchidae from the genera in Merliniidae; only the position of the genus *Quinisulcius* Siddiqi, 1971, in Merliniidae is questionable. Our results also rejected the concept of the "large genus idea" for *Tylenchorhynchus* as proposed by Fortuner and Luc (1987), which is in agreement with the results obtained from our phylogenetic relationships (Ghaderi et al., 2014). Several genera including *Bitylenchus*, *Telotylenchus*, *Quinisulcius*, and *Neodolichorhynchus* have been considered as synonyms of *Tylenchorhynchus* by Fortuner and Luc (1987), but our findings showed that these genera formed clusters distinct from *Tylenchorhynchus*.

In our HCA dendrogram, the genus *Bitylenchus* is supported as a sister taxon to the genus *Sauertylenchus*, and separated from *Tylenchorhynchus*. Fortuner and Luc (1987) transferred *Sauertylenchus* to the subfamily Merliniinae because of having a distinct perioral disc and long stylet, but Gomez-Barcina et al. (1992) and Siddiqi (2000) claimed that this genus is very close to *Bitylenchus*, though with minor but reliable differences in the lip region structure and stylet length. The genus *Neodolichorhynchus*, with cuticular longitudinal ridges on its whole body as a unique diagnostic character, was located at the base of the Telotylenchidae cluster in our study. Similar results were obtained in our recent phylogenetic studies (Ghaderi et al., 2014), but the genus also showed a sister relationship with *Trophurus* and *Macrotrophurus*.

The genera with thick cuticle at tail end (*Trophurus*, *Paratrophurus*, and *Macrotrophurus*) showed close relationships with the each other. There is no molecular information on *Paratrophurus*, but the phylogenetic position of *Trophurus* and *Macrotrophurus* (Ghaderi et al., 2014) is supported here by numerical taxonomy approach. However, the representatives of the thick-tailed genera including *Trophurus*, *Macrotrophurus*, and *Paratrophurus* did not group together in the SSU rDNA tree in Carta et al. (2010).

In the subfamily Merliniinae from Merliniidae, the only genera having no deirids (*Scutylenchus* and *Geocenamus*) formed a separate and distinct cluster from other genera including *Merlinius*. Anderson (1977) and Sturhan (2012) considered *Scutylenchus* as a junior synonym of *Merlinius* or *Geocenamus*, respectively. Siddiqi (1979, 2000) revalidated *Scutylenchus* as a genus with having longitudinal striae on the body cuticle and lacking deirids. Decraemer and Hunt (2006, 2013) and Geraert (2011) considered all the three genera as *Geocenamus*. The results of our study were congruent more with the opinion of Sturhan (2012) for considering *Merlinius* as a separate genus, but support the synonymy of *Scutylenchus* with *Geocenamus*.

The genus *Paramerlinius* showed a sister relationship with *Amplimerlinius*, but was located in a different cluster from other related genera including *Nagelus* and *Merlinius*. Based on a detailed comprehensive study (Sturhan, 2012), *Paramerlinius* was proposed for accommodating several species with a heavy sclerotized cephalic framework, previously attributed to *Merlinius* and *Nagelus*. Morphologically, *Paramerlinius* is also very close to *Amplimerlinius*, but it differs by the presence of a refractive inner cuticle layer and the absence of a distinct hyaline at female tail end. The results obtained from our cluster analysis are in agreement with classification schemes based on morphological (Sturhan, 2012) and molecular (Ghaderi et al., 2014) information.

The genera *Macrotylenchus* and *Pratylenchoides*, which formed a cluster at the base of the dendrogram (Fig. 3), have several morphological affinities with each other. Only the length of stylet is much larger in the first genus (higher than 100 *vs* less than 35 μm). Such close relationship can also be observed between other genera with different length of stylets in the HCA dendrogram (Fig. 3). For example, the genera with short stylet, *Bitylenchus* and *Paratrophurus*, clustered with the genera with longer stylet, *Sauertylenchus* and *Macrotrophurus*. From this result, it may be concluded that stylet length may not be an adequate discriminant character at the genus rank.

The studied species of *Amplimerlinius* and *Pratylenchoides* can be distinguished from the each other in accordance with important diagnostic characters mentioned as authentic taxonomic characters (Ryss, 2007; Geraert, 2011, 2013). In the genus *Amplimerlinius*, species with 25 to3 1 μm stylet length (including *A. macrurus* and *A. uramanatiensis*) formed a basal cluster distinct from other clusters consisting of the species with 20 to 25 μm stylet length (including *A. globigerus* and *A. paraglobigerus*). This method was not successful for separation of the two very closely related species of *Amplimerlinius,* i.e., *A. globigerus* and *A. paraglobigerus*, because these species can be distinguished only based on some few morphological differences in the number of head annuli, shape of cephalic framework, and body posture.

HCA may be considered as a useful tool for discrimination of the different groups of *Pratylenchoides* species, which have been defined in previously published taxonomic works (Baldwin et al., 1983; Ryss, 2007; Ghaderi and Karegar, 2014a). *P. crenicauda* and *P. erzurumensis* (group 2 of Ghaderi and Karegar [2014a]; with pharyngeal glands overlapping about one time of the corresponding body diameter) formed a cluster distinct from *P. ritteri* and *P. utahensis* (group 3 of Ghaderi and Karegar [2014a]; with pharyngeal glands overlapping about two to three times of the corresponding body diameter) (Fig. 5). The species of *Geocenamus* and *Merlinius* cannot be identified by HCA, but interestingly this approach was useful for detection of the mixed populations of *M. brevidens* (Allen, 1955) Siddiqi, 1970, and *M. nanus* (Allen, 1955) Siddiqi, 1970. The first species has a larger stylet range (13–16 μm *vs* 11–14 μm), a more developed basal ring of its

cephalic framework and smooth (*vs* striated) tail terminus in females.

Generally speaking, HCA can be successfully used together with morphological and molecular methods for more accurate identification of different populations of the plant-parasitic nematodes of the families Merliniidae and Telotylenchidae at genus or species rank. However, obtaining the reliable results requires the most important and stable diagnostic characters to be selected, defined, and then coded in an appropriate way. HCA has already been used as a supplementary tool for identification of nematodes in several genera including *Helicotylenchus* (Fortuner et al., 1984), *Xiphinema* (Lamberti and Ciancio, 1993), *Longidorus* (Ye and Robbins, 2004), and *Criconemoides* (Chenari Bouket, 2013).

Value of STEPDISC analysis: A STEPDISC analysis was carried out to investigate relationships between 35 morphometric characters relating to genera in Merliniidae and Telotylenchidae, and species in *Amplimerlinius*, *Geocenamus*, *Merlinius*, and *Pratylenchoides*. However, this was unsuccessful for grouping species in *Geocenamus* and *Merlinius*.

The CAN1 vector distinguished the two groups in *Amplimerlinius*, and the CAN2 and CAN3 vectors further separated subgroups *A. macrurus* from *A. uramanatiensis*, and *A. globigerus* from *A. paraglobigerus*. According to the already published morphological studies (Geraert, 2011; Ghaderi and Karegar, 2014b), the group1 species, *A. macrurus* and *A. uramanatiensis*, can be distinguished from the group2 species, *A. globigerus* and *A. paraglobigerus*, by having a larger stylet in females and males. Stylet length, *m*, and stylet length/tail length % have higher CAN1 scores in our multivariate analysis, but the two latter characters showed large amount of intraspecific variation and cannot be used for reliable species identification in this genus. Certain characters such as DGO, anterior end-anus distance, phasmid from anus/tail length %, and body width had higher loading values on CAN2 and CAN3 vectors (Table 6), but using these characters for taxonomic purposes needs to be studied in a more comprehensive survey including more populations from different geographical locations. In the taxonomic literature (Geraert, 2011; Ghaderi and Karegar, 2014b), the separation of the species in each subgroup 1 or 2 is usually based on qualitative characters (e.g., the number of head annuli, the shape of cephalic framework, tail shape, tail terminus striation), and morphometric data have a little impact.

In the genus *Pratylenchoides*, CAN1 vector identified two clusters labeled as group1 (*P. crenicauda* and *P. erzurumensis*) and group2 (*P. ritteri* and *P. utahensis*). According to the taxonomic literature on the genus (Ryss, 2007; Geraert, 2013; Ghaderi and Karegar, 2014a), the species in group1 can be separated from the species in group2 by more anterior position of the subventral pharyngeal gland nuclei (at least one of the nuclei is anterior to or near the pharyngo-intestinal valve *vs* both nuclei posterior to the valve) and shorter overlapping of the glands on the intestine (0.5–1.5 *vs* 1.5–3.0 times the corresponding body diameter). The characters with higher CAN1 scores (the number of tail annuli, tail length/stylet length %. and lip region width) have not been considered as reliable diagnostic characters in taxonomy of the genus. Although CAN1 vector was sufficient to distinguish between group1 and group2, CAN2 vector was also necessary to further separate *P. crenicauda* and *P. erzurumensis* from each other. From the characters with higher loadings on CAN2 vector, *b* ratio, the number of tail annuli, and phasmid from anus/tail length % can be considered as important diagnostic characters from the taxonomic point of view. These two species can be further separated by morphology of their tail termini, shape of sperm cells, and male head (Geraert, 2013; Ghaderi and Karegar, 2014a).

The results of our STEPDISC analysis did not support the separation of the genera included in Telotylenchidae from the genera in Merliniidae, because these genera are primarily separated due to differences in qualitative characters (as shown in our HCA dendrogram; Fig. 3), and do not rely on quantitative morphometric data. Individuals belong to the species of *Bitylenchus* and *Neodolichorhynchus* grouped in clusters (G2 and G4, respectively) distinct from the other cluster (G3) including *Tylenchorhynchus* species; this is in accordance with our HCA analysis and can be considered as additional evidence for rejection of the concept of the "large genus idea" for *Tylenchorhynchus*. The representatives of the genus *Neodolichorhynchus* showed a close relationship with those of *Trophurus* in our STEPDISC analysis and support the sister relationship of these two genera in phylogenetic relationships (Ghaderi et al., 2014). Furthermore, *Tylenchorhynchus* and *Paratrophurus* shared the same cluster (G3) in our study. Arias (1970) characterized *Paratrophurus* by smooth, slender lip region, and distinct thickening of the tail terminus. Lopez (1986) and Castillo et al. (1989) considered only the latter as a reliable diagnostic character, and Siddiqi (2000) noted that regarding all morphological characters, including en face view, *Paratrophurus* is similar to *Tylenchorhynchus*.

Taking the family Merliniidae into consideration, the genera *Nagelus* and *Geocenamus* (together with *Trophurus* and *Neodolichorhynchus*) formed a diverse group (G4) but in a separate cluster from the other group (G6) including closely related genera, *Paramerlinius* and *Amplimerlinius*, which is in agreement with our HCA analysis, and with classification schemes based on morphological (Sturhan, 2012) and molecular (Ghaderi et al., 2014) information. The representatives of the genus *Geocenamus* with tessellated cuticle (formerly known as *Scutylenchus*) formed a separate and distinct cluster from *Merlinius*, in congruence with HCA and Sturhan (2012), for considering *Merlinius* as a separate genus from

Geocenamus. The genus *Pratylenchoides* formed a cluster distinct from the studied genera in Merliniinae, including *Amplimerlinius*, *Paramerlinius*, *Geocenamus*, and *Nagelus*. Our findings support the monophyletic nature of *Pratylenchoides*, as well discussed in Azizi et al. (2016). However, possible affinities of the genus should be discussed with *Macrotylenchus* in future morphological or molecular studies.

Conclusions

Generally speaking, the results of the present study revealed that certain morphometric characters such as vulva position, stylet length, *m*, *a*, *b*, and *c* ratios have acceptable reliability for taxonomic works in the families Merliniidae and Telotylenchidae, but using of certain characters including *c'* ratio and the number of tail annuli for taxonomic purposes should be limited in these families. Analysis of the correlation among diagnostic characters also revealed a close relationship between the pairs of the studied characters, but few of them namely body length with tail length, and tail length with the number of tail annuli showed very poor correlation.

Multivariate analyses, HCA and STEPDISC, rejected the concept of the "large genus idea" for *Tylenchorhynchus* and considered *Bitylenchus* and *Neodolichorhynchus* as separate genera from *Tylenchorhynchus*. Furthermore, these approaches considered *Merlinius* as a separate genus from *Scutylenchus* and *Geocenamus*, but support the synonymy of the two latter genera. Monophyletic nature of *Pratylenchoides* was also supported in accordance with already available morphological and molecular studies. The multivariate data analysis approach showed robust enough to summarize relationship between morphometric characters and group genera, species, and populations of the nematodes and in particular help to identify the genera and species of *Amplimerlinius* and *Pratylenchoides*. However, additional and more comprehensive studies on numerical methods will lead to provide objective and stable classifications for certain groups of plant-parasitic nematodes having taxonomic problems including Merliniidae and Telotylenchidae.

Literature Cited

Abdollahi, M. 2009. Analysis of cyst and cone top morphometrics of Indian populations of maize cyst nematode. Journal of Plant Protection Research 49:41–47.

Akyazi, F., Felek, A. F., Čermák, V., Čudejková, M., Foit, J., Yildiz, S., and Háněl, L. 2015. Description of *Paratylenchus (Gracilacus) straeleni* (De Coninck, 1931) Oostenbrink, 1960 (Nematoda: Criconematoidea, Tylenchulidae) from huzelnut in Turkey and its comparison with other world populations. Helminthologia 52:270–279.

Anderson, R. V. 1977. Additional diagnostic characters and relationships of *Merlinius laminatus* (Wu, 1969) Siddiqi, 1970 (Nematoda: Merliniinae). Canadian Journal of Zoology 55:1923–1926.

Andràssy, I. 2007. Free-living nematodes of Hungary, II (Nematoda errantia). Budapest, Hungary: Hungarian Natural History Museum.

Arias, M. 1970. *Paratrophurus loofi* n. gen., n. sp. (Telotylenchidae) from Spain. Nematologica 16:47–50.

Azizi, K., Eskandari, A., Karegar, A., Ghaderi, R., van den Elsen, S., Holterman, M., and Helder, J. 2016. Morphological and molecular data support the monophyletic nature of the genus *Pratylenchoides* Winslow, 1958 (Nematoda: Merliniidae) and reveal its intrageneric structuring. Nematology 18:1165–1183.

Baldwin, G., Luc, M., and Bell, A. H. 1983. Contribution to the study of the genus *Pratylenchoides* Winslow (Nematoda: Tylenchida). Revue de Nématologie 6:111–125.

Bert, W., Leliaert, F., Vierstraete, A. R., Van Fleteren, J. R., and Borgonie, G. 2008. Molecular phylogeny of the Tylenchina and evolution of the female gonoduct (Nematoda: Rhabditida). Molecular Phylogenetics and Evolution 48:728–744.

Blackith, R. M., and Blackith, R. E. 1976. A multivariate study of *Tylenchus* Bastian 1865 (Nematoda, Tylenchidae) and some related genera. Nematologica 22:235–259.

Brzeski, M. W. 1998. Nematodes of Tylenchina in Poland and temperate Europe. Warszawa, Poland: Muzeum I Instytut Zoologii PAN.

Cantalapiedra-Navarrete, C., Navas-Cortés, J., Liébanas, G., Vovlas, N., Subbotin, S. A., Palomares-Rius, J., and Castillo, P. 2013. Comparative molecular and morphological characterisations in the nematode genus *Rotylenchus: Rotylenchus paravitis* n. sp., an example of cryptic speciation. Zoologischer Anzeiger 252:246–268.

Carta, L. K., Skantar, A. M., and Handoo, Z. A. 2010. Molecular rDNA phylogeny of Telotylenchidae Siddiqi, 1960 and evaluation of tail termini. Journal of Nematology 42:359–369.

Castillo, P., Siddiqi, M. R., and Gomez Barcina, A. 1989. Studies on the genus *Paratrophurus* Arias (Nematoda: Tylenchina) with descriptions of two new species. Nematologia Mediterranea 17:83–95.

Chenari Bouket, A. 2013. HCA of *Criconemoides* species (Nematoda: Criconematidae), with a proposal for unknown species identification. Archives of Phytopathology and Plant Protection 47:90–105.

De Grisse, A. 1969. Redescription ou modification de quelques techniques utilisees dans L'etude des nematodes phytoparasitaires. Meded Riijksfaculteit der landbouwwetenschappen Gent 34:351–369.

Decraemer, W., and Hunt, D. J. 2006. Structure and classification. Pp. 3–32 *in* R. N. Perry and M. Moens, eds. Plant nematology. Wallingford, Oxon, UK: CABI Publishing.

Decraemer, W., and Hunt, D. J. 2013. Structure and classification, Pp. 3–39 *in* R. N. Perry and M. Moens, eds. Plant nematology. Wallingford, Oxon, UK: CABI Publishing.

Diederich, J., Fortuner, R., and Milton, J. 1997. Constraction and integration of large character sets for nematode morpho-anatomical data. Fundamental and Applied Nematology 20:409–424.

Fortuner, R. 1982. On the genus *Ditylenchus* Filipjev, 1936 (Nematoda: Tylenchida). Revue de Nematologie 5:17–38.

Fortuner, R. 1984. Morphometrical variability in *Helicotylenchus* Steiner, 1945. 5: On the validity of ratios. Revue de Nématologie 7:137–146.

Fortuner, R. 1993. Comparison of measurements and number of annuli in *Caloosia paradoxa* (Nemata: Hemicycliophorinae). Fundamental and Applied Nematology 16:121–127.

Fortuner, R., and Luc, M. 1987. A reappraisal of Tylenchina (Nemata). 6. The family Belonolaimidae Whitehead, 1960. Revue de Nématologie 10:183–202.

Fortuner, R., and Maggenti, A. R. 1991. A statistical approach to the objective differentiation of *Hirschmanniella oryzae* from *H. belli* (Namata: Pratylenchidae). Revue de Nématologie 14:165–180.

Fortuner, R., Maggenti, A. R., and Whittaker, L. M. 1984. Morphometrical variability in *Helicotylenchus* Steiner, 1945. 4: Study of field populations of *H. pseudorobustus* and related species. Revue de Nématologie 7:121–135.

Geraert, E. 2006. Nematology monographs and perspectives, vol. 4: Functional and detailed morphology of the Tylenchida (Nematoda). Leiden, the Netherlands: Koninklijke Brill NV.

Geraert, E. 2011. The Dolichodoridae of the world, identification of the family Dolichodoridae (Nematoda: Tylenchida). Ghent, Belgium: Academia Press.

Geraert, E. 2013. The Pratylenchidae of the world, identification of the family Pratylenchidae (Nematoda: Tylenchida). Ghent, Belgium: Academia Press.

Ghaderi, R., and Karegar, A. 2014a. Contribution to a revision of the genus *Pratylenchoides* (Nematoda: Merliniidae), with redescription of *P. erzurumensis* Yüksel, 1977 from Iran. Zootaxa 3900:339–369.

Ghaderi, R., and Karegar, A. 2014b. Description of *Amplimerlinius uramanatiensis* sp. n. (Nematoda: Dolichodoridae) and observations on three other species of the genus from Iran. Zootaxa 3869:17–32.

Ghaderi, R., and Karegar, A. 2016. One new and three known species of *Geocenamus* Thorne & Malek, 1968 (Nematoda: Merliniidae) from Iran. Zootaxa 4079:151–178.

Ghaderi, R., Karegar, A., Niknam, G., and Subbotin, S. A. 2014. Phylogenetic relationships of Telotylenchidae Siddiqi, 1960 and Merliniidae Siddiqi, 1971 (Nematoda: Tylenchida) from Iran as inferred from the analysis of the D2-D3 expansion fragments of 28S rRNA gene sequences. Nematology 16:863–877.

Gómez-Barcina, A., Siddiqi, M. R., and Castillo, P. 1992. The genus *Bitylenchus* Filipjev, 1934 (Nematoda: Tylenchida) with descriptions of two new species from Spain. Journal of the Helminthological Society of Washington 59:96–110.

Goodacre, R., Vaidyanathan, S., Dunn, W. B., Harrigan, G. G., and Kell, D. B. 2004. Metabolomics by numbers: Acquiring and understanding global metabolite data. Trends in Biotechnology 22:245–252.

Gozel, U., Lamberti, F., Duncan, L., Agostinelli, A., Rosso, L., Nguyen, K., and Adams, B. J. 2006. Molecular and morphological consilience in the characterisation and delimitation of five nematode species from Florida belonging to the *Xiphinema americanum*-group. Nematology 8:521–532.

Holterman, M., Karssen, G., van den Elsen, S., van Megen, H., Bakker, J., and Helder, J. 2009. Small subunit rDNA-based phylogeny of the Tylenchida sheds light on relationships among some high–impact plant-parasitic nematodes and the evolution of plant feeding. Phytophathology 99:227–235.

Hunt, D. J., Bert, W., and Siddiqi, M. R. 2013. Tylenchidae and Dolichodoridae. Pp. 209–250 *in* R. H. Manzanilla-López, and N. Marbán-Mendoza, eds. Practical plant nematology. Montecillo, Mexico: Bibliotheca Basica de Agricultura.

Johnson, D. E. 1998. Applied multivariate methods for data analysts. Michigan: Duxbury Press.

Johnson, R. A., and Wichem, D. W. 2002. Applied multivariate statistical analysis. 5th ed. Upper Saddle River, NJ: Prentice Hall Publication.

Lamberti, F., and Ciancio, A. 1993. Diversity of *Xiphinema americanum*-group species and hierarchical cluster analysis of morphometrics. Journal of Nematology 25:332–343.

Lamberti, F., Molinari, S., Moens, M., and Brown, D. J. F. 2002. The *Xiphinema americanum*-group. II. Morphometric relationships. Russian Journal of Nematology 10:99–112.

Lopez, R. C. 1986. Nematodes asociados al arroz en Costa Rica. I. *Paratrophurus costarricensis* sp.n. Nematropica 16:177–184.

Maggenti, A. R. 1991. Nemata: Higher classification. Pp. 147–187 *in* W. R. Nickle, ed. Manual of agricultural nematology. New York: CRC Press.

Mokaram Hesar, A., Mahdikhani Moghadam, E., and Tanha Maafi, Z. 2011. Morphometrical and genetic diversity of *Meloidogyne javanica* isolates from the north east of Iran. Journal of Nematode Morphology and Systematics 14:1–11.

Panahandeh, Y., Pourjam, E., Atighi, M. R., and Pedram, M. 2014. Morphological and molecular characterization of three species of the genus *Pratylenchoides* Winslow, 1958 (Tylenchina, Merliniidae, Pratylenchoidinae) from Iran. Journal of Crop Protection 3(Supplementary):691–709.

Quicke, D. L. J. 1993. Principles and techniques of contemporary taxonomy. London, UK: Chapman & Hall.

Ryss, A. Y. 2007. Taxonomy and evolution of the genus *Pratylenchoides* (Nematoda: Pratylenchidae). Parazitologyia 41:161–194. [In Russian].

Siddiqi, M. R. 1979. Taxonomy of the plant nematode subfamily Merliniinae Siddiqi, 1970, with descriptions of *Merlinius processus* n. sp. *M. loofi* n. sp. and *Amplimerlinius globigerus* n. sp. from Europe. Systematic Parasitology 1:43–59.

Siddiqi, M. R. 2000. Tylenchida, parasites of plants and insects. 2nd ed. Wallingford, Oxon: CABI Publishing.

Sneath, P. H. A., and Sokal, R. R. 1973. Numerical taxonomy. San Francisco, CA: W. H. Freeman.

Sturhan, D. 2011. On lateral fields and deirids in Merliniinae (Telotylenchidae) and the genus *Pratylenchoides* (Pratylenchidae). Journal of Nematode Morphology and Systematics 14:179–182.

Sturhan, D. 2012. Contribution to a revision of the family Merliniidae Ryss, 1998, with proposal of Pratylenchoidinae subfam. n., *Paramerlinius* gen. n., *Macrotylenchus* gen. n. and description of *M. hylophilus* sp. n. (Tylenchida). Journal of Nematode Morphology and Systematics 15:127–147.

Subbotin, S. A., Sturhan, D., Chizhov, V. N., Vovlas, N., and Baldwin, J. G. 2006. Phylogenetic analysis of Tylenchida Thorne, 1949 as inferred from D2 and D3 expansion fragments of the 28S rRNA gene sequences. Nematology 8:455–474.

Subbotin, S. A., Vovlas, N., Crozzoli, R., Sturhan, D., Lamberti, F., Moens, M., and Baldwin, J. 2005. Phylogeny of Criconematina Siddiqi, 1980 (Nematoda: Tylenchida) based on morphology and D2-D3 expansion segments of the 28s-rRNA gene sequences with application of a secondary structure model. Nematology 7:927–944.

van Megen, H., van den Elsen, S., Holterman, M., Karssen, G., Mooyman, P., Bongers, T., Holovachov, O., Bakker, J., and Helder, J. 2009. A phylogenetic tree of nematodes based on about 1200 full-length small subunit ribosomal DNA sequences. Nematology 11:927–950.

Whitehead, A. G., and Hemming, J. R. 1965. A comparison of some quantitative methods of extracting vermiform nematodes from soil. Annals Applied Biology 55:25–38.

Ye, W., and Robbins, R. T. 2004. Cluster analysis of *Longidorus* species (Nematoda: Longidoridae), a new approach in species identification. Journal of Nematology 36:207–219.

Ye, W., and Robbins, R. T. 2005. Morphological observation on *Longidorus crassus* Thorne, 1974 (Nematoda: Longidoridae) and its intraspecies variation. Journal of Nematology 37:83–93.

Zancada, M. C., and Lima, M. B. 1985. Numerical taxonomy of the genera *Rotylenchus* Filipjev, 1936 and *Orientylus* Jairajpuri & Siddiqi, 1977 (Nematoda: Tylenchida). Nematologica 31:44–61.