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Guidelines for species descriptions of free-living aquatic nematodes: characters, measurements and their presentation in taxonomic publications

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Abstract

Free-living aquatic nematodes are abundant, diverse and of general environmental importance. However, knowledge of species distributions of both marine and freshwater nematodes is sparse. Species distribution data are crucial for evaluating environmental impacts from human activities and to conduct integrated nematode community assessments. Basic knowledge on taxonomy and species descriptions is lacking for many regions due to decreasing taxonomic expertise, yet it is essential for biodiversity research and for building molecular sequence libraries for the application of methods such as environmental DNA. In order to encourage and facilitate taxonomic and descriptive work on this understudied group,

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we present here a framework for nematode species description. We begin by providing a brief overview of nematology history, then provide suggestions of microscopic methods that should be used and provide a list of characters essential for morphometric species descriptions. Finally, we briefly discuss common molecular sequencing approaches that are commonly used in nematode taxonomic literature.

Key words: Nematoda, Enoplea, Chromadorea, taxonomy, morphology, species description

General approach to defining nematode morphospecies

Morphological characters continue to be the main source of taxonomic information for the majority of free-living aquatic nematode species, and as such remain important for species descriptions, identifications, evolutionary studies, and ecological analyses (Decraemer & Backeljau 2015). General rules for species descriptions are established by the International Code of Zoological Nomenclature (ICZN, 2024). General rules and recommendations for avoiding most common mistakes in species descriptions were recently published by Braby *et al.* (2024).

Progress in microscopy and nematology has led to more accurate and comprehensive descriptions of morphological characters and their intraspecific variability. Goodey (1959) was among the first to publish a list of all the morphological characters that are needed for species descriptions. Standardization of species description for aquatic nematodes was discussed at the First Workshop on the Systematics of Free-living Nematodes in Gent (Date: August 1977) and a list of characters important for species description and identification was subsequently published (Coomans *et al.* 1978). Later, an emended and concise version of the list of these characters was included in the Appendix to the Proceedings of the First Meeting on Free-Living Nematodes in USSR (Platonova & Tsalolikhin 1981). Lorenzen (1981 and 1994) introduced a comprehensive extension and improvement of characters and character states to be observed and described for free-living marine nematodes. A brief but not exhaustive list for recording numerical and non-numerical taxonomic data for species descriptions was provided in Platt & Warwick (1983, 1988) and Warwick *et al.* (1998). These publications, though valuable, are not always consistent, and do not always include all the characters required for species descriptions.

Over the last decades, the number of new species descriptions has increased as did the number of morphological characters used for species delimitation (Hodda 2022). The quality of recent species descriptions varies greatly and they often do not allow complete comparison and diagnosis from congeners. Here, using our experience describing species, reviewing articles, and maintaining the Nemys database (Nemys eds. 2024), we, the active and former Nemys/WoRMS editors, present a brief checklist of characters and measurements that should be taken into account for new aquatic species descriptions.

Light microscopy (LM) remains the basis for the morphological description and identification of free-living aquatic nematodes. Indeed, microscopic observation of morphological characters is still essential for diagnosis of proposed new species from those described and named previously. Electron microscopy, such as scanning (SEM) and transmission (TEM), are widely used to observe ultrastructural and internal details and structures, providing additional information that enhances our understanding of morphological characters and their functions (*e.g.* Tchesunov 2015, Tchesunov *et al.* 2012). In recent times, confocal microscopy (CM) is also employed in combination with light or electron microscopy to assist in the interpretation of characters (Semprucci *et al.* 2016). In order to conduct ecological and faunal studies, which rely on light microscopy observations, it is imperative that species descriptions, or at the very least, differential diagnoses, provide light microscopy observations. Additional morphological characteristics using SEM or CM are highly informative and can be included in the diagnosis, if it is clearly mentioned that these are only visible using SEM or CM.

The theoretical limit of resolution for the light microscope is about 0.2–0.4 μm (Heintzmann & Ficz 2006, Weisenburger & Sandoghdar 2015), and the practical limit for measurement is estimated as 0.5 μm. It should be noted that person-to-person variability in morphological measurements of nematodes using LM is 2–5% (Mazurkiewicz *et al.* 2016). The method of fixation of specimens also affects measurements through shrinking of nematodes, especially when fixatives include alcohol.

Drawings made using a light microscope equipped with a *camera lucida*, remain the most accessible and best method to represent the 3-dimensional morphology of nematodes into a 2-dimentional plane and interpretation of characters. Drawings may now be made digitally based on light micrographs instead of using a *camera lucida*, but this may result in a lower level of detail and accuracy due to optical aberrations and loss of resolution with

pixellation (especially in JPEG files). There are examples of very good drawings which can be used as references, and some useful recommendations are given by Ye & Hunt (2021).

For a new species name to be available, a description and a diagnosis must be based on a physical specimen deposited in a museum and labelled so that they are unambiguously identifiable (Article 72.10 of ICZN). That particular specimen is called the *holotype* (chosen among the other specimens as the most representative and with the best visible diagnostic characters), and is the name bearer and the universal reference for that name. All other specimens from the type locality included in the description are regarded as *paratypes* (unless explicitly excluded). For the description of aquatic free-living nematodes, the *holotype* is usually a male because of more specific characters enabling it to be distinguished among other species within the same genus. However, species descriptions based on a single gender are to be avoided if at all possible (except parthenogenetic species). Historically, many species have been described based on females only: many of them are now considered *species inquirendae* until male specimens are found and formally described. If possible, juveniles should be included in the description of the type series.

Here, we provide a complete list of characters to be included in species descriptions to help researchers ensure that they provide a comprehensive species description. For the detailed explanation of nematodes anatomy and morphology, description of terms and possible variations in organization, one should refer to general articles (Decraemer *et al.* 2014) and the most recent descriptions of closely-related species. At the time of writing, the most complete and comprehensive book on nematodes is that of Schmidt-Rhaesa (2014).

A species description is concise and written in telegraphic style (i.e., no conjugated verbs). It should include the description of a number of male and female characters and character states in a consistent order using correct and precise terminology. A description should begin with the description of the gender of the holotype and indicate variations of characters and measurements in paratypes. The description should be done in the following order: (1) general appearance, external structures from anterior to posterior, from LM to SEM or confocal observations, (2) internal structures grouped per system: digestive system, structures in pseudocoel (nerve ring, presence of pseudocoelomocytes), secretory-excretory system, reproductive system, and (3) tail region. It is followed by the description of the other gender and juveniles if present, focusing on any differences between the sexes and/or life stages.

Species description

Basic information

Reference to higher taxa (family, order) should be included in the title of manuscript or placed before the description. It is recommended to register new species to Zoobank and refer to it in the species description.

Species name: scientific name is indicated as *species nova* (**sp. nov.** or **sp. n.** or **n. sp.**). The name must be given following the rules defined by the International Code of Zoological Nomenclature.

Material examined: number of specimens used for species description by gender and age (with separation of juveniles by stage of development and of females (gravid or not), when possible).

Deposition of the type series: museum or institute (name, city, country), type of preservation (slide or other); collection number/slide number; specify, if different for holotype and paratypes, or the type series is split and stored separately to avoid risk of loss.

Type locality and distribution: geographical coordinates; region of the world (sea or country and province), exact locality (bay, bight, river, lake, nearest city, etc.); sampling date; collector's name; research vessel, cruise and station numbers (with reference to publication, if the cruise report had been published or it is stored in accessible database).

Habitat: biotope, sediment physical characteristics, water depth (depth range), associated plants or animals, collection date(s).

Etymology: the origin of the species epithet and its meaning, and the grammatical gender of the species epithet.

Basic measurements

Traditionally, nematode species descriptions begin with basic determination of body size, including total body length and several morphometric indices. The first set of relative quantitative characters was developed by de Man (1886) and includes three body measurement indices that are still used to characterize species:

a = total body length / maximum body diameter
b = total body length / pharynx length
c = total body length / tail length

In early papers (late 19th to beginning of 20th century) these indices were often referred to using the Greek alphabet: α, β and γ for a, b and c, respectively.

Later on, the index V was added:

V = distance from anterior end to vulva / total body length (expressed as %)

Several other indices were developed using anterior end to anus/cloaca distance or anal/cloacal diameter as a denominator: a', b', c' and V', the latter two being still commonly used.

The a', b' and V' are used for specimens with long tails that are broken:

V' = distance from anterior end to vulva / distance from anterior end to anus (expressed as %);
a' = length from anterior end to anal or cloacal opening / maximum body diameter,
b' = length from anterior end to anal or cloacal opening / pharynx length;
c' = tail length / body diameter at anus or cloacal aperture.

The exact form of these indices varied over time and from paper to paper. In the late 19th century, Cobb's formula was also proposed (Cobb 1890a, 1890b). Like de Man's indices Cobb's formula was placed at the beginning of the descriptions. It includes some important morphometrics: distances from head end to several structures written in the numerator and the corresponding body diameters in the denominator; the formula starts with gender sign (and number of specimens) and the total body length is given at the end in mm or μm. One should be careful when reading these formulas, because in some texts it has been presented in other forms, such as the percentage of total length instead of absolute measurements in the numerator.

Nowadays this formula is no longer in use. Instead of Cobb's formula a much clearer and more comprehensive list of all measurements is now used and is normally given in a table. The measurements to be included in tables are listed in section "Measurements" below.

Description of morphological characters

We assume researchers intending to describe new species are familiar with general nematode morphology as well as with the peculiarities of the taxon under study and the relevant terminology.

Habitus: body shape: e.g., long, short, stout, filiform, fusiform, elongate, Ɛ (epsilon)-shaped; presentation on the slide: e.g., ventrally- or dorsally-curved, S-shaped. Degree of tapering anteriorly or posteriorly. Body coloration (if present).

Cuticle: surface appearance, e.g., smooth, with transverse striation, annulations, ornamentation (e.g., punctations, spines, longitudinal bars), lateral differentiation (longitudinal ridges, lateral alae and fields, etc.), longitudinal differentiation (i.e., differences in ornamentation between anterior, middle, and/or posterior body regions), layering (two-, three-layered under LM, aspect of the different layers); presence of body pores and setae, their type, number and position.

Cephalic region: shape (e.g., rounded, truncated); continuous or set off by constriction or depression, presence or absence of cephalic capsule (as an area where basal lamina of the pharynx accreted with somatic cuticle in Enoplida or conspicuously thickened, non-annulated and thickened cuticle in Chromadorea); lips—shape, number.

Cephalic sensilla: basic set in three circles of 6+6+4 sensilla: typically anterior most circle of six inner/internal labial sensilla, second circle of six outer/external labial sensilla, third circle of four cephalic sensilla (posterior to the lips). The sensilla may be too small to be seen under LM, papilliform, setiform or pore-like; setiform sensilla may be jointed.

In some taxa, the posterior two circles can merge, resulting in a second circle of 10 sensilla: the outer labial sensilla can move posteriorly and appear at the level as the cephalic sensilla or conversely the cephalic sensilla can move forward to the level of the outer labial sensilla. The length of sensilla and their relative length to the corresponding body diameter at the level of cephalic setae need to be given.

Somatic sensilla: shape (i.e., setose, papillose, or pores), arrangement (e.g., mostly in rows or irregular), length, number of longitudinal rows (if arranged in rows), differentiation in density/length/number of longitudinal rows between body regions, presence of associated glands. Subcephalic sensilla situated in cephalic or anterior pharyngeal region—if present and differ from somatic setae. Cervical setae—somatic setae anterior to the nerve ring. The patterns of somatic setae of the anterior region are rather variable and the terminology is not well settled.

Amphids: shape, size, relative size (in relation to the corresponding body diameter), position (in µm from the anterior extremity to the anterior edge of the amphid aperture and relative to the cephalic sensilla and buccal cavity); any difference in shape and size of amphideal fovea (amphid cavity) relative to amphideal aperture (amphid opening); *corpus gelatum* (protruding or not); other peculiarities (number of turns in spiral amphid and direction of winding (dorsally, ventrally), location on cuticularised plaque); sexual dimorphism.

Ocelli or pigment spots (if present): position, structure (presence of lenses), shape and size.

Mouth opening (usually terminal), but may be offset towards the periphery.

Buccal cavity (if present): shape and relative size; dimensions; degree of cuticularization of the walls and teeth; subdivisions (cheilostom and pharyngostom (= gymnostom + stegostom), if visible); armature: teeth (their number, shape and structure (solid or hollow), relative size, position), presence of denticles, mandibles, stylet, spear-like tooth.

Pharynx (=oesophagus): muscular or muscular and glandular; undivided (largely cylindrical) or subdivided into a corpus and terminal bulb or into a *procorpus*, *metacorpus* (median- or anterior bulb), isthmus and terminal bulb (postcorpus), or multiple bulbs along posterior part. For each of bulb: with or without lumen wall thickening.

Cardia: Presence/absence (type of pharyngo-intestinal junction, if cardia absent); if present: size, shape, embedded in pharynx or separate; surrounded by intestine or not; presence or absence of cardiac glands.

Intestine: general shape, presence or absence of anterior ventricular region or posterior prerectum, inclusions, content, shape of lumen wall cells, presence or absence of microvilli (if visible); trophosome.

Nerve ring: position from anterior end and location with respect to pharynx.

Secretory-Excretory (SE) system: position of SE pore, ampulla (present/absent), length of cuticle-lined terminal duct, number of supporting cells, position and size of ventral gland(s) (= renette cell).

Reproductive system—male:

Gonad(s): monorchic (single) or diorchic (double), opposite or in parallel arrangement (in case of diorchic), testes outstretched or reflexed; position relative to intestine (left, right, ventral). Description of the parts of the reproductive system: germinal zone, *vesicula seminalis*, *vas deferens*, ejaculatory glands; spermatids; spermatozoa (also often observed in female reproductive tract).

Copulatory apparatus: Spicules: number, shape, size, equal or unequal, simple or composite; head (=*capitulum* or *manubrium*) offset or not, shaft (= *calamus*) differentiated or not; blade (= *lamina*) with or without *velum*. Gubernaculum: shape, presence or absence of apophyse(s), dorsally or caudally oriented, shape of distal part and distal denticles (if present), simple or complex (including *corpus* = main part, *cuneus* = central projection between the spicules and *crura* = lateral guiding pieces). Copulatory muscles, protractor and retractor muscles of spicules and gubernaculum.

Supplements: precloacal/postcloacal; shape, size, number, position, distribution, length of the mid-ventral row, distance from the cloacal opening, differences in shape and size along the row.

Reproductive system—female:

Genital branches: Number: single (monodelphic) or double (didelphic), shape (outstretched, reflexed), position relative to intestine (left, right, ventral). Ovaries (relative size, germinal and ripening zones, oocytes arrangement); genital tract (oviduct, sphincter, *uterus*). Presence, location and shape of spermatheca / sperm cells. Uterine egg (shape, size, differentiations, if any). Vulva (shape, orientation, differentiations; lips, *epiptygma*). Vagina (length,

shape, differentiation: *pars distalis*, *pars refringens*, *pars proximalis*); vaginal constrictor and dilator muscles; vaginal glands.

Eggs: their size and stage of development; ovoviviparity.

Demanian system (in Oncholaimids): type and structure.

Tail: shape (e.g., conical, conico-cylindrical, clavate, elongate, filiform, rounded), flagellum (if present: often partly or wholly lost during processing), relative size, caudal glands (number and position, restricted to tail or not), spinneret (shape, if present), caudal and (sub)terminal setae.

Measurements

The list of measurements includes a number of mandatory characters used in descriptions of all nematodes, as well as taxon-specific characters. The choice of additional characters to be included in the body measurements table strongly depends on the taxon in question—it is advisable to include in the table all the characters that are diagnostic, i.e., that are used to differentiate species in this taxon. Most of measurements should be present in a table, but some of them may be mentioned in the text description instead. Body measurements should be given in the table or in the text, but not both. Round up the measurements according to LM resolution and avoid extra decimals (see more about resolution in Appendix). Necessity of presentation of individual measurements for the whole type series is discussed by Brito de Jesus *et al.* (2023). Examples of basic measurements are given, for example, in Warwick *et al.* (1998). In more complicated cases particularly of measurements of head and caudal regions one may refer to Andrássy (2011, p. 3), Peña-Santiago *et al.* (2014), Ye & Hunt (2021), Zell (1993) and Zullini *et al.* (2001).

Basic measurements

Total body length

Maximum body diameter

de Man indices:

a = total body length / maximum body diameter

b = total body length / pharynx length

c = total body length / tail length (and its variant c′ = tail length / body diameter at anus or cloacal aperture)

and vulva indices:

V = anterior end-to-vulva distance / total body length (%)

V′ = anterior end-to-vulva distance / anterior end-to-anus distance (%)

Other various ratios relevant to described species can be added in the text or in the table and has to be properly described in the text. Do not use the same abbreviations and symbols that are already in use for other measurements.

Cephalic and pharyngeal region

Cephalic diameter: body diameter at the level of cephalic sensilla (if present as a 2nd or 3rd circle).

Lips height (if distinct)

Cephalic capsule length and width (if present)

Cuticle: thickness; annulation and differentiation (punctations, lateral fields etc.—if measurable).

Anterior sensilla by circle: length, distance from the anterior extremity.

Somatic sensilla: length, distance from the anterior if in groups.

Amphideal aperture and fovea: diameter or length and width (for each if different), distance from the anterior end, % of corresponding body diameter (c.b.d).

Buccal cavity: length and width (in external outline), total and by parts, if distinct. Teeth (or other buccal armature—onchs, mandibles…): size (separately for dorsal and ventrosublateral, if different).

Pharynx: length, width; cardial bulb: length and width, diameter at the level of cardia/pharyngo-intestinal junction.

Cardia length and width (if present)

Nerve ring: the distance from the anterior apex to the middle of the nerve ring—absolute value and in relation to pharynx length expressed in %.

Deirid: distance from the anterior end to deirid—absolute value and in relation to pharynx length expressed in %.

Secretory-excretory system: distance from the anterior end to secretory-excretory pore—absolute value and in relation to pharynx length expressed in %, size of ampulla and duct, distance for the excretory pore to ventral gland, size of ventral gland, distance from the anterior and corresponding body diameter.

Reproductive system of male

Testis: length (for each if diorchic), length of each zone (if possible), size and shape of spermatids/spermatozoa, noting any dimorphism.

Distance from the anterior tip of testis to cloacal opening in relation to total body length expressed in % (indicated as "T"). This parameter is optional, depending on the degree of maturity.

Spicules: length (for each if unequal) by arch (mandatory) and by chord (optional), preferably both; size of *capitulum* (if present).

Gubernaculum: length, length of apophysis or apophyses (if paired).

Precloacal and postcloacal supplements and papillae: number, size (sizes, if different), length of the row (distance from the anteriormost supplement to cloacal opening), distance from the cloacal opening.

Reproductive system of female

Vulva: Distance from the anterior end in absolute units, distance in relation to total body length expressed in % (V%) and in relation to anterior end-to-anus distance expressed in % (V′).

Genital branch: Length of each genital branch in relation to total body length expressed in %, Length of each genital branch via flexure when present and expressed as % of total body length, G1 for anterior genital branch; G2 for posterior genital branch in case of didelphic system.

If one of the genital branches is reduced to uterine sac (anterior/pre-vulval or posterior/post-vulval)—length of the sac as an absolute value and in relation to vulval body diameter expressed as a decimal.

Vagina: length in absolute value and in relation to vulval body diameter expressed as a decimal.

Egg: length and width if the character is diagnostic, egg length/width ratio, fertilized eggs in uterus/uteri.

Tail region

Anal body diameter or diameter at cloacal opening

Rectum: length as an absolute value and in relation to anal body diameter expressed as a decimal.

Tail: length total and by parts if present. Flagellum (filiform part), if present (often broken and that should be mentioned also for total length; a', b' and c' are suggested to use in that case instead of original de Man indices).

Number of tail rings: indicate how counted (dorsally, ventrally).

Caudal setae: length.

Terminal setae: length, position of setae base from tail terminus.

Note: many taxa have a number of taxon-specific characters not present in other nematodes (like number of body rings or desmens in the Desmoscolecida and metanemes in the Enoplida) that may need to be included in the table, but cannot practically be covered in this review—latest species descriptions and redescriptions should be consulted.

Illustrations

Scale bars must be present on every figure.

Line drawings

Always illustrate the holotype and another gender (if available) as follows.

General view: the total figure of both genders to represent proportions and the position of main systems (digestive, reproductive etc.). Information of the orientation (right or left view) should be given. Traditionally, the body oriented with anterior end towards the top of the plate, posterior end towards the bottom. Position illustrations straight, not oblique.

Detail of anterior end: (at highest magnification possible) with buccal cavity and armature, anterior sensilla, amphideal fovea (and aperture, if different), anterior pharynx and cuticular structure (the latter can be shown in separate figure in case of complicated surface structures like annulation, amphid, etc).

Details of pharynx and secretory-excretory system

Detail of specific diagnostic features: such as cuticular structure and differentiation along the body, somatic sensilla.

Reproductive system (male and female)

Copulatory apparatus (highest magnification)

Different parts of reproductive system of male and female with indication of position of testis/testes and ovary/ovaries and details of male copulatory apparatus at highest magnification.

Tail region of male and female: with details of male copulatory system (spicules and gubernaculum, pre- and postcloacal supplements, sensilla).

Microphotographs

LM, SEM, TEM, 3D models and other visualizations of the observation may be added to the text or as supplementary materials. Light microphotographs cannot replace drawings but can be useful to show the appearance of certain structures. LM microphotographs are recommended as an objective control for the interpretation of the drawing.

Tables

Presenting the overview of all morphometric measurements. All of the measurements of each specimen should be present separately in the table or in e-supplement. List of the examined material and sampling sites' information may be summarized in a separate table if necessary. Give range and average with standard error when relevant number of specimens is available.

Genetic information

Morphological descriptions of nematode species are increasingly complemented by genetic data. The integration of genetic information into taxonomy, phylogeny, and ecology could bring significant changes, such as an increase in the identification of cryptic species, shifts in system topology, and revised estimates of local and global diversity. Although this remains a topic of active debate, we present here the minimal set of genetic data that should ideally accompany species descriptions. The most critical point is to ensure that this information is related to the same species, which has been described by morphology.

For nematode taxonomy, researchers often employ molecular techniques such as polymerase chain reaction (PCR) to amplify and sequence specific regions of genes. The sequences can then be compared among different nematode species to assess their genetic similarities and differences, aiding in the classification and identification of these organisms.

It is important to note that the specific regions targeted for sequencing may vary depending on the research goals and the taxa under investigation. Use of both ribosomal RNA (rRNA) and mitochondrial cytochrome c oxidase subunit I (COI) gene sequences has been common in nematode taxonomy and phylogenetic studies. The most commonly used sequences are:

Ribosomal RNA (rRNA) Sequences:

Target Genes: The small subunit ribosomal RNA (18S or SSU) and large subunit ribosomal RNA (28S or LSU) are commonly targeted for analysis.

Advantages:

- Universality: These genes are highly conserved across different organisms, making them suitable for studying a wide range of taxa.
- Conserved and Variable Regions: The conserved regions provide a stable backbone for phylogenetic analysis, while variable regions (e.g., D2–D3) can offer insights into species-specific differences.

Cytochrome c Oxidase Subunit I (COI) Sequences:

Target Gene: COI is a mitochondrial gene commonly used for DNA barcoding.

Advantages:

- Maternal Inheritance: Mitochondrial genes, including COI, are maternally inherited, allowing for the tracking of maternal lineages.
- Variable Regions: COI has variable regions that are useful for distinguishing closely related species.
- DNA Barcoding: COI sequences are often used as a molecular barcode for species identification.

Combined Approach:

Complementary Information: Using both ribosomal and mitochondrial markers can provide complementary information, as mitochondrial genes like COI evolve faster than nuclear ribosomal genes.

Resolution: The combination of markers can enhance resolution at different taxonomic levels, from higher-order relationships to species identification.

Differential diagnosis

This is an important part of the description of a new species, which briefly summarizes the most important characters that distinguishing a new species from known species within the genus; it also confirms its allocation within the genus—diagnostic characters, which approves that, must be mention here.

Concluding remarks or Discussion

The last section should contain a comparison of the new species with other members of the genus or closely related species to confirm the novelty of the finding. Taxonomic significance of characters, new characters included in the description and other discussion points may be included in the section. Other pertinent information that may be included for a comprehensive approach includes biogeography, behavior and phylogeny. Also, this section is devoted to comment and discuss relevant aspects of the morphology, taxonomy, geographical distribution, etc. of the new species described. The updated list of species or the identification key may be added here. A good species description should include the genus diagnosis and a list of all species in the genus. This will help readers evaluate the completeness and quality of the description.

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Appendix

Precision and rounding off (De Ley, personal communication)
In practice, it is impossible to measure nematodes under a light microscope with accuracy greater than 0.5 µm, even with a high-quality 100x objective. For lower magnifications, the measurement error becomes significantly greater. It is therefore meaningless to write down measurements with decimals other than .0 or .5! In practice, you should round off measurements more or less as follows:

- Objective 4x: measure in the range of 1000–5000 µm; round off to 50 µm
- Objective 10x: measure in the range of 500–2000 µm; round off to 10 µm
- Objective 20x–25x: measure in the range of 100–1000 µm; round off to 5 µm
- Objective 40–65x: measure in the range of 20–150 µm; round off to 1 µm
- Objective 100x: measure in the range of 1–50 µm; round off to 0.5 µm

Ratios (a, b, c, etc.) and percentages (V, G, etc.) are derived from two measurements and are therefore subject to lower accuracy than either of the two measurements. In practice, round off as follows:

- use two decimals for ratios below 1 (*e.g.* 0.346 should be 0.35)
- use one decimal for ratios between 1–10 (*e.g.* 4.64 should be 4.6)
- do not use decimals for ratios greater than 10 (*e.g.* 10.78 should be 11)
- do not use decimals for percentage values (*e.g.* 55.6% should be 56%)

When you draw, stay clear from the edge of your field of view: all lenses (even the ones that are supposedly corrected) cause some distortion at the edges.

When to calculate statistics—and when not
Mean and standard deviation are useful for characterizing variation in populations. When calculating these, you should keep in mind that they depend on the assumption that you have measured individuals from one and the same natural population. Do *not* calculate means etc. of measurements from specimens pooled from different samples, even when they were collected in the same locality at different times. Also, there is no point in calculating statistics (especially standard deviations) for fewer than five specimens (in fact, statistical theory requires that you should try to measure at least thirty specimens!).

The *coefficient of variation* (cv) is a useful aid in assessing the correctness of your measurements: calculate it to check for any exceptional values in a series of data, and re-measure the specimen(s) that have such a value to make sure you did not make a mistake. The *cv* also allows you to compare variability and reliability of different characters: the lower a *cv* is, the more the character in question could be important in identification.

*cv= standard deviation * 100 / average*